

Molecular Oncology

ISSN 1574-7891
Volume 19 Supplement 1
June 2025

A journal for discovery-driven translational cancer research



2025.eacr.org

WILEY

www.moloncol.org

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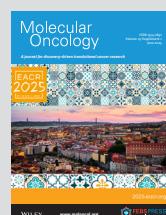
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Abstracts submitted to the 'EACR 2025 Congress: Innovative Cancer Science', from 16–19 June 2025 and accepted by the Congress Organising Committee are published in this Supplement of *Molecular Oncology*, an affiliated journal of the European Association for Cancer Research (EACR).

Online ISSN: 1878-0261

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Proffered Papers

10-minute talks awarded for the highest scored abstracts, embedded in the scientific symposia sessions. These presentations are not accompanied by a poster.

Posters in the Spotlight

Tuesday 17 June, 18:40-20:00 and
Wednesday 18 June, 18:40-20:00

Dedicated sessions taking place in the spotlight area within the Exhibition Hall. Poster presenters with high-scoring abstracts will give short presentations of up to 5 minutes each. Their posters will also be available to view during the Poster Discussion Sessions.

PROFFERED PAPER PRESENTATIONS

Top Abstracts

EACR25-0851

Oncostatin M cytokine signalling orchestrates immune remodelling and metabolism within the breast tumour microenvironment

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Introduction

Currently, there are not efficient therapies with curative intent for advanced breast cancer (BC). Chronic inflammation is a well-established hallmark of cancer and a major driver for metastasis, which is the leading cause of death in BC patients. Understanding how inflammatory signals orchestrate pro-malignant effects in the tumour microenvironment (TME) is key to designing new therapeutic strategies to target tumour-promoting inflammation.

Material and method

Cytokines are the main regulators of inflammation. We recently characterized the cytokine Oncostatin M (OSM), as a central node for multicellular interactions within the breast TME¹. In the current unpublished work, we examine the effect of OSM on tumour immune remodelling and metabolism within the TME, by combining molecular biology techniques, flow cytometry, seahorse analyses and multiomics (including single cell RNA sequencing, proteomics and metabolomics) on patients' data, murine models and cell lines.

Result and discussion

Our findings identified myeloid cells as the primary source of OSM in human breast cancer and healthy breast. In vivo studies showed that activation of the OSM receptor OSMR remodels the breast tumour immune landscape by increasing macrophage and neutrophil recruitment, in addition to decreasing the number of CD8+ T cells. The opposite effect is observed when the OSM/OSMR pathway is blocked in OSMR KO mice. In addition, OSM increases the expression of key chemokines and inhibitory immune checkpoint regulators, and the phagocytosis-inhibiting signals, indicating a relevant role for OSM/OSMR signalling in immune regulation. Furthermore, OSM stimulation enhances metabolic reprogramming of cancer cells, CAFs and tumours, increasing glycolysis and hypoxia-related gene expression. Finally, our findings reveal a common mechanism via hypoxia inducible factors for both OSM-induced metabolic reprogramming and immune evasion.

Conclusion

This work unravels the mechanisms by which OSM influences the immune landscape and metabolic configuration of the TME and lays the groundwork for further development of targeted interventions to disrupt this pro-tumoral signalling.

EACR25-1471

¹⁷⁷Lu-Trastuzumab as a promising therapy for resistant brain metastases in HER2+ breast cancer

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Introduction

Breast cancer (BC) is the most prevalent malignancy in women, with HER2 amplification detected in 25–30% of metastatic cases. While HER2-targeted therapies such as trastuzumab have significantly improved patient survival, their efficacy in HER2+ brain metastases (BrM) is often compromised by acquired resistance and limited blood-brain barrier (BBB) permeability. This study investigates the potential of trastuzumab radiolabeled with the β^- -emitting radionuclide $[^{177}\text{Lu}]$ [Lu-DOTA-Trastuzumab] as a strategy to overcome resistance in HER2+ BrM.

Material and method

HER2+ BC cell lines and their brain-tropic derivatives were evaluated for HER2 expression and sensitivity to trastuzumab and $[^{177}\text{Lu}]$ [Lu-DOTA-Trastuzumab]. In vivo models were established via orthotopic implantation of HER2+ BC cells for primary tumor development and intracardiac injection to generate BrM. Tumor progression was monitored using magnetic resonance imaging (MRI), while HER2 expression was assessed through $[^{89}\text{Zr}]$ Zr-DFO-Trastuzumab positron emission tomography (PET) imaging. BBB permeability across metastatic lesions was evaluated using dynamic contrast-enhanced MRI (DCE-MRI).

Result and discussion

Brain-tropic HER2+ cells maintained HER2 expression but exhibited resistance to trastuzumab. In contrast, $[^{177}\text{Lu}]$ [Lu-DOTA-Trastuzumab induced significant DNA damage and cytotoxicity in vitro. PET imaging confirmed specific radiotracer uptake in HER2+ primary tumors and BrM. A single dose of $[^{177}\text{Lu}]$ [Lu-DOTA-Trastuzumab effectively suppressed primary tumor growth and led to complete BrM remission in 40% of treated animals. BBB permeability varied across metastatic lesions, potentially influencing radiotracer uptake and therapeutic response.

Conclusion

These findings highlight $[^{177}\text{Lu}]$ [Lu-DOTA-Trastuzumab as a promising therapeutic strategy to overcome trastuzumab resistance in HER2+ BrM. Its potent anti-tumor effects and ability to target brain metastases underscore its potential to improve outcomes in patients with metastatic HER2+ BC.

EACR25-1517**IL-1R8 acts as an immune checkpoint in CD8+ T cells, limiting cytokine-induced anti-tumor immune responses**

R. Garuti¹, D. Supino², A. Mariancini¹, E. Magrini², L. Minutte², J. Zeleznjak³, E. Bonavita², S. Jaillon², A. Mantovani², C. Garlanda²

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Introduction

IL-1R8 is an atypical Interleukin-1 receptor (ILR) family member that controls inflammation by dampening ILR and Toll-like receptor (TLR) downstream pathways. Studies conducted on IL-1R8-deficient NK cells highlighted its pivotal role as immune checkpoint and negative regulator of IL-18-dependent activation. In particular, IL-1R8-deficiency has been shown to trigger NK cell-mediated immune surveillance against hematogenous metastasis, liver cancer and viral dissemination. Currently, genetic and pharmacological targeting of ILR and IL-18 are under investigation to improve CD8+ T cell-based immunotherapy. The relevance of IL-1R8 in mitigating IL-18-driven activation and, conversely, the pathways induced by IL-1R8 loss-of-function in T lymphocytes are still elusive.

Material and method

Combining transcriptomic analysis, high dimension flow cytometry, genetic silencing/inhibition, and *in vivo* models, we investigated IL-1R8 expression by CD8+ T cells and its function in Tumor Infiltrating Lymphocytes (TILs) and T-cell based immunotherapies.

Result and discussion

IL-1R8-deficiency led to anti-tumor immune resistance against colon carcinoma and fibrosarcoma by enhancing CD8+ TIL fitness, synergizing with the immune checkpoint inhibitor anti-PD-1. TILs exhibited accelerated maturation/proliferation kinetic, induction of IL-2R complex and increased tumoricidal potential independently of antigen presenting cells (APCs). Mechanistically, we found that IL-1R8 controlled both IL-2/EOMES/IL-2R signaling axis and IL-18-mediated Type-1 polarization thus affecting the production of antitumor molecules Interferon-γ (IFNγ) and Granzyme B (GZMB) in antigen-specific and polyclonal T cell responses. Relevant to T cell therapy, we reported increased antigen-induced proliferation for IL-1R8-deficient OT-I cells *in vitro* and superior tumoricidal potential of IL-1R8-deficient OT-I T cells *in vivo*. In human, we demonstrated that IL-1R8 is rapidly induced in early-mature precursors and the central memory stage of CD8+ T cells in healthy donors and cancer patients. Finally, an original anti-human IL-1R8 inhibitor and CRISPR-Cas9 mediated IL-1R8 ablation potentiated both primary human T lymphocytes and CAR-T cells against B-cell lymphoma.

Conclusion

Overall, the study revealed a profound impact of IL-1R8 deficiency or blockade on CD8+ T lymphocyte immunobiology, which promoted cytokine-induced responses and

conferred superior anti-tumor potential. Thus, IL-1R8 is a promising and targetable immune checkpoint of both NK and CD8+ T cells to be exploited for implementing cancer immunotherapy.

EACR25-1932**Genome-Wide CRISPR Screening Identifies Lipid-Sensing Chemosensor Receptors as Regulators of Tumor-Associated Macrophage Function in Cancer**

G. Marelli¹, S. Puccio¹, P. Colombo¹, M. Lazzeri¹, L. Morosi¹, E. Bonavita¹, F. Bertoni², L. Walsh³, E. Lugli¹, D. Di Mitri¹

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Introduction

The infiltration of macrophages is considered a hallmark of cancer progression, and re-education of tumor-associated macrophages (TAMs) toward an anti-tumor phenotype is a promising strategy in cancer immunotherapy. However, the mechanisms governing macrophage education by cancer cells remain largely elusive, posing challenges for therapeutic exploitation. Understanding the molecular pathways involved in TAM plasticity could lead to novel therapeutic approaches to enhance anti-tumor immunity.

Material and method

To gain deeper insights into TAM plasticity, we conducted an unbiased genome-wide CRISPR screening on primary macrophages, allowing us to identify key regulators involved in macrophage polarization within the tumor microenvironment. The functional impact of selected candidate genes was assessed *in vitro* and *in vivo* using murine cancer models, while human prostate cancer tissues were analyzed using innovative spatial strategies, such as spatial lipidomics and imaging mass cytometry, to confirm translational relevance.

Result and discussion

Our investigation confirmed the role of known regulators, in orchestrating TAMs responses and unveiled novel insights into TAMs behavior. Notably, we identified olfactory and vomeronasal receptors, commonly referred to as chemosensors, as major drivers of macrophage tumor-supportive functions. Both the genetic deletion and the pharmacological inhibition of selected chemosensors in TAMs *in vivo* led to cancer regression and an increased infiltration of tumor-reactive CD8+ lymphocytes, suggesting a role in shaping the immune landscape of the tumor microenvironment. In human prostate cancer tissues, we found that palmitic acid binds to Olfactory Receptor 51E2 (OR51E2) expressed by TAMs, thereby enhancing their pro-tumoral phenotype. To further validate these findings, we conducted spatial analysis, which confirmed the presence of palmitic acid in close proximity to TAMs within prostate cancer patient samples. This supports the notion that lipid mediators contribute to macrophage-driven immune suppression in tumors.

Conclusion

Collectively, our findings highlight the role of chemo-sensors in macrophage sensing of the lipid-enriched tumor microenvironment. By demonstrating their impact on macrophage function and tumor progression, this study identifies chemosensor receptors as potential therapeutic targets for enhancing anti-tumor immunity. Targeting these receptors could provide a novel strategy to reprogram TAMs and improve cancer immunotherapy outcomes.

EACR25-2439

A novel spatial DNA sequencing method for the detection of somatic mutations from hundreds of regions in archival tissue sections

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Introduction

Somatic mutations are typically identified in cancer using bulk sequencing which results in loss of histological context. Spatial transcriptomics has been used to profile copy number alterations, but it is limited by low genomic resolution and high noise. We hypothesised that a low cost high-throughput spatial DNA sequencing method would enable more accurate characterisation of genomic changes in tissue sections.

Material and method

We developed a novel method called Adaptive Resolution Multiscale Spatial (ARMS) DNA sequencing to characterise the genome at sub-millimetre resolution, while preserving the histopathological context in formalin fixed paraffin embedded (FFPE) tissue sections. FFPE sections (4 µm) from prostate cancer patient samples were laser captured as 0.09–0.25 mm² tiles. Uniquely barcoded whole genome libraries from the tiles were sequenced on the Illumina platform. Copy number calling was performed using CopyKit. The Segment Anything Model 2 (SAM2) was fine-tuned to segment prostate epithelium, from which tissue (nuclear and gland) features were extracted.

Result and discussion

We successfully derived whole genome copy number profiles at 50 kb resolution in more than 1000 tiles from 3 patient samples. Copy number calls were confirmed by bulk whole genome sequencing data from adjacent tissue. Furthermore, principal component analysis showed distinct grouping of the tiles suggesting spatially distinct genomic profiles. Tiles with similar copy number profiles were clustered to identify subclones, which in turn were used to build phylogenetic trees. Mapping the subclones to spatial transcriptomics from adjacent sections revealed significantly altered gene expression between the subclones. We identified 2 previously undiscovered (by bulk WGS) subclones through spatial DNA sequencing. Prostate gland clustering independent of genomic status identified morphologically distinct regions (> 3 in each

patient), which were also genetically distinct. Nuclei in different prostate cancer regions (high grade vs. low grade) were also more intensely stained (1.5-fold, $p < 0.0001$).

Conclusion

We have developed a novel spatial DNA sequencing method, which facilitates cost-effective profiling of somatic mutations from whole slides. When it is integrated with histopathological data and spatial transcriptomics, it reveals a more complete picture of cancer evolution and enables the identification of ‘early’ and ‘late’ morphologies. Integration of spatial genomics with digital pathology has the potential to reveal hidden relationships between genotype and phenotype.

Symposium: Next-generation Immunotherapy Modalities

EACR25-0734

Synergistic effect of thiopurine 6TG and immunocheckpoint blockade to inhibit melanoma growth

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Introduction

Significant developments in cancer immunotherapy, such as immune checkpoint inhibitors (ICI), have revolutionized the treatment of metastatic patients. Despite these breakthroughs, a group of patients does not respond favorably to ICI, partly due to a lack of cancer neoepitopes. Mutations acquired during tumorigenesis can increase the number of neoepitopes on tumor cells, eliciting a cytotoxic T-cell response that is then boosted by ICI therapy. We use a compound called thiopurine 6-thioguanine (6TG) to trigger random mutations in the tumor in order to enhance the number of neoepitopes produced by tumor cells. In preclinical models of low-mutational burden melanoma, we demonstrated that 6TG-induced mutations enhanced T cell-mediated tumor suppression and significantly improved responsiveness to anti-PD-1 therapy. However, while this strategy augmented immune activation, it remained insufficient to achieve complete tumor regression (Nazerai et al. 2023).

Material and method

YUMM1.1 (Yale University Murine Melanoma) cells have been co-cultured with mouse splenocytes; cell viability and flow cytometry analyses have been performed to evaluate T-cell mediated cell death of tumor cells treated or not by 6TG. YUMM1.1-based syngeneic mouse model has been used to test the effect of 6TG with anti-CTLA-4 monotherapy or combined with anti-PD-1. Tumor growth kinetics and immune memory have been assessed.

Result and discussion

We explored additional ICI combinations to optimize treatment outcomes, specifically evaluating anti-CTLA-4 alone or in combination with anti-PD-1 in 6TG-treated tumors. Our findings revealed that anti-CTLA-4 – particularly when combined with anti-PD-1 – achieved complete tumor remission, but only when treatment was initiated at smaller tumor volumes. In contrast, efficacy declined when therapy began at later stages, emphasizing the critical importance of early intervention.

Additionally, we found that mice experiencing complete tumor regression after 6TG + ICI treatment developed long-term immune memory. When re-exposed to tumor cells, these mice effectively suppressed tumor growth, suggesting durable protection against recurrence.

Conclusion

These findings (Nazerai et al. 2025) have significant clinical implications, highlighting the potential of 6TG-based combinatorial strategies to extend the benefits of immunotherapy to low-TMB patients and overcome current resistance.

EACR25-2222

Systematic identification of recurrent immunogenic neoantigens in treatment-resistant tumors

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Introduction

Cancer immunotherapy revolutionized cancer treatment paradigms. Yet, only around 20% of the patients respond to immunotherapy. Neoantigens have emerged as promising targets in cancer immunotherapy since they are tumor-unique and can mediate an effective tumor-directed T-cell response. Recurrent neoantigens, derived from hotspot mutations, are becoming increasingly attractive targets for immunotherapy as they are shared among large groups of patients and may pave the way toward “off-the-shelf” cancer therapies. However, identifying immunogenic ‘HLA-peptide’ pairs represents a significant challenge. HLA genes are the most polymorphic region in the human genome and are highly variable. Our study aimed to uncover recurrent neoantigens presented by HLA class I molecules, focusing on genes linked to treatment resistance. This research is crucial because new approaches that generate long-lasting therapeutic responses are critically needed, especially for cancer patients with tumors that have evolved resistance to existing therapies.

Material and method

We have developed a novel analysis pipeline, SpotNeoMet, emphasizing the identification of recurrent, clonal, driver mutation-derived neoantigens specifically presented on metastatic tumors resistant to specific targeted therapies. Analysis of large patient cohorts from the Hartwig Medical Foundation dataset and the Cancer

Genome Atlas followed by systematic HLA immuno-peptidomics.

Result and discussion

In this study, using SpotNeoMet, we discovered three recurrently presented novel neo-peptides derived from the Androgen Receptor (AR) H875Y mutation, the most common castration-resistant prostate cancer mutation. We validated these neoantigens as highly immunogenic and isolated their cognate T-cell receptors (TCRs) from healthy donor PBMCs. Further characterization revealed that these TCRs are highly sensitive and specific to their targets, demonstrating remarkable efficiency in recognizing and eliminating prostate cancer cells presenting these neo-peptides.

Conclusion

The emergence of resistance to targeted treatments in metastatic cancer patients underscores the urgent need for innovative therapeutic approaches. Our study introduces SpotNeoMet and using AR H875Y mutations as a proof of concept, we demonstrate the robustness of our pipeline in developing a T cell-based immunotherapy.

SpotNeoMet promises a systematic method to uncover ‘HLA-peptide’ pairs and their cognate TCRs across treatment-resistant cancers. Importantly, our data suggest that resistance drivers can be transformed into therapeutic opportunities through various immunotherapy modalities.

Symposium: Early-Onset Cancers

EACR25-1102

Geographic and age-related variations in mutational processes in colorectal cancer

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Introduction

Colorectal cancer incidence rates vary geographically and have changed over time. Notably, in the past two decades, the incidence of early-onset colorectal cancer, affecting individuals under the age of 50 years, has doubled in many countries. The reasons for this increase are unknown.

Material and method

We investigated whether mutational processes contributed to geographic and age-related differences by whole-genome sequencing 981 colorectal cancer genomes from 11 countries, and examining their

mutational signature dynamics and driver mutations in cancer driver genes.

Result and discussion

No major differences were found in microsatellite unstable cancers, but variations in mutation burden and signatures were observed in the 802 microsatellite-stable cases. Multiple signatures, most with unknown etiologies, exhibited varying prevalence in Argentina, Brazil, Colombia, Russia, and Thailand, indicating geographically diverse levels of mutagenic exposure. Signatures SBS88 and ID18, caused by the bacteria-produced mutagen colibactin, had higher mutation loads in countries with higher colorectal cancer incidence rates. SBS88 and ID18 were also enriched in early-onset colorectal cancers, being 3.3 times more common in individuals diagnosed before age 40 than in those over 70, and were imprinted early during colorectal cancer development. Colibactin exposure was further linked to APC driver mutations, with ID18 responsible for about 25% of APC driver indels in colibactin-positive cases.

Conclusion

This study reveals geographic and age-related variations in colorectal cancer mutational processes, and suggests that early-life mutagenic exposure to colibactin-producing bacteria may contribute to the rising incidence of early-onset colorectal cancer.

EACR25-1472

Fibroblast specific endoglin deletion increases colorectal cancer tumorigenesis in a subset-specific manner

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Introduction

Growing evidence highlights the heterogeneity of fibroblast subsets, with distinct populations playing different roles in the initiation, progression, and metastasis of colorectal cancer (CRC). While we previously reported that endoglin expression on cancer-associated fibroblasts promotes metastatic spread in CRC, its role in early-stage tumor development is unknown. Here, we investigated whether and how the fibroblast subset-specific deletion of endoglin influences tumorigenesis in a chemically induced colitis-associated CRC model.

Material and method

We generated two inducible fibroblast-specific endoglin (ENG) knockout mice Collagen1α1 CreERT2.ENGfl/fl (ENGCol1α1^{-/-}) and Collagen1α2-CreERT.ENGfl/fl (ENGCol1α2^{-/-}). Cre-mediated recombination in mice was induced by oral administration of tamoxifen for three consecutive days. Polyp formation was induced by a single injection of azoxymethane (AOM), followed by three cycles of dextran sodium sulphate (DSS). Immunohistochemistry (IHC) staining was performed to evaluate the stromal compartment and immune infiltration in polyps. Flow cytometry was used to analyze immune cell composition in colons.

Result and discussion

ENGCol1α1^{-/-} mice developed significantly more colonic polyps than non-induced controls (average 20

vs. 6, respectively, $P < 0.0001$), whereas ENGCol1α2^{-/-} showed no significant difference in polyp count (average 16 vs. 13, respectively, $P = 0.1557$). Immunohistochemical analysis revealed an increase in total stroma content (vimentin-positive) and a higher number of activated α-smooth muscle actin-positive fibroblasts in ENGCol1α1^{-/-} mice, whereas no significant changes were observed in ENGCol1α2^{-/-} mice. Moreover, ENGCol1α1^{-/-} mice showed increased infiltration of F4/80⁺ macrophages and Ly6G⁺ neutrophils, compared to controls. These findings suggest that Collagen1α1 subset-specific endoglin deletion enhances polyp formation, stroma expansion, and myeloid infiltration in AOM/DSS-induced polyps. To investigate early immune changes, which might be instrumental in polyp initiation, mice were sacrificed after the first DSS cycle. Flow cytometry analysis showed an increase in F4/80⁺ Ly6C⁺ macrophages in ENGCol1α1^{-/-} colons, whereas their number decreased in ENGCol1α2^{-/-} colons, despite no change in the overall CD45⁺ immune cell population. Furthermore, the number of F4/80⁺ CD206⁺ M2-like macrophages was also increased in ENGCol1α1^{-/-} colons, suggesting their potential role in shaping a protumorigenic microenvironment.

Conclusion

Collagen1α1-specific endoglin deletion promotes polyp formation by enhancing stromal expansion and myeloid infiltration, whereas Collagen1α2-specific deletion does not impact tumor development. These findings highlight the intriguing and opposite roles that different fibroblast subsets can play in tumorigenesis.

Symposium: Brain Metastasis

EACR25-1283

Reshaping Tumor-Vascular-Immune Crosstalk by VEGFR-3 Modulation in Breast Cancer Brain Metastases

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Introduction

Brain metastases affect 10–30% of metastatic breast cancer patients. Limited treatment options and poor clinical outcomes highlight the unmet need for effective therapies. The lymphatic vasculature plays a critical role in cancer progression, serving as the primary route for metastasis and promoting an immunosuppressive microenvironment by inhibiting cytotoxic T cells and dendritic cell maturation. However, its role in brain metastases remains unclear. Here, we investigate whether modulating VEGFR-3, a key receptor in endothelial lymphatic cells, can normalize lymphatic vasculature, influence immune cell infiltration in the tumor microenvironment (TME), and reduce tumor growth and metastases.

Material and method

VEGFR-3 expression was assessed in EO771 and 4T1 breast cancer (BC) and 2H-11 lymphatic endothelial cell

lines. Cell tolerance to VEGFR-3 inhibitor (VEGFR-3i), SAR131675, was evaluated in vitro using increasing compound concentrations. VEGFR-3i was tested ex vivo in 2D and 3D cultures of BC TME-derived cells. BC and breast brain metastases (BBM) in vivo models were generated via orthotopic inoculation of murine EO771 and 4T1 cell lines. For BBM, the primary tumor was surgically removed, mimicking clinical conditions, followed by intracranial inoculation. VEGFR-3i was administered orally, intravenously, or intraperitoneally. Lymph nodes (draining and non-draining), primary tumors, and BBM were collected at various time points to assess VEGFR-3 expression and perform immune profiling via spectral flow cytometry.

Result and discussion

In vitro studies showed minimal VEGFR-3 expression among cell lines, as expected, with a dose-dependent reduction in metabolic activity following SAR131675 treatment. Cytotoxic effects occurred only at higher concentrations, with triple-negative 4T1 being the most sensitive. In 2D primary cultures, VEGFR-3 expression was higher in BBM than primary BC, underscoring its critical role in metastases. In 3D-spheroid models, BBM displayed increased aggressiveness compared to BC, as evidenced by greater growth and sprouting. However, VEGFR-3 modulation effectively controlled BC spheroid growth and sprouting, with a moderate effect on BBM. In vivo, VEGFR3i reduced tumor volume and reprogrammed the TME.

Conclusion

These findings suggest that normalizing lymphatic vasculature through VEGFR3 modulation can reshape the tumor-immuno-lymphatic landscape, fostering a more effective anti-tumor immune response and potentially enhancing immunotherapy efficacy.

EACR25-1678

Loss of Kmt2c or Kmt2d drives brain metastasis via KDM6A-dependent upregulation of MMP3

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Introduction

Breast cancer is the most common cancer type worldwide accounting for more than 2 million newly diagnosed cases in 2020. Despite recent development of new therapies, disease-free survival rates of triple-negative breast cancer (TNBC) patients are still relatively low. The histone modifier KMT2C and KMT2D are among the most commonly mutated genes across all cancer types including TNBC. However, their direct role in promoting metastases which could be exploited for a targeted therapy approach is still unknown.

Material and method

To unravel functional implications in metastases we generated CRISPR/Cas9-mediated Kmt2c and Kmt2d KO derivatives of murine TNBC cells and used them in immunocompetent mouse models. We performed

scRNA-seq to characterize the specific immuno-microenvironment of KO tumors and combined histone mass spectrometry, RNA-seq and ChIP-seq to identify metastasis-driving events. Functional studies using genetic or pharmacologic inhibition of metastases driver were combined with experimental mouse models to validate our findings.

Result and discussion

Loss of Kmt2c or Kmt2d did not impact tumor growth in vivo, however, KO tumors were highly metastatic to multiple organs. Despite profound changes in immune cell populations in primary tumors, metastatic phenotypes were independent of a functional immune system. Tumor cell intrinsic analysis showed direct target of Kmt2c/d, H3K4me1 levels were not altered, however, H3K27me3 was reduced and H3K27ac was increased in KO cells. In accordance, ChIP-seq for these histone modifications revealed KO-specific changes and ChIP-seq peaks for KDM6A, another histone modifier, were highly enriched. By integrating our multi-omics data we identified the matrix metalloproteinase Mmp3 as a main target upregulated upon Kmt2c/d KO which we also validated in KMT2C KO patient samples. Functional data showed that MMP3 expression is independent of H3K4me1 levels but depends on H3K27 demethylase KDM6A or acetyltransferase p300 activity. Finally, genetic or pharmacologic perturbation of KDM6A decreases MMP3 expression eventually preventing Kmt2c/d KO cells from metastasizing to the brain.

Conclusion

This study revealed that mutations in Kmt2c and Kmt2d are sufficient to induce brain metastases through MMP3 upregulation and that KDM6A inhibition might be a novel strategy to treat or prevent metastasis.

Symposium: Clinical Cancer Biomarkers

EACR25-0504

Epigenetic regulation shapes the tumor microenvironment in NSCLC and impacts the outcome of immune checkpoint blockade therapy

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Introduction

Immune Checkpoint Inhibitors (ICI) have revolutionized advanced Non-Small Cell Lung Cancer (NSCLC) treatment, but low response rates highlight the need to understand response mechanisms. This work explored the

impact of epigenetics and viral mimicry in the immunogenicity of the tumor microenvironment (TME) and ICI outcome in NSCLC.

Material and method

We collected primary tumors from 30 advanced NSCLC patients before first-line anti-PD1, and evaluated the epigenetic (MethylationEPIC array), transcriptomic (RNAseq) and somatic mutation profiles (WES). We evaluated differential methylation and expression between patients with disease control at 6 months (DC, 12) and non-responder patients (NR, 18). Epigenetic silencing was assessed by integrating methylation and transcriptomics, defining genes as silenced if >30% promoter methylation correlated with lower expression (Wilcoxon $p < 0.05$).

Result and discussion

A random forest classifier integrating 156 differentially methylated CpGs, 49 differentially methylated regions and 123 differentially expressed genes discriminated DC patients (AUC 0.972). RNAseq confirmed the functional consequences of DNA methylation, revealing signaling pathways enriched in both differentially methylated and expressed genes. Pro-proliferative signaling such as Ras, NF-kB or Wnt were downregulated in DC, while CD8+ T cell differentiation and activation was upregulated. NR patients showed a global upregulation of the PI3K-Akt pathway. Despite the mutational profile of our cohort did not explain the expression differences, we found aberrant methylation of the PI3K-Akt pathway, with some of its dependent genes epigenetically silenced. Collins et al (JITC 2022) reported that PI3K-Akt activation enables ICI resistance and immune evasion. Concordantly, we found that PIK3CA and AKT3 expression correlated with the expression of immunosuppressive cytokines. Isoyama et al (JITC 2021) showed that combining PI3K inhibitors with ICI can enhance antitumor immunity by turning a ‘cold’ into a ‘hot’ TME. In line with our findings, the baseline activation of PI3K-Akt pathway predicts a poor ICI response in NSCLC. We also report that the tumor load of transposable elements (TE) derived from RNAseq positively correlated with the expression of antiviral responses, IFN I and III, and JAK/STAT pathway activation. DC patients with longer progression free survival (PFS) had the highest TE load, while those with low TE load and weak antiviral response show shorter PFS, suggesting the presence of viral mimicry which was proposed to enhance tumor immunogenicity, favoring ICI response (Ng et al, Nature 2023).

Conclusion

DNA methylation shape the TME and serve as predictive baseline biomarkers for ICI response in NSCLC.

Precisely, PI3K-Akt pathway is affected by epigenetic silencing and its activation may indicate a poor response to anti-PD1, while a viral mimicry state may enhance tumor immunogenicity and ICI response.

EACR25-0818

Monitoring genetic and epigenetic evolution of metastatic colorectal cancer through longitudinal ctDNA profiling

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Introduction

Circulating tumour DNA (ctDNA) is a valuable resource for monitoring the evolving molecular landscape of cancers under the selective pressure of therapy. Here, we are evaluating ctDNA molecular dynamics in metastatic colorectal cancer (CRC) patients enrolled into the MAYA trial, whereby patients with microsatellite-stable and O6-methylguanine-DNA methyltransferase silenced tumours receive temozolamide (TMZ) as immune sensitizing strategy followed by immunotherapy.

Material and method

ctDNA was isolated from serially collected plasma samples at baseline, post-TMZ induction, during immunotherapy and, when available, at disease progression. Whole genome ctDNA and germline DNA libraries were generated by using NEBNext® Ultra™ II kit and indexed with Unique Dual Index (UDI) UMI Adaptors. Pools with up to 9 ctDNA and germline DNA libraries were subjected to targeted exome capture by using Twist® Human Comprehensive Exome kit. Additionally, NEBNext® Enzymatic Methyl-seq ctDNA libraries were pooled and enriched for cancer-specific methylated regions using a targeted panel.

Result and discussion

A total of 95 plasma samples from 28 patients were initially profiled through deep whole exome sequencing. To reconstruct the subclonal architecture of longitudinal ctDNA samples, we developed LOBSTER, a computational tool, adapted from the previously established method MOBSTER (Caravagna et al., 2020). By using this approach, we were able to successfully discern subclonal versus clonal mutations and most importantly we identified, in every patient, clones which were expanding under treatment. In 40% of patients, these expanding clones were driven by mutations in mismatch repair genes, in particular MSH6. Notably, we observed the emergence of the SBS11 signature, characteristic of temozolamide exposure, along with an increased tumor mutational burden exclusively in MSH6-mutant patients. This suggests that the acquisition of MSH6 mutations serves as a biomarker of temozolamide-induced genetic alterations. Next, we investigated transcription factor binding site accessibility (TFBS), inferred from low-pass whole genome sequencing data, and identified an epigenetic rewiring, particularly in patients who initially responded to immunotherapy. Furthermore, distinct methylation patterns were also observed throughout immunotherapy and at disease progression, suggesting that epigenetic changes, in addition to the expansion of clones driven by genetic events, might contribute to tumour evolution and disease progression.

Conclusion

Taken together, these results underscore the potential clinical utility of longitudinal ctDNA analysis for studying real-time colorectal cancer genetic and epigenetic evolution.

Symposium: Molecular Mechanisms of Metastasis

EACR25-0080

Circulating tumor cells shed a new class of "shearosome" large extracellular vesicles in capillary bifurcations that drive metastatic processes

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Introduction

Circulating tumor cells (CTCs) and their clusters are the drivers of metastasis, but we have an incomplete understanding of how they interact with capillary beds. One open question is why are CTCs isolated downstream of capillary beds smaller than those isolated upstream of capillary beds? Is it possible that forces within capillaries may reduce the size of CTCs in the microvasculature?

Material and method

We designed 13 variants of microfluidic microchannel models that mimic the geometric variations present in human capillary bifurcations. These devices were then used to explore how small cell lung cancer patient CTCs, CTC-derived explant (CDX) cells and cancer cell lines of breast, pancreatic and ovarian cancer origins behaved within these constrictions under physiological conditions. We then used a fluorescent microscopy, scanning electron microscopy, transmission electron microscopy, proteomics, RNAseq, flow cytometry and microfluidic models of endothelial microvascular networks to characterise CTC transit and their products.

Result and discussion

We observed the shedding of 1-11 micron diameter nuclei-free fragments by all tested cells within microfluidic bifurcations. Shedding of these fragments dependent on the size of CTCs, diameter constrictions and presence of bifurcations. Importantly, shedding reduced cell sizes up to 61%, which facilitated the transit of CTCs through bifurcations. We then used published MISEV guidelines to demonstrate that shed fragments are a novel subclass of large extracellular vesicles (LEVs), "shearosomes", that require shear stress for their biogenesis and whose proteome was enriched for proteins associated with immune-related pathways. Shearosomes exhibited functions characteristic of previously identified EVs including a) cell-directed internalization by endothelial and immune cells, and b) intercellular communication abilities relevant to pre-metastatic niche formation and metastasis such as disruption of endothelial barrier integrity in 2D and microfluidic models of microvasculature, polarization of monocytes towards M2 tumor-promoting macrophages lineages.

Conclusion

These findings suggest that CTCs may routinely shed a new class of highly reactive large extracellular vesicle that drive key processes involved in the formation of pre-metastatic niches and cancer metastasis.

EACR25-0182

Cockle transmissible cancers characterized by horizontal transfers and instability of mitogenomes

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Introduction

Transmissible cancers, or contagious cancers, are a disease in which cancer cells are transmitted from one individual to another, establishing new tumors in the recipient host. Initially identified in dogs and Tasmanian devils, these cancers have also been found in at least ten bivalve species, including cockles (*Cerastoderma edule*). In bivalves, the disease affects their hemocytes and manifests as hemic neoplasia (HN), a leukemia-like condition where cancer cells spread through the host and the marine environment until they colonize new hosts.

Material and method

This study provides an evolutionary perspective of transmissible cancers in cockles through the genomic and transcriptomic analysis of 7,800 individuals.

Result and discussion

Our findings emphasize the role of mitochondrial genome evolution in these cancers, revealing multiple horizontal mitochondrial DNA (mtDNA) transfers and recurrent amplifications in the control region. Unlike nuclear genomes, mtDNA is compact, encoding essential genes for oxidative metabolism so it is highly conserved. In cockles, mtDNA is maternally inherited, as supported by our analysis of 481 healthy individuals. Phylogenetic analysis of mtDNA revealed nine distinct monophyletic cancer lineages, demonstrating no overlap with their matched-normal haplotypes or evidence of non-transmissible cancer. While these nine mitochondrial lineages were identified, only two lineages were observed in nuclear DNA consistently corresponding to two histological phenotypes that were observed. This suggests that mitochondrial diversity does not directly correlate with nuclear genomes and phenotypic variation. To resolve this complexity, we developed a mtDNA deconvolution method based on tumor purity and allele frequency. This approach confirmed the presence of nine mtDNA cancer lineages, indicating that mitochondria from transient hosts have been repeatedly captured, likely avoiding the extinction of these transmissible cancer cells. Further investigation of mtDNA sequencing revealed three independent amplifications spanning the control region of the D-loop in three independent lineages, absent in healthy cockles. These amplifications share a

conserved start motif and overlapping microhomology at their boundaries, indicative of errors in DNA break repair. While their evolutionary significance remains uncertain, they may reflect selfish mitochondrial selection or confer a selective advantage to cancer cells. Interestingly, similar D-loop amplifications have been observed in other transmissible cancers, such as those in soft-shell clams, and even in human cancers.

Conclusion

Our findings provide compelling evidence for the dynamic evolution of mtDNA in bivalve transmissible cancers, offering new insights into their survival strategies and the potential mechanisms by which they survive in natural populations.

Symposium: Clonal Hematopoiesis

EACR25-0607

Unveiling the role of migratory dendritic cells in liver cancer

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Introduction

The liver immune milieu balances immune activation against pathogens with tolerance toward microbiota and food-derived antigens. However, this inherent tolerogenicity can impair effective anti-tumor immunity. Hepatocellular carcinoma (HCC) is a leading cause of cancer-related mortality worldwide, with most patients ineligible for surgery and resistant to immunotherapy. A prerequisite for efficient anti-tumor immunity is antigen presentation to T cells. Conventional dendritic cells (cDCs), particularly mature/activated cDCs (mDCs), can migrate to lymph nodes, cross-present tumor antigens, and secrete immuno-stimulatory signals that orchestrate cytotoxic responses. However, the role of mDCs in HCC immunity remains poorly understood.

Material and method

To evaluate the proficiency of mDCs in antigen cross-presentation, we performed *in vitro* assays using bone marrow-derived dendritic cells loaded with tumor-derived material from OVA-expressing cells. We compared mDCs with other cDC subsets by assessing their ability to process and present tumor antigens to OT-I T cells. *In vivo*, we utilized a murine HCC line (Hep53.4) implanted subcutaneously (s.c.) to study mDC function in tumor growth and antigen trafficking to the draining lymph node. To further investigate the role of mDCs in HCC, we employed two gene-targeted mouse models: one enabling mDC tracking (mDC-RED) and another allowing their inducible depletion (mDC-DTR). Two-photon microscopy was used to examine mDC localization in liver tumors. Additionally, we established autochthonous HCC models induced either by carcinogen

exposure or genetic manipulation. The impact of different oncogenic drivers on the tumor immune landscape was analyzed using flow cytometry and scRNAseq.

Result and discussion

We observed that mDCs exhibit superior CD8⁺ T cell activation capacity compared to other cDC subsets *in vitro*. In the Hep53.4 s.c model, we demonstrated that migratory DCs are the only subset capable of trafficking tumor-derived material to the tumor-draining lymph node. In an orthotopic Hep53.4 model, we observed that while mDCs accumulate near the tumor, they are actively excluded from the tumor mass. Similarly, in chemically induced HCC, mDC abundance is reduced, yet they remain the primary source of immuno-stimulatory cytokines within the tumor microenvironment. In β-catenin-driven HCC, we observed a profound reduction in mDC interactions with other immune cells, along with their complete exclusion from the tumor mass.

Conclusion

Our findings highlight a potential role for mDCs in orchestrating effective anti-tumor immune responses. However, their abundance and function appear to be shaped by the tumor microenvironment. Ongoing studies are aimed at further dissecting the mechanisms that regulate cDC activation, migration, and antigen presentation in HCC.

EACR25-2072

Bulk and spatial B cell receptor repertoire analysis to characterize antibody maturation in relation to tertiary lymphoid structures and response to immunotherapy

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Introduction

Recent studies have described B cells and tertiary lymphoid structures (TLS) as biomarkers of response to immune checkpoint inhibitors (ICI), notably in clear cell renal cell carcinoma (ccRCC). B cells are activated into TLS leading to the generation of IgG- and IgA-producing plasma cells (PCs). An elevated IgG tumor cell coverage predicts response of ccRCC patients treated with ICI (Meylan et al, *Immunity*, 2022). We are now dissecting that intra-tumoral antibody response focusing on class-switch recombination (CSR) and the identification of antibodies associated with response to ICI.

Material and method

From the BioniKK cohort which includes 199 patients with metastatic ccRCC treated in majority with ICI (159), we profiled BCR repertoire for both heavy (IGH) and light chains from 95 pre-treatment frozen tumors as well as pre- and post-ICI blood samples from 30 patients. TLS in tumors were assessed by immunohistochemistry and TLS transcriptomic signatures. Spatial BCR repertoires were also analyzed from previously generated Visium spatial transcriptomics on 12 frozen ccRCC sections, and with the SpatialVDJ method (Engblom et al, *Science*, 2024) for 4 ccRCC tumors. This approach enabled the targeted and more comprehensive spatial profiling of

full-length BCR repertoires with an increasing resolution, notably for IGH.

Result and discussion

BCR reconstructed from bulk RNAseq allowed to identify hundreds of unique IGH present in multiple isotypes per tumor suggesting that CSR can occur in tumors. The main CSR events are from IgG isotypes to other IgG, suggesting switch from memory B cells but some CSR from IgM were also identified suggesting that naïve B cells could also switch within the tumors. The highest numbers of IGH with multiple isotypes were found in TLS containing tumors, especially within tumors with the highest TLS signature scores, strongly suggesting that CSR occurs in TLS. Untargeted spatial transcriptomics indicated that the same IGH is found on different isotypes inside and outside TLS areas. Using targeted BCR capture with SpatialVDJ, we profiled hundreds of unique IGH which are distributed over more than a thousand spots consistent with B cells or PC gene expression. Grouping closely related IGH in clones, we were able to find amplified clones with more than hundreds individual cells distributed over the tissue within and outside TLS areas in different isotypes supporting the role of TLS in CSR. Analysis of tumors revealed that certain IGH clonotypes are shared between several responding patients and that same IGH clonotypes can be found in the tumor and the blood of the same patient blood before and after ICI treatment.

Conclusion

Overall, our results strongly suggest the involvement of TLS in antibody selection, amplification and CSR described through fine BCR repertoire analysis with potential response to ICI. Identification of BCR repertoire in responding patients may help uncover novel therapeutic antibodies.

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Introduction

Tumor-initiating mutations can occur years before cancer is diagnosed, offering an opportunity for early detection and prevention. In clear cell renal cell carcinoma (ccRCC), VHL is inactivated in 90% of cases, and PBRM1 mutations are found in 50%. Despite their role in carcinogenesis, the progression from VHL inactivation to cancer can take decades. This study uses a genetic mouse model with VHL/PBRM1 inactivation and single-cell sequencing to explore early ccRCC progression and identify potential intervention targets.

Material and method

We used a mouse model in which tamoxifen induces inactivation of Vhl and Pbrm1 in renal epithelial cells. This inactivation led to a spectrum of renal lesions, including tumours, appearing around 20 months post-treatment. Single-cell RNA-sequencing (scRNA-seq) and chromatin accessibility (scATAC-seq) were performed at multiple time points to explore transcriptional and chromatin changes.

Result and discussion

Using scRNA-seq, we identified a set of cells with VHL inactivation, primarily in Proximal Tubule (PT) S1 cells, possible origin of ccRCC. The transition to transitional states involved a wave of transcriptomic changes driven by transcription factors (TFs). scATAC-seq confirmed these TFs as key regulators of KO1-specific gene programs. Analysis of scRNA-seq data from an acute kidney injury (AKI) mouse model revealed similarities between early progression of Vhl/Pbrm1 null renal epithelial cells and AKI, with the same TFs activated. The key difference was that, unlike AKI, the TF expression in the mutant renal epithelium remained unresolved and continuously active. Further analysis of a large population-based dataset showed a strong association between AKI, CKD, and renal cancer, including ccRCC. Interestingly, some of these TFs are activated in normal mouse and human PT cells with age.

Conclusion

VHL and PBRM1 are the most commonly mutated tumor suppressors in ccRCC, and unlike other ccRCC driver genes, they are often clonally inactivated in human ccRCC. However, the majority of Vhl/Pbrm1 null cells in the mouse renal epithelium remain transcriptionally stable for months, with most mutant cells never progressing to tumor formation. The similarities and differences between the pre-tumor ccRCC and renal injury signatures suggest that ccRCC may arise from an improper response to kidney injury. This offers a perspective on cancer progression, possibly helping to explain the long latency between the genetic event and tumor formation, where intrinsic cellular responses to environmental insults, accumulating over time with aging, together with preceding genetic mutations, drive tumor development.

Symposium: RNA Biology in Cancer

EACR25-0509

Transcriptional state transitions link tissue injury and early carcinogenesis in the kidney

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EACR25-1366**SpliceSeek: a CRISPR-based platform for uncovering T-cell alternative splicing isoforms for immunotherapy**

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Introduction

RNA splicing generates isoform diversity that plays a critical role in T cell biology. However, the functional roles of many alternative splice isoforms remain unexplored. To bridge this gap, we constructed a splicing atlas for CD8 T-cells, uncovering a rich landscape of isoforms across distinct subsets. Building on these insights, we developed SpliceSeek, a CRISPR-based platform, to investigate the functional relevance of isoforms identified in the atlas. This study aims to uncover the relationship between splicing diversity and T-cell functionality and identify isoforms with therapeutic potential.

Material and method

CD8 T cells from healthy donors were activated in vitro and sequenced using single-cell short-read (Illumina) and long-read (PacBio) platforms to create a comprehensive expression and splicing atlas. Functional analysis was performed using SpliceSeek, a new developed CRISPR screen targeting splice sites of 150 immune receptor. Transduced T cells expressing the NY-ESO-1 TCR and SpliceSeek constructs were sorted based on IFNg production after co-culture with tumor cells, and functional isoforms were identified through sequencing.

Result and discussion

The splicing atlas revealed that isoform-based clustering mirrored expression-based clusters, demonstrating their complementary roles in defining T cell subsets. For example, in effector T cells (Teff), both clustering approaches enriched similar pathways, though splicing analysis identified distinct genes driving these pathways. Using SpliceSeek, we explored the functional impact of isoforms of immune receptors. LRRN3 emerged as a top candidate, significantly enhancing IFNg secretion, GZMb production, and tumor-killing capacity in-vitro and in-vivo. This isoform-specific effect highlights the ability of SpliceSeek to validate atlas-derived hypotheses and uncover therapeutic targets.

Conclusion

This study establishes a powerful pipeline connecting splicing atlas discovery with functional validation. The integration of splicing and expression data provides a comprehensive view of CD8 T-cell biology, and SpliceSeek successfully identifies isoforms like LRRN3 with significant therapeutic potential, paving the way for splicing-based cancer immunotherapies.

Symposium: Immune Cell Heterogeneity in the Cancer Ecosystem**EACR25-1514****Characterization of a population of CD4⁻CD8⁻ Double-Negative T Cells with anti-tumor properties**

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Introduction

T cells are essential in the anti-tumor immune response, with T cell-based therapies emerging as key strategies in cancer immunotherapy. While most studies focused on conventional CD4+ and CD8+ T cells, unconventional T cells (UTCs), such as invariant natural killer T cells (iNKT) and mucosal-associated invariant T cells (MAIT), are gaining recognition for their pivotal role in tumor immunity. Recently, our group identified a subset of UTCs, double-negative CD4⁻CD8⁻ TCRαβ⁺ T cells (DNTαβ), as key players of immune resistance in murine sarcoma (Ponzetta A, Cell. 2019). These cells express a TCRαβ, lack CD4 and CD8 expression and are not recognized by antigen-loaded CD1d and MR1 tetramers, distinguishing them from iNKT and MAIT. However, the biology and functional role of DNTαβ in steady-state and cancer remain poorly understood.

Material and method

Transcriptomic analyses and multiparametric flow cytometry were used to characterize DNTαβ in tissues. We assessed the capacity to proliferate, produce cytokines, exert cytotoxic activity against tumor cells and modulate immune response in vitro.

Result and discussion

DNTαβ were present across multiple tissues of healthy mice at different ages, with the spleen serving as their main reservoir. Unlike most UTCs, DNTαβ express a polyclonal TCRαβ repertoire. Remarkably, their development is independent of both classical and non-classical MHC molecules, as evidenced by their persistence in Cd1d, Mr1, β2m, and MhcII deficient mice. Transcriptomic analysis further revealed that DNTαβ possess a unique gene signature associated with T cell activation. Additionally, they express innate-like molecules and show elevated levels of IL-1 family

receptors (IL1R1, IL18R1). Consistently, DNT $\alpha\beta$ exhibit enhanced ability to proliferate and produce effector molecules (IFN- γ , GRZB) upon cytokine stimulation only compared to conventional T cells. In particular, IL-18 is required for the effective activation of DNT $\alpha\beta$ to produce IFN- γ , and their polarization is altered in IL-18-/- mice. DNT $\alpha\beta$ were found in the immune infiltrates of different murine cancer models. Interestingly, conditioned medium from cytokine-stimulated DNT $\alpha\beta$ induced macrophages polarization towards an anti-tumor M1-like phenotype. Additionally, DNT $\alpha\beta$ showed cytotoxic activity against cancer cells in vitro. Finally, a population of DNT $\alpha\beta$ with similar phenotypic and functional characteristics to their murine counterparts was found in the peripheral blood of healthy donors and in human tumors. Notably, analysis of The Cancer Genome Atlas (TCGA) database revealed that, in sarcoma patients, a DNT $\alpha\beta$ -associated gene signature correlated with a type 1 immune response and favorable prognosis.

Conclusion

We uncovered the biology and functional properties of a previously understudied population of UTC, paving the way for their use as therapeutic targets.

EACR25-2250

Rewiring T Cell Networks to Overcome Immune Evasion in Ovarian Cancer Relapse

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Introduction

Ovarian cancer (OC) remains a major clinical challenge due to its high recurrence rates and resistance to standard therapies. High-grade serous ovarian cancer (HGSOC), the most common and aggressive OC subtype, often presents with tumor-infiltrating lymphocytes (TILs), yet immune checkpoint blockade (ICB) therapies have shown limited efficacy. Our studies aims to dissect the spatiotemporal evolution of the OC tumor micro-environment (TME), reprogram T cell networks, and develop next-generation immunotherapies to restore anti-tumor immunity. Our research has uncovered critical T cell networks that regulate immune responses in OC, revealing that tumor evolution and chemotherapy-induced microenvironmental shifts contribute to TIL dysfunction and immune evasion.

Material and method

We integrate high-resolution spatial proteomics, single-cell transcriptomics, and computational modeling to map TIL dynamics across primary, recurrent, and treatment-

resistant OC. Preclinical models, including syngeneic BRCA1-mutant and wild-type mouse models of OC recurrence, are employed to investigate tumor-intrinsic and TME-driven mechanisms of immune suppression during OC relapse.

Result and discussion

We utilized digital pathology multiplex immunofluorescence (mIF) to classify 697 ovarian cancer (OC) specimens from five independent cohorts, offering the most extensive CD8+ T cell-based immune profiling to date. Our predictive algorithm identified distinct tumor immune phenotypes in OC – i.e. purely inflamed, mixed inflamed, excluded, and desert – based on TIL infiltration and spatial organization, which correlate with HRD status and predict therapeutic outcomes. We observed significant immune and molecular heterogeneity between these tumor immune phenotypes and their dynamics during disease recurrence. Inflamed HRD tumors, maintained T cell-myeloid niches post-chemotherapy. In preclinical models, this was associated with restoration of genomic rearrangements. Recurrent murine HRD tumors upregulated the immunosuppressive PGE2-EP2/4 pathway and targeting of COX-driven PGE2 production during chemotherapy significantly prolonged relapse and survival in preclinical mouse models, identifying a key vulnerability for the recurrence of human HRD OCs. In contrast, homologous recombination repair proficient (HRP) OC tumors evolved into T-cell excluded or desert phenotypes, characterized by malignant cells over-expressing Nduf4L2/Galectins and Trem2/ApoE over-active tumor-associated macrophages. Our data suggest that therapeutically targeting of TREM2 overexpressing TAMs may improve anti-tumor immune responses and delay recurrence after first-line CTX in HRP OC.

Conclusion

Our work highlights novel immune biomarkers and therapeutic targets that can be exploited to retune anti-tumor T cell responses, paving the way for clinically actionable strategies in recurrent OC and other immunoresistant malignancies.

Symposium: Non-invasive Diagnostics

EACR25-0255

Unravelling Principles of Adaptive Chemoresistance through Dynamic Transcriptional Patterns in Individual Breast Cancer Patients

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Introduction

Recent studies have highlighted the importance of non-genetic adaptive mechanisms through which tumors acquire resistance by rewiring transcriptional programs. To investigate the emergence of adaptive resistance in breast cancer, we employed a patient-oriented approach, analyzing drug-induced transcriptional changes relative to the dysregulated state of the primary tumor.

Material and method

We collected matched triplets of archived tumor biopsies from breast cancer patients who underwent neo-adjuvant chemotherapy, including pre-treatment, post-treatment and adjacent normal epithelium samples. Whole-transcriptome mRNA sequencing was performed for each sample. A longitudinal pattern analysis algorithm was developed to track dynamic gene expression remodeling at the individual patient level. Enrichment analysis was used to identify rewired resistant pathways. Finally, we constructed personalized chemoresistome maps to illustrate patient-specific resistance dynamics.

Result and discussion

Personalized chemoresistome maps disclosed molecular pathways that adapt to resist chemotherapy while exposing persistent vulnerabilities in each patient. The patient-specific dynamics of gene expression modulations during chemotherapy depicted key principles underlying adaptive resistance:

1. Primed adaptive resistance state: Resistance-associated genes are already dysregulated in the primary tumors.
2. Convergence to confined resistance states: Despite the involvement of multiple resistance-related genes, they converge into distinct dysregulated modules.
3. Drug-specificity: some of these dysregulated modules are directly linked to the drug mechanism of action. Our findings suggest that to withstand the cytotoxic effects of treatment, tumor cells either maintain their "primed" dysregulated state or intensify it, specifically bypassing the drug's mechanism of action.

Conclusion

Understanding the dynamics of phenotypic alterations underlying adaptive resistance may provide crucial insights into critical stages of treatment response. These insights could pave the way for the development of personalized therapeutic strategies aimed at preventing or overcoming resistance.

EACR25-0812

KRAS pharmacological blockade elicits tumoral homologous recombination deficiency exploitable with PARP inhibitors

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Introduction

The generation of direct KRAS inhibitors (KRASi) represented a milestone in lung cancer therapy offering targeted treatment to vast patient cohorts with KRAS mutations. Nevertheless, escape mechanisms based on tumour compensatory activation, genetic evolution, or phenotypic switch limit their antitumor efficacy and restrict survival benefit. Thus, it is of paramount importance to uncover drug combinations which could maximize the antitumor effect of KRASi.

Material and method

We interrogated bulk and single cell transcriptome of human and mouse mutant KRAS (mtKRAS) lung cancer cell lines exposed to genetic and pharmacological KRAS inhibition, and used a reporter system to assess DNA repair functionality. Combination therapies consisting of KRASi and PARP inhibitors (PARPi) were tested in vitro, as well as in cell line-derived xenograft (CDX), allografts (CDA) or PDX in vivo. Integration of CRISPR screening data of genes sensitizing to PARPi with the Connectivity Map was employed to nominate KRASi-induced transcriptional nodes regulating genome instability which were functionally validated. In vitro and in vivo models of pancreatic and colorectal cancer were deployed to test the efficacy of KRASi + PARPi in additional KRAS-driven cancers.

Result and discussion

We report a commonly downregulated gene signature upon KRAS blockade related to DNA damage integrity, and show compromised homologous recombination (HR) functionality. Single cell analysis shows that tumoral populations able to tolerate pharmacological KRAS inhibition abrogate key elements of HR. This early adaptive response to KRASi is vulnerable to therapeutic attack with PARPi, eliciting a lethality in numerous lung cancer models reflected as delay of the onset of resistance in vitro and potent tumor regressions in vivo. At the cellular level, HR depletion exacerbates DNA damage in the context of PARPi which may underscore the synthetic lethal phenotype. Mechanistically, we unveil that KRASi-mediated loss of genome stability is via the transcription factor FOSL1, a known key effector of KRAS signalling. In vitro, FOSL1 levels correlate to PARPi efficacy, and FOSL1 genetic abrogation depletes vast elements of genomic integrity in addition to BRCA2 levels to sensitize to PARPi. mtKRAS lung cancer patients with high FOSL1 levels display poor prognosis when treated with DNA damaging agents. Lastly, we found that these observations span other mtKRAS cancers such as those of the pancreas and colorectum.

Conclusion

Our data provide nominate a mechanistic driven combination for hard-to-treat mtKRAS cancers consisting of KRASi and PARPi which defines a path for clinical translatability. In addition, we provide molecular and cellular data positioning the transcription factor FOSL1 as a key player in tumoral genome integrity and response to DNA damaging therapies.

Symposium: Senescence in Therapy Response

EACR25-0260

Targeting senescent macrophages inhibits metastatic dissemination in adrenocortical cancer

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Introduction

Cellular senescence plays a dual role in cancer, acting as both a barrier to tumorigenesis and a potential driver of tumor progression. Adrenocortical carcinoma (ACC) is a highly aggressive malignancy originating from steroidogenic cells of the adrenal cortex, with limited treatment options and a median overall survival of less than 15 months. The most frequent genetic alteration in ACC is the inactivation of ZNRF3, a tumor suppressor and inhibitor of WNT signaling. Previously, we demonstrated that male mice with conditional inactivation of Znrf3 in adrenocortical cells are protected from tumor development due to testosterone-dependent induction of senescence, leading to immune-mediated clearance of preneoplastic lesions. Interestingly, the most aggressive ACCs in patients frequently exhibit ZNRF3 inactivation combined with alterations in the P53/RB/CDKN1B pathway, suggesting a potential link between senescence dysregulation and tumor progression. To investigate this hypothesis, we developed a mouse model with conditional genetic ablation of both Znrf3 and Trp53 in the adrenal cortex (Znrf3/Trp53 dKO).

Material and method

Primary tumors and lung metastases from Znrf3/Trp53 dKO mice [1] were analyzed using immunohistochemistry, spectral flow cytometry, bulk RNA sequencing, single-cell sequencing, and spatial transcriptomics.

Result and discussion

Combined Znrf3 and Trp53 inactivation circumvented the initial senescence-mediated tumor clearance, leading to the development of highly aggressive, metastatic, and lethal ACC in both male and female mice within six months. Characterization of the immune micro-environment revealed that tumor progression was associated with a decline in T cell and NK cell-mediated antitumor immunity, alongside an accumulation of immunosuppressive macrophages in the most aggressive primary tumors. Single-cell sequencing of CD45⁺ immune cells showed that immunosuppressive macrophages displayed senescent characteristics, a finding confirmed by SA-βGal and p16 immunostaining. Interestingly, spatial transcriptomic analysis of lungs also revealed the presence of senescent immunosuppressive macrophages associated with glucocorticoid-producing metastases. This suggested that senescent macrophages may be involved in metastatic dissemination and homing in ACC. Supporting this hypothesis, treatment with the senolytic ABT-737 significantly reduced primary tumor growth and strongly inhibited metastatic spread.

Conclusion

These findings indicate that senescent immunosuppressive macrophages play a key role in metastatic dissemination in ACC. Their targeted clearance using senolytic therapy presents a promising therapeutic strategy for combating metastatic progression in this aggressive malignancy.

[1] Protocol validated by local ethics committee, APAFIS#27623-2021021611362535 v1

EACR25-0844

Therapy-Induced Senescence Promotes Immunogenicity in Acute Myeloid Leukemia Blasts through loss of PRC2-Mediated Regulation

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Introduction

Acute myeloid leukemia (AML) is a heterogeneous blood cancer typically treated with intensive chemotherapy; yet early treatment failure remains a major challenge. Therapy-induced senescence (TIS) is a stress response that can elicit immune-mediated responses. However, its functional role in AML eradication and immune surveillance by the adaptive immune system remains ill-defined. In this study, we investigated how senescent AML blasts interact with the immune system to uncover factors influencing AML responses to chemotherapy and immunotherapy.

Material and method

We exploited ex-vivo cultures of primary AML primary samples or cell lines undergoing senescence upon chemotherapy treatment. Multiparametric flow cytometry analysis integrated with quantitative imaging was used to measure senescent markers and human leukocyte antigen (HLA) expression in a cohort of primary AML samples at diagnosis and upon ex-vivo therapy. The mixed lymphocyte reaction assay was performed to evaluate T cell activation. Additionally, in vivo experiments using patient-derived xenografts (PDX) models were conducted to further validate the immunogenic effects of TIS. Western blot, nuclear proteomics and CUT&Tag analysis were exploited for assessing histone modifications associated with transcriptional activation or repression in senescence high and senescence low patients.

Result and discussion

Our results show that TIS boosts AML blast immunogenicity by upregulating senescence, interferon and HLA

genes, along with presenting senescence-associated self-peptides presentation. This induces an antigen-presenting cell (APC)-like phenotype in senescent leukemic blasts, enhancing the activation of autologous CD4+ and CD8+ T cells and their capacity to recognize AML cells. Of note, these findings were also confirmed in PDX models. Additionally, we found that TIS-driven immunogenicity synergizes with immune checkpoint blockade (ICB) therapies *in vivo*. Importantly, we identified the Polycomb Repressor Complex 2 (PRC2) as a key regulator of this process, with its inhibition restoring HLA expression and T cell activation in non-senescent AML blasts. These findings highlight a potent senescence-driven immune mechanism with potential to reduce AML relapse by augmenting anti-leukemic immune responses.

Conclusion

Our findings uncover a previously unrecognized role of therapy-induced senescence in enhancing the immunogenicity of AML blasts by promoting antigen presentation and T cell activation. By identifying loss of PRC2 activity as a key regulator of this process, we provide new insights into how senescence can be leveraged to boost anti-leukemic immune responses. Targeting the senescence pathway and its regulatory mechanisms could pave the way for novel senescence-based immunotherapies aimed at preventing AML relapse and improving patient outcomes.

Symposium: Novel Models of Cancer Cachexia

EACR25-1995

Oncolytic virus (CVA21) in combination with pembrolizumab increases tumour cell immunogenicity and remodels the tumour microenvironment in advanced NSCLC: a phase I/II trial

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Introduction

Immune checkpoint inhibitor-resistant advanced non-small cell lung cancer (NSCLC) is characterised by limited effective treatment options. There is an unmet need to improve outcomes for patients with this condition. Oncolytic viruses represent an attractive treatment approach due to their dual activity in inducing tumour cell lysis directly and augmenting anti-tumour immunity.

Material and method

Here, we present the safety, efficacy and translational findings from a phase I/II single-arm trial utilising CVA21 (CAVATAK®), an oncolytic coxsackie virus, in combination with pembrolizumab in patients with advanced pre-treated NSCLC. We applied whole genome sequencing and spatial proteomics with multiplex-ion beam imaging in pre- and post-treatment samples to comprehensively characterise the response to treatment.

Result and discussion

Ten patients received intravenous CVA21 and pembrolizumab, eight of whom had prior treatment with anti PD-(L)1 immune checkpoint inhibitor (ICI) therapy. Combination CVA21/pembrolizumab therapy was well tolerated with no serious treatment-related adverse events. Striking partial responses were seen in two patients with prior anti-PD-1 resistance. The clinical benefit rate was 80%. High baseline tumour mutational burden and PDL-1 expression was observed in patients with better response to treatment. Interestingly, an increase in antigen presentation and CD8 T cell infiltration was observed on treatment compared with baseline in patients with better progression-free survival, demonstrating the potential of oncolytic virus to modulate the immunogenicity of tumour cells and remodelling the tumour microenvironment.

Conclusion

The combination of CVA21 and pembrolizumab is safe and shows promising efficacy in ICI pre-treated patients with advanced NSCLC. Our translational multi-omic analysis provides insights that may enhance patient selection for future trials investigating novel immunotherapeutic strategies.

EACR25-2124

Translational control shapes Treg cell fate in the tumor microenvironment

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Introduction

Cancer immunotherapy has revolutionized oncology, yet only a fraction of patients respond. Our previous work revealed that T cells rapidly deploy translational machinery during activation within the tumor microenvironment, raising questions about how translational regulation affects T cell trajectories in tumors. Regulatory T cells (Tregs) – the master

orchestrators of immune suppression – represent a critical barrier to effective anti-tumor immunity. Despite their crucial role, the translational landscape shaping Treg function within tumors remains largely unexplored.

Material and method

We performed multifocal analysis of tumor infiltrating lymphocytes (TILs) translatomes in pleural mesothelioma (PM) and lung cancer (NSCLC). We developed CITePuro-seq technology to simultaneously capture, for the first time, single-cell translation and gene expression in human lymphocytes. Single-cell metabolic activity was analyzed using a microfluidic system with pH-sensitive fluorescence.

Result and discussion

Our discovery reveals a striking translational divergence in TILs across tumor types:

1. CD4+ T cells exhibit dramatically higher translation rates in mesothelioma compared to lung cancer, with actively translating Tregs predominating in PM,
 2. translation is not uniformly active in the tumor and
 3. specific genes are controlled at the translational level.
- Transcriptomic analysis revealed significant heterogeneity in Treg populations between PM and NSCLC. Moreover PM-associated Tregs exhibited a distinct signature enriched for translational machinery. Patient-specific Treg profiles revealed two clusters with divergent signatures. Using CITePuro-seq, we associated single-cell RNA sequencing with direct measurement of translational activity. The super-imposition of the the translational activity of CD4+ Tregs with their transcriptional profile leads to cluster highly suppressive cells and to define known and novel subtypes of Tregs by differential translation. Remarkably, our *in vitro* translational signature manifested *in vivo* in PM tumors, with tumor hypoxia emerging as a critical driver of Treg translational reprogramming and metabolic adaptation. Most surprisingly, Tregs with different translation levels exhibited functional versatility beyond immuno-suppression, revealing an unexpected plasticity.

Conclusion

Our findings establish translation as the hidden regulator shaping T cell fate in the tumor microenvironment. The differences in translational potential across TILs reveal how tumor-specific factors reprogram cellular function independently of transcription. This demonstrates why purely transcription-based analyses can be misleading. Our work identifies translational control as a previously unrecognized checkpoint in TIL programming, suggesting a promising avenue for enhancing immunotherapy.

Symposium: New Drugs on the Horizon

EACR25-0376 Inhibition of WEE1 kinase activity to overcome secondary resistance to targeted therapies in colorectal cancer

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Introduction

Molecular therapies targeting the EGFR/MAPK signaling pathway have markedly improved treatment outcomes for colorectal cancer (CRC). However, the emergence of secondary resistance – whether through pre-existing factors or adaptive mechanisms – remains a major challenge in achieving long-term therapeutic success. The DNA damage response (DDR) plays a pivotal role in enabling cancer cells to cope with therapy-induced genotoxic stress. In CRC, dysregulation of the DDR pathways contributes to the survival of tumor cells despite DNA damage, suggesting DDR as a potential therapeutic target in the context of secondary resistance.

Material and method

We generated a series of models with acquired resistance to anti-EGFR based targeted therapies, referred to as the ARes platform. To assess the impact of secondary resistance on cancer cells' dependence on DDR pathways for survival, we performed a drug screening, by exposing parental and resistant models to a range of DDR inhibitors (DDRi). *In vivo* validation of these findings was performed using xenograft mouse models.

Mechanistic studies were conducted to evaluate DNA damage levels, repair capacity, cell cycle dynamics, and replication stress in selected cell pairs. These studies employed a range of techniques, including western blotting, comet assay, immunofluorescence for foci analysis, cytofluorimetry, and fiber assay, to provide comprehensive insights into WEE1i efficacy.

Result and discussion

Pharmacological screening of the ARes platform with several DDRi revealed WEE1 as the most promising therapeutic target. *In vivo* experiments using xenograft models demonstrated that CRCs with acquired resistance to anti-EGFR therapies exhibited sustained or even enhanced sensitivity to WEE1i, consistent with *in vitro* observations. Mechanistic investigations showed that resistant cells maintain constitutive activation of the MAPK pathway and present heightened basal levels of DNA damage. In the same models, elevated RAD51 expression mediates tolerance to DNA damage and replication stress. However, we found that WEE1i treatment accelerates G2/M entry and disrupts RAD51 activity, leading to higher DNA damage accumulation and consequent cell death. Remarkably, these effects were observed even at sublethal concentrations of WEE1i, suggesting potential for synergistic strategies when combined with conventional chemotherapeutic agents.

Conclusion

Our results highlight the therapeutic potential of WEE1 inhibition in CRC patients who have developed acquired resistance to EGFR-targeted therapies. These findings provide preclinical evidence for future clinical investigations into the use of WEE1 inhibitors as part of combination therapies to overcome secondary resistance in CRC.

EACR25-0553

Reversing Acidic Immune Suppression in Cancer with PTT-4256, A First-In-Class Small Molecule Inhibitor of GPR65

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Introduction

Extracellular acidification is a recognised driver of tumour immunosuppression. Pathios identified GPR65 as the primary pH-sensing GPCR in human immune cells. Activation of GPR65 suppresses immune effector homing and function and induces a pro-tumour phenotype in macrophages: downregulating interferon and immunostimulatory genes and upregulating wound repair and remodelling factors. Consequently, acid sensing by GPR65 explains tumour resistance to current immunotherapies. Validating this target in immuno-oncology, cancer patients homozygous for the hypomorphic GPR65 variant (I231L) exhibit profoundly improved survival compared to patients with other genotypes.

Material and method

Pathios have developed PTT-4256, a first-in-class small molecule inhibitor of GPR65. The in vitro efficacy of PTT-4256 under low pH conditions was determined by cAMP screening, gene expression and cytokine release profiling of primary immune populations. Anti-tumour activity was assessed using the MC38 and B16.F10 syngeneic mouse models, whilst immunomodulatory effects in MC38 tumour-infiltrating leukocytes were profiled by targeted RNAseq. Notably, PTT-4256 is markedly more potent at human GPR65 versus the mouse receptor. To determine its full potential in patients and to develop an accurate prediction of efficacious human exposure, we employed a range of approaches including molecular pharmacology, site-directed mutagenesis, PK/PD modelling, and genetically engineered mice.

Result and discussion

PTT-4256 fully counteracts the low GPR65-dependent pH-driven immunosuppressive programme in immune cells. Molecular modelling and site-directed mutagenesis identified three key residues that explained the 1000-fold difference in potency of PTT-4256 between mouse and human GPR65. Incorporating these residues in a transgenic knock-in mouse restored human pharmacology in vitro and provided an important translational platform. PTT-4256 displays an excellent oral PK profile across species and elicits monotherapy efficacy in MC38 and B16.F10 mouse models that exceeds anti-PD1 control alongside rapid upregulation of anti-tumour NK and T cell genes.

Conclusion

GPR65 is a key checkpoint on tumour-infiltrating immune cells that links the chronically acidic tumour microenvironment to tumour-promoting immuno-

suppression. Pathios' oral small molecule GPR65 inhibitor, PTT-4256, targets fundamental biology co-opted by all solid tumours with a mechanism that is orthogonal to established anti-tumour therapies including anti-PD1. An ongoing First in Human Phase 1/2 clinical trial (RAISIC-1) to establish safety and PK started in Q4 '24.

Symposium: Normal Tissue (not Blood)

EACR25-0457

Dietary Histidine controls intestinal stem cell activity, providing a potential protective role against colorectal cancer

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Introduction

Intestinal stem cells (ISCs) are essential for maintaining tissue homeostasis by regulating the continuous intestinal turnover. They achieve this balance through self-renewal and differentiation into specialized cells in response to environmental cues such as nutrient availability.

Dysregulation of these processes is a hallmark of colorectal cancer (CRC). However, the mechanisms by which nutrient signaling modulates ISC activity during the early stages of CRC remain poorly understood

Material and method

To decipher which and how nutrients affect oncogenic ISC, we took advantage of the *Drosophila* midgut and its fully defined diet to screen for single amino acid vulnerability of wild-type ISCs and those bearing common CRC-associated mutations (Apc-Ras). Based on this screen, we further focused on one amino acid that stood out with a putative role in regulating ISC activity. Combining advanced genetic and bioinformatic tools, we dissected its metabolic pathway and validated our findings in a primary mouse crypt-derived intestinal organoid model.

Result and discussion

Our data from the single amino acid-deficient dietary screen highlighted overgrowth of Apc-Ras ISCs fed on Histidine-deprived diet compared to the normal one, suggesting that dietary Histidine exerts a protective effect against Apc-Ras-driven tumorigenesis. In the wild-type *drosophila* intestine, we demonstrate that dietary Histidine plays a critical role in regulating ISC activity by promoting their differentiation through an unexpected downstream metabolite. Mechanistic analyses indicate that this metabolite operates via two distinct functions: one activating ISC asymmetric division, and the second, mTOR-dependent, promoting enterocyte differentiation. Moreover, we found that dietary supplementation of this metabolite was reducing stemness of Apc KD ISCs and triggering their differentiation, providing a mechanistic basis for Histidine's protective role. Finally, experiments using primary crypt-derived intestinal organoids confirmed that this metabolite is sufficient to enhance

ISC differentiation, demonstrating the conservation of the mechanism in mammals.

Conclusion

These findings indicate that Histidine metabolism is essential for ISC maintaining homeostasis by triggering enterocyte lineage differentiation program. Because enhanced stemness is a hallmark of early colorectal cancer, dietary histidine supplementation could represent a novel strategy to modulate ISC behavior and counteract early tumorigenesis.

EACR25-0647

Pancreatic Cancer Initiation: Wnt Signalling and Cell Dormancy Enable Kras Mutant Cell Survival

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Introduction

How does cancer start if epithelial tissues possess robust defence mechanisms to eliminate aberrant or genetically mutant cells? The way cancer-causing mutations override tissue defences remain poorly understood. We have shown that the adult pancreas protects against early cancer by actively expelling genetically mutated cells. Cell expulsion requires cell-cell interactions between mutant cells and normal neighbours and dynamic remodelling of E-cadherin dependent cell-cell adhesions. Pancreatic cancer starts with cells carrying oncogenic KRAS mutations. This suggests some KRAS mutant cells must override cell elimination mechanisms to survive in tissues. Here we aim to understand how some Kras mutant cells survive tissue homeostasis mechanisms.

Material and method

We employed a mouse model of sporadic pancreatic cancer to explore the early stages of cancer initiation. Oncogenic mutations in Kras gene were induced in low numbers of adult pancreatic epithelial cells, which were tracked using a fluorescent reporter. Gene signatures of the surviving mutant cell populations were identified through bulk RNA sequencing. Key molecular pathways were validated in the murine pancreas via immuno-fluorescence and functionally tested through in vitro epithelial co-culture systems and in vivo inhibitor studies.

Result and discussion

We showed that when KrasG12D mutations are expressed only in few cells in the adult pancreas, most of them are eliminated. However, a small population of Kras mutant cells are not eliminated and instead remain in the tissue, eventually progressing into pre-malignant lesions. Transcriptomic analysis of retained cells identified Wnt5a signalling, cell dormancy and stemness as key features of surviving KrasG12D cells in vivo. We functionally validated these results in vitro showing that Wnt5a, but not Wnt3a, inhibits cell elimination of RasV12 cells by promoting stable E-cadherin based cell-cell adhesions at RasV12: normal cell-cell boundaries. RasV12 cells arrested in the cell cycle are not eliminated and this is rescued when Wnt signalling is inhibited. In vivo, we confirmed that cell-cell adhesions were stabilised between retained KrasG12D cells and normal

neighbours with an increase in Wnt signalling, E-cadherin and β-catenin at normal-mutant cell-cell contacts. Importantly, inhibition of Wnt signalling in mice increased KrasG12D cell elimination, showing that active Wnt signalling is a general mechanism required to promote mutant cell survival in vivo. Finally, we found expression of Wnt5a increased in human pancreatic tumours and pre-malignant lesions.

Conclusion

RAS mutant cells activate Wnt5a, stemness signatures and a dormant cell state to avoid cell elimination and survive in the adult pancreas. These findings shed light into how cell-cell interactions can activate important signatures for cancer initiation and progression at very early stages.

Symposium: Tumour Innervation

EACR25-1319

Medullary sinus macrophages limit cancer therapy efficacy by an efferocytosis-induced IL-33/ST2 axis in the tumor-draining lymph node

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Introduction

Cell death is a fundamental aspect of tissue homeostasis that can expose potentially immunogenic cell components that must be removed to prevent inflammatory autoimmunity. We have shown immune suppression in response to apoptotic cell phagocytosis (a process called efferocytosis) is dependent on tissue-resident macrophages. Indeed, if we disrupt macrophage regulatory responses post-efferocytosis, the same apoptotic cells induce inflammation suggesting macrophage responses to dying cells are a key determinant of tolerance. We predict that resident macrophages lining the lymphatic sinus inside the lymph nodes are responsible for tolerance against tumor apoptotic cells.

Material and method

Melanoma tumor-bearing mice were treated with chemotherapy or targeted therapy to induce tumor cell death. Both tumor and the tumor-draining lymph node (TDLN) were analyzed at different time points after treatment. In some experiments, we used a novel mouse model, generated in our laboratory, where IL33 was specifically deleted in a subpopulation of macrophages of TDLN, called medullary sinus macrophage (MSM) (MSM-IL33ko).

Result and discussion

Using our mouse melanoma model, we observed that after either chemotherapy or targeted therapy MSM avidly phagocytosed dying tumor cells and acquired a tolerogenic phenotype. RNA sequencing analysis revealed MSM rapidly and exclusively induced expression of the alarmin IL-33 as opposed to other MF

or dendritic cell populations in the TDLN. Importantly, genetic deletion of IL33 (MSM-IL33ko) or blockade of the IL-33 receptor ST2 with IgG, transformed responses to both chemotherapy or targeted therapy with prolonged, enhanced tumor control and reduced cancer reoccurrence. Functionally, MSM-derived IL-33 triggered accumulation and activation of regulatory T cells expressing ST2 in the TDLN which then migrated to the tumor limiting intratumoral CD8+ T cell function.

Conclusion

Our data revealed a previously undescribed tumor cell death-induced mechanism limiting anti-cancer immunity and therapy efficacy. The findings from this project have resulted in a manuscript that was recently accepted by *Cancer Cell* journal.

EACR25-1355

Spatially Resolved Analysis of Cancer-Infiltrated Nerves Identifies DRAKIN as a Marker of Injury Response with Tumor-Suppressive Potential

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Introduction

Perineural invasion (PNI), the spread of cancer cells along nerves, is a hallmark of pancreatic ductal adenocarcinoma (PDAC) and is associated with poor prognosis, local recurrence, and metastasis. However, the biological processes underlying these outcomes remain unclear.

Material and method

To address this gap, we used spatial transcriptomics (10x Genomics Visium) to profile cancer-invaded and non-invaded nerves from three PDAC patients *in situ*.

Result and discussion

Transcriptomic analysis, combined with multiplexed immunofluorescence, showed that cancer infiltration induces nerve damage, leading to changes in the nerve secretome. Among the upregulated factors, we identified DRAKIN, an axon-guidance protein involved in neural development but with a largely unexplored role in cancer. Functional assays, including *in vitro* proliferation studies and CRISPR-based screens, demonstrated that recombinant DRAKIN induces cancer cell death.

Conclusion

These findings provide new insights into the molecular changes in cancer-infiltrated nerves and highlight DRAKIN as a nerve-derived factor with anti-tumor properties.

Symposium: Cancer Genomics

EACR25-0508

Integrative multi-omic approach to identify patterns of immune selection in primary breast tumours

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Introduction

Cancer evolution is driven by selective pressures favouring clones that evade immune surveillance and adapt to their microenvironment. Radiotherapy, a standard treatment for breast cancer, reduces recurrence risk by 10–25%, depending on the stage and molecular subtype. While its success is partly attributed to immune activation, the underlying mechanisms remain unclear. We hypothesise that radiotherapy fuels immune response through DNA damage and neoantigens release, inducing the transition of immune-cold to immune-hot tumours. However, there is a lack of computational tools to identify immunoediting and stratify patients for improved outcomes. Here, we analysed data from 24 breast cancer patients enrolled in two clinical trials testing neoadjuvant radiotherapy. Genomic and transcriptomic data at multiple time points (diagnosis, pre-/post-radiotherapy, and at surgery) were used to quantify immunoediting and correlate it with clinical response. Our computational strategy enables the study of tumour-immune co-evolution across different therapeutic regimens and mutational landscapes.

Material and method

FFPE tissue samples from primary breast tumours and draining lymph nodes were subjected to WES, WTS, TCR-Seq, and multiplex IF. Standard bioinformatic pipelines were used for somatic variant calling (Sarek), transcriptome analysis (rnaseq) and HLA genotyping (hlatyping). Downstream genomics analysis included “dndscv” to measure patterns of selection, “mutSignatures” for mutational signatures, “TcellExTRECT” for T cell infiltration, and “pVACseq” for neoantigen calling. Our in-house tools, SOPRANO and NegSelect, quantified patient-specific immunoediting in samples with low TMB. Given this context, we aim to develop an integrated pipeline for immune multi-omics analysis.

Result and discussion

Preliminary genomic analysis of primary breast tumours revealed that mutations in PIK3CA, ERCC2, SPEN and HLA-C were positively selected in one of the clinical trials. Moreover, radiotherapy-treated patients showed

increased SBS3 (homologous recombination deficiency) and decreased SBS16 (chromosomal instability) compared to controls. By using the ratio of non-synonymous to synonymous mutations (dN/dS) in the immunopeptidome, we identified differences in the proportion of immune-escaped tumours between the two clinical trials. Ongoing computational work is focused on integrating data from all modalities to study genetic and non-genetic mechanisms of immune evasion, the contribution of T cell infiltration, and the potential use of immune dN/dS as a predictive marker for response to treatment.

Conclusion

We have established a bioinformatic pipeline to successfully integrate immune multiomic data from longitudinal samples collected within two neoadjuvant radiotherapy trials in breast cancer, and further research is ongoing to fully characterise the immunogenomic evolution and therapeutic implications.

EACR25-2424

Genetic background sets the trajectory of cancer evolution

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Introduction

Human cancers are heterogeneous. Their genomes evolve from genetically diverse germlines in complex and dynamic environments, including exposure to potential carcinogens. This heterogeneity of humans, our environmental exposures, and subsequent tumours makes it challenging to understand the extent to which cancer evolution is predictable.

Material and method

Addressing this limitation, we re-ran early tumour evolution hundreds of times in diverse, inbred mouse strains, capturing genetic variation comparable to and beyond that found in human populations. The sex, environment, and carcinogenic exposures were all controlled and tumours comprehensively profiled with whole genome and transcriptome sequencing.

Result and discussion

Within a strain, there was a high degree of consistency in the mutational landscape, a limited range of driver mutations, and all strains converged on the acquisition of a MAPK activating mutation with similar transcriptional disruption of that pathway. Despite these similarities in the phenotypic state of tumours, different strains took markedly divergent paths to reach that state. This included pronounced biases in the precise driver

mutations, the strain specific occurrence of whole genome duplication, and differences in subclonal selection that reflected both cancer susceptibility and tumour growth rate.

Conclusion

These results show that interactions between the germline genome and the environment are highly deterministic for the trajectory of tumour genome evolution, and even modest genetic divergence can substantially alter selection pressures during cancer development, influencing both cancer risk and the biology of the tumour that develops.

Joint EACR-ASPIC Symposium: Cancer Therapy Resistance

EACR25-0507

Dynamics and chemotherapy response of single cell-derived human breast cancer clones

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Introduction

Human breast cancers display significant genomic and phenotypic heterogeneity which are major factors in the development of treatment resistance. Recent advances have made it possible to combine clonal tracking methods with single cell RNA sequencing (scRNAseq) to associate clonal response to treatment with transcriptomic changes at the single cell level. With this approach, we aim to investigate clonal response to chemotherapy in human breast cancer patient-derived xenograft (PDX) models to understand the role of tumour cellular heterogeneity in molecular response and clonal adaptation to chemotherapy.

Material and method

A custom-built expressed lentiviral-based cellular barcoding system was used to label and track the growth of single cell-derived clones in 4 PDX models (2 ER-positive, and 2 triple-negative) serially propagated in primary and secondary tumour xenografts. Secondary xenograft replicates that contained the same barcode clones propagated from the primary were treated with Carboplatin or Paclitaxel, and compared to untreated controls.

Result and discussion

40 barcoded xenografts (treated and untreated) were analysed by DNA amplicon sequencing (DNAseq) and scRNAseq to track clonal response to chemotherapy and associate each clone with its transcriptomic profiles at single cell resolution. DNAseq identified 3260 barcode clones in total; 153/454 (34%) and 537/1102 (49%) were chemo-resistant in the 2 ER-positive models, and 480/633 (76%) and 737/1071 (69%) were chemo-resistant in the 2 triple-negative models. Strikingly while both treatments altered the clonal landscape of the 2 PDX models that showed resistance from bulk tumour growth rates after chemotherapy (1 ER-positive and 1 triple-negative), they had little impact on the clonal landscape of the 2 sensitive models. This implies that treatment selects for pre-existing resistant clones or a rapid adaptation to confer resistance in some clones. From scRNAseq a large dataset comprising ~500,000 high quality single cell RNA profiles was generated where expressed barcodes allowed single cell RNA profiles to be annotated based on response to chemotherapy.

Conclusion

We generated a robust set of clonal tracking data coupled with scRNAseq allowing for clonal response to chemotherapy to be investigated on a single cell level. We found that treatment profoundly alters the clonal landscape of resistant xenografts but has little impact on sensitive xenografts. This analysis will allow us to explore molecular pathways that distinguish chemo-resistant from chemo-sensitive clones and develop novel therapeutic strategies to overcome chemotherapy resistance in human breast cancer.

EACR25-0716

Unveiling the impact of intratumoral microbiota in the treatment efficacy of soft tissue sarcoma

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Introduction

The intratumoral microbiome has been recently recognized as an “Emerging Hallmark of Cancer” due to its presence in the tumor microenvironment. Bacterial DNA has been detected in various cancers, revealing tumor-type-specific microbiome compositions. Tumor-resident bacteria influence cancer biology by promoting progression, enhancing metastatic potential, and modulating antitumor immunity. Additionally, they may impair anticancer therapies by inactivating drugs or reducing cancer cell sensitivity. Despite this, the role of the intratumoral microbiota in soft tissue sarcomas (STSs) remains underexplored. Given STS heterogeneity, with over 100 histological subtypes, personalized treatment is complex. Doxorubicin remains the gold-standard chemotherapy for advanced STSs, though its efficacy is low. Since doxorubicin is a natural anthracycline produced by Streptomycetaceae, some bacteria may have evolved mechanisms to inactivate it. This study investigates STS microbiota and its potential impact on doxorubicin efficacy.

Material and method

Bacterial presence in STSs was assessed using Fluorescence In Situ Hybridization (FISH) targeting the 16S rRNA gene. Microbiome profiling via 16S rRNA sequencing compared tumor-associated microbiota with adjacent healthy tissues. Additionally, we isolated 46 bacterial strains from STSs, identifying facultative and obligate anaerobes adapted to hypoxic tumor conditions. Functional assays exposed these isolates to doxorubicin to evaluate drug inactivation assessed also with mass spectrometry approaches. Proteomic analysis of *Proteus vulgaris* under anaerobic conditions identified upregulated enzymes, and molecular docking simulations investigated their interaction with doxorubicin.

Result and discussion

FISH confirmed the presence of bacteria across different sarcoma histotypes. 16S rRNA sequencing revealed distinct microbiome profiles between STSs and adjacent tissues, correlating more with histological subtype than tumor location. Functional studies showed that certain bacteria, including *Porphyromonas* and *Proteus vulgaris*, inactivated doxorubicin. In a doxorubicin-sensitive fibrosarcoma mouse model, intratumoral *Porphyromonas* infection induced chemoresistance. Proteomic analysis of *P. vulgaris* revealed high expression of nitrite reductases under anaerobic conditions, and molecular docking suggested their role in doxorubicin inactivation. These findings suggest a link between bacterial nitrogen metabolism and chemoresistance.

Conclusion

Our findings highlight the role of the intratumoral microbiome in shaping therapeutic responses, showing that specific bacteria can inactivate doxorubicin and induce chemoresistance. Considering tumor-associated bacteria may be crucial for optimizing cancer treatments and overcoming microbiome-driven drug resistance.

Joint EACR-EMBO Symposium: Advanced in vitro Models

EACR25-0343

Contact-based transfer of thymidylate promotes collective tumor growth

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Introduction

Cancer pathogenesis is a cumulative outcome of multiple factors that prime a conducive environment for cells to sustain diverse stress conditions, proliferate and metastasize. Proliferation, though central to growth, is largely undermined as a unidimensional and standalone hallmark. Its mechanistic involvement in heterogeneous tumor organization, intercellular interactions, and metabolite exchanges is largely unexplored. In this study, we uncover a mechanism of tumor growth via collective proliferation, where cells exchange thymidylate (dTMP),

a nucleotide critical for DNA synthesis, rendering its synthesis indispensable in a significant portion of proliferative tumor cells. Collective proliferation rheostats key tumor phenotypes, and thereby, impacts pathophysiology of cancer.

Material and method

dTMP synthesizing de novo enzyme thymidylate synthase (TS) and/or the salvage enzyme thymidine kinase (TK1) were knocked out in cancer cells with nuclear GFP tags. A coculture system was setup between the KO cells and cells expressing TS and TK1, where the KO cells were exclusively recognized by a real-time imager Incucyte S3 based on its GFP expression. Single cell RNA sequencing and spatial transcriptomics were used to analyze the contribution of dTMP deficient cells in patient samples. A Kras-p53 driven genetic mouse model with dual knockout TS and TK1 (KPTT) was used for in vivo validation of collective growth. Molecular dynamics were studied at single cell level by quantitative image-based cytometry (QIBC).

Result and discussion

Our study elucidates a novel proliferative mechanism where cells lacking intrinsic dTMP synthesis (by TS and/or TK1) employ gap junctions to form a cellular syncytium with dTMP-proficient cancerous and several non-cancerous cell types, such as macrophages and endothelial cells. dTMP equilibration in this network allows proliferation of dTMP-deficient cells, dispensing the need of ubiquitous activation of TS and TK1. Analysis of dTMP-sharing at cellular level further hints that this mechanism influences key processes such as senescence, inflammation and genomic instability. The mechanism is also observed in clinical samples and is associated with key pathological features of cancer. Collective proliferation is validated in a genetic mouse model of lung cancer harboring a dual Ts/Tk1 tumor-specific knockout, in which tumors grow despite lacking enzymatic dTMP synthesis and tumor progression is opposed by gap junction inhibition.

Conclusion

These findings revise the current dogma of ubiquitous nucleotide biosynthesis in each proliferating cancer cell in tumors and suggest that a programmed dTMP distribution maintains collective tumor growth. Collective tumor growth allows proliferation to functionally connect with other cancer hallmarks, affecting cancer pathology, thereby not only alters the basic biology of cancer, but also opens new therapeutic options by targeting metabolite sharing.

EACR25-2342

Deconvolution of heterogeneous 3D mammary spheroid dynamics using Oblique Plane Microscopy (OPM)

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Introduction

Genetic heterogeneity is increasingly recognised as a critical factor in breast cancer progression. The dynamic process of clonal evolution in breast cancer can be driven by ageing, menstrual cycles, pregnancy, and menopause, leading to a diverse genetic landscape. Malignancy can arise in these clones through secondary mutations, field cancerisation, or competitive behaviour. The heterogeneous composition of each tumour, under selective pressure, can shift the competitive advantage, leading to resistance of certain clones and recurrence of the disease with limited therapeutic options.

Material and method

In this project, we developed a mammary spheroid system that enables longitudinal observation and analysis of clonal competition from the onset of mutational heterogeneity to the progression to the invasive stage, facilitating the study of drug responses in a heterogeneous background. Using oblique plane microscopy (OPM), a light-sheet microscopy technique that illuminates a tilted plane of the specimen and collects fluorescence with the same high numerical aperture objective, we captured high-content, 3D time-lapse imaging of up to 180 spheroids over 8 days at 8-hour intervals. Machine-learning-based nuclear segmentation with Cellpose allowed for precise nuclei counting and categorisation into up to three different oncogenes, each associated with a unique fluorescent protein. This segmentation tool enabled measurements of fluorescence intensity, nuclear size, and nuclear count for each oncogene, quantifying clonal competition over time.

Result and discussion

The ability to track individual clones over time revealed shifts in competitive advantage and clonal composition under selective pressure. Our findings indicate that rare clones, such as BRAF mutant clones, may be shed early and evade detection through classical sequencing approaches, potentially explaining its low frequency in primary breast cancer and increased frequency in circulating tumour cells as well as secondary sites. High-content imaging with OPM provided detailed spatial information on heterogeneous spheroid architecture and clonal localisation, offering insights into clonal competition or resistance mechanisms. In addition, utilising this pipeline, we established a drug screening platform, validated with a proof-of-principle drug combination.

Conclusion

We developed a genetic model of heterogeneity and demonstrated the utility of high-content longitudinal imaging using OPM, combined with machine learning, to analyse clonal dynamics. Our platform offers a valuable tool for studying tumour evolution and competition in heterogeneous background, rare clones behaviour, and resistance mechanisms, supporting the development of more effective drug discovery strategies.

Symposium: Tumour Dormancy

EACR25-0988

Neuroblastoma metastatic cells adopt an embryonic diapause-like state in the fetal bone marrow driven by mTOR activity

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Introduction

Neuroblastoma (NB) is a deadly pediatric cancer of the peripheral sympathetic nervous system. NBs arise from an embryonic transitory cell lineage: the sympathoadrenal cells of the neural crest (NC). NB primary tumors typically form in the abdomen along the sympathetic chain and in the adrenal gland. Over 50% of NB patients are diagnosed with extensive metastases, particularly in the bone marrow (BM), precluding early therapeutic intervention and therefore representing a major clinical challenge. Although both tumor initiation and metastatic onset towards the BM have been proposed to occur during fetal life, in a temporal sequence that parallels the fetal development of the NC, why BM-infiltrating NB cells remain undetected up until large metastatic colonization of the BM and diagnosis of the disease is unknown.

Material and method

We combine multiscale analyses of patient samples with functional experiments in innovative and adapted models that reproduce the fetal (FBM) and pediatric (PBM) bone marrow microenvironment, in the context of NB metastasis. We exploit an avian graft model that faithfully recapitulates NB metastatic spread from sympathoadrenal tumors, with NB cells primarily reaching the fetal BM through the dorsal aorta (DA) and peripheral nerves. Complementary to this fetal model of BM metastasis, we use a standardized 3D model of human BM, implemented with primary fetal and pediatric mesenchymal stem cells (MSC) to decipher the complex and dynamic ecosystem provided by the fetal and pediatric BM upon NB seeding.

Result and discussion

Upon reaching the fetal BM, single cell transcriptomic analyses show that NB cells initially enter a reversible diapause-like resting state. Through a series of functional assays, we demonstrate that NB cells exposed to fetal BM-MSCs or to their secretome only are driven into this dormant state, whereas pediatric BM-MSCs have the opposite effect, promoting diapause exit, cell cycling and metabolic activity. Moreover, we describe that fetal BM-MSCs drive this diapause-like state by decreasing the activity of the growth-regulating mTOR signaling pathway, whereas pediatric BM-MSCs reactivate this pathway. Such regulation appears to be mediated by specific MSC secreted factors that impact tyrosine kinase receptors (RTKs) and integrin signaling.

Conclusion

These findings explore for the first time the spatio-temporal dynamics of NB metastasis in the BM,

revealing a feto-pediatric transition from a diapause-like resting state to an awaken pediatric state, and therefore provide a major insight in the understanding of the dynamics of NB metastases and of the role the BM compartment plays in this process.

EACR25-2481

Translational Reprogramming Drives Immune Evasion in Melanoma Persister Cells Through MIF Secretion

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Introduction

Metastatic melanoma often relapses following targeted therapy (TT) or immune checkpoint inhibitors (ICI), suggesting that melanoma persister cells (MPC) evade immune surveillance. We investigated how translational reprogramming in MPC contributes to tumor persistence and immune evasion.

Material and method

We analyzed supernatants from melanoma cell lines (parental vs. MPC) using chemokine arrays and ELISA, and assessed their impact on lymphocytes and monocytes through metabolic and functional assays. MIF secretion was measured in patient biopsies before and after treatment initiation. CRISPR-Cas9 and siRNA approaches were used to study MIF regulation and its transporter ABCA7. Polysome profiling was performed to assess translational regulation in MPC.

Result and discussion

MPC exhibited globally reduced translation but increased translation of specific mRNAs encoding proteins involved in chromatin remodeling and immune modulation. MIF was identified as a key immunosuppressive factor highly secreted by MPC, leading to reduced immune cell fitness. MIF knockout in MPC abrogated these effects, confirming its role in immune evasion. The transporter ABCA7 was upregulated at the translational level in MPC and identified as a key regulator of MIF secretion. Additionally, low immune infiltration and poor patient outcomes correlated with high expression of MPC markers in biopsies. Targeting eukaryotic initiation factor 4A (eIF4A) in combination with TT delayed resistance and improved survival.

Conclusion

MPC persistence is driven by translational reprogramming, which enhances MIF secretion through ABCA7, fostering an immunosuppressive tumor microenvironment. Targeting translation may represent a promising therapeutic avenue to counteract melanoma persistence and resistance.

Symposium: AI Tools in Cancer Research

EACR25-0439

Spatial and multi-omic profiling of HGSC to identify immune vulnerabilities for precision immunotherapy

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Introduction

Tumorigenesis and cancer progression rely on malignant cells evading immune system. Despite promising immunotherapies, many high-grade serous carcinoma (HGSC) patients do not respond, highlighting the need to explore spatial immune dynamics. We aim to use spatial multi-omic profiling, and machine learning to uncover immune evasion strategies, identifying prognostic markers and therapeutic targets for precision oncology.

Material and method

This project used samples collected during Oncosys-Ova study. We analyzed 42 FFPE tumor samples, including 16 paired chemo-naïve and treated samples, alongside clinical and sequencing data. Spatial single-cell protein analysis was performed using t-CycIF imaging of FFPE samples, targeting ten key markers for tumor, stromal, and immune cells. Additionally, we conducted Nanostring GeoMX spatial transcriptomics on regions of interest (ROIs) from adjacent FFPE slides, guided by t-CycIF imaging.

Result and discussion

We developed a new method to choose ROIs in GeoMX, using t-CycIF to improve the identification of cell hubs through specific immune markers. In this project, we focused on the following immune markers: CD4 (CD4+ T cells), CD8 (CD8+ T cells), CD11c (dendritic cells), Iba1 (macrophages), and NKG2a (natural killer cells). Raw images underwent pre-processing to reduce noise and enhance true cell signal clustering. Overlaps of pre-processed true signals were identified across different marker combinations, and the most informative ROIs were selected through automated selection based on their size, as well as subsequent manual inspection in Napari viewer. We acquired 10 ROIs in 14 samples and a varying quantity in additional 28 samples (median 17 ROIs), prioritizing diversity in selected ROIs. Spatial transcriptomics (GeoMX) analysis was performed adjacent to the t-CycIF slide for selected ROIs to assess gene expression profiles in tumor and stromal compartments. We analyzed 213 compartments in 14 samples and approximately 800 more compartments from the remaining 28 samples. Our multi-omic study integrated gene and protein expression profiles from tumor, immune, and stromal cells in around 1000 compartments across 42 samples. Combining these datasets with patient genomic and clinical data enabled a detailed characterization of the spatial molecular landscape of HGSC tumors. Such protein and

transcriptomic atlas reveal immune hubs and their interactions with cancer and stromal cells, offering insights into immune escape mechanisms. Preliminary data indicate that spatial tumor microenvironment features correlate with clinical outcomes, highlighting potential immune vulnerabilities and therapeutic targets, which could be uncovered with the help of machine learning.

Conclusion

Our study aims to provide insights into the spatial immuno-genomic landscape of HGSC, informing the development of targeted immunotherapeutic strategies for improved patient outcomes.

EACR25-1681

High-throughput drug screening in breast cancer PTCs enables clinically relevant drug discovery for personalised medicine

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Introduction

Traditional drug discovery for precision medicine relies on high-throughput drug screening (HTDS) on cancer cell lines (CCLs). However, CCLs have lost their resemblance to primary tumours, which leads to a modest conversion rate of clinically useful discoveries. Patient-derived Xenografts (PDXs) more faithfully recapitulate the original tumour phenotype than CCLs, but their application in drug screens is limited by logistical constraints specific to in vivo drug testing. Here, we performed HTDS in PDX-derived tumour cells (PTCs) and demonstrate their superiority to CCLs and PDXs for clinically relevant drug discovery in precision medicine.

Material and method

We treated PTCs from 34 different breast cancer PDXs with 67 compounds at 7 concentrations in 3 technical replicates, generating a total of 47 838 drug response data points. The compounds were either approved cancer treatments or drugs targeting key cancer pathways. Viability was measured by Cell-Titer-Glo (CTG) after 7 days and area under the dose-response curve (AUC) was calculated to determine therapy effectiveness. Tumours were profiled by shallow whole genome sequencing, whole exome sequencing, single-cell RNA sequencing, methylation-sequencing, protein mass cytometry and reverse phase protein array, yielding an average of >200 000 features per PDX. Machine learning was used to extract integrative multiomic signatures predictive of drug response. These were validated by testing their ability to predict drug response in real-world clinical trials.

Result and discussion

First, we described the landscape of drug response in breast cancer PDTCs. As expected, drugs sharing similar biological targets displayed similar activities. This held true both at the scale of drug class (e.g. DDR vs mitogenic inhibitors), and drug target (e.g. ERK/MEK inhibitors vs PI3K inhibitors). Subsequently, we characterised the multiomic profile of PDXs and linked it with drug response. We found that ER status associated with resistance to DDR agents, but not to mitogenic signalling, while PI3K activation led to resistance to drugs targeting mitogenic signalling, but did not alter sensitivity to cytotoxic chemotherapy. We showed that a multiomic machine learning model trained on PDTCs could predict drug response in clinical trials. Moreover, this PDTC-based model was superior to an equivalent model trained on CCLs – for example, at predicting pathological complete response in a trial of neoadjuvant anthracycline of 168 patients ($p < 0.01$).

Conclusion

We showed that PDTC drug screens result in higher rates of clinically relevant drug discoveries than traditional CCL HTDS, without the logistical impairments linked to screening PDXs *in vivo*. This approach could be used to accelerate the implementation of precision medicine across all cancers.

Symposium: DNA Repair and Immunosurveillance

EACR25-0256

Exploit neutrophil heterogeneity to induce synthetic lethality in breast cancer

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Introduction

About 80% of breast cancers are hormone-dependent. Although anti-hormonal therapy remains a treatment mainstay, a considerable fraction of these patients does not respond to the therapy and ultimately progress with advanced cancer. Tumor evolution is one of the primary mechanisms responsible for acquiring therapy-resistant and more aggressive cancer clones. Whether the tumor microenvironment through immune-mediated mechanisms might promote the development of more aggressive cancer types is crucial for identifying additional therapeutic opportunities.

Material and method

To achieve these goals, my team has set up innovative state of the art methodologies, used different *in vivo* mouse models of breast cancer and performed validation in the patients.

Result and discussion

Recent results from my lab identified a subset of immature neutrophils named Neutrophil Precursors (PreNeu) that are enriched and can proliferate in the tumors of highly proliferative hormone-dependent breast cancer patients, an aggressive tumor type characterized by poor prognosis. We demonstrated that these cells directly promote cancer genomic instability, favoring the

generation of therapy-resistant cancer clones.

Mechanistically, we found that the oncometabolite succinate, secreted by tumor-associated PreNeu, impairs homologous DNA repair, promoting error-prone DNA repair through non-homologous end-joining regulated by PARP-1. Consequently, breast cancer cells acquire genomic instability, resulting in tumor progression and resistance to endocrine therapies. Selective inhibition of these pathways induces increased tumor cell kill *in vitro* and *in vivo*. Intra-tumour PreNeu score correlates with copy number alterations in highly proliferative hormone-dependent tumors from breast cancer patients. Having found that the PreNeu induce dependence of the tumor cells on alternative DNA repair mechanisms mediated by PARP, we tested and demonstrated that the presence of PreNeu in the tumor sensitizes the tumors to PARP inhibitors. Treatment with PARP inhibitors counteracts the pro-tumorigenic effect of these neutrophils and reverses endocrine resistance.

Conclusion

Our data add novel insights into the neutrophil heterogeneity in breast cancer, describing an unexpected new function for this immune subset as cellular mediator of synthetic lethality in tumors. For a clinical prospective, these data demonstrate that olaparib treatment can be effective not only in tumors that harbor genetic homologous recombination deficiency *per se* but also in tumor contexts in which the homologous recombination is altered by the presence of tumor-associated PreNeu.

EACR25-0468

Intratumoral STING pathway activation enhances anti-tumor immune responses and therapeutic efficacy of ATR inhibition

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Introduction

Small cell lung cancer (SCLC) is the most aggressive lung cancer subtype. Recent trials of immune checkpoint blockade (ICB) combined with chemotherapy have observed modest benefits. This study explores the therapeutic potential of targeting ATR, the key activator of the replication stress response, in SCLC.

Material and method

In this study, we performed genetic and pharmacological inhibition of ATR in a panel of human and murine SCLC cell lines. Furthermore, we investigated the effect of ATR inhibition either alone or in combination with PD-L1 blockade in multiple immunocompetent mouse models of SCLC. The downstream effects of ATR inhibition were assessed by bulk RNA sequencing, multicolor flow cytometry, western blot analysis, and real-time qRT-PCR. SCLC clinical samples from treatment-naïve patients and patients treated with an ATR inhibitor were analyzed by single-cell and bulk RNA sequencing to ascertain the effect of ATR on immune subsets.

Result and discussion

In multiple immunocompetent SCLC mouse models, ATR inhibition (ATRi) remarkably enhanced the anti-tumor effect of PD-L1 blockade. We next tested ATR inhibition either alone or in combination with PD-L1 in the second-line regimen for SCLC. We observed that

ATR inhibition in combination with PD-L1 blockade significantly reduced tumor volume and prolonged survival of aggressive mouse models compared to PD-L1 alone. Targeting ATR enhanced the expression of PD-L1, activated the cGAS/STING pathway, induced the expression of Type I and II interferon pathways, and caused significant infiltration of cytotoxic and memory/effector T-cells into tumors. Interestingly, ATRi also led to significant induction of MHC class I in SCLC in vitro and in vivo models. Analysis of pre- and post-treatment clinical samples from a proof-of-concept study of a first-in-class ATR inhibitor, M6620 (VX970, berzosertib), and TOP1 inhibitor topotecan, in patients with relapsed SCLCs, validated the induction of MHC class I and interferon pathway genes, for the first time in this disease. Single-cell analysis of patient samples revealed how ATR inhibition modulated antigen presentation and confirmed the immunosuppressed phenotype in SCLC.

Conclusion

Our findings highlight ATRi as a potentially transformative vulnerability of SCLC, paving the way for combination clinical trials with anti-PD-L1. Given the increasing importance of immunotherapy for the management of SCLC and the fact that ATR inhibitors are already in clinical trials, combining an ATR inhibitor with PD-L1 blockade may offer a particularly attractive strategy for treating SCLC and contribute to the rapid translation of this combination into the clinic.

Symposium: Prevention and Early Detection

EACR25-0817

Tumour genotype shapes blood biomarker expression for use in pancreatic cancer detection and diagnosis

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Introduction

Typically diagnosed late, when systemic metastasis has already occurred, pancreatic ductal adenocarcinoma (PDAC) has one of the worst 5-year survival rates of any cancer type. For many patients with advanced disease, current chemotherapy regimens offer only modest benefit despite significant toxicity and surgical resection, the only treatment option with curative potential, is not possible. Therefore, while new treatments are much needed, diagnosing patients at an ‘earlier’ disease stage when surgery remains possible and the window of opportunity for treatment response is longer will be critical to improving patient outcomes. In this regard, the identification of biomarkers present in biospecimens that can be easily sampled from patients remains the focus of considerable research, however success has not been forthcoming.

Material and method

Using a suite of novel genetically defined murine isogenic models of early PDAC, engineered using CRISPR-Cas9 gene editing to represent the most common mutational backgrounds found in the human disease, coupled with the multi-omics analysis of blood, we sought to address whether common driver gene mutations in PDAC, and thus the genetic heterogeneity inherent to the disease, may represent an important confounding factor in the identification of a one-size-fits-all biomarker suitable for early detection.

Result and discussion

We show that both loss of Cdkn2a and / or Smad4 on the background of a KrasG12D p53^{-/-} genotype has profound effects on the profile of differentially expressed RNA species including protein coding RNAs, lncRNAs, snoRNAs, scRNAs, snRNAs and miRNAs, and on plasma protein expression, when compared to both healthy controls and chemically induced pancreatitis.

In addition, we find that loss of Smad4, a genomic event that occurs following progression from PanIN to PDAC, substantially limits the availability of blood biomarkers.

Conclusion

These findings identify the need to move towards genotype-specific biomarker signatures and uncover a potential role for SMAD4 loss in limiting opportunities for the early detection of pancreatic cancer.

EACR25-1604

Multi-omic spatial analysis reveals reshaping of tumour-immune dynamics at the transition to invasive colorectal cancer

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Introduction

The introduction of bowel cancer screening has led to an increase in the detection and removal of pre-malignant adenomas, however many of these would never progress to colorectal cancer (CRC) within a patient’s lifetime. This implies a key evolutionary bottleneck at the transition from adenoma to carcinoma, with immune surveillance likely playing a central role in suppressing the outgrowth of invasive cells. Here we analysed a cohort of “ca-in-ads” (adenomas with a small focus of cancer) to provide a unique snapshot of the invasive transition. We combined genomics, spatial transcriptomics, digital pathology and multiplex imaging, concurrently deriving the distribution of tumour clones, their gene expression and their interplay with the immune microenvironment.

Material and method

We performed multi-region low-coverage whole genome sequencing (lcWGS) and T-cell receptor sequencing (TCRseq) on archival ca-in-ads from 40 patients. For a subset of cases we performed spatial transcriptomics using the 10x Genomics Visium platform, including custom probes to detect T-cell clonotypes of interest. We applied a deep learning cell classifier to haematoxylin and eosin (H&E) stained sections to determine the abundance and spatial distribution of eight cell types, with detailed immunophenotyping on selected cases (using a 50-marker panel on Akoya's PhenoCyclerFusion platform).

Result and discussion

We found a significant increase in copy number alterations in carcinoma regions relative to adenomas, and this was accompanied by a large shift in the composition of the local T-cell repertoire. TCR sequencing revealed expanded T-cell clonotypes that were adenoma-specific or carcinoma-specific, and we validated the spatial exclusivity of these using a custom Visium assay. Digital pathology and cyclic immuno-fluorescence revealed significantly increased infiltration of macrophage and neutrophil populations in regions of invasive cancer.

Conclusion

Taken together our analysis suggests a striking reshaping of tumour-immune dynamics at the transition to an invasive phenotype in CRC. Understanding what governs this invasive shift could highlight new avenues for cancer prevention or identify individuals at risk of CRC.

MYCN copy numbers, accelerating intratumor genetic heterogeneity. We hypothesized that ecDNA-driven genetic heterogeneity fosters phenotypic diversity, enabling neuroblastoma cells to adapt to treatment-induced selective pressures. We investigated the impact of ecDNA-driven heterogeneity on phenotypic diversity and therapeutic response across primary tumors, patient-derived xenografts, and cell lines.

Material and method

MYCN copy numbers were quantified from neuroblastoma cell lines, patient-derived xenografts and neuroblastomas -/+ genotoxic therapies using fluorescence in situ hybridization (FISH). These data were integrated with mathematical models of oncogene copy number-dependent fitness, single-cell ecDNA quantification, and phenotypic analyses.

Result and discussion

Pronounced ecDNA-driven intratumor heterogeneity was observed in all 95 MYCN-amplified neuroblastoma samples. FISH-guided spatial proteomics revealed cells with high MYCN copy numbers exhibited protein signatures associated with replication stress and DNA damage, whereas senescence-associated pathways were enhanced in cells with low MYCN copy numbers. Single-cell ImmunoFISH confirmed varying ecDNA copy numbers influenced cell fate under chemotherapy. High-MYCN cells underwent apoptosis, while low-MYCN cells persisted as senescent or resumed proliferation after drug withdrawal. These ecDNA-mediated changes drive tumor phenotype and therapy response, as low-MYCN cells contribute to therapy resistance. Consequently, a senolytic "one-two punch" using doxo-rubicin and navitoclax achieved significant tumor growth control in neuroblastoma PDX models.

Conclusion

This study establishes how ecDNA-driven MYCN variability underpins tumor heterogeneity and therapy resistance. Conventional treatments eliminate high-MYCN cells, but low-MYCN cells survive, persist in senescence, and regain proliferative capacity. Refining therapeutic strategies to target low-MYCN cells may improve outcomes for high-risk MYCN-amplified neuroblastomas.

Symposium: EC DNA

EACR25-0364

Heterogeneous oncogene dosage driven by extrachromosomal DNA determines neuroblastoma response to therapy

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Introduction

Despite initial responsiveness to neoadjuvant chemotherapy, neuroblastomas with MYCN amplification frequently relapse, implicating dynamic processes in treatment resistance. In neuroblastomas, MYCN amplification predominantly occurs on extra-chromosomal DNA (ecDNA), which, due to the lack of centromeres, segregates unevenly during cell division. This uneven segregation generates cells with variable

EACR25-2262

Investigating the impact of extrachromosomal DNA amplification on cell fate decisions and therapy resistance

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Introduction

Despite advances in precision oncology, persisting treatment resistance necessitates a better understanding of therapy response mechanisms. In various tumours, the extrachromosomal amplification of oncogenes accelerates intratumoral heterogeneity development. Due to the lack of a centromere, ecDNA can undergo random segregation during cell division, enabling rapid copy number changes. While gradual selection pressures during tumor evolution are known to shape ecDNA composition, the effect of acute selection pressures on both ecDNA copy number and resulting cell fate

decisions, such as apoptosis, senescence or resistance, remains poorly understood.

Material and method

To investigate the relationship between ecDNA copy number dynamics and cell fate decisions, we utilized neuroblastoma and astrocytoma cell lines and patient-derived xenografts (PDX) harboring MDM2, CDK4 or MYCN amplifications, either in linear or extra-chromosomal form. These models were subjected to different targeted and cytotoxic therapies. To precisely capture rapid ecDNA copy number changes in relation to cell fate outcomes, we employed fluorescence *in situ* hybridization (FISH) for real-time ecDNA assessment. Cell fate responses were characterized using ATP-based viability assays and senescence-associated β -galactosidase (SA- β -Gal) stainings. To functionally link ecDNA copy number dynamics to cell fate on a single cell level, we coupled FISH with immunofluorescence (IF) for selected markers, such as H3K9me3, Cleaved Caspase-3, p21 and TP53.

Result and discussion

We show that both cytotoxic and targeted therapies induce ecDNA copy number changes and that cell fate decisions are tightly linked to ecDNA dynamics primarily by eliminating cells with defined ecDNA copy numbers. Importantly, cytotoxic and targeted therapies can influence ecDNA copy numbers differently as exemplified in neuroblastoma cells with extra-chromosomal MDM2 amplification and its impact on TP53 activation. MDM2 copy numbers, which strongly correlate with MDM2 protein levels, rise upon doxorubicin exposure to suppress TP53-mediated apoptosis to promote cell survival and fuel treatment resistance. Conversely, treatment with the MDM2 inhibitor Nutlin-3a leads to a marked decrease in MDM2 ecDNA due to higher TP53 activation in high MDM2 copy number cells and their subsequent cell death.

Conclusion

Our study demonstrates that ecDNA dynamics are intricately linked to cell fate outcomes as exemplified by a clear association of ecDNA copy number changes and TP53-mediated apoptosis in MDM2-amplified neuroblastoma cells. The results highlight how rapid alterations in ecDNA copy number heterogeneity serve as a reservoir for promoting resistance, emphasizing the need for combined ecDNA and cell fate-oriented therapeutic strategies to prevent relapse and improve treatment outcome in ecDNA-driven cancers.

POSTER PRESENTATIONS (Tuesday/Wednesday)

Bioinformatics and Computational Biology

EACR25-0123

Deciphering Tumor-Immune Crosstalk Through Multiplexed Imaging-Powered Deep Visual Proteomics (mipDVP)

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Introduction

The tumor microenvironment (TME), a dynamic ecosystem of tumor, immune, and stromal cells, dictates cancer progression and therapy response. Understanding its complexity is critical for advancing immunotherapy, yet conventional methods lack the spatial resolution and multiplexing depth to fully resolve cell-specific interactions. We introduce multiplexed imaging-powered Deep Visual Proteomics (mipDVP), an integrative platform combining high-plex immunofluorescence imaging, automated single-cell laser microdissection, and ultrasensitive mass spectrometry. This approach enables spatially resolved, single-cell-type proteomic profiling to investigate how tumor-immune interactions drive immune evasion.

Material and method

Using the MACSima Imaging Platform, we spatially mapped cell populations in formalin-fixed, paraffin-embedded tissues from colorectal and tonsil cancers (14–22 markers). Targeted single-cell-type laser microdissection (Leica LMD7) isolated ~100–200 cells per population (e.g., tumor cells, macrophages, cytotoxic T lymphocytes [CTLs]), followed by data-independent acquisition (DIA) mass spectrometry (timsTOF/Astral platforms).

Result and discussion

This workflow quantified 4,000–6,000 protein groups per population, achieving proteomic depth previously unattainable in spatially resolved studies:

1. Colorectal cancer (A cold tumor case): Macrophages formed spatially compartmentalized immunosuppressive barriers, limiting CTL infiltration. Proteomics revealed M2-like polarization and impaired antigen presentation in macrophages, while tumor-adjacent CTLs showed suppressed cytotoxicity.
2. Tonsil Cancer (A hot tumor case): CTLs localized near tumor cells exhibited hypoxia-driven metabolic adaptation, enhancing their cytotoxic function. Tumor cells proximal to CTLs displayed heterogeneous expression of immune-regulatory proteins, suggesting adaptive resistance.

Conclusion

mipDVP bridges the gap between spatial biology and proteomics, unveiling mechanisms of immune evasion and activation. By identifying metabolic checkpoints and

immunosuppressive drivers, this platform pinpoints therapeutic targets and predictive biomarkers. Its ability to decode TME heterogeneity at single-cell-type resolution positions mipDVP as a tool for precision oncology, guiding therapies tailored to a tumor's unique immune context.

EACR25-0176

HALO Breast IHC AI: Demonstration of its Application as a Training Tool for Students

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Introduction

Introduction: Immunohistochemical assessment of HER2, ER, PR, and Ki67 is essential in diagnosing invasive breast carcinomas and guides treatment, prognosis, and patient management. Here, we show how Indica Lab's clinical software can be utilised to undertake research projects and train medical students at the University of Bern; focussing on a recent study comparing breast cancer IHC scoring by undergraduates to that of trained pathologists, when unassisted or assisted by HALO Breast IHC AI.

Material and method

Fifty cases (250 slides) stained for ER, PR, HER2, and Ki67 were scored twice by three students and two certified pathologists. Initial scores were obtained using manual digital pathology methods (Visual Dx), followed by reassessment with AI assistance by HALO Breast IHC AI after a 4-week washout period (AI-Assisted Dx). Accuracy and agreement of the student scores were compared to those of the pathologists.

Result and discussion

AI-Assisted Dx produced greater agreement at the clinical cutoff for all four biomarkers for both students (ER: 85% to 91%, PR: 19% to 73%, HER2: 47% to 82%, Ki67: 58% to 98% agreement) and pathologists (ER: 98% to 100%, PR: 44% to 90%, HER2: 71% to 100%, Ki67: 75% to 98% agreement). Similarly, Fleiss' Kappa for all biomarkers increased for AI-Assisted Dx by an average of 0.42 for both students and pathologists. When both groups were combined, agreement at the clinical cutoff also showed improvement (ER: 85% to 91%, PR: 20% to 57%, HER2: 35% to 80%, Ki67: 51% to 98% agreement) indicating that student scores more closely aligned with pathologists when assisted by HALO Breast IHC AI.

Conclusion

HALO Breast IHC AI provides support for both students and certified pathologists, improving agreement and consensus scores across groups with different levels of experience. HALO Breast IHC AI can objectively standardise scores and be an effective training tool for students.

EACR25-0192

From Transcriptomic Data to Therapeutic Potential: Drug Repositioning for Papillary Thyroid Carcinoma

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Introduction

Papillary thyroid carcinoma (PTC) is the most prevalent type of thyroid malignancy, with an increasing incidence worldwide. Although conventional treatments are effective for many patients, some individuals encounter resistance or lack appropriate therapeutic options. This highlights the necessity for alternative therapeutic strategies. Drug repositioning has emerged as a promising approach in oncology, providing a faster and cost-effective manner for new treatments. This study aimed to identify potential repositioned drug candidates for PTC treatment and conduct their preliminary in vitro evaluation using a PTC cell line.

Material and method

Four microarray datasets (GSE6339, GSE3467, GSE27155, and GSE138198) were analyzed to identify differentially expressed genes (DEGs) between normal and PTC tissues. Drug repositioning was conducted based on commonly upregulated and downregulated genes using L1000CDS² search engine. The gene expression signatures method was employed to identify potential therapeutic molecules, leading to the identification of 50 repositioned drug candidates. Selected candidates were tested on the MDA-T32 PTC cell line to evaluate IC₅₀ values.

Result and discussion

The IC₅₀ values of the tested drugs varied considerably against MDA-T32 cells. NSC632839 exhibited the highest cytotoxicity, with an IC₅₀ value of 178.9 nM, followed by MP7 (2.166 μM), Iodophenpropit dihydrobromide (7.325 μM), and H89 (10.85 μM), indicating their strong inhibitory effects on MDA-T32 cell viability. Other tested compounds, including Cinnamyl-3, 4-dihydroxy-α-cyanocinnamate (14.59 μM), L741,626 (18.05 μM), and L732,138 (68.56 μM), demonstrated moderate cytotoxicity, whereas Clofibrate acid showed the lowest potency (3.926 mM) against PTC.

Conclusion

These findings indicate that NSC632839 and MP7 may serve as promising candidates for further evaluations. Future studies should focus on elucidating mechanisms of action and validating the therapeutic potential of those repositioned drugs.

EACR25-0205

A clinically interpretable machine learning model using 19 proteins to predict recurrence/metastasis in OSCC patients

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Introduction

Oral squamous cell carcinoma (OSCC) is a lethal disease with increasing incidence globally and poor prognosis (5-year survival ~50%), due to late diagnosis and high rates of recurrence and metastasis. The aim of this study is to develop a predictive model to identify the risk of recurrence or metastasis in patients who have undergone curative resection surgery for OSCC, in order to assist in personalised treatment planning and the implementation of better post-treatment monitoring.

Material and method

Clinical information from OSCC patients, including 27 clinical, diagnostic and treatment characteristics, and immunohistochemical images showing expression levels and patterns for 19 proteins, were used to develop and implement predictive models. Two machine learning approaches were compared: Decision trees and neural networks. The decision tree model was implemented using Scikit-learn, with hyperparameters tuned to control complexity and prevent overfitting. The neural network used a multimodal architecture combining convolutional layers for image feature extraction and fully connected layers for clinical data fusion to capture complex patterns in multimodal data. The models were trained on a 70-30 split of training and test data.

Result and discussion

A total of 200 patients (mean age = 61.4 ± 14.3 years), 63 patients had cancer recurrence/metastasis within the 3-year follow-up after undergoing curative resective surgery. For outcome prediction, the predictive models were evaluated which showed that decision tree achieved significantly higher accuracy than neural network (75% vs. 63%). The decision tree model also provided higher interpretability, with feature importance analysis revealing eight features contributed significantly to the prediction model. These were the expression levels of six proteins (CA9, E-cadherin, B-catenin, P-cadherin, Slug, and Versican) and two clinical features (invasion depth, age at diagnosis). These proteins promote tumour cell migration and invasion by regulating epithelial-mesenchymal transition, enhancing hypoxia adaptation, and remodelling tumour microenvironment, which the model has identified as potential biomarkers of OSCC progression.

Conclusion

The decision tree model exhibited superior performance and interpretability, and is a robust model for predicting OSCC recurrence and metastasis, though it is limited by the relatively small sample size. Future validation in multicentre cohorts is warranted. The proposed model has the potential to serve as a valuable tool for clinicians to improve post-treatment monitoring for OSCC patients.

EACR25-0282

MetaboDynamics: a Bioconductor package for the probabilistic analysis of longitudinal metabolomics data

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Introduction

Deregulated metabolism is one of the emerging hallmarks of cancer. While modern experimental methods can measure many metabolites over time and under different conditions, it is still challenging to analyze this kind of complex, noisy data. Current tools often rely on pairwise comparisons between time points or experimental conditions, limiting our understanding of dynamic metabolic changes. Additionally over-representation analysis is often restricted to pathways missing broader changes in the metabolome. To address this gap we developed “MetaboDynamics” [1], an R-package now available on Bioconductor.

Material and method

The MetaboDynamics workflow starts with concentration tables of metabolites over multiple time points and experimental conditions such as radiation doses. We use this data to fit a hierarchical Bayesian model, a model type known to avoid over- and under-fitting. From the fit we obtain estimates of concentration changes between time points and thus dynamics profiles for all included metabolites. These profiles can be used as input for clustering. Once clusters of metabolites with similar dynamics under one experimental condition are identified these clusters can be analyzed in three different ways:

- 1) By quantifying the probability of a pathway or biological function, represented by a KEGG functional module, being over-represented in a dynamics cluster using a hypergeometric probabilistic model
- 2) By comparing the dynamics profiles of clusters or groups of metabolites from different experimental conditions with a Bayesian model
- 3) By comparing the metabolite composition of metabolite clusters from different experimental conditions

Result and discussion

We applied MetaboDynamics to study the longitudinal effects (0-24 h) of different radiation doses (2 Gy & 10 Gy) on the metabolome of cancer cells in vitro. Estimated concentration differences between time points revealed consistent changes in amino acid concentrations over time. Clustering identified metabolite groups with similar dynamics, and functional enrichment analysis highlighted biological functions such as amino acid, nucleotide, and energy metabolism. Combining dynamics and metabolite composition comparisons revealed clusters with similar metabolite compositions but differing dynamics between radiation doses. This suggests radiation dose-dependent changes in cancer cell metabolism in vitro. These results demonstrate the utility of MetaboDynamics in uncovering complex metabolic changes and providing new insights into longitudinal effects of experimental conditions or treatments on metabolism.

Conclusion

MetaboDynamics provides a robust and interpretable framework for analyzing complex metabolomics data, offering insights into dynamics and functional enrichment that traditional methods may overlook.

[1] doi: 10.18129/B9.bioc.MetaboDynamics, URL: bioconductor.org/packages/MetaboDynamics

EACR25-0319

Identification of Olveremabatinib as a Potential Inhibitor for ROR1+ Triple-negative Breast Cancer

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Introduction

Receptor tyrosine kinase-like orphan receptor 1 (ROR1) is an attractive target in breast cancer research due to its overexpression. This study aims to identify potential ROR1 inhibitors for investigation in triple-negative breast cancers through high-throughput virtual screening, molecular docking, molecular dynamics (MD) simulations, and cell-based in vitro validation. The binding affinity and stability of a potential inhibitor, Olveremabatinib, were compared with Ponatinib, a known ROR1 inhibitor.

Material and method

Virtual screening was performed using the Glide SP docking module in Schrödinger 2022.2, screening over 9,000 compounds from DrugBank against ROR1 (PDB ID: 6TU9). The best-scoring compounds were further analyzed for their binding interactions, including hydrogen bonding, hydrophobic contacts, and π - π stacking. MD simulations were conducted using Gromacs v2023.3, with input files prepared via the CHARMM-GUI server. Protein-ligand complexes were solvated in a TIP3P water box, and the system was equilibrated under NVT and NPT conditions at 303.15 K and 1 bar. A 300 ns MD simulation was performed for Olveremabatinib and Ponatinib, and key analyses such as root mean square deviation (RMSD), root mean square fluctuation (RMSF), and interaction stability were conducted. Additionally, Olveremabatinib and Ponatinib were tested on ROR1+ MDA-MB-231 and ROR1- MCF-7 breast cancer cells using the WST-1 assay in a time- and dose-dependent manner.

Result and discussion

Olveremabatinib demonstrated the best docking score (-14.511 kcal/mol) among all screened compounds, including Ponatinib (-14.023 kcal/mol), indicating its strong binding affinity to ROR1. Olveremabatinib formed dual hydrogen bonds with Ile555, in addition to π - π stacking with Phe552 and a π -cation interaction with His613, which were absent in Ponatinib. Hydrophobic interactions with Leu487, Leu530, and Val536 further contributed to its stability. MD simulations confirmed these findings, with Olveremabatinib maintaining key hydrogen bonds with Ile555 and Asp633. RMSD analysis showed lower fluctuations, while RMSF indicated minimal flexibility in critical binding site residues. Cell viability assays demonstrated that Olveremabatinib had higher selectivity and inhibitory activity in ROR1+ cells than in ROR1- cells. It appeared to be more selective than Ponatinib against ROR1+ cells while demonstrating

comparable effectiveness. The long-lasting effects of Olveremabatinib across decreasing doses were especially remarkable.

Conclusion

This study highlights Olveremabatinib as a promising ROR1 inhibitor with strong and persistent interactions, making it a viable lead compound for further preclinical validation and development of targeted therapies against ROR1-driven triple-negative breast cancers.

EACR25-0320

Evolution of phenotypic plasticity leads to tumor heterogeneity with implications for therapy

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Introduction

Intratumor heterogeneity is a major challenge in cancer treatment, arising from both somatic evolution and phenotypic plasticity. While genetic diversity in tumors has been widely studied, the interplay between evolutionary changes and phenotypic plasticity remains poorly understood. In glioblastoma, the most aggressive brain tumor, plasticity between migration and proliferation plays a crucial role in tumor progression. We investigate how somatic evolution shapes phenotypic plasticity, particularly how tumor cells respond to microenvironmental changes.

Material and method

We develop a spatially explicit model that tracks both genetic and phenotypic states of individual tumor cells. In our model, cells switch between migratory and proliferative states, with switching dynamics influenced by inherited genotypes, mutations, and local microenvironmental conditions. We use analytical and computational approaches to predict evolutionary outcomes and quantify resulting heterogeneity patterns.

Result and discussion

Our simulations reveal that tumor cells at the periphery tend to evolve toward a migratory phenotype, whereas cells within the tumor bulk favor proliferation. Strikingly, this pattern emerges from different genetic configurations, highlighting the role of microenvironment-driven selection. We further show that tumors display varying levels of genetic and phenotypic heterogeneity, with phenotypic heterogeneity emerging as a strong predictor of tumor recurrence time after treatment. Unlike genetic heterogeneity, higher phenotypic heterogeneity correlates with worse treatment outcomes.

Conclusion

Our study provides a mechanistic explanation for the heterogeneous recurrence patterns observed in glioblastoma. We show that phenotypic heterogeneity, rather than genetic heterogeneity, is a key determinant of treatment success. This insight may help refine therapeutic strategies by targeting tumor plasticity to improve patient outcomes.

EACR25-0321**Integrated Genomic and Structural Analysis Reveals Novel Insights into Polymerase Proofreading Deficiency Drivers***D. Dayan¹, Y. Maruvka²*¹*Technion - Israel Institute of Technology, Biotechnology and Food Engineering, Haifa, Israel*²*Technion - Israel Institute of Technology, Lori Loka Center for Life Science and Engineering, Haifa, Israel***Introduction**

Polymerase proofreading deficiency (PPD), caused by mutations in POLE and POLD1 genes, leads to extremely high tumor mutation burdens and distinct mutational signatures. While PPD tumors show promising responses to immunotherapy, identifying the specific variants responsible remains challenging. Current variant classification efforts are incomplete, with many variants of unknown significance, including well-known drivers like POLE P286R and V411L.

Material and method

We analyzed 235,161 sequenced tumors from three major databases (GENIE, TCGA, and CPC) using an iterative SVM approach that incorporated both mutation burden and signature analysis. For structural characterization, we leveraged AlphaFold2 to generate 160 models for each variant, developing a novel metric based on pLDDT scores to assess structural impact. We further employed AlphaFold3 to analyze magnesium ion binding in specific variants. Clinical correlations were examined across different driver clusters.

Result and discussion

We identified 567 PPD tumors across 13 cancer types, with 515 harboring driver variants. Our analysis revealed novel POLE drivers outside the exonuclease domain, challenging traditional assumptions about variant pathogenicity. Structural analysis achieved an AUC of 0.85 in distinguishing drivers from passengers, outperforming existing tools like AlphaMissense (AUC = 0.73) and MutPred2 (AUC = 0.63). We identified three distinct structural regions affected by PPD variants and classified drivers into six clusters based on their structural impact. These clusters showed significant associations with MMR status, mutation burden, and signature profiles. Clinical analysis revealed age-related correlations specific to male colorectal cancer patients and endometrial cancer cases. Our integrated approach provides novel insights into PPD variant classification and their functional impacts. The characterization of distinct structural clusters suggest multiple mechanisms through which PPD variants affect polymerase function. The differential associations of clusters with clinical features and molecular characteristics indicate potential prognostic and therapeutic implications. Saturation analysis suggests that while additional drivers may be discovered with larger datasets, the current list captures most clinically relevant variants.

Conclusion

This comprehensive analysis of PPD drivers combines genomic evidence with structural insights to advance our understanding of polymerase proofreading deficiency.

Our findings provide a framework for variant classification and suggest that different PPD drivers may operate through distinct mechanisms, potentially influencing clinical outcomes. The established relationships between structural impacts and molecular features offer new perspectives for personalized therapeutic approaches in PPD tumors.

EACR25-0400**Deep Learning-Based PET Synthesis from CT Scans: Advancing Abdominal Lymph Node Metastasis Assessment***W. Gong¹, Y. Qu², M. Zhou³, F. Li¹, H. Zhang¹, H. Wang⁴, A. Liu¹, P. Sun¹*¹*Yantai Yuhuangding Hospital, Department of Oncology, Yantai, China*²*Yantai Yuhuangding Hospital, Radiology Department, Yantai, China*³*Yantai Yuhuangding Hospital, Nuclear Medicine Department, Yantai, China*⁴*Yantai Yuhuangding Hospital, Information Technology Department, Yantai, China***Introduction**

Abdominal lymph node metastasis (ALNM) plays a critical role in cancer staging, treatment decision-making and defining radiotherapy target boundaries. However, traditional CT imaging lacks functional metabolic data, making it difficult to distinguish benign lymph nodes from malignant ones especially the tiny lesions. PET/CT is commonly used to identify ALNM, offering both metabolic and anatomical data. Despite its benefits, widespread use is limited by high costs, radiation exposure, and limited availability. With advances in deep learning, particularly Generative Adversarial Networks (GANs), generating synthetic PET-like images from CT scans provides a feasible computational strategy for PET imaging, potentially complementing standard imaging workflows. This study explores a deep learning model to generate PET-like images from CT to enhance ALNM assessment and improve clinical decision-making for cancer patients.

Material and method

A deep learning model was derived from Reg-GAN. The model was trained on a training set using paired CT and PET/CT scan images of patients with suspected ALNM. After training, the PET/CT images were synthesized with an estimated average approximately 40 seconds per patient. The diagnostic quality of contrast, sharpness, and lesion visibility was assessed by three independent radiologists using a 5-point Likert scale, ensuring the confidentiality of the image type.

Result and discussion

The synthetic PET images closely resembled real PET scans, with radiologists rating nearly 85% as "good" or "excellent" (Likert score ≥ 4), with a high inter-reader agreement ($\kappa = 0.82$). The generated images improved lesion detection, particularly those with a diameter ≤ 10 mm, which are often missed by conventional CT or MRI. Unlike previous studies on PET synthesis focusing on lung or brain cancer imaging (CT-to-PET, MRI-to-PET), this model is specially designed for ALNM, where metabolic imaging significantly influences clinical

decisions. The results support AI-generated PET synthesis as a practical adjunct to CT, improving diagnostic capabilities, particularly in preoperative planning and follow-up assessments of lymph node involvement.

Conclusion

This study demonstrates the potential of PET synthesis generated from CT images using deep learning, providing a cost-effective and radiation-free option for ALNM assessment without direct PET acquisition. The method may improve surgical strategies and radiotherapy target delineation. To enhance its applicability in diagnosis and cancer management, future research will focus on multi-center validation and integration with multimodal biomarkers.

EACR25-0449

nf-hlamajority: a Nextflow pipeline for consensus MHC class I genotyping and its application to neoantigen identification in breast and lung cancer stromal cells

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Introduction

Cancer-associated fibroblasts (CAFs) are a heterogeneous cell type found in the tumour microenvironment. CAFs can support tumour growth and metastasis and contribute to therapeutic resistance, making them a potential therapeutic target. Here, we aim to identify neoantigens resulting from somatic mutations in CAFs. HLA genotyping, a critical step for neoantigen prediction, can be performed using DNA sequencing data with various tools available. Claeys et al. (PMID:37161318) found that a majority voting approach improved HLA typing performance. No end-to-end pipeline exists to apply this approach, making it difficult for non-informatics to implement. The objectives of this study were to:

- 1) develop a Nextflow pipeline implementing majority voting for MHC class I typing from DNA sequencing, and
- 2) use HLA calls from this pipeline to identify neoantigens in CAFs.

Material and method

CAFs and corresponding tumour-associated normal fibroblasts (TANs) were cultured from the tissue of 11 patients with breast cancer (10 Luminal A, one triple-negative) and 10 patients with lung cancer (six adenocarcinoma, two squamous cell carcinoma, two of unknown subtype). Whole-exome sequencing (WES) and bulk RNA sequencing were carried out on all samples. Using our pipeline, nf-hlamajority, we carried out HLA typing on our patient WES data and WES data from 12 NCI-60 Human Tumor Cell lines using Optitype, Polysolver, HLA-LA, and Kourami. For each HLA gene, the pipeline assigned the HLA genotype called by the

highest number of tools. These HLA genotypes were used as input to Landscape of Effective Neoantigens Software (LENS), along with the WES and RNA-sequencing data, to identify CAF-specific neoantigens (PMID: 37184881).

Result and discussion

Results from the NCI-60 dataset showed 97% accuracy, with 68 out of 70 HLA calls matching PCR-based genotyping. LENS identified potential neoantigens resulting from missense mutations, with more high-confidence expressed mutations observed in lung cancer CAFs compared to breast cancer CAFs (Welch's Two Sample t-test, $p = 0.017$). All missense mutations were private, although two lung cancer CAF samples had a mutation in the COASY gene. Interestingly, this gene and other genes harbouring mutations are implicated in lipid metabolic pathways. CAFs contribute to lipid metabolism within the TME, thus impacting cancer progression and tumour immunogenicity.

Conclusion

In this study, we have developed an automated pipeline for consensus HLA genotyping which we envisage will be useful to the research community. nf-hlamajority has helped us identify candidate neoantigens in breast and lung cancer CAFs. Future work will focus on validation using T-cell immunogenicity assays and investigating the CAF subpopulation distribution of our candidate neoantigens using single-cell RNA sequencing. This will improve our understanding of the potential of targeting CAF neoantigens to enhance the efficacy of anti-cancer therapies.

EACR25-0451

Integrative analysis of the TBXT-driven transcriptome and proteome in chordoma

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Introduction

Chordoma is a rare spinal neoplasm driven by over-expression of the embryonic transcription factor (TF) TBXT. Pharmacologic inhibition of TBXT is challenging, and its molecular network in chordoma is not well studied. Using designed ankyrin repeat proteins that specifically bind TBXT and prevent its binding to DNA (T-DARPin), we profiled and integrated the transcriptomes and proteomes of chordoma cells upon TBXT inhibition to identify TBXT-regulated genes and proteins that might represent novel, targetable vulnerabilities.

Material and method

Chordoma cells were transduced with vectors encoding a non-targeting control DARPin or three T-DARPin in

biological triplicates. Total RNA was sequenced using Illumina TruSeq Stranded RNA Kit (paired end, 100 cycles), and raw data were processed with an in-house workflow that uses STAR for alignment and featureCounts to estimate gene expression. Proteomes were analyzed via liquid chromatography-tandem mass spectrometry and raw data were processed with MaxQuant or Spectronaut to estimate relative protein abundance. Gene and protein annotations were imported from GENCODE and UniProt. A custom workflow based on the R packages DESeq2, DEP, and limma was developed to analyze, integrate, and annotate differentially expressed genes (DEGs) and proteins (DEPs). Functional annotation analysis was performed via pre-ranked gene set enrichment analysis (GSEA). Potential drug targets were selected based on The Cancer Druggable Gene Atlas.

Result and discussion

TBXT inhibition induces widespread changes in the transcriptome and proteome. We found 3,271 DEGs common to all three T-DARpins and 960 DEPs common to at least two. In addition to known TBXT targets and chordoma-associated genes, IGFBP3 stood out as the most upregulated protein. 393 DEPs/DEGs are targetable by clinically approved or experimental drugs. GSEA indicated suppression of pathways associated with cell division and DNA replication and repair upon TBXT inhibition, in line with TBXT's oncogenic role in chordoma. We also observed downregulation of embryonic progenitor cell signatures and upregulation of differentiated cell type signatures, suggesting alteration of cell identity, and downregulation of interferon response and JAK-STAT signaling pathways, which we found to be active in tumors of chordoma patients. Proteome profiling of cells with combined TBXT and IGFBP3 inhibition showed that part of the TBXT regulome is modulated by IGFBP3, particularly the interferon response pathway. Accordingly, chordoma cells were sensitive to JAK2 inhibition, suggesting a novel treatment option for chordoma patients using clinically approved JAK2 inhibitors.

Conclusion

DARPin-mediated TF inhibition combined with integrative multi-omics represents a novel strategy to investigate the regulatory networks of cancers driven by aberrant TF activity, which can lead to novel entry points for targeted therapies.

EACR25-0461

Mutational signatures as footprints of tumors' metastatic tropism

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Introduction

Tumors vary in their metastatic pattern. Metastases (mets) often exhibit distinct mutational landscapes compared with primary tumors. Mutational signatures – patterns of mutations reflecting underlying sources of cancer mutagenesis – have been extensively studied in primary tumors. However, their role in metastatic disease remains less well understood. In this study, we performed a

comprehensive analysis of mutational signature enrichment in primary tumors and metastases across multiple metastatic sites and cancer types.

Material and method

We analyzed more than 14,000 samples from the MSK-MET and MSK-IMPACT cohorts. Dominant mutational signatures were identified using MESiCA (Yaacov et al., 2024), a machine learning tool we developed for targeted gene panels. Briefly, MESiCA uses a neural embedding model that learns to represent mutation and signatures as vectors. For each new sample, observed mutations are compared to reference signature values. MESiCA detected the presence of APOBEC, SBS1, SBS5, MMR, POLE, and UV signatures. Two-sided Fisher's exact test was used to assess associations between signatures and metastatic samples, as well as with different metastatic sites.

Result and discussion

Mutational signature landscape between mets and primary tumor specimens was compared, stratified by cancer type. Of the total, 7,793 samples were primary and 5,294 were mets. APOBEC, SBS5, and UV signatures were enriched in mets samples, whereas SBS1, MMR, and POLE were enriched in primary tumor. The Tobacco signature showed no enrichment. Notably, APOBEC was enriched in mets originated from head and neck cancer (Odds Ratio [OR] 4.77), non-small cell lung cancer (OR 1.34), and thyroid cancer (OR 1.94). SBS5 was enriched in colorectal cancer mets (OR 1.40) and UV in melanoma mets (OR 2.47). Conversely, SBS1 was enriched in primary cervical (OR 0.17) and colorectal cancer (OR 0.71). MMR and POLE were linked to primary endometrial and colorectal cancer (OR 0.70 and OR 0.20, respectively). In site-specific analyses (comparing each metastatic site against the other 19 sites in a pan-cancer approach), mets harboring APOBEC were significantly associated with bladder, bone, brain, head and neck, mediastinum, and pleura (OR 1.3–1.91), but negatively associated with gastrointestinal sites (biliary tract, bowel, intra-abdominal, and liver; OR 0.58–0.71). UV signatures were highly enriched in skin (OR 4.56) and brain mets (OR 1.33). POLE showed no significant enrichment. All reported associations were significant at an FDR-corrected $p < 0.05$.

Conclusion

This large-scale analysis reveals distinct enrichment patterns of mutational signatures between primary and metastatic specimens and across different metastatic sites. Our findings demonstrate site-specific preferences for certain signatures, suggesting that mutational processes may influence metastatic tropism and could help in guiding clinical strategies.

EACR25-0475

Classification of Small Blue Round Cell Tumors via mass spectrometry imaging of peptide and glycan content

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Introduction

Small blue round cell tumors (SBRCT) is an umbrella term referring to tumors of similar histological presentation but may have completely different origins, e.g. Ewing sarcoma or nephroblastoma. Correct diagnosis is therefore essential due to significantly different treatment approaches and prognosis for each tumor entity. Matrix-assisted laser desorption/ionization mass spectrometry imaging (MALDI-MSI) is a technique enabling visualization and analysis of the spatial distribution of different molecular classes, such as peptides, glycans, lipids, and metabolites. We used MALDI-MSI of tryptic peptides and N-glycans from archival SBRCT samples to create classification algorithms that could reliably distinguish the different entities and identify discriminatory features between the tumor types.

Material and method

Six different SBRCT types were assembled into a tissue microarray (TMA; n = 26): acute lymphoblastic leukemia/lymphoma (ALL, n = 5), Ewing sarcoma (EWS, n = 5), neuroendocrine carcinoma (NEC, n = 5), nephroblastoma (NEPB, n = 3), neuroblastoma (NEUB, n = 3), and rhabdomyosarcoma (RMS, n = 5). TMA sections underwent on-tissue enzymatic digestion using either N-glycosidase F or trypsin, followed by matrix application and measurement on a mass spectrometer (rapifleX, Bruker). Following measurement, the sections were H&E stained, digitized and histopathologically annotated (QuPath, v.0.4.3). Data analysis was performed using SCiLS Lab (Bruker, v.2024a), Weave (v.1.0, Aspect Analytics), and Python (v.3.9). Weave was used for image co-registration, combining the peptide and glycan data into a single dataset. This enabled comparison of analytes and histology, and interactive browsing of data analysis results through a web-based report.

Result and discussion

MALDI-MSI data was employed for the training and validation of four classification algorithms (gradient boosting, support vector machine, k-nearest neighbor, and linear discriminant analysis) using 10-fold cross-validation on a pixel level. Multiclass classification was implemented as a one vs. rest (one entity vs. all other entities) and one vs. one (one entity vs. every other entity) approach. For the one vs. rest strategy, the mean accuracy of all classifiers was 90.5% with a mean accuracy of 88.7% for peptide analysis, 92.4% for glycan analysis, and 95% for combined peptides and glycans. For the one vs. one approach, mean accuracy of all classifiers was 92.4% with a mean accuracy of 88.8% for peptide analysis, 96.1% for glycan analysis and 97% for the combined dataset.

Conclusion

In summary, MALDI-MSI of peptides and glycans, particularly in combined form, shows potential in distinguishing between different SBRCT entities. Future work will expand the patient cohort size and explore alternative statistical methods to further improve classification performance.

EACR25-0476

Weave: a software package for integrated spatial multi-omics visualization and data analysis

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Introduction

There has been enormous progress in single-omics spatial technologies, which has revolutionized our understanding of the tumor microenvironment (TME). While the data generated from these methods have helped to unravel cellular intricacies within spatial context at the genomic, transcriptomic, metabolomic and protein levels, users are increasingly combining different omics readouts to obtain a holistic view of TME heterogeneity and complexity. However, spatial multi-omics data analysis presents specific bioinformatics challenges, as the data is typically acquired at different spatial resolutions, using a variety of platforms, and generates large data volumes. We present Weave, a cloud-based software for spatial omics bioinformatics, enabling efficient integration and joint visualization of different spatial-omics assays.

Material and method

Human lung cancer sections were sequentially analyzed with spatial transcriptomics using a cancer panel targeting 289 genes (Xenium, 10X Genomics), followed by multiplexed immunofluorescence using a 40-antibody panel (COMET, Lunaphore), and then H&E staining. The H&E images were digitized (Axioscan 7, Zeiss), and pathology annotation performed in QuPath. Cell segmentation was performed on the Xenium dataset using DAPI-based nuclear expansion (10X Genomics), and on the COMET dataset using CellSAM. All data were co-registered at full resolution using a non-rigid spline-based algorithm, then visualized in a web-based viewer (Weave, Aspect Analytics).

Result and discussion

Weave software was developed to address several spatial omics bioinformatics challenges. This software fully supports joint visualization of different spatial omics assays, and multiple common downstream multimodal analysis pipelines. The cloud-based software allows interactive browsing of datasets at full resolution via web-browsers, enabling communication of results between collaborators, and removes limitations of location or operating system. For the use case presented here, we utilized an advanced integration pipeline to match readouts across the COMET, Xenium and H&E data, accounting for different sized measurement regions and spatial resolutions. Pathology annotations and cell segmentation results were integrated and overlaid as additional visualization layers. We conducted correlation analysis to identify which transcript-protein pairs had similar spatial expression and if this correlation was affected by cell segmentation approach. Some pairs had high correlation regardless of cell segmentation, while proteins from complexes derived from multiple genes (e.g. CD3) yielded variable correlations.

Conclusion

As spatial multi-omics is increasingly used to investigate TME biology, we present software addressing the need for appropriate bioinformatics solutions.

EACR25-0534

Optimizing Chemoradiotherapy for Malignant Gliomas Using a Validated Mathematical Model and In Silico Trials

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Introduction

Malignant gliomas (MGs), including glioblastomas, are among the most aggressive cancers, characterized by rapid progression and resistance to standard therapies such as temozolomide (TMZ) and radiotherapy. Standard treatments, such as the Stupp protocol, offer limited survival benefits and high toxicity. This study aims to optimize chemoradiotherapy (CRT) protocols using a validated mathematical model and in silico trials to optimize patient outcomes. Finally, our model can be adapted to other cancers with similar resistance mechanisms, suggesting broader utility.

Material and method

We developed a mathematical model using ordinary differential equations to simulate MG dynamics and CRT response. The model integrates key biological processes, such as cell proliferation, tumor dormancy, and resistance mechanisms. Key features include Ki-67, the role of persister cells in the development of TMZ resistance and the impact of treatment timing on survival. We calibrated and validated the model using experimental data from a mouse subventricular zone (SVZ) model developed through retroviral expression of EGFR-wt in primary cultures of neural stem cells from mice. For calibration, we administered 3 daily sessions of 3 Gy each, beginning 40 days post-tumor injection, and monitored tumor progression via bioluminescence using the In Vivo Imaging System (IVIS), which tracks tumor cell viability. In addition, survival data from prior TMZ experiments were utilized. To validate the model, we compared *in vivo* and *in silico* survival data from a combined CRT treatment protocol adapted from the Stupp protocol, with 3 consecutive concomitant CRT daily sessions (3 Gy and 3.33 mg/kg of TMZ) followed by a 2-day break and 3 subsequent adjuvant TMZ sessions (6.66 mg/kg daily) separated by 3 days. Finally, we translated the model and the optimal treatment into humans.

Result and discussion

Our *in silico* simulations revealed that concomitant CRT protocols, incorporating treatment breaks, resulted in a 17% improvement in median overall survival (OS) in mice compared to the standard Stupp protocol, with reduced toxicity. Translating these findings to human contexts, extended treatment protocols with concomitant

CRT or concomitant CRT plus adjuvant TMZ separated by treatment breaks resulted in a median OS of up to 48 months, a 280% increase over the standard Stupp protocol. These results underscore the potential of optimized protocols to enhance OS and reduce side effects.

Conclusion

This study highlights the power of mathematical modeling and *in silico* trials in optimizing oncology, providing valuable insights into the design of more effective CRT regimens for MGs. Our findings indicate that extended treatment schedules with treatment breaks could significantly improve OS and quality of life. Clinical trials are required to confirm our findings in real-world settings.

EACR25-0572

Multi-Cancer Early Detection and Tumor localization in Women Using Metabolomics-Based Machine Learning Models

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Introduction

Cancer poses a significant health threat to women in China, with lung, colorectal, gastric, breast, cervical, kidney, and reproductive tract malignancies exhibiting persistently high incidence and mortality rates, covering 61.2% of female cancer-related deaths. Current female-specific screening paradigms are limited to individual cancers (e.g., mammography for breast, Pap tests for cervical) and lack accessibility in underserved populations. Herein, we developed and validated a metabolomics-based machine-learning model for simultaneous multi-cancer screening and tumor localization in women.

Material and method

The 1,360 participants were enrolled in this study, including 1,097 cancer patients spanning eight cancer types (breast, ovarian, endometrial, cervical, lung, gastric, colorectal, and renal cancers) and 263 non-cancer controls (176 healthy controls and 89 benign cases) across seven centers. Plasma samples were analyzed using GC-MS and LC-MS multi-platforms. The cohort was randomly divided into discovery ($N = 1159$) and validation ($N = 201$) sets. A two-stage machine learning pipeline was applied: (1) a multi-cancer screening model (with 96.0% specificity) to differentiate cancer patients

from non-cancer controls, and (2) a tumor localization classifier to predict the origin of cancers. Performance was assessed in the validation cohort using sensitivity/specificity for cancer detection and the accuracy of the top one predicted origin (TPO1) and top two predicted origins (TPO2) for tumor origin classification.

Result and discussion

In the validation set, the model showed consistent performance, with a specificity of 95.2% (95% CI: 83.8% to 99.4%). Sensitivity for detecting early-stage cancers (I-II) was 45.9% (95% CI: 37.3% to 54.7%) across eight cancer types. Sensitivity improved with advancing cancer stages: 41.4% at stage I, 54.2% at stage II, 64.1% at stage III, and 84.2% at stage IV. For tumor localization, the accuracy of TPO1 and TPO2 was 74.1% and 85.5%, respectively. Additionally, the model achieved an accuracy of 89.4% in predicting the top one origin of the three most common cancers in women – lung, colorectal, and breast cancer – in terms of both incidence and mortality. Age and menopause status were unlikely to affect the model's accuracy, making it applicable across diverse populations.

Conclusion

This study highlights the potential of metabolomics-based machine learning models as a non-invasive tool for multi-cancer screening and precise tumor localization in women. With validation across large-scale datasets, our approach offers a simple, cost-effective method for improving early detection and clinical outcomes. Future work will expand the model by incorporating more cancer types and developing gender-neutral screening solutions.

EACR25-0678

Computational identification of pan-cancer multi-omic metabolic signatures as prognostic biomarkers and therapeutic targets

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Introduction

Metabolic reprogramming is a key factor in tumor progression and immune evasion, influencing prognosis and therapeutic response. Here, we conducted a multiomic analysis to identify metabolic signatures as potential prognostic biomarkers and therapeutic targets, covering energy, carbohydrate, lipid, amino acid, nucleotide, vitamin, and cofactor metabolism.

Material and method

We developed algorithms to integrate omic data from enzyme-coding genes, mature miRNAs, lncRNAs, and transcript isoforms, correlating them with tumor phenotypic features and clinical outcomes across 33 TCGA tumor types. Only targets with statistically significant associations ($p.\text{adj} < 0.05$) were selected for further analyses. We applied differential expression, Cox regression, log-rank, and Spearman correlations with immune infiltrates to define metabolic signatures. We adopted an innovative strategy: candidates belonging to

the same metabolic pathway with consistent results across all analyses were aggregated, and common molecular interactions among signature elements were identified. We developed the CancerMetabolismGPSShiny, an interactive tool that facilitates the exploration of our database results through visualizations and report outputs, paving the way for new discoveries in precision oncology.

Result and discussion

We identified 251,687 multi-omic signatures associated with 33 tumor types, distributed across carbohydrate (23.87%), lipid (23.32%), amino acid (22.38%), vitamin and cofactor (13.49%), nucleotide (6.17%), energy (4.81%), and other amino acid (5.96%) pathways. After selecting for signature clinical potential (risky, protective, and poorer prognosis) in all survival metrics, we identify 2,949 signatures, including 744 whose elements have known pharmacological interactions, in 20 tumor types. For example, in Liver Hepatocellular Carcinoma (LIHC), we identified the signature (ADH4 + TAT) involved in tyrosine metabolism and interacting with hsa-miR-376c-3p. This signature is downregulated in LIHC ($p = 3.5e-21$), with a negative correlation with stemness ($\rho = -0.3$, $p.\text{adj} = 1.01e-40$) and a protective effect in Cox regression analyses for OS ($p = 0.000008$), DSS ($p = 0.0005$), DFI ($p = 0.008$), and PFI ($p = 0.002$). Log-rank tests confirmed that higher expression is associated with improved prognosis ($p < 0.005$ across all survival metrics). This signature is linked to a cold immune phenotype and dual-profile (pro- and antitumor) immune cells. ADH4 and TAT interact with agonistic drugs (e.g., linolenic acid, griffolic acid) and inhibitors (e.g., nitrefazole, RO-24-7429), highlighting their therapeutic potential.

Conclusion

Our findings underscore the value of metabolic signatures as prognostic biomarkers and therapeutic targets, while the data available in CancerMetabolismGPSShiny can foster new hypotheses, experimental validation, and the development of tumor metabolism-based therapeutic strategies.

EACR25-0721

Describing Molecular Alterations in Breast Cancer Through a Multi-Omics Lens

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Introduction

The identification of breast cancer-related genetic mutations and gene expression profiles has contributed to better patient stratification for treatment modalities and improved patient outcomes. However, the molecular landscape of cancer expands beyond genetic components. The alterations in protein and metabolite profiles could be considered as more precise determinants of breast cancer phenotype. Nevertheless, such monitoring is limited as studies focusing on multi-omics profiling of tumor tissue are rather scarce due to limitations in robust

protocols. Moreover, the benefits of implementation of multi-omics profiling for single patients are also not fully understood. Our goal was to establish a robust protocol for multi-omics tissue profiling and to assess the relevance of molecular landscape revealed across different layers of alterations.

Material and method

This is part of Breast Cancer Omics Research Qatar (B.CORQ) project in which specimens from 200 patients (100 benign and 100 malignant) of Arab and Asian ethnicity were collected and processed. Tumor and adjacent tissues were collected during the operation and processed. Different omics including untargeted metabolomics, targeted lipidomics, proteomics, transcriptomics, methylation and genomics were conducted on those samples.

Result and discussion

The genetic and transcriptomic outcomes aligned with histopathological tumor characterization. Altered gene expression profiling, in agreement with proteomic analysis, revealed strong alterations of peroxisome proliferator-activated receptors (PPARs), actin cytoskeleton organization and metabolic process. Among 647 metabolites identified by untargeted metabolomics 289 distinguished tumor tissue from adjacent normal tissue. These differentiating metabolites were distributed across various pathways including metabolism of methionine, carnitine and aspartate as well as purine metabolism. The targeted lipidomic profiling revealed sphingolipids, phosphatidylcholines as well as triacylglycerols as the main lipid classes differentiating tumors from adjacent tissue. These metabolic differences were linked to enzymes showing corresponding alterations in gene expression and protein abundance.

Conclusion

This study advances the integration of multi-omics approaches into the clinical pipeline. It also highlights interactions across various omics layers within the dysregulated tumor molecular network, offering insights that could aid in optimizing treatment strategies.

EACR25-0724

Using the CancerHubs approach and web application to define novel cancer-related protein interaction hubs

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Introduction

Discovering the players and understanding the molecular mechanisms involved in cancer initiation and progression are fundamental issues for developing effective targeted therapies. Conventional methods for predicting protein involvement in cancer typically focus on isolated analyses of single-gene aberrations or transcript level correlations with patient survival. These methods are usually performed separately and concentrate on a single gene/protein at a time, ignoring nucleotide changes outside coding regions or the simultaneous mutations in genes within the same interaction network.

Material and method

To address these limitations, we have recently developed CancerHubs, a systematic data mining and elaboration approach that integrates unbiased mutational data, clinical outcome predictions and interactomics to define novel cancer-related protein hubs. The approach is based on a newly introduced metric termed the 'network score', which is able to predict the level of involvement of a certain gene in a particular cancer by defining the number of mutated interactors its encoded protein has.

Result and discussion

Here, we will present the web application we have implemented based on the CancerHubs approach. We will describe the application functionalities, including data input processes, network visualization and output interpretation and will demonstrate how the CancerHubs web application enables the users to easily navigate through complex molecular interactions, generate testable hypotheses, and prioritize candidate proteins for further study.

Conclusion

By providing an intuitive and robust tool for cancer research at a systems-level, CancerHubs empowers researchers to uncover hidden biological insights and accelerate the discovery of novel therapeutic targets.

EACR25-0725

Radiomics-based survival prediction in glioblastoma using the RaSPr score

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Introduction

Glioblastoma (GBM) is an aggressive brain tumor with significant survival variability, necessitating advanced prognostic tools for personalized treatment. Radiomic features extracted from medical imaging offer a non-invasive approach to characterizing tumor heterogeneity and predicting clinical outcomes. This study aimed to use radiomic analysis to assess survival variability in GBM and develop a predictive tool to support clinical decision-making.

Material and method

We analyzed a cohort of GBM patients from the TCGA-GBM dataset, extracting radiomic features from contrast-enhanced T1-weighted MRI (T1ce) scans. A total of 1,213 radiomic features were extracted, and feature selection was performed in two stages, first with univariate analysis ($P < 0.05$) followed by LASSO regression to refine the set. The selected features were weighted using LASSO coefficients to develop the "Radiomics Survival Predictor (RaSPr)" score, stratifying patients into low- and high-risk categories. Kaplan-Meier analysis was performed to evaluate the survival prediction efficiency. Independent datasets, UCSF-GBM and UPENN-GBM, were used for validation. Differential gene expression (DEG) analysis provided biological insights into tumor characteristics, and the RaSPr score was integrated into our self-developed "RadGLO" web platform for personalized survival predictions.

Result and discussion

From the initial 1,213 radiomic features, 8 key features were identified to construct the Radiomics Survival

Predictor (RaSPr) score, stratifying patients into low- and high-risk categories. Kaplan-Meier analysis showed that high RaSPr scores were associated with worse survival ($P < 0.05$), demonstrating its predictive power. We validated RaSPr using the UCSF-GBM and UPENN-GBM datasets, confirming its robustness and generalizability across cohorts. Differential gene expression analysis revealed significant upregulation of MDM2 in high RaSPr patients. MDM2, a negative regulator of the tumor suppressor protein p53 that promotes tumorigenesis, links radiomic risk with molecular markers. To enhance clinical adoption, RaSPr is integrated into "RadGLO" [1], allowing clinicians and researchers to submit MRI images or radiomic features for personalized survival risk predictions.

Conclusion

These findings underscore the potential of RaSPr score in personalizing GBM prognosis and guiding risk-based treatment strategies. By offering a non-invasive, efficient method for survival risk assessment, RaSPr can significantly enhance clinical decision-making and facilitate tailored treatment plans to improve patient outcomes. Integrating RaSPr into the 'RadGLO' web platform further enhances accessibility, providing clinicians and researchers with an easy-to-use tool for submitting MRI images or radiomic features and receiving personalized survival predictions.

[1] URL: project.iith.ac.in/cgntlab/radglo

EACR25-0729

Multimodal inference of morphogenomic phenotypes using morphology and expression data optimal clustering (MEDOC)

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Introduction

Spatial omics integrates three key types of information: cellular location, gene expression, and morphology. While current algorithms primarily combine gene expression with spatial location, they often neglect morphological features.

Material and method

In this study, we present Morphology and Expression Data Optimal Clustering (MEDOC), a novel algorithm that incorporates morphology into spatial omics analysis. MEDOC transforms contour representations and their derivatives into the Fourier domain, selects optimal shape features, and applies graph clustering for improved cell classification. To benchmark MEDOC, we evaluated its performance on a synthetic dataset containing 18 distinct shapes and a bacterial dataset comprising four different species. MEDOC outperformed several recently published algorithms in both accuracy and computational efficiency.

Result and discussion

To demonstrate its biological and clinical applications, we applied MEDOC to the morphogenomic analysis of bone marrow samples from chronic myelomonocytic leukemia (CMML), a rare hematologic malignancy characterized by sustained peripheral blood moncytosis, bone marrow dysplasia, and overlapping features of myelodysplastic syndromes (MDS) and myeloproliferative neoplasms (MPN). Increased monoblast levels – immature monocytes with round nuclei – are a known risk factor in CMML, yet their underlying molecular drivers remain unclear. Since suspension cells, such as bone marrow mononuclear cells (BMMCs), are not compatible with conventional spatial omics workflows designed for adherent cells, we developed a new immobilization protocol. This enabled MERFISH spatial RNA analysis of 492 genes in ~25,000 cells across a 16 mm² region of interest within 34 hours. We then applied this assay to samples from 12 CMML patients and 8 healthy controls. MEDOC analysis of this dataset identified multiple nuclear shape-associated genes involved in myeloid cell maturation, revealing shared differentiation pathways between cancerous and healthy bone marrow. Additionally, we successfully distinguished a promonocyte population, a monoblast subtype that was previously difficult to classify. These findings suggest that integrating morphological analysis with gene expression using MEDOC could enhance CMML patient stratification and uncover the molecular underpinnings of clinically relevant malignant cell morphologies.

Conclusion

More broadly, MEDOC provides a fast, scalable, size, reflection, and rotation-invariant approach for extracting novel morphogenomic phenotypes from any large-scale, cellular-resolution spatial omics dataset with an imaging component.

EACR25-0737

AI-Powered Nuclei Segmentation in Cervical Cytology: Advances with Deep Learning Models

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Introduction

Cervical cancer remains a significant public health concern worldwide, with early detection playing a crucial role in reducing mortality rates. Whole Slide Imaging (WSI) in cervical cytology enables digital analysis of cellular morphology but presents challenges in nuclei segmentation, essential for accurate diagnoses. This study aims to evaluate and compare deep learning (DL) models for nuclei segmentation in cervical cytology images.

Material and method

We implemented three DL models: U-Net, Vision Transformer (ViT), and Detectron2 (Mask R-CNN architecture). The models were trained on the CNseg dataset and evaluated on the ISBI 2014 dataset and an independent institutional dataset. Preprocessing steps included annotation with QuPath, patch extraction, and

exclusion of regions with less than 60% cytologic material. Performance was assessed using the Dice Similarity Coefficient (DSC), Intersection over Union (IoU), and Panoptic Quality (PQ).

Result and discussion

The models achieved high segmentation accuracy on public datasets, with Detectron2 obtaining a PQ of 0.98 on ISBI 2014, surpassing previously reported methods. On the CNseg dataset, U-Net performed best (DSC: 0.86, PQ: 0.86). Performance decreased on the institutional dataset (U-Net PQ: 0.62), likely due to variations in image acquisition and blurred nuclei. Notably, the models detected nuclei in challenging conditions, indicating potential for improving diagnostic workflows.

Conclusion

Deep learning models, particularly U-Net and Detectron2, provide accurate and efficient nuclei segmentation in cervical cytology WSIs. Despite domain shifts affecting performance, these models offer promising tools for automated screening and diagnostic applications, supporting the development of reliable AI-driven cytology solutions

EACR25-0756

A Smart Platform for Cancer Research: Integrating Consensus Meta-Analysis of Omics Data with Ontology-Based Network Analysis

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Introduction

The rapid growth of omics and ontology data, coupled with advancements in large language models (LLMs) and deep learning, has opened new opportunities for data-driven drug target and biomarker discovery. By integrating these technologies, we aim to streamline cancer research through comprehensive data mining and network-based analysis.

Material and method

We integrated diverse omics and ontology datasets from public sources into the Q-omics data mining platform [1]. Meta-analysis and data consensus were performed across datasets and incorporated into Q-omics' smart databases. A LLM-powered "Text-to-Data Mining" interface was implemented, enabling researchers to perform complex analyses without computational expertise. For functional insights, we developed NetCrafter [2], a novel ontology-based network analysis algorithm, and incorporated it into the Q-omics workflow. Graph neural networks (GNNs) such as message-passing neural networks (MPNNs) were used to identify network hotspots, facilitating drug target discovery.

Result and discussion

We identified over 29 billion significant cross-associations across heterogeneous datasets, encompassing over 1 billion multi-modal omics data points. Consensus analysis on cross-associated data across pan-cancer datasets provides a valuable resource for identifying reliable drug targets, biomarkers, and underlying mechanisms. Genes with tumor-specific

overexpression and unfavorable prognoses show high consistency across cancer lineages. Lineage consensus in gene perturbation data (CRISPR/shRNA) demonstrates strong reproducibility, reinforcing the reliability of functional screening approaches. In addition, advanced data mining applications within Q-omics enable the identification of synthetic lethal gene pairs and tumor-specific neoantigens, supporting novel therapeutic strategies. Ontology-based network analysis provides immediate functional insights from gene lists derived from RNA/protein expression or perturbation experiments (CRISPR/shRNA). Ontological networks highlight pan-cancer consensus functions associated with drug response and patient survival and serve as valuable input for MPNN models, aiding hotspot identification for drug target discovery.

Conclusion

By integrating multi-omics meta-data with LLMs and deep learning, Q-omics pioneers an innovative "Text-to-Data Mining" platform, enabling researchers to effortlessly identify anticancer targets, biomarkers, and mechanistic insights—without computational expertise. As omics datasets expand and AI technology advances, Q-omics [1] serves as a transformative resource, driving progress in cancer research and drug development.

[1] URL: qomics.io

[2] URL: netcrafter.sookmyung.ac.kr

EACR25-0770

Panoptic Segmentation and Spatial Analysis of Whole Slide Images: A Unified Open-Source Framework

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Introduction

Histopathological features like tissue architecture, nuclear morphology, and stromal organization are key prognostic factors in cancer. However, extracting these features from hematoxylin & eosin (H&E) stained Whole Slide Images (WSIs) reproducibly remains challenging. Existing computational tools prioritize AI-driven predictive modeling but lack structured feature extraction tools directly linked to biological and clinical insights. Panoptic segmentation, which simultaneously segments nuclei and tissues, offers a powerful yet underutilized solution. In this work, we present two open-source Python libraries, cellseg_models.pytorch, for panoptic segmentation of WSIs, and cellseg_gsontools for spatially resolved and clinically relevant feature extraction at WSI scale.

Material and method

We demonstrate the utility of our Python libraries by extracting clinically relevant biological phenomena in two use-cases. First, we characterize the spatial heterogeneity of desmoplastic reaction in the stromal compartment of a high-grade serous ovarian cancer (HGSC), a phenomenon linked to EMT and poor outcomes in HGSC. Additionally, we examine immune

infiltration patterns in Human Papilloma Virus (HPV)-induced cervical intraepithelial neoplasia grade 2 (CIN2), where immune activation influences the lesion regression potential due to the viral etiology of the disease. For these analyses, we utilized 50 H&E-stained WSIs from HGSC omental tissue sections from the DECIDER trial (NCT04846933) and 163 CIN2 diagnostic biopsies collected as routine diagnostic biopsies at the Helsinki University Hospital. Panoptic segmentation models were applied to delineate nuclei and tissues, enabling spatially resolved feature extraction. Stromal organization in HGSC was assessed through clustering of tissue intensity and collagen fiber orientation features, while immune infiltration in CIN2 was quantified through immune cell densities within lesions and at the lesion-stroma interface.

Result and discussion

Our results demonstrate that `cellseg_models.pytorch` and `cellseg_gson tools` enable robust extraction of interpretable histopathological features at the whole-slide scale. By leveraging these tools, we successfully quantified clinically relevant features in both HGSC and CIN2. These examples showcase the versatility of our framework in spatial histopathological analysis, extending beyond HGSC and CIN2 to studying tumor microenvironments, stromal remodeling, immune infiltration, and morphometric changes in diverse disease contexts.

Conclusion

Our computational framework provides a scalable and interpretable solution for extracting spatially resolved histopathological features from WSIs, addressing a key gap in digital pathology. By integrating panoptic segmentation with structured spatial analysis, we enable reproducible quantification of tissue organization, immune infiltration, and stromal remodeling.

EACR25-0789

Comprehensive machine learning analysis of high-dimensional radiomics datasets by PYRAMID

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Introduction

The identification of novel quantitative imaging biomarkers, including those derived from radiomics, facilitates personalized medicine. Radiomics has been successfully applied to predict patient outcomes, histopathological and molecular features. However, the absence of standardized protocols for its analytical process, spanning from data transformation to predictive modeling, poses a significant challenge in establishing

uniform and reproducible approaches. To address these issues, we present PYthon Radiomics And MachIne learning Data analysis (PYRAMID), a Python framework for a reproducible radiomics analysis workflow.

Material and method

PYRAMID is a python framework for radiomics analysis, built on scikit-learn and imbalanced-learn, providing a comprehensive machine learning workflow. It comprises five modules: DaTrax (data transformation/normalization), SLIC (class imbalance handling), FeatX (feature selection), HyperTune (model training), and PREDICT (validation). By implementing several state-of-the-art methods in each module, a complete PYRAMID run tests up to 16,200 method combinations (pipelines). PYRAMID was evaluated on multiple TCIA and RadiomicsHub datasets, benchmarking AUC, F1, and average precision (AP) against results from original papers and challenges.

Result and discussion

Here, we report on the application of PYRAMID to the RADCURE dataset, including pre-treatment CT scans from patients with head and neck cancer. Considering 2,175 patients with available tumor segmentation and clinical information, we extracted 851 radiomics features per patient by pyradiomics and split the dataset into training ($n = 1,605$) and validation ($n = 570$) sets. Using radiomics-only features to predict the binarized 2-year survival, we applied PYRAMID evaluating all possible pipelines. Considering the top 5% pipelines in terms of F1-score, a median AUC of 0.75 (0.68-0.78) and median AP of 0.40 (0.30-0.47) on the validation set were obtained exceeding the performance of baseline radiomics model of the challenge (AUC = 0.71, AP = 0.33). These pipelines were enriched for robust scaling and uniform distribution transformation for data preprocessing, AllKNN for sampling, and Gradient Boost and ElasticNet for ML. Notably, pipelines lacking data transformation or class imbalance correction were significantly underrepresented in the top performing 5%, highlighting the importance of preprocessing of radiomics data.

Conclusion

PYRAMID is a toolkit for standardized radiomics workflow analysis. Applied on RADCURE, it achieved performance superior to original results, enabled the identification of best-performing methods and pipelines and suggested best-practice strategies. Further results from additional publicly available datasets, spanning various classification tasks and imaging types, will be presented at the congress.

EACR25-0836

Developing an artificial intelligence model for clinical benefit prediction in breast cancer

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Introduction

Understanding the complex interplay of features influencing treatment outcomes is crucial for prognostic modelling and clinical decision making. We aimed to analyze the clinical benefit of adjuvant chemotherapy in breast cancer patients at 5-year survival and establish a web-based novel tool.

Material and method

We utilized the Surveillance, Epidemiology, and End Results database to retrieve data of breast cancer patients after the year 2000. The inclusion criteria were adult patients (≥ 18 years) with available overall survival data. After excluding incomplete records, duplicate patient ID's and censored data for 5 years, 1,090,297 cases with 18 parameters were analyzed. We developed a gradient boosting machine learning model using chemotherapy and non-chemotherapy breast cancer patient groups to predict the clinical benefit from adjuvant chemotherapy. These models were trained to estimate the survival event probabilities of breast cancer patients.

Result and discussion

The gradient boosting model demonstrated high accuracy of 0.77 and 0.84, balanced accuracy of 0.81 and 0.87 and AUC of 0.86 and 0.91 in chemotherapy and non-chemotherapy models, respectively. Sensitivity was 0.83 and 0.84 and the specificity reached 0.71 and 0.84 in chemotherapy and non-chemotherapy models, respectively. Among all the parameters, lymph node positivity was found to be the most important parameter in both the chemotherapy and non-chemotherapy models. Internal test validation showed that the gradient boosting model could predict 82% of the correct cases in the chemotherapy model and 69% of the correct cases in the non-chemotherapy model. Our web-based platform was set up at www.recurrenceonline.com/clinical to enable real-time predictions.

Conclusion

Our online tool offers a user-friendly interface for real-time monitoring of the clinical benefit of adjuvant chemotherapy in breast cancer patients. The algorithm has the potential to be used in clinical settings and assist clinicians in making more informed treatment decisions.

EACR25-0970

Artificial intelligence-based pathological analysis for Gleason grade and tumor quantification in prostate cancers

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Introduction

Prostate cancer poses diagnostic challenges due to its multifocal nature and often small tumor volumes. The global shortage of pathologists exacerbates these issues, resulting in inconsistent and delayed diagnoses. While artificial intelligence (AI)-based image analysis has demonstrated the potential to improve diagnostic accuracy in various areas, its application in whole radical prostatectomy (RP) specimens remains underexplored. This study evaluates the clinical feasibility of AI-based pathological analysis for Gleason grading and tumor quantification, aiming to enhance prostate cancer diagnosis.

Material and method

We enrolled patients who underwent RP from 2011 to 2021 with more than one year of follow-up. Patients with fewer than 10 specimen slides or cancers other than prostatic adenocarcinoma were excluded. All slides were digitized and analyzed using an AI-based algorithm. Algorithm-based outputs, including International Society of Urological Pathology (ISUP) grade groups (GGs), tumor volumes (TVs), and percent tumor volumes (PTVs), were compared to those obtained by pathologists. We evaluated their prognostic performance for predicting biochemical progression-free survival (BPFS) in comparison with pathologist assessments.

Result and discussion

A total of 29,646 H&E-stained slides from 992 patients were analyzed. Post-RP, 348 patients experienced biochemical recurrence, with 178 occurring within 1 year. Pathologists identified cancer in 986 cases and assigned GGs (pGG) to 980. The AI-based algorithm identified cancer and assigned GGs (aGG) to all cases. GGs by pathologists and by the algorithm showed fair agreement (linear-weighted kappa: 0.374). Their prognostic performance for predicting BPFS showed little difference, with concordance index (c-index) of 0.654 and 0.644 for pGG and aGG, respectively ($p = 0.52$). Median TV and PTV determined by pathologists (pTV, pPTV) were 3.8 mL and 15.0%, while those calculated by the algorithm (aTV, aPTV) were 1.0 mL and 3.7%, respectively. Their Pearson correlation coefficient was 0.830 for TV and 0.846 for PTV. In predicting BPFS, algorithm-based TV and PTV measurements demonstrated superior performance compared to pathologist-based correspondences, with c-index of 0.657 and 0.672 against 0.622 and 0.641, respectively.

Conclusion

The AI-based algorithm and pathologists showed comparable power for predicting BPFS while the algorithm exhibited superior performance in tumor quantification, suggesting its potential as an effective tool for enhancing pathological diagnosis and prognosis.

EACR25-0985

Machine Learning Algorithms for Prediction of Bone Metastasis Risk in Patients with Lung Cancer

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Introduction

Lung cancer is the leading cause of cancer deaths in Hong Kong, accounting for 26.1% of all cancer deaths in 2023. The cause is often metastasis, even in patients who have had their primary lung tumours resected.

Approximately 30% to 40% of patients with advanced lung cancer are estimated to experience bone metastases. The aim of this study was therefore to optimise the accuracy and explainability of machine learning models to identify lung cancer patients with high risk for bone metastasis.

Material and method

The patient cohort comprised 1864 patients diagnosed with primary lung malignancy at a territory-wide tertiary referral centre between 2016 and 2021. A total of 25 variables, spanning sociodemographic, clinical, pathological, and treatment information, were considered as potential predictors of bone metastasis. The outcome was binary, indicating the presence or absence of bone metastasis followed up for a minimum of 1 year. All patient health records were extracted from the clinical management system (CMS) of the hospital. For optimisation of model performance and explainability, this study utilised various ensemble machine learning techniques, including Random Forest and Boosting methods. The performance of these ensemble methods was compared to two simpler baseline models, logistic regression and radial basis function (RBF) kernel support vector machine (SVM), which have shown the highest performance out of other commonly used machine learning classifiers. Principal component analysis (PCA) was performed for dimensionality reduction and feature importance, and probability calibration was performed to enhance model usability.

Result and discussion

Both Random Forest and Boosting type ensemble methods outperformed the baseline models across all three primary evaluation metrics. PCA also revealed that lung cancer histological type, T-staging, American Joint Committee on Cancer staging, differentiation grade and lymphovascular staging were the five input features with the greatest contribution to explained variance in the prediction of bone metastasis.

Conclusion

The optimised accuracy and interpretability of the machine learning models developed in this study can enable clinicians to better understand the parameters used to generate the prediction, which is key to its implementation within a clinical setting. The analysis and identification of feature importance can also allow for more targeted collection of input variables, resulting in heightened model cost-effectiveness.

EACR25-1047

Single-nuclei transcriptome profiling reveals vascular endothelial cell heterogeneity induced by VEGFA/BRAF targeting in murine melanoma model

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Introduction

Malignant melanoma is an aggressive cancer, with high risk of metastasis and mortality. The introduction of targeted therapies and immunotherapy has represented the most significant advances in the treatment of melanoma; however, the onset of resistance remains a challenge to overcome. Vascular Endothelial Growth Factor A (VEGFA) is a key promoter of tumor angiogenesis and immunosuppression and is an attractive target for combinatorial therapy. We have demonstrated that VEGFA removal improved the antitumor efficacy of BRAF inhibitor (BRAFi), through vessel normalization and M1-macrophages and CD8+ T cells infiltration, boosting immune checkpoint blockade. Single cell biology is a powerful tool to decipher cancer cell heterogeneity and complex tumor microenvironment (TME) at a molecular level. However, this approach requires enzymatic cell dissociation that can influence cellular coverage. By contrast, single nucleus RNA sequencing (snRNA-seq) has substantial advantages including compatibility with frozen samples and the elimination of a dissociation-induced, transcriptional stress response.

Material and method

Single-nuclei transcriptome profiling was conducted on samples obtained from D4M syngeneic melanoma model at varying treatment regimen. We performed high-quality snRNA-seq from 8 frozen tissue specimens, a biological replicate for each condition, that were preserved in liquid nitrogen. Approximately 10,000 nuclei were partitioned in each snRNA-seq experiment using used 10x Genomics technology and sequenced (150 bp PE) by the Illumina NextSeq 500 platform. The integrated Seurat object consisted of 37,104 high-quality nuclei (8,510 from control; 10,920 from BRAFi; 4,549 from anti-VEGFA; 13,125 from BRAFi + anti-VEGFA). Raw sequencing data were processed using Cell Ranger and analyzed with the Seurat package in R. Cell-cell interaction based on ligand-receptor expression levels was investigated using CellChat bioinformatic tool. We used Azimuth and loupeR for cell annotation.

Result and discussion

In this study, we have used single snRNA-seq as a profiling strategy to further investigate how VEGFA targeting regulates the TME in melanoma, with the aim to identify cellular and molecular changes that regulate the interplay between different cell populations.

Unsupervised Seurat-based clustering identified 20 clusters which were further annotated into six major cell types (malignant, myeloid, dendritic, T cells, fibroblasts, endothelial, adipocytes). We found significant heterogeneity within melanoma cancer cells (9 clusters) and myeloid cell (5 clusters) that display distinct global transcriptional program and different enrichment in different condition.

Conclusion

The current study offers a deep insight into the biology of melanoma highlighting TME reprogramming through target therapy treatment, underlying further discovery of new TME biomarkers which may be potentially druggable.

EACR25-1099

Investigating molecular evolution of tumor heterogeneity at recurrence unveils patient-specific trajectories

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Introduction

Tumor heterogeneity is one of the major barriers to the treatment of many tumor types. In glioblastoma (GBM) – the most aggressive brain tumor – the survival of patients following standard therapy is around 14 months, largely due to the inherent heterogeneity and phenotypic plasticity. Investigations of paired primary-recurrent tumours and preclinical models derived thereof using multi-omics profiling can provide clinically relevant insights into tumour evolution at the individual patient level.

Material and method

To understand the impact of treatment on the intratumoral heterogeneity of tumor cells, we applied bulk and single-cell RNA-seq, spatial transcriptomics, epigenomics (DNA methylation arrays), genomics (targeted DNA-seq) and proteomics (LC-MS) in GBM patient-derived orthotopic xenograft models generated from 8 longitudinally matched treatment naïve and treatment-exposed recurrent patient tumors. We performed comprehensive integrative multi-omics analysis including state-of-the-art machine learning tools to assess the molecular evolution of GBM tumors at recurrence.

Result and discussion

Multi-omics analysis revealed a presence of diverse molecular profiles, representing various genetic, epigenetic and transcriptomic subtypes of glioblastomas observed in patients. A machine learning-based independent component analysis (ICA) identified independent biological signals from heterogeneous cellular populations representing distinct biological processes that drive glioblastoma recurrence, without relying on prior knowledge of cell types or states.

Conclusion

Our GBM PDOX models provide a platform for high-quality pre-clinical research and improved clinical relevance in the development and testing of novel therapeutics.

EACR25-1175

Unified Whole Genome Sequencing for Hematological Malignancies

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Introduction

Acute myeloid leukemia (AML) or myelodysplastic syndromes (MDS) rely on genetic testing and molecular profiling for risk stratification. Comprehensive genomic analysis of hematologic malignancies requires the evaluation of multiple variant types, including small variants (single nucleotide variants and insertions/deletions < 50 bp), copy number alterations (CNAs), structural variants (SVs) such as translocations, Internal Tandem Duplications (ITD) and loss of heterozygosity (LoH) calculation. Chromosomal evaluation by karyotyping, microarray, and FISH, and sequencing methods such as single gene and gene panel testing are currently employed to detect across these variant classes, resulting in high costs, high operational complexity, need to support multiple molecular technologies, and potential for conflicting results. Enabled by continued sequencing cost reductions, recent studies using tumor-only whole-genome sequencing (WGS) have demonstrated that a single assay can provide results that are equivalent to or better risk stratification than conventional testing approaches with lower sample failure rates (Duncavage et al., 2021).

Material and method

We present a complete solution for analysis and interpretation of hematological whole genome samples. The workflow leverages DRAGEN(TM) secondary analysis to detect all variant types relevant to hematological malignancy research. To establish performance of the pipeline, we performed PCR free WGS at an average ~220 X coverage on reference cancer cell lines and 53 AML clinical samples.

Result and discussion

VAF LoD was established for small variants and SVs at 5% and 7.3%, respectively. We observed an overall accuracy of > 95%, correctly calling 73/73 clinically relevant small variants (including 4 FLT3-ITD), structural variants, and > 96% of copy number events across this cohort. SV and CNA evaluation included 2 inversions sized approximately 45 and 56 Mb in length, 8 translocations, 22 CNAs ranging from 4.5 Mb to 100 Mb, and 7 whole chromosome events. The secondary analysis results are directly ingested into Connected Insights for variant interpretation, automatic curation including AML subtyping and risk stratification based on common guidelines such as WHO 2022 and ELN 2022. Evidence maps and decision trees can be tailored to a laboratory's preference based on clinical trials or hem-specific sources such as CKB, OncoKB and more. Powerful visualizations including interactive Circos plots enable exploring of complex structural rearrangements.

Conclusion

The DRAGEN Heme pipeline is a tumor only WGS pipeline for genomic profiling of hematological malignancy samples. The analytical performance of this approach resulted in high accuracy for variants commonly assessed in AML samples. We demonstrate its ease of use to advance hematological malignancy clinical research and its accessibility for all labs looking to drive informatics forward.

EACR25-1184

Predicting drug response from omics data using MultiOmic2Drug

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Introduction

Recent clinical trials have proven the importance of leveraging patients' molecular profiles (omics) for the personalisation of cancer treatments (Sicklick et al. 2019; Rodon et al., 2019). However, the complex and heterogeneous nature of omics motivates the application of deep learning methods. These methods hold great promise in uncovering biomarkers and patterns that are essential for the treatment of hard-to-treat cancers, such as pancreatic ductal adenocarcinoma (PDAC). PDAC is considered to be a lethal cancer, associated with a very low 10-year survival rate of 5%. In this work, we present MultiOmic2Drug (MO2D), a deep learning neural network aimed at the prediction of drug response through the integration of multiple omic layers.

Material and method

As a proof of concept, our model, MO2D, was trained using molecular data of pan-cancer cell lines deposited in Depmap (cancer dependency map). In particular, we used copy number variation (CNV), transcriptomics and proteomics (MS/MS) data of the cancer cell lines in combination with in-vitro drug response results.

Result and discussion

By inspecting the latent space, we assessed that the model has learnt to predict the drug response.

Quantitatively, the model is being evaluated with a MSE (Mean Squared Error) loss and the performance ranges between 0.15 and 0.3 on unseen data.

Conclusion

Our MO2D demonstrated through its latent space its ability to learn the drug response. Additionally, we will externally validate MO2D on validation datasets, and the predictions of MO2D will be tested in-vitro.

EACR25-1225

DTFD-MIL-CN: A Cost-Effective and Accurate Deep Learning Model for Hormone Receptor-Positive, HER2-Negative Breast Cancer Distant

Recurrence Risk

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Introduction

EndoPredict is a multigene assay that effectively assesses the long-term recurrence risk in patients with hormone receptor (HR)-positive, HER2-negative breast cancer. This tool aids in identifying low-risk patients who may safely forgo chemotherapy while guiding high-risk patients toward more aggressive treatment strategies. However, its high cost limits widespread clinical application. This study aims to develop a deep learning model that integrates clinical features and hematoxylin and eosin (HE)-stained pathological images to stratify breast cancer patients into risk categories, providing a cost-effective alternative to EndoPredict.

Material and method

This study included patients diagnosed with early-stage HR-positive, HER2-negative breast cancer who underwent EndoPredict testing at multiple oncology centers, including the Cancer Hospital, Chinese Academy of Medical Sciences, between January 2021 and June 2024. A total of 295 cases were collected, of which 254 were retained after excluding samples with poor-quality pathological images or missing clinical data. A deep learning model, DTFD-MIL-CN (Double-Tier Feature Distillation Multiple Instance Learning with Clinical and Nuclear Features), was employed, incorporating a dual-attention learning mechanism and hierarchical classification structure for feature modeling. Specifically, ResNet-50, pre-trained on a large-scale image dataset, was used to extract 1,024-dimensional feature vectors from HE-stained image patches. Additionally, a HoVer-Net-based model was applied to extract nuclear features, including eccentricity, equivalent diameter, and Euler number. These morphological features were then integrated with clinical variables such as age, estrogen receptor status, and progesterone receptor status to construct a multimodal analytical framework.

Result and discussion

A ten-fold cross-validation strategy was implemented to maximize sample utility and enhance model robustness. Specifically, 80% of the dataset was used for cross-validation, while the remaining 20% served as an independent test set. During cross-validation, the model demonstrated strong performance, achieving an average area under the curve (AUC) of 0.852. Furthermore, the model weights from the best-performing fold were applied to the independent test set, yielding an AUC of 0.846, indicating favorable classification performance.

Conclusion

The DTFD-MIL-CN model effectively predicts distant recurrence risk in HR-positive, HER2-negative breast cancer patients. Compared to EndoPredict, it offers a more cost-effective alternative with high accuracy and generalization capability. This model holds promise as an efficient and economical tool for individualized risk assessment in patients with early-stage Luminal-type breast cancer, thereby supporting clinical decision-making and optimizing treatment strategies.

EACR25-1229

Deep transfer learning for predicting BRAF V600E mutation status in colorectal

cancer using image-derived features associated with microsatellite instability

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Introduction

Microsatellite instability (MSI) and BRAF V600E mutation are key molecular biomarkers in colorectal cancer (CRC) with significant implications for diagnosis, prognosis, and treatment. MSI results from mismatch repair system defects, leading to genetic hypermutability and increased mutation burden. MSI and the BRAF V600E mutation often co-occur due to shared molecular pathways, highlighting a complex biological relationship between these biomarkers. Transfer learning repurposes pre-trained models for related tasks, allowing the extraction of relevant features for new biomarkers from pathology images. We hypothesise that histomorphological patterns associated with MSI, as extracted through deep learning (DL) from digitised haematoxylin and eosin (H&E)-stained pathology slides, may harbour relevant features indicative of BRAF V600E mutational status.

Material and method

We utilised a DL model pre-trained for predicting MSI status on whole slide images (WSIs) of H&E-stained primary CRC slides ($n = 1707$). This model was used to extract image-based features for a separate cohort of 701 CRC WSIs with known MSI and BRAF V600E status. A multi-layer perceptron classifier was trained on the image-derived features using 5-fold cross-validation. The model's performance was assessed using the area under the receiver operating characteristic curve (AUC). The statistical association between MSI and BRAF V600E mutation was evaluated with a chi-squared test.

Result and discussion

The BRAF V600E mutation was present in 14.98% (105/701) of the cohort. MSI-high tumours accounted for 12.84% (90/701) of the cases. Among these, 63.33% (57/90) harboured the BRAF V600E mutation, compared to just 7.86% (48/611) in MSI-low and stable cases. The chi-squared test ($\chi^2 = 185.25$, $p < 1e-05$) confirmed a statistically significant relationship between MSI and BRAF V600E mutation. The strength of this relationship was quantified using Cramér's V, which indicated a moderately strong association ($V = 0.51$). Using transfer learning, we achieved an average AUC of 84.77% ($\pm 3.14\%$), with values ranging from 80.95% to 88.20% across 5 folds. These results suggest that MSI-related histomorphological features identified with DL are informative of BRAF V600E mutation status.

Conclusion

This study highlights the potential of deep transfer learning for capturing morphological patterns associated with MSI and BRAF V600E mutation using only H&E-stained WSIs. The ability to extract biologically relevant

features from pathology slides enables rapid, cost-effective biomarker assessment, which could enhance clinical management and inform treatment decisions. Future work will investigate the biological interplay between MSI and the BRAF V600E mutation, investigating the role of DL in bridging the gap between morphology and underlying molecular mechanisms.

EACR25-1234

Novel clustering of glioblastoma patients with EGFR mutation by unsupervised machine learning

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Introduction

Glioblastomas (GB) are a subtype of brain tumors originating in glial cells. The most malignant primary brain tumor, five-year survival rates lie at only 6.9% and average survival is estimated at 15 months with standard treatment (surgery plus radio and/or chemotherapy). However, there is great heterogeneity amongst patients, with survival ranging from some months after diagnosis to years post-surgery. The desire to classify these phenotypically diverse cases has led to extensive research into the molecular background of GB patients. This includes the identification of several frequently occurring mutations, such as in PI3K, EGFR or TP53. Alterations in expression of epidermal growth factor receptor (EGFR) have been found in over 50% of GB cases. Over-expression of the gene is associated to more aggressive GB, with multiple mechanisms leading to EGFR pathway activation. The existence of different variants, and incomplete understanding of all the signaling pathways that are affected as a consequence, have limited the applications of treatments targeting this protein.

Material and method

The presented project applies unsupervised machine learning methods to transcriptomic data of GB patients to specifically cluster these altered EGFR patients.

Result and discussion

Application of our clustering algorithm has identified several subgroups of cases with differential patterns in tumor-associated biological processes. Geneset enrichment analysis (GSEA) shows significant differences in stemness, oxidation state, neuronal function and tumor expansion pathways.

Conclusion

Elucidating the biological basis that separates these patients can lead to better understanding of tumor progression as well as of the differences in response to treatments, with a final goal of more personalized tumor management.

EACR25-1252

MetaAnalysisOnline's Network Tool: Simplifying Network Meta-Analysis for All Researchers

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Introduction

Network meta-analysis (NMA) is a powerful statistical method that enables indirect comparisons of multiple treatments by integrating data from various studies. However, conducting NMA often requires advanced statistical expertise and specialized software, creating barriers for researchers and clinicians. This study introduces metaanalysisonline.com etwork, a user-friendly web-based platform designed to streamline network meta-analysis without requiring programming skills.

Material and method

MetaAnalysisOnline.com supports NMA using both fixed-effects and random-effects models. The platform automates complex calculations and provides essential visualization tools, including treatment rankings, league tables, inconsistency and heterogeneity tests, and split comparisons. The system integrates statistical methodologies from the netmeta package in R, ensuring accuracy and transparency. The web-based interface allows users to input study results, define treatment networks, and generate interactive visualizations without coding expertise.

Result and discussion

The online tool is freely accessible and allows researchers to input data directly from commonly used spreadsheet applications such as Excel. It generates key outputs such as network and forest plots, funnel plot, and league tables to compare multiple treatments effectively. In addition, the platform performs sensitivity analyses and inconsistency assessments to ensure the robustness of findings. By providing comprehensive statistical outputs and interactive visualizations, MetaAnalysisOnline.com enhances the interpretation of complex treatment networks.

Conclusion

Metaanalysisonline.com simplifies network meta-analysis by automating statistical calculations and visualization, making advanced evidence synthesis more accessible and reproducible for researchers and clinicians.

EACR25-1255

Spatial Transcriptomics Reveals Immune-Tumor Interactions Associated with Pembrolizumab Response in NSCLC

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Introduction

Immunotherapy has transformed the treatment landscape for non-small cell lung carcinoma (NSCLC), yet predicting which patients will respond to immune checkpoint inhibitors (ICIs) remains a major clinical challenge. Despite the use of PD-L1 expression as a main predictive biomarker, its power is limited. This highlights the need for better patient stratification strategies. Spatial transcriptomics (ST) offers a novel approach by integrating histology with spatially resolved whole-transcriptome profiling, preserving tissue architecture and enabling in-depth characterization of the tumor microenvironment (TME). This study aims to identify transcriptomic and spatial patterns of immune and tumor cells associated with pembrolizumab response in PD-L1 high NSCLC patients.

Material and method

A total of 16 NSCLC patients (PD-L1 ≥ 50%) treated with pembrolizumab as a first line therapy were selected divided into responders (n = 8) and non-responders (n = 8). In each group, there were 4 adenocarcinoma and 4 squamous cell carcinoma samples. FFPE small biopsy samples were analyzed using 10x Genomics Visium spatial transcriptomics, integrating H&E-stained sections to ensure accurate spatial mapping. Space Ranger, scampy, squidpy, and cell2location were used for spatial clustering and immune cell deconvolution. TLS characterization, immune exclusion assessment, and spatial proximity mapping of immune-tumor interactions was performed.

Result and discussion

Responders exhibited fragmented tumor architecture, with cancer cells interspersed among immune cells, suggesting immune-mediated tumor disruption. In contrast, non-responders displayed dense tumor clusters, potentially creating immune-privileged niches. T cells in responders localized within tumor regions, enhancing tumor-immune interactions, whereas in non-responders, T cells were confined to stromal areas, indicating an immune-excluded phenotype. Plasma cell enrichment in responders suggests a B-cell-mediated antitumor response, whereas myeloid-dominated non-responders may exhibit immunosuppressive signaling, limiting ICI efficacy.

Conclusion

Spatial transcriptomics reveals key tumor-immune architectural differences that may influence pembrolizumab response in NSCLC, even in small diagnostic biopsies. Understanding immune infiltration patterns, tumor fragmentation, and myeloid-driven suppression could refine patient stratification and guide immuno-therapy strategies. Further validation is needed to establish these features as predictive biomarkers for immunotherapy efficacy.

EACR25-1257

Machine Learning Meets Man's Best Friend: Uncovering Prognostic Factors in Canine Mammary Tumors

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Introduction

Canine mammary tumors (CMTs) are the most prevalent type of cancer in dogs. Approximately 45% of canine mammary carcinoma cases result in metastasis and death within one year after diagnosis, although survival time can vary greatly between dogs. This variation is attributed to the highly heterogeneous nature of the disease, which presents with diverse clinical manifestations, histopathological subtypes and molecular properties. Currently, prognosis for CMT patients is primarily based on histopathologic examination and TNM staging, which includes assessment of tumor size, lymph node involvement and metastasis. While molecular markers such as estrogen receptor (ER) protein levels and gene expression are recognized as prognostic factors of CMT, they are not routinely used in clinical settings for CMTs as they are for human breast cancer.

Material and method

In this study, we applied a machine learning approach to a publicly available dataset of 146 canine mammary tumors to explore prognostic factors, particularly the potential of gene expression data to improve survival predictions. We assessed prognostic factors including gene expression and clinical data such as age, breed, ER status and lymphatic invasion. Three Cox proportional hazard models with different data types were fit: one with only clinical variables (Clinical model), one with gene expression data (GEX) and one combining both data types (Clinical + GEX). To evaluate the models' risk discrimination capabilities, we used Uno's C-index and the time-dependent Area Under the Curve (ROC-AUC). We performed differential gene expression analysis, and gene set enrichment analysis (GSEA) to investigate gene expression differences between risk groups established using the Clinical model.

Result and discussion

Our results indicated that risk discrimination was most effective at 6 months post-diagnosis, with the Clinical model outperforming the other two models, indicating the essential role of clinical data in survival prediction. At this time point, the median ROC-AUC was 0.811 for the Clinical model, 0.727 for the GEX model, and 0.768 for the Clinical + GEX model. The GSEA revealed significant differential expression of 24 out of 50 Hallmark signatures between the two risk groups, highlighting key pathways involved in canine mammary tumor pathogenesis.

Conclusion

Clinical data is crucial for predicting the survival of individuals with CMT while addition of gene expression offers limited extra value. Moreover, risk groups identified through prognostication based on clinical

variables show significant differences in the expression of Hallmark signatures.

EACR25-1306

Enhancing Mutational Signature Detection in Whole Exome Sequencing: Insights from Esophageal Squamous-Cell Carcinoma

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Introduction

Mutational signatures represent distinct mutation patterns that arise from biological processes such as DNA damage, repair mechanisms, and environmental factors. These signatures play a crucial role in cancer research, offering insights into tumor etiology, early detection, and potential therapeutic targets. Whole genome sequencing (WGS) is the gold standard for identifying mutational signatures due to its comprehensive genomic coverage. However, whole exome sequencing (WES), which is widely used in clinical settings due to its cost-effectiveness, presents challenges in accurately capturing these signatures due to its lower mutation detection capacity. Enhancing WES's ability to reliably identify mutational signatures is essential for expanding its clinical applicability.

Material and method

In this study, we analyzed 552 esophageal squamous-cell carcinoma (ESCC) samples to assess the differences in mutational signature detection between WGS and WES. We generated down-sampled WES data from the WGS samples to simulate real-world exome sequencing conditions. Mutational signatures were identified and compared across three major signature types: single base substitutions (SBS), insertions and deletions (ID), and doublet base substitutions (DBS). We evaluated the detection sensitivity of WES relative to WGS and examined the extent of information loss across different signature classes.

Result and discussion

Our analysis revealed that WES exhibited a 50% reduction in detection sensitivity across all mutational signature types compared to WGS. This loss of sensitivity was observed for SBS, ID, and DBS signatures, indicating a significant limitation in using WES for comprehensive mutational signature analysis.

Conclusion

These results highlight the need for advanced methodologies, such as deep learning approaches, to improve the precision and sensitivity of mutational signature identification in WES, ultimately enhancing its clinical utility.

EACR25-1353

Deciphering the Molecular Impact of Asbestos: Gene Expression Profiles in Malignant Pleural Mesothelioma

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Introduction

The incidence of malignant pleural mesothelioma (MPM) has risen significantly due to extensive asbestos exposure, particularly since the mid-20th century. Despite advancements in cancer therapies, a definitive cure for MPM remains elusive, largely because the molecular mechanisms driving asbestos-related carcinogenesis are not yet fully understood. This exploratory study investigates gene expression alterations specifically associated with mesothelioma patients who have a documented history of asbestos exposure, aiming to provide a foundation for future research focused on identifying novel prognostic and predictive biomarkers.

Material and method

Publicly available RNA sequencing datasets were analyzed using a bioinformatics pipeline to perform differential gene expression analysis. Furthermore, functional enrichment analysis was conducted to identify significantly overrepresented Gene Ontology (GO) terms related to biological processes, molecular functions, and cellular components, offering insights into the molecular pathways implicated in MPM pathogenesis.

Result and discussion

The analysis identified a set of differentially expressed genes (DEGs) in MPM patients with confirmed asbestos exposure, along with key enriched GO terms. These biological annotations highlight processes such as ion homeostasis and oxidative stress response, shedding light on the cellular disruptions triggered by asbestos exposure.

Conclusion

The findings of this study enhance our understanding of the molecular alterations underlying asbestos-induced carcinogenesis in MPM. By pinpointing specific DEGs and enriched GO categories, this research provides a valuable basis for future studies aimed at discovering biomarkers and therapeutic targets to improve patient outcomes.

EACR25-1395

Pan-cancer analysis of signaling pathways regulatory connection with pyrimidine metabolism and its chemoresistance role

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Introduction

Cancer is often characterized with the accumulation of mutations and deregulated signaling pathways or metabolic processes, which in turn facilitates uncontrolled proliferative ability to the cell with high

dependency on nucleotide metabolisms. Notably, aberrant expression levels in the rate-limiting enzymes of pyrimidine metabolism (PyMet) attributes 80% of cancer types, and the understanding of the intricate signaling pathways or metabolic processes behind PyMet is quite limited. Therefore, a comprehensive portrait of the signaling pathways and metabolisms connections with PyMet is essential to exploit therapeutic strategies for cancer.

Material and method

A pan-cancer analysis of PyMet's interaction with signaling pathways and metabolic processes was performed using a pathway-based approach in around 10,000 gene expression profiles of 32 cancer types utilizing a large collection of gene-sets representing signaling pathways and metabolic processes. The identified interactions were further validated in vitro using pyrimidine metabolic inhibitor (brequinar) treatments and genetic knockdowns. In addition, PyMet inhibition was explored for the synergistic activity with commonly used chemotherapeutic drugs such as cisplatin and doxorubicin using cell lines and mouse derived KrasG12D p53Δ/Δ lung tumor organoids to improve chemosensitivity of the drugs.

Result and discussion

Pan-cancer analysis with pathway-based approach has identified a strong inter-dependency of regulatory connections of PyMet including TERT, MTOR, DAX1, HOXA1, TP53 and TNC which in turn was tightly linked to chemoresistance. These PyMet-signaling interactions were further validated in vitro by inhibiting thymidylate synthase activity using knockdown approach and with brequinar treatment. Strikingly, chemoresistance gene signatures of widely used clinical drugs such as doxorubicin and cisplatin showed an inverse association pattern with brequinar treatment profile. In fact, brequinar treatment in cell lines and mouse-derived lung tumor organoids displayed a synergistic sensitization of cells to chemotherapeutic drugs implying targeting PyMet can overcome chemoresistance in cancer.

Conclusion

The study highlights the complex signaling and metabolic cascades governing pyrimidine metabolism and its role in chemoresistance, and thereby providing an effective tool for advancing PyMet targeting strategy in cancer. The analysis as an accessible resource is available at: www.pype.combio.sdu.dk

EACR25-1406

Single Cell Transcriptomics Reveals Hallmark Enrichment in Luminal A and Triple Negative Breast Cancers

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Introduction

The cancer hallmark framework offers valuable insights into the mechanisms underlying tumor diversity. To explore how these hallmarks differ among breast cancer (BC) subtypes and normal samples, we analyzed single-cell RNA-seq data of Luminal A (LumA), triple-negative breast cancer (TNBC), and normal tissues. We also

assessed gene expression trajectories and cell-cell communications that may influence tumorigenesis.

Material and method

Single-cell RNA-seq data with 10x Genomics protocols were utilized from NCBI GEO. In total, we integrated 47 LumA samples (164,091 cells), 24 TNBC samples (82,456 cells), and 16 normal breast samples (63,241 cells) into datasets. Data were processed using the Python scanpy pipeline; cell types were annotated via the CellMarker2.0 database, and tumor cells were identified with inferCNV. The CancerHallmarks.com platform was utilized to characterize cancer hallmark gene enrichment in the top 5000 genes. Pseudotime analyses with Monocle3 captured differentiation trajectories, and cell-cell communication networks were inferred with CellChat V2.

Result and discussion

Cell type distributions revealed unique micro-environments: LumA contained abundant epithelial and cytotoxic T cells, whereas TNBC had a higher proportion of B cells, macrophages, and cancer stem cells (CSCs). The proportion of tumor cells was 31% in LumA and 23% in TNBC samples. Tumor cells were characterized by distinct hallmark enrichment: in LumA, the hallmarks “reprogramming energy metabolism” and “resisting cell death” were particularly enriched ($p < 0.0001$), while in TNBC, additional hallmarks, including “evading immune distraction”, “tumor-promoting inflammation” and “replicative immortality” were also significant ($p < 0.01$). Subclustering of CSCs identified two principal groups in LumA and four in TNBC, reflecting broader heterogeneity in TNBC. Pseudotime trajectories showed that key mitochondrial and ribosomal genes, as well as MALAT1, FTH1, TPT1, and EEF1A1, vary significantly along differentiation pathways in both LumA and TNBC. CellChat V2 detected marked shifts in signaling pathways such as MK and VISFATIN and immune-related signals (e.g., CXCL, CCL, IL2) between normal and tumor cells. These suggest that tumor cells shift communication dynamics, favoring angiogenesis and reduced immune visibility, potentially enhancing tumor survival.

Conclusion

We demonstrate distinct hallmark enrichments and altered signaling pathways by integrating single-cell gene expression data from multiple BC subtypes. Our findings underscore the importance of investigating dynamic cell states and communication networks.

EACR25-1409 Enabling Clinical and Early-Phase Drug Development Applications through Single-Sample Tumor Archetype Assignment

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Introduction

Tumor archetypes provide a biologically driven framework for classifying solid tumors through the lens of the tumor microenvironment (TME). Emerging studies have identified pan-cancer TME subtypes and established their association with overall survival, response to immunotherapy, and specific genomic alterations, highlighting the potential of TME classification for refining patient stratification and optimizing precision oncology strategies. However, computational approaches for deriving tumor archetypes are often tailored to specific datasets or require a representative input cohort as a preprocessing step, limiting their applicability to individual samples or small cohorts, which are common scenarios in clinical practice and early-phase trials.

Material and method

We adopted as our working model the pan-cancer taxonomy proposed by Bagaev et al., which categorizes tumors into four distinct TME subtypes: immune-enriched, fibrotic, immune-enriched and fibrotic, and desert. Focusing on colorectal, lung, and breast cancer, and using RNA-seq data from TCGA and Roche clinical trials, we systematically assessed the robustness and generalizability of Bagaev tumor archetypes across indications and cohorts. We further trained a set of indication-specific single-sample machine learning classifiers that leveraged within-sample pairwise gene expression relationships to assign archetype labels to out-of-sample and previously unseen data.

Result and discussion

The replicability of tumor archetypes and their associated gene signature enrichment profiles varied across indications and cohorts. Moreover, the assigned archetype label for a given sample was highly dependent on the cohort's composition. This is problematic, as archetype assignment should not be influenced by the specific cohort in which samples are evaluated. We found that our classifiers achieved robust and reliable results comparable to cohort-based clustering outcomes when all four archetypes were well-represented, and particularly excelled in settings with limited samples or under-represented archetypes. In cases of disagreement, our classifiers showed stronger alignment with H&E evaluation of TME features and maintained performance despite batch effects and variable sequencing depths, underscoring their practical utility for real-world clinical applications.

Conclusion

We offer a robust and generalizable framework for TME-based tumor archetype assignment in individual patients. This framework addresses the limitations of cohort-dependent methods, making it particularly well-suited for clinical settings and early-phase drug development applications. Future work will focus on addressing TME gene signature redundancies and exploring the interpretability and utility of soft-assignments using class-label probabilities.

EACR25-1476 Linking *in situ* phenotyping of intestinal stem cells to functional analyses of patient-derived organoids as a risk

stratification strategy during follow-up monitoring of CRC patients

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Introduction

Intestinal stem cells (ISCs) are crucial for high-turnover tissue regeneration and homeostasis of the colonic crypt, however, their dysregulation is a major factor in developing colorectal cancer (CRC). Two types of ISCs have been identified: cycling LGR5+ SCs at the base and a reserve pool of SCs in the +4 position of the crypt. To maintain cell proliferation and self-renewal, SCs express high levels of β-Catenin, a central component of the Wnt signaling pathway. Here, we aim to phenotype the colonic SC compartment at both the single-cell level *in situ* and the functional level *ex vivo* to identify CRC patients at high risk of recurrence during follow-up monitoring.

Material and method

In situ phenotyping of ISCs was performed using the spatial single-cell proteomics Immuno-Oncology panel (CosMxTM from Bruker). Colonic tissues of patients during CRC follow-up versus normal controls were analyzed. Therefore, ISCs were defined as Vimentinneg β-Cateninpos cells, and the two subtypes were distinguished based on their spatial position within the crypt. To link these data to functional analyses, the *ex vivo* proliferative potential of ISCs was studied using patient-derived 3D organoids (PDOs). PDOs were generated from colonic crypts that were isolated from biopsy samples collected during regular follow-up colonoscopies. The proliferation and differentiation of PDOs were analyzed using live cell imaging and RNA sequencing, respectively.

Result and discussion

In-depth characterization revealed different phenotypes of Vimentin neg β-Catenin pos ISCs between follow-up CRC patients and controls. In preliminary experiments, these phenotypes could be functionally validated using PDO cultures.

Conclusion

Our findings suggest that the proliferative capacity of colonic PDOs highly depends on the phenotype of the ISC compartment. In the long term, characterizing the stem cell profile could contribute to risk stratification of CRC follow-up patients and identification of therapeutic or metabolic targets for the prevention of recurrences as part of tertiary prevention.

EACR25-1478

Good Models or Good Riddance: A Guide for Researchers on Choosing Optimal Cell Lines for Translational Ovarian Cancer Research

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Introduction

Epithelial ovarian cancer (EOC) poses a significant global health challenge, accounting for over 200,000 deaths per year. EOC comprises five histological subtypes that respond differently to chemotherapy and have distinct prognoses. EOC cell lines serve as essential tools for cancer research; however, subtype specificity and molecular similarity to primary tumours are often overlooked, hindering applications in preclinical research. Here, we address the challenge of selecting appropriate EOC cell line models for subtype-specific research. We also identify long non-coding RNAs (lncRNAs) upregulated in high-grade serous ovarian carcinoma (HGSOC) cell lines. A subset of these lncRNAs demonstrated strong correlations with genes involved in the epithelial-to-mesenchymal transition (EMT).

Material and method

Non-negative matrix factorization (NMF) was performed on 56 EOC cell lines. The mutational, copy number and gene expression landscapes of these cell lines was then characterized. The gene expression profiles of these 56 cell lines were compared with those of 93 primary tumour samples, stratified by subtype. Differential gene expression analysis was performed to identify deregulated lncRNAs in HGSOC cell lines. Finally, Spearman's correlation test was carried out to identify lncRNAs displaying a similar expression pattern to 37 EMT-related genes.

Result and discussion

Non-negative matrix factorization separated 56 EOC cell lines into five stable clusters. The five groups of cell lines identified by NMF each shared the major mutational and copy number characteristics of each of the five subtypes of ovarian cancer, suggesting that these groupings were reflective of histological subtype. Integration of gene expression data identified EOC cell lines most suited to transcriptomic studies. We also highlight that A2780, used in over 90% of EOC preclinical studies, is an unsuitable model of ovarian carcinoma of any subtype. We then focused on HGSOC cell lines to identify potential therapeutic or diagnostic biomarkers. lncRNAs have been highlighted as auspicious biomarkers in cancer diagnosis and prognosis, due to their tissue-specific expression and stability in circulation. We identified 229 lncRNAs differentially expressed in HSGOC cell lines compared to control samples. Interestingly, nine of these lncRNAs displayed similar expression patterns to several EMT-related genes, marking them as prospective candidates for *in vitro* diagnostic and prognostic testing.

Conclusion

Ultimately, this study underscores the critical importance of selecting subtype-specific EOC cell line models for computational and laboratory-based investigations. Additionally, we pinpointed subtype-specific lncRNAs exhibiting strong correlations with EMT-related genes. A greater understanding of the diverse subtypes of ovarian will lead to more targeted therapies, novel diagnostics and increased survival for patients affected by EOC.

EACR25-1565

Artificial Intelligence-Based Polyp Size Measurement: Multinational Validation Across Endoscopic Datasets

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Introduction

Colon polyp size measurement is crucial for predicting colon cancer risk. However, precise measurement remains challenging due to variability among clinicians and non-calibrated equipment. Recent studies have aimed to improve accuracy by incorporating Artificial Intelligence (AI) models. However, the models have not been extensively validated across diverse clinical datasets. This study evaluates an AI-based colon polyp size measurement system using real-world clinical data from multiple countries.

Material and method

The Real-Colon (Italy, UK, USA, Austria, Japan), PICCOLO (Spain), and SNUH (Korea) datasets were utilized, with polyp sizes validated by gastro-enterologists. Paired images, including standard and instrument-referenced frames, were measured by three physicians. A CASCADE model generated segmentation masks. Depth maps were created using a deep learning estimation model and underwent iterative grid optimization for each dataset. These maps were converted into 3D point clouds, from which 2D projections were derived to calculate colon polyp diameters.

Result and discussion

We conducted binary classification at 5 mm and 10 mm clinical thresholds using three datasets: Real-Colon (131 polyps, 2,097 images), PICCOLO (68 polyps, 2,968 images), and SNUH (76 polyps, 165 images). The system showed robust performance across all datasets. At the 5 mm threshold, accuracies were 80.96% (F1: 0.8812) for PICCOLO, 81.29% (F1: 0.4988) for Real-Colon, and 76.97% (F1: 0.7246) for SNUH. At the 10 mm threshold, accuracies were 72.57% (F1: 0.7140) for PICCOLO, 98.06% (F1: 0.5176) for Real-Colon, and 90.91% (F1: 0.7368) for SNUH.

Conclusion

This study marks the first comprehensive evaluation of a colon polyp size measurement algorithm on multi-

institutional data from various countries, demonstrating its broad applicability across diverse clinical settings and populations, and confirming the potential for clinical application of AI-based polyp size assessments across varied datasets.

EACR25-1570

Prediction of Rectal Cancer Complete Response to Chemoradiotherapy by Multiscale Imaging and Deep Learning Analyses (PRECORMIDEL)

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Introduction

Colorectal cancer (CRC) is the third most common cancer worldwide, accounting for 9.6% of cancer-related deaths. Among CRC, rectal cancer (RC) has significant morbidity and mortality. Neoadjuvant chemoradiotherapy (nCRT) is a standard treatment for locally advanced RC, yet response to therapy varies among patients. Pathological response to nCRT is a critical prognostic factor, but its prediction remains a challenge. Biomarkers have been explored, but their clinical applicability is limited. We hypothesize that key features within patient biopsies can predict treatment response and that deep learning (DL), as a powerful and unbiased analytical approach, can effectively analyze histological images to make these predictions.

Material and method

This study applies DL algorithms to predict nCRT response using high-resolution Hematoxylin and Eosin (H&E) images from 20 cases (10 "responder" patients with persistent complete response after nCRT, and 10 "non-responders" who had to undergo salvage surgery) of a larger dataset of 120 patients identified in the Watch&Wait cohort of the Champalimaud Foundation. Image preprocessing involved patch extraction, followed by patch-level classification using pretrained ResNet18 and VGG19. Patient-level predictions were generated through a majority-vote aggregation. The model performance was assessed using accuracy, F1 score, precision, and recall at both patch and patient levels. These metrics allow for evaluating the model's ability to correctly classify both individual patches and patient outcomes.

Result and discussion

Preliminary results show that ResNet18 outperforms VGG19, achieving an accuracy of 87%, F1 score of 88%, precision of 99%, and recall of 78%. The superior precision of ResNet18 underscores its ability to reduce false positives, while recall values indicate room for improvement in minimizing false negatives. These results are supported by a dataset with well-defined, high-resolution images acquired through consistent protocols. Despite the small sample size, the reliability of ResNet18's predictions suggests that our data-driven methodology plays a key role. Analyzing the whole dataset, incorporating immunohistochemistry images, and

refining model architectures will be key to improving performance.

Conclusion

These preliminary findings demonstrate the potential of DL for predicting nCRT response in RC, with ResNet18 showing strong performance across key metrics. The high quality and rigor of the dataset likely contribute more to these results than model complexity alone. While the results are promising, further validation with external datasets is necessary. This approach could pave the way for personalized treatment strategies by identifying patients most likely to respond to nCRT while also providing insights into the cellular mechanisms underlying these response differences.

EACR25-1610

Development of a mathematical model for early prediction of relapses in colorectal cancer

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Introduction

Colorectal cancer (CRC) is the second leading cause of cancer-related deaths and the third most common cancer worldwide. While primary tumors are effectively managed in most cases, approximately 20–40% of patients experience recurrence within the first two to three years after treatment. Cancer stem cells (CSCs) are most responsible for relapse due to their unique characteristics, including their low division rate, their self-renewal capacity, and their ability to evade the primary tumor, travel through the bloodstream (due to its epithelial-mesenchymal transition capacity), and colonize other locations, both proximally and distally (metastasis). Although several biomarkers have been described in the literature for the detection of CSCs, none are exclusive to this cell population. This limitation highlights the need for a combination of biomarkers to improve early relapse prediction.

Material and method

The presented project applies supervised machine learning methods to generate a mathematical model for relapse prediction. The expression levels of a combination of CSCs biomarkers together with the clinicopathological characteristics of the patients were the inputs for the developed mathematical model.

Result and discussion

Different supervised machine learning models were applied to develop a mathematical model for relapse prediction, with Logistic Regression yielding the best results. This model achieved a specificity of 82.4% and a sensitivity of 82.6%, offering reliable predictive capabilities. We also analyzed the contribution of each biomarker to the model to better understand their roles in relapse prediction.

Conclusion

The development of a mathematical model for early relapse prediction contributes to advancing precision medicine and improving the quality of life for colorectal cancer patients by enabling more targeted and effective interventions, maximizing response rates, minimizing unnecessary treatments, and reducing side effects.

EACR25-1680

Deciphering the Role of Stress Granules: Dampening Inflammation in Cancer through Mitochondrial RNA Sequestration

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Introduction

Stress granules (SGs) are dynamic, membraneless cytoplasmic assemblies formed in response to various cellular stressors, including hypoxia, nutrient deprivation, and chemotherapy, that are commonly encountered in the tumour microenvironment. Initially linked to global translational suppression, SGs are now recognized for their role in the selective sequestration of proteins and mRNAs, a process that modulates stress responses and influences cell survival. In cancer, SGs have been associated with metastasis and chemoresistance, highlighting their potential impact on tumour progression.

Material and method

We performed an unbiased analysis of publicly available transcriptomes of SGs, collected by either differential centrifugation or proximity RNA labelling, from diverse human cell types, stress conditions, and isolation methodologies. To address methodological discrepancies and elucidate the functional implications of SG composition, we developed, using machine learning, an SG gene signature technique. This signature was subsequently employed to assess prognostic outcomes in cancer survival and therapeutic response.

Result and discussion

Our analyses suggest that SG transcript profiles derived via differential centrifugation are strongly influenced by inherent methodological biases, resulting in minimal concordance with datasets obtained through proximity labelling. Notably, the former naturally biases for heavier and consequently longer RNAs. Despite these methodological challenges, after removal of that bias, an enriched sequestration of mitochondrially encoded RNAs in SGs was consistently observed across studies. We hypothesize that these RNAs have immunogenic properties and that their sequestration within SGs mitigates the immunogenic response, thereby reducing inflammation and apoptosis. While the machine learning-derived SG gene signature was often elevated in tumours compared to normal tissues, its performance as a prognostic tool was not superior to that of random gene sets, a limitation of many other cancer-related signatures. This suggests that, although SG-mediated dampening of the inflammatory response may contribute to cancer cell survival and therapy resistance, its translation into a robust prognostic marker remains challenging.

Conclusion

This study highlights the dual challenges of methodological variability and biological complexity in SG research. Our findings support a model in which SGs selectively sequester immunogenic mitochondrially encoded RNAs, attenuating inflammatory responses and apoptosis, which may promote tumour progression. While our developed SG signatures imply the presence of these structures in cancer tissues, its limited prognostic

performance calls for further refinement and the need for standardised isolation techniques to clarify the precise role of SGs in cancer biology.

EACR25-1696

A semi-automated approach for detecting minimal residual disease with single-tube by flow cytometry in pediatric acute myeloid leukemia

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Introduction

Acute Myeloid Leukemia (AML) demands precise diagnostics and prognostics. Flow cytometry (FC) is essential for identifying neoplastic markers, while Minimal Residual Disease (MRD) detection in Bone Marrow (BM) evaluates treatment response. This study explores the use of the semi-automated t-SNE algorithm with HOVON single-tube FC for MRD detection in pediatric AML, comparing its accuracy and efficiency to manual analysis.

Material and method

This retrospective study analyzed cytomorphological markers in leukemic cells from 8 pediatric AML patients at a Brazilian public hospital between 2021-2024. Children aged ≤ 18 years with AML who maintained positive MRD ($\geq 0.1\%$ blasts in the BM) were included. Clinical data were collected by reviewing medical records. Acquisition for MRD detection in BM samples was performed using an FACSCanto cytometer, using a combination of monoclonal antibodies. In this study, BM aspirates were prepared for cytometric analysis by centrifugation or massive lysis of red blood cells with ammonium chloride solution, known as Bulk Lyse, increasing the sensitivity of the method and allowing the recovery of enough leukocytes to perform the labeling and acquisition of ≥ 1.5 million cells per tube. The 5 control samples were labeled with a combination of 46 specific monoclonal antibodies, totaling 6 tubes (8 colors) to identify residual leukemic cells and differentiate normal/residual cells regenerating in the BM during treatment. The 8 test samples were labeled only from a single tube adapted from the HOVON group to the panel used for monitoring MRD in AML. FlowJo software was used to apply the automated data clustering methodology by t-SNE. The local ethics committee approved the study.

Result and discussion

Analyses were performed on 8 patients, 1 male and 7 female. The mean age was 10.75 years and 5 BM aspirates from children with AML were included as

controls. Single-tube analysis (CD7/CD13/CD34/CD117/CD33/HLA-DRA/CD45) facilitated the characterization of myeloid compartments at immature stages, including pre-leukemic CD34/CD33 stem cells associated with leukemia recurrence. The inclusion of CD56 as a cross-lineage marker proved useful for detecting MRD. The automated cluster mapping methodology, specifically t-SNE, successfully identified expected MRD populations, demonstrating consistency with manual gating results. Control samples showed no MRD populations, aligning with this methodology. A comparison of manual and semi-automated analyses revealed a 100% agreement between t-SNE and manual gating results. Statistical evaluation showed no significant differences in mean detection rates and Pearson's correlation indicated no bias between the methods.

Conclusion

The findings support the integration of t-SNE methodology into monitoring workflows. This study highlights t-SNE as a complementary tool, providing clinicians with a risk stratification method based on machine learning.

EACR25-1735

Accelerating real world data collection using Large Language Models in rare neoplasms, a bone sarcoma example

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Introduction

Real-world data collection in oncology poses many challenges due to an extensive number of important variables, the rapidly changing treatment landscape, and mostly unstructured source data. Large Language Models (LLMs) have proven capable of extracting information across various domains, yet their size and computational requirements still limit their practical use. However, the popularization of smaller LLMs' variants and model quantization methods have opened doors for local, controlled inference without the necessity for substantial computing infrastructure. This study evaluates the performance of multiple small LLMs as information extractors on Polish language free-text medical notes.

Material and method

Electronic health records (EHRs) of 302 consecutive bone sarcoma patients treated in a reference center between 2016 and 2022 have been selected. 5 variables (pathology type, tumor size, localization, grade, and type of primary resection) for each EHR have been extracted by an oncologist experienced in sarcoma treatment.

4 LLMs (Llama 3.1 8B, DeepSeek-R1 8B, Mixtral 8x7B,

Bielik-11B-v2.3-Instruct) have been chosen for the study, with the temperature set to 0. A zero-shot prompting technique with or without additional background information has been used to query the models with the task of returning the value for a given variable using an XML tag. Accuracy was defined as a percent of values concordant with the human-extracted values.

Additionally, among non-concordant values, we distinguished valid results, i.e., of expected format and containing a keyword/phrase from per-variable, expert-devised list. Finally, we've designed a voting strategy that selects the value obtained in more than half of valid results as the ensemble result.

Result and discussion

The average accuracy across the models ranged between 19.2% and 34.2%, with performance on distinct prompts varying greatly. In particular, the tumor localization values turned out to be the easiest to assess by LLMs, with accuracy up to 70.4%. The models had better accuracy when given background information (OR: 1.31, 95% CI: 1.25-1.38). The majority of non-concordant values were non-valid values. Applying the voting strategy improved performance significantly, with 75.8% overall accuracy.

Conclusion

Our study highlights the potential of using lightweight LLMs to automate data extraction from oncologic notes, which could significantly accelerate clinical research. A singular, small LLM is not yet sufficient for real use cases in non-English settings; however, prompt engineering and ensemble methods can greatly improve performance.

EACR25-1741

Pan-Cancer Analysis Identifies Dorsomorphin Dihydrochloride as an Anticancer Drug Targeting the Notch Signaling Pathway

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Introduction

Cancer is characterized by the uncontrolled and continuous division of cells. Identifying specific biomarkers for tumor treatment has become increasingly important, particularly with the discovery of oncogenes and tumor suppressor genes. Various biochemical signaling pathways contribute to cancer development, and one critical pathway is the Notch signaling pathway.

Material and method

This study applies advanced bioinformatics approaches to analyze transcriptome and clinical data from nine tumor types in TCGA, focusing on two main goals: identifying biomarkers linking Notch signaling to carcinogenesis and exploring the potential of drug repositioning to target this pathway.

Result and discussion

The results reveal CA4, CFD, DPT, FHL1, TMEM100, TMEM132A, and VEGFD as critical biomarkers associated with Notch signaling in tumorigenesis. In particular, dorsomorphin dihydrochloride was successfully repositioned as a drug targeting pan-cancer

Notch biomarkers, offering a promising therapeutic strategy for cancers influenced by this pathway.

Conclusion

This study highlights the significant role of drug repositioning, demonstrating how existing drugs can be repurposed to target key biomarkers like those involved in Notch signaling. In precision medicine, these biomarkers, combined with repositioned drugs like dorsomorphin dihydrochloride, hold the potential to revolutionize patient-specific therapies, reducing both the time and cost of developing new treatments.

EACR25-1799

Proteomic Analysis of the Pd(II) Complex-Induced Changes in HeLa Cervical Cancer Line

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Introduction

Palladium(II) complexes have emerged as potential anticancer agents due to the metal core similarity with widely used platinum(II) complexes. However, the precise molecular mechanisms remain unclear. In this study, we investigate the effects of a Pd(II) complex on HeLa cells at the protein level.

Material and method

HeLa cells were treated with 50 µg/ml dichloro(1,2-diaminocyclohexane)palladium(II), [Pd(dach)Cl₂] for 48 hours, followed by comparative proteomics analysis using label-free data-independent LC-MS/MS acquisition coupled with ion mobility. A bioinformatics approach using STRING and Cytoscape plug-in ClueGO was applied to perform functional enrichment analysis of the proteins with statistically significant differential abundance, highlighting key cellular processes and molecular pathways affected by treatment.

Result and discussion

We have identified 121 differentially abundant proteins (Anova < 0.05) in Pd(II) treated HeLa cells compared to controls. An enrichment analysis of GO annotations indicated ATP hydrolysis activity and RNA binding as top molecular functions. The most affected biological processes were RNA splicing, protein-RNA complex organization, and cytoplasmic translation. KEGG pathways enrichment revealed a significant association with the spliceosome and ribosome pathways, suggesting RNA processing and stability disruptions. Identification of hub genes in the protein-protein interaction network of differentially abundant proteins revealed that the top 6 highly essential proteins in this network were ribosomal (RPL4, RPS9, RPL32, RPS15A, RPS25, RPL36AL).

Conclusion

This study provides novel insights into the changes in HeLa cell proteins induced by a Pd(II) complex. The enrichment of RNA-processing pathways suggests a potential mechanism of action, warranting further

investigation into the therapeutic relevance of Pd(II) complexes in cancer treatment.

EACR25-1837

MOKCa-3D Database: Functional and structural analysis of gain-of-function and loss-of-function missense mutations in cancer

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Introduction

Determining the functional consequence of missense mutations acquired in the development of cancer is critical to the understanding of the evolution and the therapeutic vulnerabilities of an individual tumour. Over three million unique missense mutations have been reported in the COSMIC database of cancer somatic mutations, with little functional annotation accompanying each mutation. We have designed the MOKCa-3D database [1] to enable the contextualization and interpretation of cancer somatic missense mutations including the structural impact of the mutation on the 3D structure, and whether the mutation results in a gain or loss of the protein's function.

Material and method

For each protein, a sequence feature viewer enables interactive visualization of the amino acid sequence, missense mutations, post-translational modification sites (PTMs), protein domains, active sites, binding sites, protein-protein interaction sites, and mutational frequency. The mutation-level page concisely presents functional insights for each individual mutation, and an interactive MOL star viewer highlights mutated residue on an AlphaFold protein structural model. The SAAP structural impact analysis pipeline was used to identify the structural impact of the mutation.

Result and discussion

MOKCa-3D concisely presents functional insights and structural impacts of cancer somatic missense mutations enabling users to interpret their functional consequences.

Conclusion

MOKCa-3D is freely accessible and easy to navigate, making it usable by the widest range of researchers.

[1] URL: bioinformaticslab.sussex.ac.uk/MOKCa-3D

EACR25-1839

The S-RACE Platform: Empowering Healthcare Real-World Data with Artificial Intelligence (AI) Using a Cloud-Based Approach

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Introduction

Prognostic and predictive clinical decision support systems based on Real-World Evidence (RWE) data are crucial in clinical research. These systems help clinicians optimize therapeutic choices, reduce adverse events, and enhance personalized medicine. AI plays a key role in these advancements, as machine learning algorithms uncover hidden patterns beyond human capability, leading to novel clinical insights. However, the clinical translation of such algorithms depends heavily on data quality. RWE data are often unstructured, sparse, and poorly curated, requiring extensive manual processing. To address this, we developed an end-to-end data engineering pipeline to import RWE from our IT system and create machine learning models to tackle urgent clinical questions.

Material and method

The S-RACE Cloud-based platform, developed with Microsoft, has three main functionalities: a universal data platform (ingestion), a clinician AI hub (exploration), and a data science lab (modeling). The universal data platform lets investigators select patient cohorts, define data sources, and retrieve DICOM images. An on-prem anonymization engine processes data before securely transferring it. AI technologies, including Microsoft Cognitive Health Services, use natural language processing (NLP) and medical ontologies to extract relevant clinical information. Processed RWE, standardized using FHIR, are stored in a data lake and linked to specific use cases. Preliminary analyses are conducted via Microsoft Power BI, while data modeling is performed using Microsoft Azure Machine Learning Studio. Explainability techniques enhance model interpretability, and standardized templates automate documentation. Validated models will be shared internally and with the broader clinical and research communities via the clinician AI hub.

Result and discussion

We have integrated five major hospital IT systems into the platform including electronic health records, PACS, blood lab tests, pathology and cancer registries leading to a total of 5000 cancer patients' data imported. Currently, ten clinical use cases (prostate cancer, metastatic lung cancer, colorectal cancer, pancreatic cancer, liver cancer, lung cancer screening, diabetes, multiple sclerosis, cardiovascular diseases) are active, with AI models under development and validation. One oncological case study has been concluded: we have developed one web-based risk score to preoperatively predict kidney cancer mortality [1].

Conclusion

The S-RACE platform is a scalable AI-driven solution to leveraging RWE in clinical decision-making. By integrating hospital IT systems, automating data processing, and enabling AI modeling, the platform enhances research and fosters data-driven personalized medicine. Future work will focus on expanding validated AI models and facilitating their clinical adoption.

[1] URL: app-s-race-model-uc2-dev-webapp.unisr.it

EACR25-1880

Monte Carlo Simulation of Gold Nanoparticles in Glioblastoma

Radiotherapy: Impact on Survival and DNA damage under Co-60 γ -radiation and 160 kVp X-rays

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Introduction

Gold nanoparticles (AuNPs) can enhance radiotherapy effects in tumors without raising patient dose, being Monte Carlo simulations (MCS) a very useful tool to study the effect of AuNPs as radiosensitizers.

Material and method

Voxelized cell geometry of GBM cell model was implemented on TOPAS MC, and MCS results estimated the SF using local effect model (LEM) and microdosimetric kinetic model (MKM). Different concentrations of AuNPs were evaluated. Additionally, DNA damage caused by direct and indirect interactions during the physical and chemical stages was assessed. Strand breaks (SBs) on opposite DNA strands within 10 base pairs were classified as double-strand breaks (DSBs); otherwise, they were single-strand breaks (SSBs). DSBs were categorized as direct (both SBs from direct interactions), indirect (both from indirect interactions), or hybrid (one direct, one indirect).

Result and discussion

Regarding the survival fraction, the results, obtained using LEM, were benchmarked against experimental results, demonstrating a good agreement. The SF obtained either in the absence or in the presence of AuNPs with LEM were compared with the ones obtained using MKM. The study showed that selecting the appropriate domain size is crucial in MKM, as it directly affects the calculation of the microdosimetric quantity y^-_D (dose-mean lineal energy). Four domain sizes were considered: 5, 10, 15, and 20 nm. The percentual difference in SF at 2 Gy between LEM and MKM follows a consistent trend, regardless of the irradiation type. The highest difference is observed when the domain size is set to 5 nm, decreasing as the domain size increases. This difference is also more pronounced in the presence of AuNPs compared to cases where no AuNPs were considered. Furthermore, the differences obtained for X-ray irradiation are significantly larger than those observed for Co-60 exposure. The results of MCS for DNA damage demonstrate that X-rays, in combination

with AuNP, induce a substantial increase in DSBs, with nearly four times higher DSBs production. This increase is primarily driven by indirect mechanisms. In contrast, after γ -ray exposure, the production of DSB does not increase so significantly as for the X-ray exposure. In this case, the indirect damage is negligible. These results agree with the experimental findings.

Conclusion

The results reinforce the potential of AuNPs as effective radiosensitizers, particularly for kilovoltage X-ray therapy. The findings highlight the importance of optimizing parameters in advanced radiobiological models, such as MKM, to better understand the micro- and nanoscale effects of AuNPs.

Funding and Acknowledgments: This work was supported by FCT: UID/Multi/04349/2020 to C2TN and UIDP/50007/2020 and 2023.15783.PEX to LIP. JA and ART are supported by the ProtoTera PhD fellowship SFRH/BD/151146/2021 and PRT/BD/154914/2023, respectively. CGIP is supported by the PhD fellowship 2020.07119.BD also from FCT.

EACR25-1936

Deep learning in digital histopathology for clinically applicable biomarker discovery in prostate cancer

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Introduction

Prostate cancer is a leading cause of cancer-related mortality, with disease heterogeneity complicating risk stratification and treatment decision-making. AI models developed using gene expression or digital histopathology have demonstrated significant prognostic value for cancer patients. However, the biological interpretation of these AI models, and the integration of these multi-modal data types, could enhance clinical applicability and prognostic value, respectively. This research aims to use functional genomics to improve AI model explainability, and deep learning to refine prostate cancer risk stratification.

Material and method

This study will utilise two in-house prostate cancer cohorts: the FASTMAN cohort, which includes 466 locally advanced patients, and the FIR cohort, which consists of 159 intermediate-risk patients. This study will also utilise the 499 patients within the TCGA-PRAD cohort. Self-supervised learning (SSL) will be used on digital prostate histopathology whole-slide images (WSIs) to extract morphological features without reliance on extensive annotations. Functional genomics through gene expression will be used to biologically interpret the ArteriaAI Prostate Test, and hypertension status. SSL-derived histopathology features, gene expression, and clinicopathological factors will be integrated using deep learning autoencoders for patient subgroup discovery.

Result and discussion

Through the exploration of different methods within the analysis pipeline of SSL methods in digital histopathology, we will provide a benchmark for other researchers to avail to, as well as developing SSL-derived histopathology features for our curated prostate cancer patient cohorts. The biological interpretation of the ArterAI Prostate Test will enhance its explainability for greater trust towards future use in clinical practice. Hypertension status was significantly associated with better outcomes in disease progression and overall survival for locally advanced prostate cancer patients treated with radiotherapy and ADT. We anticipate that the integration of the described multimodal data types will reveal novel patient subgroups with improved patient stratification.

Conclusion

Through SSL digital histopathology analysis, functional genomics, and multimodal data integration, this research aims to enhance risk stratification and characterize the underlying biology associated with prognostic AI models and hypertension status in prostate cancer patients

EACR25-1940

Unravelling HR-positive, HER2-negative Breast Cancer and its Tumor Microenvironment Heterogeneity Using Spatial Transcriptomics

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Introduction

HR-positive, HER2-negative (HR+/HER2-) breast cancer exhibits significant molecular and cellular heterogeneity, influencing prognosis and treatment response. We applied spatial transcriptomics (ST) to dissect tumor microenvironment (TME) heterogeneity and its impact on clinical outcomes.

Material and method

Spatial transcriptomics (Visium 10X) was performed on 86 HR+/HER2- of no special type of frozen tumor samples, and relative H&E slides were morphologically annotated. Single-cell deconvolution of ST spots was performed to increase the resolution of Visium data. Tumor and TME compartments were identified using morphological annotation, and differences in the abundance of deconvoluted cell types between two intrinsic molecular subtypes, LumA and LumB, were investigated. Unsupervised clustering of spatially resolved transcriptomes was performed to identify distinct spatial subtypes across samples. Differential gene expression and pathway enrichment analyses were conducted to explore functional differences among clusters.

Result and discussion

As expected, LumB tumors exhibited worse relapse-free survival compared to LumA tumors ($p = 0.012$). Distinct spatial patterns emerged between LumA and LumB subtypes. LumA tumors were more enriched for CXCL12+ endothelial cells, naive B cells, and multiple CAF subtypes across both tumor and TME zones compared to LumB tumors, whereas LumB tumors exhibited higher infiltration of exhausted T cells (LAG3+), CXCL10+ macrophages, and lipid-associated macrophages (FABP5+) within TME regions compared to LumA tumors. Unsupervised clustering revealed 24 distinct clusters with different transcriptomic patterns, cellular compositions and histological features. Clusters that were significantly associated with poor prognosis are validated using external datasets METABRIC and SCAN-B. Cluster 6, present in all patients, was enriched in stromal cells, epithelial cells, and fat tissue. Cluster 11, (78 patients), represents areas of necrosis and fat tissue within the tumor and was enriched in lipid-associated macrophages, inflammatory response and IFN- α /IFN- γ signaling pathways. Similarly, Cluster 12 (71 patients), consists of tumor regions exhibiting necrosis, associated with IFN- γ stimulated macrophages and enriched in IFN- α /IFN- γ signaling, suggesting immune activation in response to tumor cell death. Lastly, cluster 17 (seven patients) marked tumor regions. These findings highlight the role of intra-tumor heterogeneity and immune composition in prognosis.

Conclusion

These findings enhance the understanding of the TME in HR+/HER2- breast cancer, highlighting its critical role in tumor progression. Further validation is needed to determine how these spatial clusters and immune interactions influence treatment response and long-term patient outcomes.

EACR25-1986

Multiomic network-based modelling in Polish High-Grade Serous Ovarian Cancer (HGSOC) patients reveals prognostic importance of RAD21 and miR-4516

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Introduction

Given that ovarian cancer is often diagnosed at an advanced stage, there is a high demand for translational studies discovering advanced clinical biomarkers. The aim of the study was to develop prognostic models for

High-Grade Serous Ovarian Cancer (HGSOC) Overall Survival (OS) based on the multilayered omics networks.

Material and method

A non-TCGA-related (The Cancer Genome Atlas Program) independent Polish patient cohort constituted of 66 women with HGSOC, and 43 non-HGSOC controls. Multiomic analyses covering: Whole Exome (WES), total RNA, smallRNA and methylome (RRBS) sequencing, were performed on Illumina platform for each of the samples, and standardized data processing was applied. To reduce the dimensionality of data, networks of correlated profile modules were created using WGCNA (Weighted Correlation Network Analysis). Time-to-event analyses related to OS were performed utilizing log-rank tests and multivariate Cox PH models. Feature selection and modelling were conducted using machine learning approaches. A performance of the binary classifier for survival during follow-up period was assessed using AUROC, with internal cross-validations (CV) using LOOCV and 5-fold CV.

Result and discussion

The mortality rate was 35% with median follow-up ca. 4.5 years. 12% of patients carried heterozygous germline pathogenic BRCA1/2 variants. Network analysis allowed for identification of 28 RNA and 15 miRNA WGCNA modules. Final prognostic model for OS was developed using Cox regression with elastic net regularization ($\alpha = 0.5$; $\lambda = 0.18$), and following variables were selected in the order of importance: M15 RNA module, M3 miRNA module, BRCA1/2 germline status, BMI, and two cell signatures. Co-expression and interactome data reveal that RNA module M15 is driven by RAD21, responsible for DNA repair and playing an oncogenic role, while miRNA module M3 is miR-4516 related - targeting genes involved in cell cycle regulation and p53 signalling pathway (FDR-adjusted p-value = 0.03). Patients stratified by the median model outcome differed significantly in their hazard ratios (HR = 9.1; p = 0.004; 95% CI = 2.0-40.9). Median survival time in the high-risk group was 33 mo. (95% CI = 18-40 mo.), whereas in the low-risk group median survival was not reached (NR; 95% CI = 66-NR mo.). Selected features were fuelled into logistic regression model for mortality prediction during post-surgery follow-up period, which was characterised by AUC = 0.74 (95% CI = 0.62-0.975).

Conclusion

Multiomic network-based approach allows for development of significantly prognostic models in oncology setting. Obtained results highlight the cell cycle-related pathways, whose expressional aberrations are associated with survival outcomes in HGSOC. In the next steps of model development we are planning to incorporate additional multiomic layers including different genome-wide DNA methylation features.

EACR25-2023

integrative Molecular Tumor Board (iMTB): A Decision-Support Tool for Precision Cancer Medicine in iCAN

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Introduction

As part of the Finnish digital cancer precision medicine flagship (iCAN) initiative, a substantial collection of molecular profiling and clinical data is being gathered from individual cancer patients (current participants: n = 3811). To identify clinically relevant and actionable results from molecular data of each patient and to present the findings in a concise and well-structured report, we have established a reporting platform, integrative Molecular Tumor Board (iMTB). This system consolidates clinically relevant molecular profiling findings and facilitates the integration of these findings to the Biobank and clinical workflows.

Material and method

The iMTB currently leverages the Personal Cancer Genome Reporter (PCGR) tool (Nakken et al., 2018) for exome sequencing data, analyzing somatic mutations, copy number alterations (amplifications and deletions), and germline variants. Additionally, new modules have been integrated for RNA-seq data and cancer fusion proteins. The iMTB operates in a pan-cancer framework within iCAN but is also adaptable for other translational studies. Within iCAN, iMTB reports have been implemented across various cancer types, including pediatric cancers, urological cancers, rare tumors, breast cancer, and ovarian cancer.

Result and discussion

This workflow is currently being piloted for pediatric solid tumors (n = 86) and rare cancers (n = 113) at the Helsinki University Hospital (HUS) through the iCAN Flagship initiative and associated studies. At the request of clinicians and with the patient's consent, the iMTB reports are delivered to clinics via the Helsinki Biobank (HBB). Our pilot study highlights the translational impact of molecular profiling, such as identifying pathogenic germline variants to be further validated in diagnostic laboratories. These findings demonstrate that iMTB addresses a critical gap, enhancing Helsinki Biobank's and the research community's ability to effectively translate research findings into clinical practice, ultimately benefiting the patients.

Conclusion

Looking ahead, we aim to further enhance the iMTB tool through open science collaborations and continuous development. This initiative will continue to support the visualization of complex omics data, streamline research efforts, and facilitate the translation of preclinical cancer research into clinically applicable tools, ultimately advancing precision medicine and improving patient outcomes.

EACR25-2050

In-silico Approach to Uncover Biomarkers and Therapeutic Targets in Metastatic Oral Squamous Cell Carcinoma

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Introduction

Oral squamous cell carcinoma (OSCC) is the most prevalent malignant neoplasm in the oral cavity, accounting for approximately 95% of cases. In Brazil, it poses a significant public health challenge due to its high incidence, morbidity, and mortality, particularly in advanced stages with metastasis. Effective therapeutic options remain limited, underscoring the need for novel molecular targets and drug repositioning strategies. This study aims to identify biomarkers and therapeutic targets associated with metastatic OSCC using bioinformatics approaches.

Material and method

Transcriptomic data from The Cancer Genome Atlas (TCGA) and Gene Expression Omnibus (GEO) were analyzed to distinguish tumor (OSCC) and non-tumor samples. We applied the Limma-Voom statistical method to identify differentially expressed genes between tumor and non-tumor samples. Subsequently, we performed gene co-expression network analysis to detect gene modules highly correlated with clinical relevance. Protein-protein interaction analysis was conducted with the modules of interest, followed by functional characterization of each module through gene ontology and signaling pathway analyses.

Result and discussion

Preliminary findings revealed distinct co-expression modules associated with tumor progression, invasion, and metastasis. Specific modules, particularly those enriched in migration-related genes, extracellular matrix remodeling, and epithelial-mesenchymal transition, were strongly correlated with OSCC aggressiveness. Overlap analysis with a curated metastasis-associated gene panel further confirmed the relevance of these findings.

Conclusion

Future steps include implementing machine learning models to predict drug-target interactions facilitating the identification of candidate drugs for therapeutic repositioning. Integrating bioinformatics and AI-driven approaches enhances precision oncology efforts, providing insights into OSCC molecular mechanisms and developing more effective treatment strategies.

EACR25-2052

Unveiling the spatial regulation of chemoresistance in ovarian cancer

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Introduction

High-grade serous ovarian cancer (HGSC) is the most aggressive subtype, causing over 150,000 deaths annually. Its genetic heterogeneity complicates classification and drives chemoresistance, worsening clinical outcomes (ref). Beyond genetics, the tumor microenvironment (TME) promotes chemoresistance through immune evasion, angiogenesis, and cell interactions. Transcriptional and post-transcriptional networks further shape tumor adaptation. A comprehensive understanding requires integrating multiple omics approaches. Traditional omics technologies, when analyzed independently, provide valuable but limited insights, capturing only individual layers of tumor biology, while spatial omics can add context by preserving molecular organization. However, multimodal data integration faces challenges like resolution differences, tissue misalignment, and spatial variability. Overcoming these barriers is key to deciphering HGSC progression and therapeutic resistance.

Material and method

We utilize a dataset from Launonen et al. comprising 22 HGSC tumor samples (treatment-naïve and chemotherapy-treated) from 16 patients, analyzed with t-CyCIF imaging, scRNA-seq, and bulk RNA-seq. To integrate multimodal data and identify biologically relevant patterns, we will employ CAVACHON from Hsieh et al., a probabilistic learning framework based on a generalized hierarchical variational autoencoder. This method integrates prior biological knowledge about the relationships between different data types and allows the extraction of variability specific to each modality while capturing their interdependencies. CAVACHON will be applied to integrate scRNA-seq and t-CyCIF data, enabling cell clustering, modality-specific differential expression analysis, and disentangling of shared and modality-specific molecular programs. As a final step, bulk RNA-seq will be used to validate the results.

Result and discussion

We analyzed 22 tumor samples from 16 patients, identifying 6,402,172 cells with t-CyCIF and annotating 68,019 single cells with scRNA-seq. Seven major cell types – B cells, CD4+ and CD8+ T cells, regulatory T cells, myeloid cells, stromal cells, and tumor cells – were consistently shared across both modalities. Applying CAVACHON, we aim to uncover non-canonical cell clusters with unique regulatory patterns, providing insights into tumor heterogeneity, chemoresistance, and immune evasion. Integrating spatial and molecular data will further elucidate spatially confined tumor cell states and microenvironmental influences on disease progression.

Conclusion

This study leverages spatial omics, multimodal integration, and network analysis to dissect aberrant tumor cell states and their TME interactions. By identifying chemoresistant subpopulations and characterizing spatially confined molecular programs, we aim to reveal therapeutic vulnerabilities, advancing precision oncology for HGSC.

EACR25-2056

Machine Learning-Driven Drug Repositioning for Oral Squamous Cell Carcinoma: A Predictive Approach Using Affinity2Vec

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Introduction

Drug repositioning aims to identify new therapeutic applications for existing drugs, offering advantages such as reduced development time, cost savings, and lower risks, as these drugs have already undergone clinical trials. Artificial intelligence (AI) has advanced this field in recent years by processing large volumes of data and integrating scientific studies, medical records, and molecular information to uncover relevant patterns. In this study, we explore the application of machine learning to identify promising drug candidates for Oral Squamous Cell Carcinoma (OSCC). By leveraging state-of-the-art computational techniques, we aim to discover novel drug candidates through an in-depth analysis of drug-target interactions.

Material and method

We curated a comprehensive dataset using publicly available drug-target affinity data from ChEMBL to achieve this. This allowed us to explore a broad spectrum of drug classes and target families, potentially uncovering new repositioning opportunities. We applied stringent filtering criteria to refine the dataset and focus on molecular interactions relevant to our objectives. Specifically, we selected data points that met the following criteria: classified as binding assays, originating from *Homo sapiens*, involving single proteins as targets, and containing non-null pChEMBL values and maximum clinical trial phase information. We implemented Affinity2Vec, a model designed to predict

drug-target affinity scores using a regressor based on the Extreme Gradient Boosting (XGBoost) algorithm. This model takes as input an embedding representing a drug-target pair, which is generated by concatenating two distinct representations:

(i) the drug representation obtained via the Seq2Seq algorithm applied to the SMILES 2D structure of the drug and

(ii) the protein representation derived from the ProtVec algorithm applied to the FASTA sequence of the protein.

Result and discussion

The regressor was trained on 15,364 drug-target affinity scores from our dataset. Once trained, the model was used to predict the affinities between all 3,340 drugs and the 31 OSCC-related target proteins, resulting in a ranked list of 103,540 drug-protein pairs based on predicted affinity scores. The model achieved a mean squared error of 1.05 in the training set and a concordance index of 0.722. Finally, using the trained model, we generated a ranked list of predicted affinities for the 31 target proteins across the 3,340 drugs in our dataset.

Conclusion

In conclusion, in silico tools enabled the identification and ranking of promising drugs for repositioning in treating oral squamous cell carcinoma. Additionally, the database containing target-drug activities could be utilized to train future OSCC research models.

EACR25-2076

Gene expression profile in colon cancer therapeutic resistance and its relationship with the tumor microenvironment

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Introduction

Colon cancer is a common disease, treated with few chemotherapeutic agents with similar treatment sequencing despite its heterogeneity. A significant proportion of patients are diagnosed with metastasis, and resistance to antineoplastic drugs is associated with disease progression and therapeutic failure. It is known that the tumor microenvironment plays an essential role in cancer progression, contributing to processes that may be associated with therapeutic resistance mechanisms in colon cancer. In this study, we aim to identify a gene expression signature and its relationship with immune cell infiltration in colon cancer, contributing to the identification of potential resistance biomarkers.

Material and method

An in silico study was conducted using RNA-seq data from The Cancer Genome Atlas Program (TCGA) samples, subdivided into two groups (treatment-resistant and non-resistant), taking into account the molecular subgroups (CMS1, CMS2, CMS3, and CMS4). The following algorithms were used:

i. Limma was applied to identify differentially expressed genes;

- ii. WGCNA was applied to construct co-expression networks;
- iii. CIBERSORT was applied to estimate the proportion of infiltrating immune cells; and iv. TIMER was applied to explore the relationship between core genes and immune cell content.

Result and discussion

Twenty differentially expressed genes (DEGs) were found, with 18 related to the group considered resistant to oncologic treatment and presenting poorer overall survival. T CD4 memory resting cells and M0 and M2 macrophages were found in more significant proportions in the analyzed samples and more infiltrated in the tumor microenvironment, the higher the expression of some of these resistance DEGs. Additionally, these genes correlate with biological aspects of neuronal differentiation, axogenesis, and synaptic transmission.

Conclusion

The gene expression signature suggests the presence of differentially expressed synaptic membrane genes, which may be involved in neuronal pathways that influence the tumor microenvironment, potentially serving as future biomarkers. Furthermore, the presence of M0 and M2 macrophages and T CD4 memory resting cells suggests a potential interaction that may play a role in therapeutic resistance.

EACR25-2117

Evaluating computational and enzymatic strategies for artifact removal in FFPE-derived NGS data

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Introduction

Formalin-fixed, paraffin-embedded (FFPE) samples are invaluable in biomedical research and clinical diagnostics, particularly in genomic studies such as next-generation sequencing (NGS). However, the formaldehyde fixation process introduces DNA damages, primarily through deamination of cytosines to uracil, which by sequencing can be recognized as modifications of the C>T or G>A type that can confound mutation detection. To mitigate these effects, computational tools such as ideafix, microSEC, FFPolish, and SOBdetector have been developed to filter out sequencing artifacts.

Material and method

In this study, we evaluated the effectiveness of these tools across three datasets: (1) 36 matched WES FFPE and fresh-frozen samples from TCGA, (2) 21 matched WGS samples from CGCI, and (3) our custom WES dataset, including fresh-frozen samples, formalin-treated samples, both treated and untreated with the NEBNext® FFPE DNA Repair v2 Module. All samples were analyzed using the four computational tools, and filtered results were compared against fresh-frozen samples as the gold standard using som.py. We considered only variants with DP > 10 in our analysis.

Result and discussion

Our results indicate that SOBdetector achieved the highest sensitivity for somatic SNV detection, while FFPolish demonstrated the highest precision. However, the high precision of FFPolish came at the cost of an elevated false-positive rate in the artifact detection process. This effect was particularly pronounced in the classification of variants from fresh-frozen samples, where a significant proportion were identified as artifacts. Importantly, enzymatic repair using NEBNext outperformed all computational methods, demonstrating its effectiveness in mitigating fixation-induced damage. Additionally, our findings reveal that formalin fixation leads to an increase in C>A or C>T variants in WES data, with some specificity to cancer type, and that the unamplified WGS showed no clear variant preference.

Conclusion

Together, these results emphasize the necessity of both enzymatic and computational artifact removal strategies to improve the accuracy of FFPE-based genomic analyses, ultimately enhancing mutation detection in cancer genomics.

EACR25-2134

Spatially resolved OMICs defines specific immune-tumor niches in esophageal adenocarcinoma responders to neoadjuvant chemotherapy

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Introduction

Esophageal adenocarcinoma (EAC) is a highly aggressive malignancy, with neoadjuvant chemotherapy (nCT) followed by surgery as the standard of care. However, fewer than 30% of patients achieve a pathological complete response (CR), which is strongly associated with improved survival. A deeper understanding of the tumor microenvironment (TME) in responders (CRs) and non-responders (NRs) is critical for patient stratification and therapeutic advancements.

Material and method

Building on our prior discovery that pre-existing immunity correlates with nCT response, we performed single-cell spatial transcriptomics (1000 x Spatial Molecular Imager-SMI) on treatment-naïve EACs ($n = 3$ CRs vs. $n = 3$ NRs) and profiled two independent EAC cohorts ($n = 75$) using multiplexed immunofluorescence (mIF), digital spatial transcriptomics (DSP), and bulk RNA sequencing (RNAseq).

Result and discussion

Our SMI analysis revealed distinct immune-tumoral niches exclusively enriched in CR tumors, characterized by dense clusters of dendritic cells (DCs), T and B cells, and granulocytes, supporting a robust pre-existing immune activation state. In contrast, NR tumors exhibited augmented M2-like macrophages, pro-tumoral cancer-associated fibroblasts, endothelial cells, and proangiogenic signatures, driven by distinct ligand-receptor interactions and spatial organization. These findings were further corroborated by DSP analysis of 20 EACs, which confirmed heightened neutrophil degranulation and DC activity in CRs, while NRs displayed dominant angiogenic and lipid metabolism signatures. Notably, immune cells in CRs showed upregulation of antigen presentation, immune activation, and M1-like differentiation pathways, whereas NRs were enriched for immunosuppressive pathways and M2-like macrophage signatures. Ongoing integration with mIF and bulk RNAseq (> 50 EACs) aims to further delineate these immune landscapes.

Conclusion

This study uncovers previously unrecognized features of the immune microenvironment in EAC responders, marked by clusters of antigen-presenting cells cross talking with adaptive (T and B cell) and innate (granulocyte) immune responses, in stark contrast to the immunosuppressive, pro-angiogenic, and lipid metabolism-driven TME in NRs.

EACR25-2249

Comprehensive Analysis of Fusion Transcripts in HCC: Implications for Gene Expression and Tumor Progression

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Introduction

Fusion transcripts (FTs) are chimeric RNA molecules formed either through chromosomal rearrangements or as a result of transcriptional events. These transcripts have been increasingly recognized for their crucial role in

tumorigenesis. Hepatocellular carcinoma (HCC), a leading cause of cancer-related mortality, remains poorly understood in terms of its FT landscape. Therefore, this study aims to systematically identify and characterize tumor-specific FTs in HCC. By uncovering novel fusion events and assessing their impact on gene expression, we aim to establish a comprehensive framework for understanding their role in HCC progression.

Material and method

Eight independent datasets comprising 328 samples in our meta cohort were analyzed using STAR-Fusion, identifying tumor-specific FTs. Differential gene expression (DEG) analysis was conducted between HCC tumors and normal adjacent liver tissues (NAT) to identify key fusion partners in the meta cohort. Validation of key fusion partners was performed using an in silico cohort of 436 samples and two wet lab validation cohorts comprising 42 samples. The expression patterns of fusion partner genes were further examined both in silico and in vitro to determine their potential role in tumorigenesis.

Result and discussion

We detected 4268 FTs in HCC and 2762 FTs in paired NAT samples. Among these FTs that are expressed in either tumor or NAT tissues, 124 of them exist in at least five samples (recurrent FTs). Importantly, a total of 81 recurrent FTs were exclusively detected in tumor samples. Upon analyzing the FTs found at least five times in tumor samples but absent in paired NAT samples, we identified a total of 15 FTs; among the identified fusion partners, 72% were protein-coding genes, 17% were classified as noncoding RNAs, and 11% were pseudogenes. DEG analysis revealed that fusion partner genes, including liver-associated genes such as ALB, APOA2, IGF2, MT2A, SERPINA1 and H19, were frequently involved in fusion events and significantly downregulated in HCC tumors compared to NAT.

Detailed characterization of FTs shows that they have variants with distinct structural modifications that may impact their functional output, and some of them are significantly associated with more aggressive HCC phenotypes. These findings were confirmed with an in silico cohort. Wet lab validation experiments with patient samples verified reduced levels of fusion partners.

Conclusion

This study provides a comprehensive analysis of the FT landscape in HCC, identifying novel tumor-specific fusions and characterizing their expression patterns. The downregulation of key fusion partner genes in aggressive HCC suggests their relevance as potential biomarkers. These findings highlight FTs as promising diagnostic and prognostic biomarkers for HCC, which may ultimately contribute to improved patient management and therapeutic strategies.

This study is supported by the TUBITAK project, 120C216.

EACR25-2256

TNMv2: online tool to perform stage specific gene expression analysis

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Introduction

We present an updated version of TNMplot, a web-based platform integrating RNA-Seq and gene-chip data from 56,938 samples. TNMplot enables users to select a gene and tumor type for comprehensive gene expression analysis, supporting over 20 tumor types with publication-ready visualizations.

Material and method

RNA sequencing data were sourced from the Genomic Data Commons (GDC), including adult tumor samples from TCGA, pediatric samples from the TARGET project, and normal reference data from the GTEx portal. Gene chip-based data, comprising over 33,000 manually curated samples, were obtained from the NCBI GEO database. TNMplot facilitates differential expression analysis across normal, primary tumor, and metastatic tissues, supported by an expanded clinical dataset for stage-specific tumor analysis.

Result and discussion

The updated platform offers advanced tools, such as a pan-cancer dot matrix for simultaneous visualization of multiple tissue types and genes. It also includes correlation analysis between pairs of genes or multiple genes, along with correlation profile computation. The platform provides a unique feature for identifying progression-related genes through the recently added stage-based expression comparison. This analysis includes data from over 4,500 patient samples, encompassing breast ($n = 2,331$), colorectal ($n = 648$), skin ($n = 82$), prostate ($n = 61$), and lung ($n = 1,399$) cancer cases. The tool also has the ability to analyze gene expression using both RNA-seq and gene chip approaches, enabling internal validation across diverse patient populations. In addition to stage-specific expression estimation, this dual-platform capability is unique among comparable tools.

Conclusion

In conclusion, the updated TNMplot platform provides a comprehensive starting point for transcriptomic analysis for oncology-related basic, pharmacological, and translational research.

EACR25-2265

Unraveling Osteosarcoma Heterogeneity: molecular subtyping of early-stage osteosarcoma

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Introduction

Osteosarcoma (OS) represents a highly heterogeneous bone malignancy. Identifying molecular subtypes can improve the understanding of tumor biology and contribute to future targeted therapies.

Material and method

Non-negative matrix factorization (NMF) was used to classify 102 OS samples, derived from patients who did not have metastases and did not receive chemotherapy, into three subtypes (S1, S2, S3) based on gene expression profiles. Identification of key biological processes and hub genes associated with each subtype was done using functional enrichment analysis and weighted gene coexpression network analysis (WGCNA). Tumor micro-

environment (TME) analysis was conducted using ESTIMATE and CIBERSORT algorithms.

Result and discussion

S1 exhibited enrichment in cell cycle regulation and RNA metabolism processes, S2 in extracellular matrix organization and angiogenesis, and S3 in translation, ribosome biogenesis, and immune evasion. WGCNA identified subtype-specific gene modules. Processes enriched in the S1 group of OS are mostly connected to cellular transport, Golgi apparatus organelle functions, and cytokinesis, while the S2 correlated modules have shown enrichment mostly in extracellular matrix organization, adhesion, platelet-derived growth factor and integrin-mediated signaling. S3 subtype has shown considerable enrichment in Golgi apparatus processes, response to endoplasmic reticulum stress and bone forming processes such as ossification, bone development, etc. Analysis of the ESTIMATE score revealed differences between groups, with the lowest score exhibited by the S1 group, indicating poor prognosis for this subtype. CIBERSORT algorithm results showed different fractions of immune cells in each subtype, and correlation analyses highlighted distinct immune cell interactions in each subtype. S1 had more T follicular helper cells, eosinophils, and CD8 T cells, with the lowest M2 macrophage fraction. S2 had more resting mast cells, memory B cells, resting NK cells, and activated dendritic cells but fewer T gamma delta cells. Correlation analysis showed co-infiltration of naive T cells, T gamma delta cells, and resting dendritic cells in S1, while CD8 T cells correlated with neutrophils. In S2, activated NK and mast cells were linked, whereas S3 showed co-infiltration of M0 macrophages and naive CD4 T cells.

Conclusion

This study identifies three distinct OS subtypes with unique molecular signatures and immune profiles, providing potential biomarkers and therapeutic targets for personalized treatment strategies.

Acknowledgments: Supported by the Ministry of Science, Technological Development and Innovation of the Republic of Serbia (grant no. 451-03-136/2025-03/200042), and the Science Fund of the Republic of Serbia (grant no. 7503)

EACR25-2278

Assessing the maturation stages of PMN-MDSC to dissect the immunosuppressive mechanisms within TME

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Introduction

The tumor microenvironment (TME) presents significant obstacles to the success of cancer therapies, and understanding its complexities is crucial for developing more targeted therapies. One of the key immuno-suppressive cellular subsets in the TME consists of myeloid-derived suppressor cells (MDSCs), especially the polymorpho-nuclear (PMN) type. PMN-MDSCs are immature neutrophils that primarily reside in the bone marrow (BM). In pathological conditions like cancer, infection, or

inflammation, these cells migrate to the peripheral blood (PB) and inflamed tissues. PMN-MDSCs suppress the immune system by blocking the activity of important immune cells, like T cells and NK cells, thereby promoting tumor growth and enabling immune evasion. High levels of MDSCs in tumors are associated with worse prognosis and resistance to cancer immunotherapy.

Material and method

PMN-MDSCs were isolated from peripheral blood and bone marrow of healthy donors mobilized with the stimulating factor G-CSF, as well as from peripheral blood of lung tumor patients. After isolating peripheral blood mononuclear cells, PMN-MDSCs were separated using immunomagnetic beads and prepared for single-cell RNA sequencing (scRNA-seq). Data analysis was performed using custom scripts based on Scanpy and Seurat tools.

Result and discussion

This study aims to understand the differentiation and immunosuppressive function of PMN-MDSCs. Based on their origin (i.e., healthy donor bone marrow, healthy donor peripheral blood, or peripheral blood from tumor patients), we identified different subpopulations of MDSCs, each with distinct gene expression patterns and immunosuppressive characteristics. By combining RNA-velocity and pseudotime analyses on integrated scRNA-seq data, we observed that PMN-MDSCs in the bone marrow show the highest level of cellular plasticity, proliferation, and stemness-related properties. In contrast, PMN-MDSCs from the peripheral blood of lung cancer patients appear to be more differentiated and acquire more mature immunosuppressive traits.

Conclusion

In this study, we identified distinct subsets of PMN-MDSCs that express specific genes related to immunosuppression. By comparing PMN-MDSCs from healthy donor bone marrow, healthy donor peripheral blood, and peripheral blood from lung tumor patients, we were able to reconstruct the differentiation trajectory of these cells in relation to their immunosuppressive functions.

EACR25-2312 Modelling Somatic Alterations with Protein Language Models

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Introduction

Cancer is a genetic disease where somatic alterations accumulate in a cell and result in uncontrolled proliferation and invasiveness. Some genes are much more likely to be altered and are used in the clinic to guide prognosis and treatment. Yet, there is a large variability in the way in which patients respond to treatment even when carrying alterations in well-known genes. The cause of this variability is unknown, part of it comes from the complexity that arises in somatic alterations. Some genes like KRAS are mutated in several cancers, yet some prognostic differences have been reported depending on the protein position and the substitution amino acid. Furthermore, sometimes the

mutant gene is also amplified, making it hard for mathematical models to capture these intricacies. Some artificial intelligence models known as protein language models have been proposed to model germline alterations. By training on millions of protein sequences, they are able to determine accurately if a variant is likely to be pathogenic. Here, we propose using pre-trained protein language models to generate embeddings for somatic alterations that can be used to build machine learning models for cancer.

Material and method

In order to repurpose protein language models, we built a custom somatic embedding layer that combines fusions, mutations and copy number alterations to combine somatic alterations. We applied our somatic embedding layer in a transformer architecture and fine-tuned the embeddings of the protein language models using publicly available datasets of drug response in cell-lines and progression free survival in pancreatic, colon and breast cancer.

Result and discussion

We show that our architecture can be used to predict drug response and survival in publicly available datasets more accurately than other models. Our architecture is able to not only capture subtle differences between similar substitutions, like in the case of KRAS mutants, but also to combine the effect of multiple alterations on the same cell or patient. Furthermore, using explainable AI techniques, we were able to dissect why our model's predictions.

Conclusion

Our results show the potential applications of artificial intelligence to model somatic alterations in cancer. While in this work we focus on two problems, drug response in cell-lines and progression free survival, our architecture has the potential to be applied to other settings, including patient stratification and optimal treatment prediction.

EACR25-2347

Leveraging SHAP Values from Machine Learning and Mutational Signatures in Supervised Clustering to Enhance Disease-Specific Survival Prognosis in Endometrial Carcinoma

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Introduction

Endometrial carcinoma is a leading cause of gynecologic cancer deaths, with prognosis varying significantly. Current risk stratification tools are limited, prompting the need for more precise methods. This project applies machine learning and SHAP values to improve the interpretability of mutational signatures, identifying clinically relevant subgroups. By integrating biological insights with machine learning, it aims to enhance patient stratification and guide personalized therapeutic strategies, improving survival outcomes.

Material and method

Somatic mutation data from 448 endometrial carcinoma (UCEC) samples in TCGA were analyzed using the COSMIC mutational signature framework via the SigProfilerAssignmentR package. Four machine learning models – Random Forest, Support Vector Machines, Logistic Regression, and LightGBM – were trained on clinical variables and mutational signature exposures. LightGBM achieved the best performance based on accuracy and AUC metrics. SHAP values quantified each mutational signature's contribution to survival predictions, offering interpretability. These values were clustered using DBSCAN, with parameters optimized for clinical and biological relevance. Stability analysis identified robust cluster configurations. Clinical and demographic characteristics of clusters were compared using ANOVA or chi-square tests, while survival differences were assessed via Kaplan-Meier analysis and log-rank tests.

Result and discussion

DBSCAN clustering of SHAP values identified four distinct groups (C1–C4). Cluster C1 exhibited enrichment in Signature SBS6, associated with mismatch repair deficiency. Clinically, C1 demonstrated better DSS outcomes compared to other clusters (log-rank $p < 0.01$). Additionally, patients in C1 were characterized by lower age at diagnosis but higher BMI, suggesting unique biological pathways influencing prognosis. Clustering based on SHAP values demonstrated enhanced resolution and interpretability compared to clustering performed directly on original mutational signature exposures. SHAP-based clustering captured clinically meaningful subgroups that aligned with known biological mechanisms, such as the prognostic impact of SBS6 enrichment. The integration of SHAP values into clustering workflows provides a novel approach to stratify endometrial carcinoma patients by leveraging both machine learning interpretability and biological relevance.

Conclusion

This study underscores the utility of explainable machine learning techniques in oncology research. By combining mutational signature analysis with SHAP-based clustering, we identified biologically and clinically relevant subgroups that improve survival prognosis in endometrial carcinoma. Future work should explore integrating additional omics data to further enhance predictive accuracy and clinical applicability.

EACR25-2357

Tumor Growth Dynamics and Telomere Maintenance in Sarcoma: Insights from 3D Spheroid Models

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Introduction

Sarcomas are a diverse group of malignant tumors originating from mesenchymal tissues, characterized by rapid growth, metastatic potential, and significant heterogeneity in clinical behavior and molecular profiles. Despite advancements in treatment, mortality remains high, particularly in metastatic cases, underscoring the importance of early detection. To understand tumor progression, we focused on the avascular phase, where limited nutrient diffusion leads to gradients, hypoxia, and necrosis—better replicated by 3D spheroid models than traditional 2D cultures, enabling detailed cancer stem cell (CSC) study. CSCs play a key role in tumor initiation, maintenance, and recurrence, sustaining immortality through telomere maintenance mechanisms, including telomerase-dependent mechanisms or alternative lengthening of telomeres (ALT). This study aims to deepen understanding of tumor biology and CSCs, with potential applications in developing more precise therapies.

Material and method

This study utilized patient-derived sarcoma spheroids to examine tumor growth dynamics, telomere maintenance, and length. The cells were cultured under restrictive conditions, with growth monitored over time using confocal microscopy to obtain high-resolution Z-sections. The resulting images were processed in ImageJ, binarized, and 3D volume reconstruction was performed using ReViMS software. Relative telomere length was assessed through qPCR by comparing amplified telomeric sequences to a reference single-copy gene. Telomerase presence was evaluated using the qRT-PCR method.

Result and discussion

This study employed a method utilizing precise 3D reconstruction to enhance volumetric assessments by accurately capturing the irregular morphology of spheroids, accounting for subtle shape variations caused by uneven proliferation, cell death, and mechanical constraints. Unlike previous studies that relied on diameter-based approximations assuming a spherical shape, this method offers a more accurate representation of spheroid volume. The spheroids varied in size from tens to hundreds of μm , displaying characteristic irregular shapes. The inflection point in spheroid growth was successfully identified, with growth subsequently following a sigmoidal (S-shaped) curve, consistent with patterns observed in existing literature. Additionally, telomere length and telomerase activity were assessed.

Conclusion

We reconstructed spheroid volumes from 2D Z-slice images, providing key data on tumor growth and aggressiveness. Integrating these volume measurements into predictive models could offer insights into therapy resistance and tumor progression, particularly concerning CSCs. Monitoring telomere dynamics and immortality mechanisms enhances our understanding of cancer biology, highlighting the importance of 3D spheroids in developing personalized therapeutic strategies to improve patient outcomes.

EACR25-2412

YOLO-based approach for breast cancer tumour detection and segmentation in MRI scans

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Introduction

YOLOv11 is one of the latest versions in the YOLO (You Only Look Once) model series, designed for fast and accurate real-time object detection. It introduces architectural improvements, such as advanced feature extraction and optimised training processes, resulting in higher precision with fewer parameters. YOLOv11 is particularly useful in medical image analysis, supporting tasks like instance image classification. The application of this model for automatic detection and subsequent segmentation of breast cancer tumours in MRI scans has the potential to support early diagnosis and enhance treatment effectiveness.

Material and method

For training, validation, and testing, 75 MRI images from patients with diagnosed breast cancer from the National Oncology Institute of Maria Skłodowska-Curie (Gliwice branch) were used. Tumour regions in the scans were annotated and verified by two specialists from oncology and breast radiology. The images were converted from the DICOM format to PNG for network training. The regions of interest were labelled using the LabelImg tool. Data augmentation techniques, such as image rotation, were applied while simultaneously recalculating the coordinates in the annotation files. The dataset was then divided using a stratified split with a 70/15/15 ratio for training, validation, and testing, ensuring better model generalisation. Object detection model performance was evaluated using the F1 curve, precision-recall metrics, and accuracy. After detection, segmentation was conducted using the Fuzzy C-Means Clustering method on the tumour regions identified by the network. Additionally, a DICOM RTSTRUCT file was generated, containing the segmented region of interest with the FCM mask applied, enabling direct visualization of the detected area within the original examination.

Result and discussion

The results confirmed the effectiveness of YOLOv11 in identifying tumour regions in breast MRI scans, achieving high detection precision. Moreover, applying FCM-based segmentation to the detected areas allowed for precise tumour region delineation, detailed analysis of tumour structure and determination of subtypes. Generating DICOM RTSTRUCT files facilitated seamless integration with medical systems, enhancing clinical usability.

Conclusion

In conclusion, combining YOLOv11 with segmentation techniques and appropriate data processing represents a valuable step toward supporting breast cancer diagnosis based on MRI scans, enabling precise tumour detection and analysis.

Funding: Excellence Initiative – Research University Program, project number: 32/014/SDU/10-22-71(AS).

EACR25-2422

Characterizing Aggressiveness of Prostate Cancer with Large-scale DNA Organization Image Analysis

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Introduction

Prostate cancer is a pathologically heterogeneous disease, in that patients may present with cancers that require timely treatment or may have indolent disease. In the absence of a reliable prediction method, risk assessment for disease progression and metastasis has been challenging. Typically, all grading and risk assessment is based on pathologist observation of prostate tissue architecture, PSA, and tumor stage; less emphasis has been placed on individual cell morphological characteristics. Here, we demonstrate that large-scale DNA/chromatin organization (LDO) in prostate cancer cells of patients with more advanced cancers is altered and can be used to identify patients of Gleason scores (GS) of 6 and 9, independent of other clinical variables.

Material and method

Using a multiple instance learning approach, binary labels were assigned to each nucleus in a patient core needle biopsy by a random forest classifier, and the proportion of nuclei predicted to be associated with advanced prostate cancer was used to determine each patient's GS (GS 6 or GS 9). Our patient cohort consists of 60 patients, (38 with GS6, 22 with GS9) of which 25% of patients were assigned to the holdout set. The performance of the training patient classification was computed using a 5-fold cross validation. For each train/test split in the cross-validation, the classifier was trained on the training set and then used to generate scores for the patients in the test fold.

Result and discussion

Patients in the training set were classified with a balanced accuracy of 0.851, and an F1-score of 0.815. This performance also translated to the patients in the holdout set, where the balanced accuracy and F1-scores of patient classification was 0.857 and 0.833 respectively. In multiple instance approaches where labels are assigned to each instance, there exists the risk of not being able to learn meaningful discriminations, especially if the number of nuclei critical in the patient classification is scarce. However, the consistently high balanced accuracy and F1-scores in the training patients and holdout set suggest that the classifier was able to capture the patterns of LDO necessary for patient classification.

Conclusion

We demonstrate that leveraging large-scale DNA/chromatin organization (LDO) provides a robust method for discriminating GS6 and GS9 patients independent of other clinical variables. The high balanced accuracy and F1-scores across both the training and holdout sets suggest that the critical patterns in LDO are effectively captured by the nuclear classifiers. Future studies will apply the trained classifiers to patients with GS7 and GS8 to visualize how nuclear chromatin organization may change over the course of disease progression.

EACR25-2465

The effect of common germline variants on tumor mutation burden and mutational signatures activity

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Introduction

A major component in tumorigenesis is the accumulation of somatic mutations, driven by both stochastic and systematic mutational processes, which leave distinct mutational signatures in the genome. While external and random factors contribute to these processes, inherited germline variants may also modulate their activity. However, the extent to which common germline single nucleotide polymorphisms (SNPs) influence tumor mutational burden (TMB) and mutational signatures remains largely unexplored, mainly due to the lack of a large enough dataset sufficient to detect such a weak association. Here we suggest a novel method we call GroupSig that can aggregate panel sequencing data and overcome the small amount of mutations observed in each single sample. We used that to investigate the association between genotypes in common germline variants and TMB and mutational signature activity across multiple cancer types using large-scale genomic datasets.

Material and method

We analyzed data from two cohorts: a dataset of 35,000 tumor samples from the Dana-Farber Cancer Institute (DFCI) panel sequencing cohort and a dataset of 10,000 tumor samples from The Cancer Genome Atlas (TCGA) whole-exome sequencing (WES) cohort. To address the challenge of low somatic mutation counts in panel sequencing, we developed GroupSig, a novel approach that aggregates mutations across patient groups stratified by SNP genotype, creating "super patients" to enable the use of mutational signature fitting tools. We first conducted a genome-wide association study (GWAS) in the DFCI dataset, analyzing the effect of genotype on TMB, while accounting for group sizes. We filtered SNPs based on a genome-wide significance threshold ($p < 5 \times 10^{-8}$). We then tested if the significant SNPs were correlated with the activity of specific signatures. We then repeated the analysis on the smaller TCGA dataset (~10,000) as a validation set without GroupSig, as there is a sufficient number of mutations per sample.

Result and discussion

Application of GroupSig to the DFCI cohort enabled the fitting of grouped somatic mutations data to the profiles of known single substitution signatures. We found 79

genomic loci that correlated with high TMB in the DFCI dataset and that were validated also in the TCGA dataset. Of those, 3 loci were correlated with the activity of specific signatures.

Conclusion

This study provides evidence that common germline SNPs influence mutational processes in cancer, advancing the research on hereditary cancer risk. The GroupSig approach enables large-scale assessment of these associations, even in panel sequencing datasets with low mutation counts per sample. Our findings enhance the understanding of genetic contributions to tumorigenesis and may inform cancer risk assessment and personalized therapeutic strategies.

EACR25-2486

RabAnalyser: a computational framework to measure the cell-to-cell variability of Rab GTPases organization

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Introduction

Cancer cell evolution depends on cell-to-cell heterogeneity arising from genetic and non-genetic variations, which facilitate adaptation to dynamic environments. While genomic alterations contribute to this variability, additional factors such as spatial architecture and metabolic diversity further shape cellular behaviour. Rab proteins, the largest subfamily of small GTPases, orchestrate intracellular trafficking by localizing to membrane-bound organelles and cycling between active and inactive states. In their active form, Rab proteins cluster on membranes, regulating organelle positioning, tethering, and fusion. The continuous remodelling of these organelles during cellular processes generates variability in their spatial organization, reflecting heterogeneity in Rab cluster patterns among cells. However, computational strategies to measure the heterogeneity of Rab cluster patterns are lacking.

Material and method

Here, we developed RabAnalyser, an image-based computational framework designed to investigate heterogeneity of Rab cluster patterns at the single-cell level. It extracts quantitative features from fluorescence microscopy images, capturing key attributes of Rab cluster organization. A minimal yet robust set of 11 features was identified, integrating conventional intensity and size assessments with novel geometric and spatial positioning metrics. By employing the Kolmogorov-Smirnov statistic, RabAnalyser detects distinct cell subpopulations based on Rab cluster patterns and generates as outputs: UMAP-based clustering, statistical analysis, and machine learning-based classification.

Result and discussion

We applied RabAnalyser to analyse glioblastoma cells treated with an anti-mitotic agent known to alter Rab11 distribution. Imaging of 1000 cells revealed two primary subpopulations distinguished by the elongation of Rab11 clusters, with nearly equal cells. Upon treatment, an additional subpopulation emerged, increasing overall heterogeneity and redistributing cells into three groups

characterized by differences in Rab11 cluster elongation and spatial density. These findings indicate that antimitotic treatment induces heterogeneity within isogenic cells, underscoring the role of Rab cluster organization in cellular response to perturbation.

Conclusion

This study demonstrates that RabAnalyser provides a robust methodology for quantifying cell-to-cell heterogeneity in fluorescence microscopy images with spotty signals. Moreover, the emergence of distinct subpopulations in response to treatment suggests that analysing Rab cluster organization offer insights into organelle arrangement and their implications in cancer. The elongation of Rab11 clusters may reflect underlying tubulation processes, highlighting a potential link between organelle architecture and cancer progression.

EACR25-2501

Site-specific validation of the GRANT model for renal cell carcinoma (RCC) cancer-specific survival (CSS) after surgery

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Introduction

This study evaluates the GRANTs model's performance, a prognostic score for RCC from 2017, using three implementations on two different cohorts: a rule-based approach as in the original paper and two Cox Proportional Hazards (PH) models. The assessment covers two patient cohorts

Material and method

Inclusion: RCC patients post-partial or radical nephrectomy. Exclusion: metastasis at diagnosis, metachronous cancer, Von Hippel–Lindau disease, bilateral synchronous carcinoma, neoadjuvant therapy, total cold ischemia. Follow-up data were censored at five years; patients lacking cause of death information or dying from non-kidney cancer causes were excluded. These criteria were applied to HSR (IRCCS Ospedale San Raffaele) (2536 patients) and Firenze (Azienda Ospedaliero-Universitaria Careggi) (670 patients) datasets. The rule-based GRANT model was used to determine risk groups and it was externally validated using Kaplan–Meier survival curves and log-rank tests on both cohorts. Two Cox PH models using GRANT variables underwent 100 Monte Carlo Cross Validation (MCCV) simulations for site-specific validation. These models differed by GRANT variables being binarized or continuous (e.g., age > 60 vs. continuous). We compared them using survival metrics (e.g., C-index) across cohorts. Risk stratification enabled comparison with the rule-based GRANT on the Firenze dataset.

Result and discussion

The rule-based GRANT model provided statistically significant stratification in both datasets. The Cox PH model with continuous variables showed higher C-index values than the binarized variant in both internal (HSR) and external (Firenze) validations. Among the three

models post-stratification on the Firenze dataset, the Cox PH model with continuous variables exhibited superior performance.

Conclusion

Comparison of GRANT model versions demonstrates the effectiveness of site-specific validation, with site-specific Cox PH models outperforming the rule-based version, particularly when validated on a similar demographic. The use of continuous over binarized variables enhanced prediction accuracy. While the rule-based model historically allows ready application, modern web applications enable rapid deployment of machine learning models in real-world settings

EACR25-2527

Radiomics-based classification of tumour subtypes in breast cancer using MRI imaging

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Introduction

MRI and mammography are the primary imaging examinations for the diagnostic detection of breast cancer. Those modalities also can be used not only by specialists but also by computer-aided systems that allow analysis to be carried out automatically, quickly and objectively and, above all, reproducibly and accurately. Radiomics features, that are calculated based on diagnostic images, can provide additional information that is not visible even to experienced specialists. They can also form the foundation for building diagnostic support systems based on classifiers.

Material and method

For training, validation, and testing, 75 MRI images from patients with diagnosed breast cancer from the National Oncology Institute of Maria Skłodowska-Curie (Gliwice branch) were used, who were scanned using a standard contrast agent. Tumour delineation, defining region of interest for the subsequent analysis, was performed using two step approach with detection phase based on deep learning YOLO (v11) network and final detailed segmentation in the detected area by local classification algorithm based on fuzzy c-means algorithm. Tumour regions in the scans in the training and validation of YOLO network step were annotated and verified by two specialists from oncology and breast radiology. T1-related subtractive MRI images were used to determine the ROI. T1 and T2 images were used furthermore, together with ROI definition interpolated to the corresponding space as an input to pyRadiomics (v3.1.0) Python module to compute a set of 100 radiomics features for each image set.

Result and discussion

The dataset was then divided using a stratified split with a 70/15/15 ratio for training, validation, and testing for the classification of two classes that relate to two breast cancer subtypes: Class first was subtype 1, which corresponds to the solitary tumour, and class 2 corresponds to subtypes 2, 3 and 4 (grouped, separated and replaced tumours). Other subtypes were discarded

from the analysis. Classical algorithms for classification were tested, including logistic regression, support vector machines, and random forests. Results of the model comparison are presented by the model's accuracy, precision, sensitivity and specificity.

Conclusion

In conclusion, combining YOLOv11 with segmentation techniques and appropriate data processing allowed us to calculate radiomics features and build and train classification models to stratify different cancer subtypes in breast cancer using MRI T1, T2 and subtraction images.

EACR25-2597

POSTER IN THE SPOTLIGHT

IRE1 signaling in Triple Negative Breast Cancer

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Introduction

IRE1 is a protein that senses the presence of endoplasmic reticulum stress. It is constitutively active in several cancers, including triple-negative breast cancer (TNBC). Its RNase activity allows it to cleave CUGCAG sequences found inside stem loops. Among its well-known targets is XBP1 mRNA, which has two such sequences and whose cleavage leads to the formation of an active transcription factor called XBP1s. As XBP1s has the ability to facilitate the progression of TNBC, IRE1 presents itself and its downstream targets as promising candidates for therapeutic interventions. Additionally, IRE1 can cause the degradation of other RNA molecules (including mRNAs, miRNAs, lncRNAs) in a process known as Regulated IRE1-dependent decay (RIDD).

Material and method

Omics datasets encompassing mRNA, miRNA, proteins, and metabolic products were generated from the TNBC cell line MDA-MB-231 following treatment with MKC866, an IRE1 inhibitor, at multiple time points. These datasets were analyzed using MultiOmics-Integrator, a Nextflow pipeline previously developed and published by the authors of this abstract [1]. The analysis included detailed isoform switching, RNA-seq, proteomics, and lipidomics investigations. Additionally, to further focus on IRE1 signaling, we predicted novel XBP1 and RIDD candidates, incorporating recent advancements in the field.

Result and discussion

The results provide valuable insights into IRE1 signaling in the context of TNBC pathology. By integrating data across multiple omics layers, several novel candidates downstream of the XBP1 and RIDD axis were identified. These candidates play key roles in metabolism, migration, and survival of TNBC cells. Additionally, we examined their behavior in single-cell datasets to better understand their impact on tumor microenvironment formation. Experimental validation of these candidates is currently underway. The robustness of the signature was evaluated using BRCA cell lines, and its prognostic

potential was assessed through survival analysis with TCGA samples.

Conclusion

We focused on identifying novel downstream targets of IRE1/XBP1 and IRE1/RIDD signaling pathways. We believe that our work will aid in deciphering the role of IRE1 in TNBC progression and characterization.

[1] available at github.com/ASAGlab/MOI-An-integrated-solution-for-omics-analyses

Biomarkers in Tissue and Blood

EACR25-0048

Detection of Circulating Tumour DNA (ctDNA) with ddPCR and NGS in Liposarcoma

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Introduction

Cell-free DNA (cfDNA) is naturally released during cell death processes like apoptosis or necrosis. In the tumour context, it is known as circulating tumour DNA (ctDNA). We harness the power of ddPCR to determine the copy number variation (CNV) of mouse double minute 2 (MDM2) and cyclin-dependent kinase 4 (CDK4) gene amplifications, which is one of the molecular characteristics of both well-differentiated liposarcomas (WDLPS) and dedifferentiated liposarcomas (DDLPS).

Material and method

Demographic data for all included patients were included in a prospective cross-sectional analysis. A total of 32 adult patients with primary and recurrent retroperitoneal sarcomas were assessed. Blood samples were collected prospectively during treatment. cfDNA was isolated from plasma and quantified. All patients who had tumour in situ during blood sampling and were histologically positive for MDM2 amplification via immunohistochemistry (IHC) were selected for inclusion in this study. A total of 15 plasma-derived DNA samples (Eleven non-paired samples plus four paired samples – pre- and post-surgery) were included between June 2023 - June 2024. ctDNA detection and copy number determination were performed using Bio-Rad QX200 Droplet Digital PCR (ddPCR) (Bio-Rad Laboratories) and Oxford Nanopore Technologies (ONT).

Result and discussion

MDM2 was detectable in both tissue tumour samples and plasma-derived samples (ctDNA). The average concentration was 2.5 ng/μl and 5 ng/μl, respectively. A duplex MDM2:RPP30 ddPCR assay revealed one sample had an amplified copy number of 18.9, which was confirmed by ONT. This positive sample occurred in a

patient who had unresectable disease, therefore, open and close laparotomy (stage AJCC IIIB). The remainder negative copy number samples were staged AJCC IB. For the longitudinal paired samples, there was no difference in CNV, however the median follow-up was only 40-month. On the other hand, a repeated experiment with duplex CDK4:RPP30 assay yields a lower amplified copy number of 9.87 for the same sample (E01), but overall consistent findings compared to MDM2 assay.

Conclusion

Analysis of ctDNA is a novel approach for cancer screening, diagnosis, monitoring treatment response, and identifying mutations. Our analysis shows that ctDNA detection is possible in patients with both retroperitoneal WDLPS and DDLPS using liquid biopsy ddPCR and NGS approaches, however this appears to be restricted to patients with locally advanced disease. A larger sample size to improve sensitivity in ctDNA MDM2 and CDK4 detection is required for clinical utility.

EACR25-0110

The systemic inflammation response index (SIRI) may identify less toxic treatments for specific advanced pancreatic adenocarcinoma patients

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Introduction

The systemic inflammation response index (SIRI) has been recently proposed as a prognosis biomarker which could predict oncological outcome for advanced pancreatic ductal adenocarcinoma (PDAC). The aim of this study was to try to validate SIRI in an independent cohort through univariate and multivariate analysis considering confounding factors.

Material and method

A retrospective cohort of 132 advanced and metastatic PDAC patients was collected between 2010 and 2021. Kaplan-Meier, along with univariate and multivariate Cox regression, was used to test association between overall survival (OS) and SIRI values at diagnosis, as well as to assess chemotherapy (FOLFIRINOX, Gemcitabine plus Nab-paclitaxel and Gemcitabine alone) outcomes by SIRI levels (defined as neutrophil x monocyte/lymphocyte, with a threshold of $10^9/L$).

Result and discussion

Baseline SIRI presented a non-statistically significant tendency ($p = 0.066$) of prognostic OS value on univariate analysis, which did not prevail (HR (95% CI): 1.227 (0.851-1.769), $p = 0.273$) in the presence of risk factors such as ECOG2 (HR (95% CI): 2.632 (1.501-4.615), $p = 0.001$) and liver metastases (HR (95% CI): 1.451 (1.011-2.083), $p = 0.043$). According to individualized treatments, patients with low SIRI levels had similar outcomes when comparing chemotherapy

regimens whereas patients with high SIRI levels presented a non-statistically significant tendency ($p = 0.065$) of performance similar to the general population, where FOLFIRINOX and Gemcitabine plus Nab-paclitaxel had similar outcomes and both were better than Gemcitabine alone. Our findings indicate that SIRI lacks statistically significant prognostic value in PDAC. Nevertheless, consistent with the original report, we found that patients with low SIRI levels showed no significant differences in survival related to treatment regimens, suggesting that the Gemcitabine treatment, which is less toxic, could be preferred for this group. However, for high SIRI levels, contrary to prior research claiming FOLFIRINOX superiority, no statistically significant differences were found.

Conclusion

We were not able to confirm the prognostic value of SIRI in either the univariate or the multivariate analyses. Nevertheless, we believe that further studies could help confirm its role in guiding treatment, especially in identifying less toxic regimens for patients with low SIRI levels.

EACR25-0111

The Monocyte/Hemoglobin Ratio as a New Prognostic Biomarker in Patients with Advanced Pancreatic Adenocarcinoma

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Introduction

Pancreatic ductal adenocarcinoma (PDAC) is currently one of the deadliest cancers. It is generally diagnosed at very advanced stages, resulting in a low survival rate for patients. In this context, it is crucial to find new tools that can predict the survival of patients with advanced PDAC in the presence of known risk factors, such as diabetes and liver metastases.

Material and method

Retrospective information was collected from 126 patients diagnosed with advanced and metastatic PDAC who were treated with chemotherapy at University Hospital San Pedro (Spain). Optimal cut-off points were calculated for several macrophage-based ratios: neutrophil-lymphocyte ratio (NLR), lymphocyte-monocyte ratio (LMR), platelet-lymphocyte ratio (PLR), neutrophil-hemoglobin ratio (NHR), lymphocyte-hemoglobin ratio (LHR), monocyte-hemoglobin ratio (MHR) and platelet-hemoglobin ratio (PHR). Using Kaplan-Meier curves and the log-rank test, these ratios were related to overall survival (OS), evaluated along with risk variables by the Cox proportional hazards multivariate model and validated by logistic regressions.

Result and discussion

Patients with LMR ≥ 2.38 ($p = 0.034$) and MHR < 70.87 ($p < 0.001$) showed a higher probability of survival in univariate analysis but only MHR retained prognostic power (HR (95% CI): 2.852 (1.699–4.788), $p < 0.001$) in the presence of risk factors such as ECOG grade, diabetes, and liver metastases. MHR also remained significant ($p = 0.0140$) in logistic regression. Stratified by diabetes or by liver metastases, only MHR was prognostic in both groups ($p < 0.001$, $p = 0.002$). Current research shows controversial results regarding the prognostic power of traditional macrophage-based ratios, rarely evaluated with these risk factors, highlighting the need for novel biomarkers. Hemoglobin levels, used as indicators of treatment tolerance, have been studied in colorectal cancer (MPR), lung cancer (ferritin-to-hemoglobin), and others (hemoglobin/red cell ratios), but in PDAC few references are available. For instance, hemoglobin was used as part of the HALP index (hemoglobin, albumin, lymphocytes, and platelets), showing relevance in PDAC resected cases. However, to the best of our knowledge, the prognostic value of hemoglobin in advanced and metastatic PDAC populations has not been studied, despite its demonstrated role in other cancers.

Conclusion

The new MHR ratio has shown better prognostic power than other macrophage ratios in the presence of risk factors associated with PDAC. This ratio could be used as a prognostic biomarker at diagnosis in clinical practice to aid in medical treatment decisions.

EACR25-0157

miRNAs in Serum and Extracellular Vesicles as Potential Biomarkers of Metastatic Disease in Clear Cell Renal Cell Carcinoma

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Introduction

There is still a lack of blood-based biomarkers for renal cell carcinoma (RCC). miRNAs including exosomal miRNAs, play a pivotal role in the detection of several types of cancers. Our prior research demonstrated a decrease in the levels of miR-451a, miR-10b-5p, the let-7 family, and miR-30c in primary metastatic tumors. In this study, we aimed to evaluate the significance of these miRNAs in both serum and serum extracellular vesicles (EV) for detecting clear cell RCC (ccRCC) and their potential as biomarkers of metastatic disease.

Material and method

EVs were isolated from the serum of ccRCC patients, including 13 non-metastatic, 33 metastatic (20 metachronous (metachr), 10 synchronous (syn)) as well as 18 healthy individuals, using the Size Exclusion Chromatography (SEC) kit according to the manufacturer's recommendations. The miRNeasy kit was employed to isolate miRNA from both serum and its EVs. The quality and quantity of EV isolation were analyzed using western

blot for exosome markers and nanoparticle tracking analyser for size evaluation. The expression levels of miRNAs (Let7c, miR-451a, miR-30c-5p, miR-126-3p; references: miR-361-5p, miR-1228-3p) in serum and its EVs were investigated using TaqMan real-time PCR techniques.

Result and discussion

The expression levels of miR-451a, let-7c, miR-30c-5p, and miR-126-3p showed significant differences between tumor patients and healthy controls. Let-7c and miR-126-3p were significantly decreased ($p \leq 0.05$) in serum of metachr patients compared to those with non-metastatic ccRCC whereas no significant differences were found between syn and non-metastatic ones. All 4 miRNAs were significantly lower expressed in metachr compared to healthy controls. Analysing miRNAs from EVs, let-7c and miR-126-3p showed significant differences between tumor patients and healthy controls. There was a significant lower expression ($p \leq 0.05$) of miR-451a and let-7c in EVs from all metastatic and in metachr compared to non-metastatic patients, and all 4 miRNAs differed between syn and non-metastatic patients.

Conclusion

We confirmed that miRNA expression of metastatic tumor tissues is reflected in liquid biopsy. Our results suggest that certain miRNAs in serum and their EVs could serve as promising minimally invasive diagnostic and prognostic markers in ccRCC.

EACR25-0160

miR-7974: A predictor of poor prognosis and link to autophagy in ER+ breast cancer

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Introduction

Patients with estrogen receptor-positive (ER+) breast cancer (BC) generally have better survival rates than those with ER-negative early BC; however, individual prognoses can vary greatly. Therefore, identifying biomarkers to detect patients who may require more aggressive treatment is crucial. MicroRNAs (miRNAs), a class of small non-coding RNAs, regulate gene expression and have been linked to cancer progression, making them promising candidates for diagnosis and

prognosis. In this study, we investigate miR-7974, a less-explored miRNA, and its potential role in ER+ BC.

Material and method

We extracted total RNA from 204 breast tissue samples from the Kuopio Breast Cancer Study cohort using the mirVana™ miRNA Isolation Kit. We prepared small RNA sequencing libraries with the TruSeq® Small RNA Library Prep Kit and sequenced them on an Illumina MiSeq sequencer. To investigate the correlation between miR-7974 and clinical features, as well as patient outcomes in breast cancer (BC), we used bioinformatics tools like DESeq2 and multivariate survival analyses. To study the functional role of miR-7974 in MCF-7 at the molecular and cellular levels, we utilized in vitro methods, including Western blot, qPCR, and miRNA pull-down assays, alongside in ovo chick chorioallantoic membrane (CAM) assay. Additionally, the direct target of miR-7974 was validated using another BC cell line, MDA-MB-453.

Result and discussion

Our findings show that the upregulation of miR-7974 was significantly ($P = 8.93 \times 10^{-6}$) higher in invasive local BC cases ($n = 182$) compared to benign cases ($n = 22$). The multivariate survival analyses revealed that the high expression of miR-7974 was significantly ($P < 0.05$) associated with poorer relapse-free survival (RFS) and BC-specific survival (BCSS) compared to the low expression in all invasive local BC cases. Additionally, high expression of miR-7974 was linked to poorer RFS in ER+ patients, with a hazard ratio (HR) of 8.70 and a 95% confidence interval (CI) of 3.28–23.06 ($P = 1.37 \times 10^{-5}$). We also found that miR-7974 is involved in genes related to autophagy. Specifically, MAP1LC3B, a key gene involved in autophagy, was found to be a direct target of miR-7974 ($P < 0.05$) in MCF-7 cells. Overexpression of miR-7974 in these cells resulted in anti-proliferative effects, reducing cell growth in both in vitro and in ovo models.

Conclusion

Our results suggest that miR-7974 could be a valuable prognostic biomarker for poor outcomes in ER+ BC, potentially improving survival predictions. The dual role of autophagy helps explain the mechanism behind poor survival, alongside the tumor-suppressive effect observed in both in vitro and in ovo models with high miR-7974 expression. Further research is needed to fully understand miR-7974's role in autophagy regulation and its downstream effects.

EACR25-0245

Immunohistochemistry expression of HER2, Ki-67, and Sialyl-Tn as biomarkers of poor prognosis from patients with gastric adenocarcinoma in Southeastern Mexico

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Introduction

Gastric cancer (GC) is one of the most common cancer types worldwide. Adenocarcinoma is the most frequent type of tumor in GC. Southeast Mexico has the highest rate of comorbidities in all of Mexico. In clinical practice, diagnostic and prognostic biomarkers are scarce; hence, besides the great proportion of the undiagnosed population, some affected subjects do not receive the correct treatment. The Human Epithelial Growth Factor Receptor 2 (HER2) is related to the invasion and progression of tumors, and its expression in GC is around 7–34%. Ki-67 is associated with cell proliferation and poor prognosis in patients with GC; Ki-67 is considered an excellent predictor of GC recurrence. Finally, the expression around 77% of Sialyl-Tn (sTn) is also considered a marker of low survival and high mortality in GC. Thus, the present study aims to determine the expression of HER2, Ki-67, and sTn expression as biomarkers of poor prognosis in patients with gastric adenocarcinoma who attended a reference hospital in Southeast Mexico.

Material and method

221 patients with gastric adenocarcinoma were identified, but only 30 were selected after applying inclusion criteria. All selected patients had endoscopic or gastrectomy biopsies, showing 16 intestinal and 14 diffuse adenocarcinoma subtypes. Four slides with tumor tissue were obtained from each patient. An immunohistochemistry assay was performed to assess the expression of HER2, Ki-67, and sTn, and the staining of Hematoxylin & Eosin. One specialized pathologist determined the identification and the degree of differentiation.

Result and discussion

In general, overexpression of the HER2 receptor in patients with gastric adenocarcinoma was 56.7%. Likewise, HER2 showed overexpression in the intestinal subtype (62.5%), even higher than diffuse adenocarcinoma. Ki-67 expression was predominantly expressed in the diffuse subtype (62.5%). Interestingly, all diffuse adenocarcinomas with Ki-67 expression showed poor differentiation in the tissue, most of them with advanced tumor stage and high phenotypic heterogeneity. Otherwise, sTn showed an expression of 73.3% in patients with gastric adenocarcinoma, and 72.7% of them had died. We observed that patients with sTn positive in diffuse adenocarcinoma increase 1.2-fold the risk of dying. Co-expression of three biomarkers increases 2.2-fold the risk of mortality. The expression of biomarkers was independent of the tumor stage.

Conclusion

The presence of three biomarkers in gastric adenocarcinoma indicated a poor prognosis. HER2 was overexpressed in intestinal adenocarcinoma. Ki-67 and sTn were predominantly expressed in diffuse adenocarcinoma poorly differentiated. Therefore, our findings suggest that the presence of sTn positive might be

considered a mortality biomarker for patients with gastric adenocarcinoma in Southeast Mexico.

EACR25-0249

Genome-scale cell-free DNA methylation analysis for early detection and monitoring of lung cancer

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Introduction

Lung cancer accounts for 1.8 million deaths globally every year. Due to lung cancer's non-specific symptoms, which overlap with other respiratory illnesses (e.g., tuberculosis, asthma, pulmonary emphysema, and fibrosis), most patients present at the hospital with an advanced stage of malignancy. This is responsible for poor patient survival and drives inequities in outcomes. We and others have shown that alterations in DNA methylation are not only tissue- and cancer-type specific, but, because of their abundance, can enable earlier tumour detection with high sensitivity and specificity. Although mutational studies involving cell-free DNA (cfDNA) have experienced considerable progress in their technical and clinical applications, some challenges persist. Alternatively, cfDNA methylation analysis is emerging as a promising biomarker for early lung cancer detection and management, especially when tissue biopsies and low-dose computed tomography (LDCT) scans are unsuitable or inconclusive. We aim to identify blood-based cfDNA methylation markers that can improve early detection and prognosis of lung cancer.

Material and method

In this study, we analysed the cfDNA methylomes of lung cancer patients to determine the tissue of origin and applied this information to lung cancer diagnosis and prognosis. Using cell-free reduced representation bisulfite sequencing (cfRRBS), we have prepared genome-scale methylation libraries from 6–10 ng of plasma-derived cfDNA obtained from lung cancer patients at either early or late-stage, and non-malignant controls (patients with respiratory illness, but not cancer).

Result and discussion

The resulting sequencing data was passed through our in-house bioinformatics pipeline, yielding an average of three million high-quality CpGs per sample. We used deep-learning-powered models for tissue deconvolution and correlated these findings with the clinical presentation of the patients. Differential methylation analysis revealed a subset of regions that were highly discriminatory between 1) lung cancer and non-malignant controls and 2) early and late-stage lung cancer. These promising results suggest that a highly sensitive, tumour-specific methylation signature could enable the earlier detection of lung cancer compared to the current standard of care.

Conclusion

We have successfully demonstrated that blood-based cfDNA methylation biomarkers can accurately detect the presence of lung cancer, even at an early stage. Future work will focus on extending these findings and developing a clinically relevant cfDNA-based methylation test that can be used to complement current screening and treatment monitoring strategies.

EACR25-0258

Unravelling predictors of response to dual HER2 blockade in HER2+ metastatic breast cancer using single-cell peripheral immune profiling

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Introduction

Human epidermal growth factor receptor 2-positive (HER2+) metastatic breast cancer (mBC) is an aggressive BC form treated in first line with dual HER2 blockade (trastuzumab and pertuzumab antibodies). This regimen was shown to recruit and activate immune cells in the tumor microenvironment (TME), enhancing tumor clearance. However, not all patients benefit from this therapy, and resistance develops often, highlighting the need to identify immune biomarkers of response. Since tumor-associated immune cells can migrate from circulation to the TME, blood may serve as an accessible source of biomarkers to predict response to anti-HER2 therapy. Characterizing the immune landscape of responders and non-responders could guide personalized treatments and uncover new immunotherapy targets for improved patient outcomes.

Material and method

Peripheral blood mononuclear cells (PBMC) from 8 HER2+ mBC female patients undergoing standard-of-care treatment with trastuzumab, pertuzumab and docetaxel at the Portuguese Institute of Oncology (IPOLFG) were analysed via scRNA-seq. A direct comparison between responder and non-responder patients was performed to identify putative response biomarkers at the levels of immune population frequency, functional gene expression signatures and pathways, and cell-cell communication networks.

Result and discussion

We used a unique biobank of PBMC samples from HER2+ mBC patients, collected before and along 5 years of treatment with dual HER2 blockade, to perform an in-depth transcriptomic analysis of the peripheral immune landscape of HER2+ mBC at the single-cell level. Comparison between response groups revealed responders to be enriched in circulating monocytes,

dendritic cells (DC) and central memory CD8+ T cells before the start of treatment. Upregulation of biological pathways related to antigen presentation, IFN-g signalling, and cytotoxicity was found in monocytes and NK cells from responder patients, while non-responders were enriched in pathways typically associated with poor prognosis such as hypoxia and vascular wound healing. Co-upregulation analysis revealed gene signatures shared by DC and monocytes in non-responders, and by T and B cells in responders, and highlighted KLF9 as a potential biomarker of poor response given its upregulation in both myeloid and lymphoid populations in non-responder patients.

Conclusion

With the first scRNA-seq peripheral immune profiling of HER2+ mBC patients, we show that responder and non-responder patients may be distinguished before the start of treatment. PBMC composition and gene expression profile support the potential of blood as a source of tumor-associated immune biomarkers for therapy assignment. Monocyte, DC and CD8+ T cell subsets are highlighted as potentially significant players in the clinical response to anti-HER2 antibodies, which prompts the need for further investigation into their dynamics in the TME of HER2+ breast cancers.

EACR25-0322

Czech Metastatic Colorectal Cancer Patients: their Copy Number Variation and Clinical Response to Chemotherapy and Bevacizumab

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Introduction

Bevacizumab (Avastin®) is a humanized monoclonal antibody targeting vascular endothelial growth factor A (VEGF-A), inhibiting angiogenesis in metastatic colorectal cancer (mCRC). It is primarily used in patients with RAS mutations, where cetuximab or panitumumab are ineffective. Although bevacizumab is established in mCRC treatment, no definitive DNA biomarker for its efficacy exists. Somatic mutations in NRAS, BRAF, and PIK3CA have been studied but lack validation as predictive markers. We hypothesize that somatic copy number variations (CNVs) might be key biomarkers for bevacizumab efficacy.

Material and method

We analyzed 30 mCRC patients from University Hospital Olomouc, divided into two groups based on progression-free survival (PFS): poor responders (PFS ≤ 9 months) and good responders (PFS ≥ 10 months). DNA was isolated from FFPE samples and quantified by qPCR. CNV analysis was performed using the OncoScan FFPE Assay Kit, which applies molecular inversion probes technology to detect CNVs in FFPE-derived DNA. The

data was processed using OncoScan Console and R software for segmentation and GISTIC 2 analysis. Functional annotation of genes was done using the DAVID database.

Result and discussion

Good responders showed amplifications in the 18p11.32 region and deletions in chromosomes 1p36.33, 8p11.22, 10q11.23, 14q32.33, 16p13.3, and 20p12.1. Poor responders exhibited amplifications at 8q24.21, 14q12, and 19q13.2, with no deletions above the threshold. Functional annotation revealed that good response genes were involved in ATPase activity, neuronal signaling, and transcription regulation. Poor responders had amplified genes linked to immune regulation (IFNL1, IFNL2, IFNL3), MAPK signaling (MAP3K10, MAP4K1), and differentiation (EID2, SIRT2). Genes like AGRN, MAPK8, and ARHGAP22 from good responders have been linked to angiogenesis and treatment resistance, while DVL1 and MYC in poor responders are associated with tumor proliferation.

Conclusion

This study suggests that CNVs detected by OncoScan technology could serve as biomarkers for predicting bevacizumab response in mCRC. Identifying genes linked to angiogenesis, tumorigenesis, and proliferation (e.g., AGRN, MAPK8, MYC, and DVL1) requires further validation in larger cohorts. Such findings could lead to personalized therapies, improving mCRC treatment outcomes. Future studies will validate these results using high-throughput methods like PCR to confirm their clinical utility.

EACR25-0354

Colorectal cancer patients comprehensive genomic profiling using TruSight Oncology 500

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Introduction

Comprehensive genomic profiling (CGP) provides testing of hundreds of pan-cancer biomarkers with tumor markers specific for individual tumor types. This approach brought the possibility of single nucleotide variants (SNV), insertions and deletions (INDELS), copy number variations (CNV), fusions and splice variants detection in DNA and RNA at one time, and tumor mutational burden (TMB) and microsatellite instability (MSI) additionally. The aim of this study was to validate TruSight Oncology 500 HT (TSO500) NGS assay using different types of samples and different types of bioinformatician analysis.

Material and method

We enrolled 513 colorectal cancer patient samples and commercial reference DNA standard in this study. The input amount was 40 ng of gDNA (derived from FFPE and RNA-later tissue samples). All samples were mechanically fragmented prior to library preparation. Sequencing libraries were prepared using DNA Unique

Dual Indexes. After quality control of all libraries, sequencing on the Illumina NovaSeq 6000 was performed. Bioinformatic analysis was performed using two approaches for secondary and tertiary analysis – Velsera Pierian and Illumina Connected Insights (ICI).

Result and discussion

The results showed that the TSO500 HT kit provides high sensitivity and specificity in detecting tumor markers in patients with colorectal cancer. The average total number of reads obtained was 118 thousand with an average coverage per human genome of 271, with a minimum coverage of 124, and a maximum coverage of 1127 that corresponded with different types of input material. It was found that biomarkers were significantly elevated in samples with higher amounts of cancer cells.

Conclusion

The use of the TSO500 HT kit offers new possibilities for non-invasive diagnosis and monitoring of colorectal cancer. The high reproducibility and robustness of the kit in clinical settings indicate that this tool can be valuable for personalized treatment and monitoring of oncology patients. Furthermore, the results of this research can contribute to the further development of diagnostic methods for colorectal cancer and similar oncological diseases.

EACR25-0360

AREG or EREG Overexpression Identifies a Subset of Gastro-Esophageal Adeno-carcinoma Patients Responsive to EGFR

Targeting

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Introduction

The development of EGFR-targeting drugs for gastro-esophageal adenocarcinoma (GEA) treatment has been hampered by negative results from phase II/III clinical trials testing EGFR inhibitors in combination with chemotherapy. These failures could be explained by the lack of patient selection due to the absence of reliable predictive markers of response. However, preclinical and clinical findings indicate the effectiveness of EGFR blockade in GEA patients harboring EGFR gene amplification, promising a reassessment of EGFR as a therapeutic target in GEA within the context of a precise patient selection. Retrospective analyses also suggest that

a subset of EGFR non-amplified GEA patients might benefit from EGFR targeting, highlighting the urgent need for predictive biomarkers of sensitivity.

Material and method

We screened 27 GEA primary cancer cell lines and 10 Patient-Derived Xenografts for sensitivity to anti-EGFR drugs. The molecular characterization of these models was performed to uncover potential predictive biomarkers of response. Identified biomarkers were then validated by IHC and RNA in situ hybridization in samples from patients enrolled in the COG clinical trial.

Result and discussion

Our analysis identified both in vitro and in vivo a subset of GEAs lacking EGFR gene amplification, but sensitive to EGFR inhibition. The molecular characterization of these models revealed overexpression of the EGFR ligands amphiregulin (AREG) or epiregulin (EREG) in the drug-sensitive group. To assess the translational significance of these findings, we conducted a post-hoc analysis on tumors from patients enrolled in the COG trial who were treated with the EGFR inhibitor gefitinib. Importantly, we detected a significant correlation between overall survival (OS) and AREG/EREG expression level (HR: 0,5827; CI:0,3445-0,9854, P = 0,0439). Moreover, overexpression of EGFR ligands had no predictive power in the presence of KRAS mutations.

Conclusion

We propose AREG or EREG overexpression as a new predictive marker to identify a subgroup of GEA patients who could benefit from EGFR inhibition.

EACR25-0386

LGALS3BP's role in bladder cancer: a potential urinary biomarker and therapeutic target

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Introduction

Bladder cancer remains a major public health challenge, necessitating improved diagnostic and therapeutic strategies. Liquid biopsy, leveraging urinary biomarkers, offers a non-invasive approach for early detection by capturing disease-specific molecular changes. Targeted therapies, particularly antibody-drug conjugates (ADCs), are also advancing precision medicine in bladder cancer treatment. LGALS3BP (Galectin-3-binding protein), is a secreted glycoprotein involved in various biological processes such as immune modulation, cell adhesion, and tumor progression. Elevated circulating LGALS3BP levels are linked to various cancers, making it a promising candidate also as urinary biomarker for liquid biopsy. Additionally, LGALS3BP is emerging as a

potential ADC therapy target in combination with immune checkpoint inhibitors (ICIs).

Material and method

Immunohistochemical analysis of paraffin-embedded tissues was conducted to quantify LGALS3BP expression in bladder cancer tissues. An in-house ELISA assay was developed and used to assess urinary circulating LGALS3BP levels in both healthy controls and bladder cancer patients. A panel of eight commercially available bladder cancer cell lines was utilized for in vitro characterization of LGALS3BP expression and its glycosylation status. Therapeutic studies were performed using xenograft models.

Result and discussion

LGALS3BP expression was significantly elevated in bladder cancer tissues and correlated with disease progression. Its overexpression was confirmed in urothelial cancer cell lines, with variations in glycosylation patterns. Notably, treatment with Kifunensine, a mannosidase inhibitor, enhanced 1959 therapeutic antibody reactivity, suggesting that LGALS3BP glycosylation may influence antibody recognition and protein function. Urinary LGALS3BP levels were markedly higher in patients than in healthy individuals. Of note, in-house ELISA developed by our group, based on the 1959 therapeutic antibody, demonstrated promising accuracy (67% sensitivity, 79% specificity), highlighting LGALS3BP as a potential urinary biomarker. Lastly, therapeutic studies performed with 1959-sss/DM4 confirmed LGALS3BP as an effective target for ADC therapy in bladder cancer.

Conclusion

The findings presented here strongly position LGALS3BP as a promising candidate for bladder cancer research, highlighting its potential both as a urinary biomarker and as a therapeutic target.

EACR25-0393

Assessing the potential of specific Tubulin post-translational modifications as predictive biomarkers for taxane response in breast cancer

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Introduction

Microtubules (MTs) are crucial components of the cytoskeleton and play direct roles in essential cellular processes, such as cell division and migration, intracellular transport and stress response. Taxanes (paclitaxel and docetaxel), which act by stabilizing MTs, are widely used in neoadjuvant chemotherapy (NAT) for breast cancer (BC) patients. Despite their undeniable clinical success, responsiveness is not guaranteed and taxanes bare significant side effects. We have recently identified deviations in the levels of two post-translational modifications (PTMs) of tubulin, namely α -tubulin acetylation and detyrosination, that correlate with or influence cancer cell response to taxanes in vitro, respectively. To validate these findings and determine the clinical significance of this “tubulin code” in BC, we are conducting a retro-spective clinical study to test the predictive, prognostic and therapeutic value associated with α -tubulin acetylation and/or detyrosination for precision oncology. If successful, this will allow patient stratification, towards improved therapeutic choices and outcomes.

Material and method

We have established a robust immunohistochemistry (IHC) protocol and scoring system for assessing the levels of α -tubulin acetylation and detyrosination in formalin-fixed paraffin embedded patient material. For this study, histological cuts from naïve tumor biopsies from BC patients (any molecular sub-type) who underwent NAT regimens that included taxanes were collected, along with relevant clinical and histopathological data. When available, surgically removed post-NAT material from the same patients was also analyzed.

Result and discussion

Using our established methodology, the levels of α -tubulin acetylation and detyrosination will be classified as highly positive, positive, or negative, based on visual assessment by pathologists and quantitative digital analysis. The correlation between tubulin PTM levels and the response to taxanes in NAT (complete pathological response, partial or no response), as well as other clinical and pathological factors, will be analysed. Sensitivity and specificity of the biomarkers will be evaluated using receiver operating characteristics (ROC) curve analyses.

Conclusion

With this approach, we have generated preliminary results and shown that the study of tubulin PTM levels in BC is feasible and may represent new biomarkers of therapy response. If successful, our study will enable more informed and personalized therapeutic choices for patients, by pinpointing the good and the weak responders to taxane-based therapy. We envision that our findings will establish the grounds for personalized taxane dosage-optimization and improved therapeutic efficacy, while opening the possibility to manipulate these tubulin PTMs as new therapeutic targets for enhanced treatment of specific breast cancer sub-types, with special attention to triple negative breast cancer.

EACR25-0394

Detection of high molecular weight DNA contaminations in cell-free DNA samples

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Introduction

Cell-free DNA (cfDNA) is a critical component in translational research, facilitating the identification of biomarkers and mutations through noninvasive methods. However, its low yield, complex fragmentation, and potential contamination with high molecular weight (HMW) DNA present challenges for next-generation sequencing (NGS) workflows. Quality assessment of cfDNA is essential, requiring visualization of fragmentation patterns and contamination.

Material and method

Plasma samples were processed using different cfDNA extraction kits. The extracted cfDNA samples were analyzed with the Cell-free DNA ScreenTape assay and the High Sensitivity D1000 ScreenTape assay on the Agilent TapeStation system as well as with the High Sensitivity DNA kit on the Agilent 2100 Bioanalyzer system.

Result and discussion

Total DNA and cfDNA concentration of various samples were compared. Despite some samples having a higher total DNA amount, there was no correlation with cfDNA concentration. Electropherogram comparisons of different samples revealed the presence of HMW DNA, which is also reflected in lower %cfDNA scores. Other QC methods used in comparison were unable to accurately display and quantify HMW DNA contaminations, thus failing to correctly assess cfDNA quality.

Conclusion

The Agilent TapeStation system, utilizing the Cell-free DNA ScreenTape assay, offers an objective and reliable qualification of cfDNA samples. It provides the % cfDNA quality score, indicating the proportion of cfDNA relative to HMW DNA contamination, thus ensuring accurate assessment of cfDNA quality before NGS library preparation and sequencing. Other QC methods might overlook HMW DNA contamination, leading to inaccurate yield estimations and potentially compromising downstream experiments. Accurate quantification of cfDNA, including HMW DNA contamination, is essential for reliable NGS library preparation and results.

EACR25-0473

Developing a Targeted Plasma DNA Methylation Panel for Early Prostate Cancer Detection

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Introduction

Prostate cancer (PCa) represents a major health burden globally, with over 1.4 million annual diagnoses and almost 400,000 PCa-related deaths. Current approaches to PCa screening and diagnosis are suboptimal, creating challenges for the accurate detection of clinically important PCa. Hence, there is an urgent need for novel, accessible, and clinically deployable biomarkers to optimise PCa care. Circulating tumour DNA (ctDNA) is a minimally invasive, highly specific technology that identifies the presence of a tumour using plasma-derived cell-free DNA (cfDNA). DNA methylation changes are a universal hallmark of cancer that dynamically influence gene expression. We, and others, have shown that ctDNA-based epigenetic alterations are higher in number, potentially enabling earlier tumour detection than genetic mutation analysis. Analysis of the cell-free DNA methylome, therefore, represents an exciting avenue for the development of highly accurate biomarkers for early PCa detection.

Material and method

In this multicentre, prospective discovery study, we have performed whole-genome scale DNA methylation profiling of matched patient blood and prostate biopsy tissue samples. Men scheduled to undergo prostate biopsy for clinical suspicion of PCa were recruited from several localities across New Zealand, with matched pre-biopsy peripheral blood samples and prostate biopsy tissue collected for molecular profiling. Unbiased, whole-genome scale DNA methylation profiling for both plasma cfDNA and tissue samples was performed using cell-free reduced representation bisulfite sequencing (cfRRBS).

Result and discussion

Over 200 differentially methylated regions (DMRs) differentiating malignant from benign and healthy samples have been identified from early profiling results of cfDNA samples, demonstrating significant discriminatory power for PCa detection at very early-stage disease. PCa-specific DMRs have also been identified from prostate tissue samples – interestingly, however, the most predictive tissue-derived DMRs cluster distinctly from those detected in cfDNA, even in matched patient samples.

Conclusion

Early profiling results show promise for cfRRBS as a discovery approach for cell-free DNA-based methylation biomarkers, with high prospective utility in the setting of PCa detection. Profiling results will be further refined and correlated to clinical and histopathological features of disease severity to generate a blood-based, targeted methylation panel for early and accurate PCa detection.

EACR25-0474

Liquid Biopsy-Based Biomarkers for Predicting Radiotherapy Response in

Locally Advanced Head and Neck Squamous Cell Carcinoma

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Introduction

Radiotherapy (RT) is frequently used to treat head and neck squamous cell carcinoma (HNSCC), however resistance is still a significant problem. Treatment choices and patient outcomes may be influenced by trustworthy biomarkers that forecast RT response. Exosomal RNA and circulating tumor DNA (ctDNA) have become attractive liquid biopsy-based biomarkers for tracking tumor dynamics and therapy effectiveness. Exosomal RNA and ctDNA signatures are examined in this work as potential indicators of RT response in locally progressed HNSCC.

Material and method

60 HNSCC patients undergoing adjuvant or definitive radiation therapy participated in a prospective research. Samples of plasma were taken before, during, and after RT. NGS (ultra-deep next-generation sequencing) was used to monitor ctDNA levels and TP53, NOTCH1, and HRAS mutations. Furthermore, the expression of genes linked to immunity and hypoxia was examined in exosomal RNA. Tumor regression, locoregional control, and progression-free survival (PFS) were all associated with biomarker dynamics.

Result and discussion

Shorter PFS and greater recurrence rates ($p < 0.01$) were linked to persistent ctDNA detection after RT. The 2-year locoregional control was considerably better in patients with ctDNA clearance mid-RT (HR = 0.42, $p = 0.003$). Poor response was associated with high exosomal RNA production of VEGF and HIF1A, suggesting that tumor hypoxia contributes to radioresistance. Additionally, patients with lower amounts of exosomal PD-L1 mRNA demonstrated better RT response and immune activation.

Conclusion

The potential of ctDNA and exosomal RNA as real-time indicators for RT response prediction in HNSCC is demonstrated by this work. High exosomal hypoxia markers and persistent ctDNA post-RT may be used as markers of radioresistance to inform individualized treatment plans. To enhance patient outcomes, future research should look into incorporating liquid biopsy-driven adaptive RT techniques.

EACR25-0499

Predictive Signature of Response to Neoadjuvant Chemotherapy Based on Tumor-Educated Platelet mRNA Profiles in Muscle-Invasive Bladder Cancer

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Introduction

Cisplatin-based neoadjuvant chemotherapy (NAC) followed by radical cystectomy is the standard treatment for muscle-invasive bladder cancer (MIBC). However, NAC use is limited due to its modest impact on survival, patient comorbidities, and lack of predictive biomarkers. Tumor-educated platelets (TEPs) have emerged as potential biomarkers for cancer diagnosis and treatment response through liquid biopsy. TEPs, functionally altered by tumors, internalize and transport tumor-derived mRNA, reflecting its genetic profile. This study aims to identify a TEP mRNA-based predictive signature of NAC response in MIBC patients.

Material and method

Pre-NAC blood samples from 50 MIBC patients (T2-4N0/+M0) were collected from two Spanish hospitals, along with 12 diagnostic tumor tissue samples for TEP-tumor gene expression correlation. Blood samples from 10 healthy donors served as controls. Total RNA from platelets and FFPE tissues was extracted using the mirVana™ miRNA Isolation Kit and Maxwell RSC RNA FFPE Kit (Promega), quantified via NanoDrop (Thermo Fisher). Gene expression was analyzed using the NanoString nCounter Human Tumor Signaling 360 Panel. Data preprocessing and normalization were performed in R (v4.3.1), and differential expression analysis was conducted using limma within the NanoTube framework ($|FC| > 1.2$, $p < 0.05$, $q < 0.1$) to compare complete responders (ypT0N0) and non-responders ($yp \geq T2$). Gene set enrichment analysis was performed with fgsea (implemented within NanoTube) on pathway and hallmark gene sets provided by NanoString with the panel annotation.

Result and discussion

We identified nine genes – CDCP1, SOX7, EXO1, ANGPT2, BRCA1, GREM1, GRHL2, TNS4, and FGFR2 – that were significantly overexpressed in TEPs from non-responsive MIBC patients compared to responsive patients. Notably, these genes exhibited similar expression patterns between responsive patients and the control group, suggesting their association with treatment resistance. Among them, GREM1 was the most overexpressed gene ($FC = 2.29$; $q\text{-value} = 5.4e-4$) in the TEPs of non-responders versus responders. Furthermore, our exploratory functional enrichment analysis revealed that cancer hallmarks such as evading growth suppressors, activating invasion and metastasis, and sustaining proliferative signaling, along with pathways related to the cell cycle and epithelial-mesenchymal transition, were significantly enriched in the TEPs of non-responsive patients.

Conclusion

This prospective study highlights the potential of TEPs as a non-invasive liquid biopsy tool for predicting NAC response in MIBC. We identified nine overexpressed genes in non-responders, with GREM1 showing the strongest discriminative power. TEP RNA profiles effectively distinguished between responders and non-

responders, suggesting their utility as predictive biomarkers to guide treatment decisions. Further validation in larger cohorts is warranted.

EACR25-0520

Serum metabolomic profiling unveils a metabolite marker panel for early detection of colorectal cancer

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Introduction

Early detection of colorectal cancer (CRC) is critical for identifying the disease at a curable stage. Current screening methods, such as invasive colonoscopy and stool-based tests, face limitations due to low to moderate uptake and compliance. Minimally invasive blood-based approaches, particularly those analyzing tumor-derived or tumor-relevant metabolites, offer a promising alternative for CRC screening. This study aims to identify serum metabolite markers for early-stage CRC detection and evaluate their diagnostic potential, both individually and in combination.

Material and method

In this case-control study, untargeted metabolite profiling using liquid chromatography-mass spectrometry (LC-MS) was performed on serum samples from 32 pairs of stage I/IIA CRC patients and age-, gender-matched controls with colonoscopy-verified no evidence of disease (NED). Differences in serum metabolite levels between cases and controls were analyzed by using Mann-Whitney U test. Receiver operating characteristic (ROC) curves were constructed for assessment of assay sensitivity and specificity. Multivariate logistic regression models, followed by ROC analysis, were used to evaluate the assay performance of metabolite combinations.

Result and discussion

A total of 59 metabolites were differentially expressed between CRC cases and NED controls, with 31 upregulated and 28 downregulated. Notably, nine upregulated metabolites exhibited substantially higher serum levels in CRC cases as compared to controls ($P < 0.001$). ROC analysis confirmed the discriminative power of these metabolites, with area under the curve (AUC) values ranging from 0.732 to 0.887 ($P \leq 0.001$). Combinations of two or more metabolites yielded higher AUCs and improved sensitivities as compared to individual metabolites. A five-metabolite panel demonstrated particularly strong diagnostic performance, achieving a sensitivity of 96.9% while maintaining a specificity of 90%. This represented a significant improvement over the sensitivities of the individual markers (59.4%, 50.0%, 46.9%, 53.1% and 46.9%, respectively) at the same specificity (all $P \leq 0.001$).

Conclusion

The identified five-metabolite serum panel shows promise to be developed into a minimally invasive, blood-based test for early detection of colorectal cancer.

Further validation in larger cohorts is warranted to confirm its clinical utility.

EACR25-0528

Detection rate for ESR1 mutations is higher in circulating-tumor-cell-derived genomic DNA than in paired plasma cell-free DNA samples as revealed by ddPCR

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Introduction

Plasma cell-free DNA (cfDNA) analysis to track estrogen receptor 1 (ESR1) mutations is highly beneficial for the identification of tumor molecular dynamics and the improvement of personalized treatments for patients with metastatic breast cancer (MBC). Plasma-cfDNA is, up to now, the most frequent liquid biopsy analyte used to evaluate ESR1 mutational status. Circulating tumor cell (CTC) enumeration and molecular characterization analysis provides important clinical information in patients with MBC. In this study, we investigated whether analysis of CTCs and circulating tumor DNA (ctDNA) provide similar or complementary information for the analysis of ESR1 mutations.

Material and method

We analyzed both plasma-cfDNA ($n = 90$) and paired CTC-derived genomic DNA (gDNA; $n = 42$) from 90 MBC patients for seven ESR1 mutations using the ddPLEX Mutation Detection Assay (Bio-Rad, Hercules, CA, USA).

Result and discussion

Eight out of 90 (8.9%) plasma-cfDNA samples tested with ddPCR were found positive for one ESR1 mutation, whereas 11/42 (26.2%) CTC-derived gDNA samples were found positive for at least one ESR1 mutation. Direct comparison of paired samples ($n = 42$) revealed that the ESR1 mutation rate was higher in CTC-derived gDNA (11/42, 26.2%) than in plasma-cfDNA (6/42, 14.3%) samples.

Conclusion

Our results, using this highly sensitive ddPLEX assay, reveal a higher percentage of mutations in CTC-derived gDNAs than in paired ctDNA in patients with MBC. CTC-derived gDNA analysis should be further evaluated as an important and complementary tool to ctDNA for identifying patients with ESR1 mutations and for guiding individualized therapy.

EACR25-0529

Do small extracellular vesicles within serum of different thyroid pathologies express the same miRNA as those isolated from tissue-on-chip?

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Introduction

Following the successful isolation and characterisation of small extracellular vesicles (sEV) released from thyroid cancer, Graves' disease, and benign thyroid tissue maintained on a tissue-on-chip device, these were analysed for miRNA content to determine any differences in the profile between pathologies. Now, the serum from patients of these pathologies has been analysed to identify if the miRNA profiles detected in sEV released from tissue-on-chip can be found within the blood. For the tissue-on-chip analysis, there was a cohort of 30 patients; thyroid cancer (n=10), Graves' disease (n = 10), and benign thyroid (n=10). For the serum analysis, where blood was available, a sample from the same patient was used. So far, a total of 6 serum samples per pathology have been analysed to identify potential miRNA biomarkers.

Material and method

Thyroid biopsies from 30 patients were sliced using a vibratome before being incubated on 8 separate tissue-on-chip devices with constant perfusion in EV depleted medium for 6 days. Effluent coming from the devices was collected and pooled prior to differential ultracentrifugation to isolate sEV. RNA was extracted using the miRNeasy Micro Kit (Qiagen) prior to qRT-PCR analysis. Serum from patients also underwent ultracentrifugation to isolate sEV, prior to qRT-PCR. qRT-PCR analysis of the serum sEV was completed using the panel of miRNA identified from sequencing of sEV isolated from tissue-on-chip samples.

Result and discussion

Four miRNAs were seen to be significantly differentially expressed in Graves' vs. Benign tissue-on-chip sEV: miR-7-5p ($p < 0.025$), miR-382-5p ($p < 0.025$), miR-435-5p ($p < 0.023$), miR-129-5p ($p < 0.026$). Five miRNAs were differentially expressed in Cancer vs. Benign sEV: miR-375-3p ($p < 0.002$), miR-382-5p ($p < 0.008$), miR-146b-5p ($p < 0.017$), miR-432-5p ($p < 0.000$), miR-129-5p ($p < 0.008$). To date, qRT-PCR analysis of serum has demonstrated the presence of several miRNAs of interest, including miR-7-5p and miR-432-5p. It is clear there needs to be an improvement in RNA extraction, to allow reliable qRT-PCR detection; this should enable the measurement of miRNA present at low concentrations.

Conclusion

Tissue-on-chip technology developed at the University of Hull has allowed the isolation of tissue specific sEV

which demonstrate distinct miRNA biomarker profiles between thyroid pathologies. qRT-PCR has been used to validate the miRNA identified using RNA sequencing. Some markers have been identified by qRT-PCR in serum samples, but additional optimisation of the extraction protocol is currently ongoing. The identification of these miRNA biomarkers of thyroid disease could provide a non-invasive thyroid cancer and disease progression analysis tool in the future.

EACR25-0530

THOR Methylation signatures in liquid biopsies for early breast cancer detection

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Introduction

Breast Cancer (BC) is the leading cause of cancer-related death among women worldwide. The incidence of BC in young women (<40 years old) is rising, revealing the need to develop and implement new strategies for BC diagnosis beyond current imaging-based approaches. In most BC cases, cancer cells reactivate the telomerase enzyme through upregulation of human Telomerase Reverse Transcriptase (hTERT) gene, acquiring limitless self-renewal capacity and supporting tumor progression. One mechanism responsible for this reactivation is the hypermethylation of a specific region within the hTERT promoter, called TERT Hypermethylated Oncological Region (THOR). Our group showed that THOR is significantly hypermethylated in malignant breast tissue when compared to benign tissue (40.23% vs. 12.81%). Liquid biopsy is a minimally invasive approach that provides real-time information about tumor status, particularly through circulating cell-free DNA analysis. We aim to identify THOR DNA methylation patterns exclusive to BC that could be used as liquid biopsy-based biomarkers to detect BC through a simple blood test.

Material and method

DNA derived from 59 BC tissue samples, 60 healthy breast tissue samples and 47 healthy blood samples was bisulfite treated, followed by PCR amplification of THOR region and sequenced using Illumina Next generation MiSeq. The raw DNA methylation data of the THOR region was processed through a multistep bioinformatic pipeline: data quality control and poor bases trimming, alignment, incomplete bisulfite conversion reads filtration, methylation extraction, and complete reads selection. Then, we quantified the THOR methylation patterns present in the BC tissue, healthy breast tissue and healthy blood. We selected the THOR methylation patterns exclusively detected in the BC tissue but never detected in both healthy breast tissue and healthy blood samples, thus considered BC specific. This analysis was performed in BASH, and in R.

Result and discussion

Comparing BC and healthy breast tissue samples, we identified 450 BC-specific THOR methylation patterns that were never detected in healthy breast tissue. After comparing it with healthy blood samples, we found that 19 THOR methylation patterns remained exclusive to BC

(sensitivity = 15%–75%; specificity = 100%). Notably, we also identified a panel of 13 BC-specific THOR methylation patterns present in 58 out of the 59 BC tissue samples (sensitivity = 98%, specificity = 100%).

Conclusion

Our findings underscore the potential of THOR methylation signatures to be used as a highly sensitive and specific biomarker for breast cancer detection.

EACR25-0547

STRN4/Zinedin is a favourable prognostic indicator for patients with colon cancer

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Introduction

Striatin-4 (STRN4) is a member of the STRIPAK (striatin-interacting phosphatase and kinase) complex, and regulates multiple cellular processes such as cell differentiation, growth and metabolism. STRN4 has been implicated in tumorigenesis through its involvement in the Hippo signalling pathway, autophagy, and anti-tumour immunity via the STING pathway. Its role in colon cancer remains poorly understood and its clinical relevance has not been established; it is crucial therefore to determine the clinical significance of STRN4.

Material and method

Post-surgical colon tissue samples, including 90 tumour and 79 non-cancerous tissues were obtained from patients at the University Hospital of Wales. The samples were homogenised and underwent reverse transcription to generate cDNA for transcriptional analysis. STRN4 expression was assessed across the cohort and results stratified according to clinicopathological parameters including tumour differentiation stage, TNM stage, Dukes stage, presence of metastasis, and patient survival. Expression levels were analysed based on patients' EGFR (epidermal growth factor receptor) status.

Result and discussion

STRN4 expression was significantly reduced in higher-grade tumours compared with lower-grade tumours ($p = 0.031$) and increased with higher TNM stages ($p = 0.06$, $p = 0.0079$, $p = 0.0039$ for TNM1 vs. TNM2, TNM3, and TNM4, respectively). In survival-based analysis, patients with high STRN4 levels had significantly greater overall survival than those with low expression ($p = 0.041$ by Kaplan-Meier model, hazard ratio (HR) = 0.416, 95% confidence interval (CI): 0.174–0.994). Stratification of patients by EGFR status showed that the survival benefit of STRN4 was more pronounced in patients with high EGFR expression ($p = 0.020$) compared with those with low EGFR ($p = 0.552$). STRN4 expression had a more profound impact on predicting disease-free survival (DFS) of colon cancer patients; high STRN4 expression was associated with significantly improved DFS ($p = 0.007$, HR = 0.331, 95% CI: 0.142–0.770). Multivariate analysis identified STRN4 ($p = 0.009$) and TNM staging

($p = 0.027$) as significant independent predictors of DFS with better outcomes in cases of high EGFR expression. High levels of STRN4 were associated with reduced metastasis potential for colon cancer patients (DMFS $p = 0.015$, HR = 0.342 (95% CI 0.137–0.854)) and may also indicate increased sensitivity of patients to chemotherapies.

Conclusion

The present study is the first to demonstrate that high STRN4 expression in human colon cancer is a favourable prognostic factor, associated with improved overall, disease-free, and distant metastasis-free survival. The stronger prognostic value in patients with high EGFR expression suggests a potential therapeutic implication of STRN4, warranting further investigation into STRN4 as a clinical biomarker and therapeutic target in colon cancer. Ethical approval reference number: SJT/C617/08.

EACR25-0608

Integrating tumor fraction, next generation sequencing and qPCR analysis in liquid biopsy of EGFR-mutated non-small cell lung cancer patients

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Introduction

Tumor fraction (TF) assessment in liquid biopsy (LB) is increasingly used to evaluate the actual presence of circulating tumor DNA (ctDNA). False negative LB results in fact are often associated to low or undetectable TF. The aim of our study is to evaluate the integration of TF assessment into the diagnostic workflow of non-small cell lung cancer (NSCLC) LB, with a particular focus on its utility in the interpretation of cases testing negative for actionable gene mutations, such as EGFR mutations, to enhance diagnostic accuracy.

Material and method

We collected LBs from a cohort of 30 newly diagnosed NSCLC patients, carriers of EGFR mutations determined by routine tissue analysis and enrolled in the ongoing REM trial. Plasma EGFR mutations were identified both by Next Generation Sequencing (NGS) with the 77-gene Avenir Expanded Panel and the real time Cobas EGFR Mutation Test v2 (Roche). TF was assessed by shallow whole genome sequencing (sWGS) using the KAPA HyperPrep Kit (Roche) and SAMURAI plus ichorCNA algorithms.

Result and discussion

Across 30 LB samples, sWGS revealed a mean TF of $7.0\% \pm 1.0\%$ (median = 0.0%; range 0.0 to 43.4%). TF was detectable in 13 out of 30 samples (44%), with a mean TF of $16.2\% \pm 11.1$ (median = 0.1%, range 2.1 to 43.4%). In all cases with detectable TF, EGFR mutations were detected both by the Avenir and Cobas test, with a median VAF of 21.6%. Moreover, the Avenir test

detected a second EGFR mutation in two patients and EGFR amplifications in three patients. TF was undetectable in 17 out of 30 liquid biopsies (56%). However, EGFR mutations were still identified in 15 out of these 17 TF-negative samples using the Cobas and Avenio tests, with a median VAF of 1.25%. Three samples tested negative by both methods. Notably, two patients with undetectable TF carried two EGFR mutations with VAFs of 12.4 and 14.6%, in the absence of gene amplifications, according to the results of Avenio NGS test, suggesting that EGFR mutations can still be detected even in cases with no measurable TF. This apparently false negative TF determination might be attributed to intrinsic limitations of TF calculation, which primarily relies on the detection of genomic aneuploidy and can be affected by a low presence of copy number alterations.

Conclusion

These study highlights the superior sensitivity of NGS and qPCR tests in detecting EGFR mutations, even in cases with undetectable TF, underscoring the limitations of TF assessment by sWGS and emphasizing the need for an integrated multi-modal approach in LB analysis of lung cancer samples.

Disclosure. Unconditioned research support: AstraZeneca.

EACR25-0611

The Impact of Circulating Malignant Tumour Cells on Tumour Recurrence in Oral Squamous Cell Carcinoma

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Introduction

Oral squamous cell carcinoma (OSCC) is a serious and debilitating disease, with surgical resection the mainstay of treatment. The extent to which surgical resection of OSCC disseminates cancer and is associated with metastasis is currently unknown. The aim of this study was to determine changes in numbers of malignant cells released into the systemic circulation immediately following tumour removal and over the first seven post-surgical days and to relate these numbers with cancer recurrence or metastasis over a 3-year post-surgical follow-up period.

Material and method

Patients identified at a single centre with histopathologically confirmed OSCC and who were scheduled to undergo resective surgery were invited to join the study. Peripheral blood from 54 patients were sampled at baseline (before induction of anaesthesia of the planned surgery), when the surgeon declares that the tumour had been resected, and at post-surgical day 1 and day 7. Blood was processed for circulating tumour malignant cell enumeration within 48 hours of blood taking using an immunofluorescent *in situ* hybridisation technique. White blood cells were stained CD45+, DAPI+ (4',6-diamidino-2-phenylindole) but circulating tumour malignant cells were stained CD45-, DAPI+. Clinical information at baseline and up to 3-years post-surgical treatment, including cancer recurrence or metastasis were

retrieved from the clinical management system. Spearman's correlation was used to look at relationships between outcome and tumour cell count at various timepoints.

Result and discussion

A total of 54 patients with mean (SD) age of 65.94 (11.26) years, were studied. Tumours were at various stages (I to IV) and occurred in anatomical sites including tongue, buccal mucosa and floor of mouth. Patients were followed up for recurrence, metastasis, or death for between 3 to 5 years post-surgery. 16 patients had passed away (mean age 71.00) at project census. Circulating tumour cells and circulating tumour-derived endothelial cells were detected in all but two patients. The number of circulating tumour cells, and circulating tumour-derived endothelial cells were higher at the post-surgical day 1 and day 7 timepoints, but these increases were not statistically significant. Results showed that there is no association between the number of circulating malignant cells and the clinical-pathological stage of the tumour. Presence of circulating malignant cells at any timepoint during the tumour resection journey is predictive of recurrent/metastatic disease at up to 5 years of patient follow-up, although there was no direct correlation between number of cells and disease status at 5 years.

Conclusion

Patients with any circulating malignant cells in their peripheral blood at any time during the surgical journey should be given heightened surveillance with regard to detecting recurrent/metastatic disease

EACR25-0664

Evaluation of a novel protein-based assay for multi-cancer early detection

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Introduction

Early cancer detection is critical for improving patient survival, however, current diagnostic methods often lack sensitivity, especially for asymptomatic or early-stage malignancies. Liquid biopsy technologies have emerged as promising alternatives, yet challenges remain, particularly due to the low abundance of circulating tumor DNA (ctDNA). This study evaluates the Carcimun® test, a protein-based assay, for its ability to differentiate cancer from non-malignant inflammatory conditions.

Material and method

A prospective, single-blinded study included 172 participants, categorized as healthy ($n = 80$), cancer patients ($n = 64$, nine distinct types including lung, colorectal, and pancreatic, bile duct, liver, esophageal, stomach, gastrointestinal stromal, and peritoneal cancer, stage I-III), and individuals with inflammatory conditions (fibrosis, sarcoidosis, pneumonia) or benign tumors ($n = 28$). Plasma samples were analyzed using the Carcimun® test, measuring protein-based changes via optical extinction. Sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were

determined, with a predefined extinction threshold of 120 units as a cut-off for malignancy. Statistical analysis was conducted using ANOVA and post-hoc testing.

Result and discussion

The Carcimun® test demonstrated high accuracy (95.4%), with sensitivity (90.6%) and specificity (98.2%), effectively differentiating cancer from non-malignant conditions. The PPV was 96.7%, indicating a high probability that individuals testing positive truly have cancer. The NPV was 94.6%, suggesting a strong ability to rule out malignancies when test results fall below the extinction threshold. Cancer patients exhibited significantly elevated extinction values (315.1 vs. 23.9 in healthy individuals, $p < 0.001$). Importantly, the test distinguished between inflammatory and malignant cases ($p < 0.001$), addressing a major limitation of previous liquid biopsy methods. Coagulation disturbances may affect extinction readings, potentially resulting in false-positive or false-negative findings.

Conclusion

The Carcimun® test presents a highly sensitive and specific approach to cancer detection, with enhanced differentiation from inflammatory conditions. By expanding inclusion criteria to non-malignant diseases, this study underscores the test's real-world applicability in early cancer screening. Further large-scale validations and longitudinal studies are warranted to optimize its integration into clinical practice.

EACR25-0689

Prognostic Potential of ctDNA Methylation in Prostate Cancer: Predicting Biochemical Recurrence and Overall Survival in Localized and Metastatic Disease

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Introduction

Circulating tumor DNA (ctDNA) methylation has emerged as a pivotal epigenetic biomarker reflecting tumor progression and treatment resistance. This study evaluates whether ctDNA methylation levels can serve as a prognostic marker for biochemical recurrence (BCR) in localized prostate cancer (LPCa) and overall survival (OS) in metastatic castration-resistant prostate cancer (mCRPC). Additionally, we investigate the interplay between ctDNA methylation and circulating tumor cells (CTCs) to provide deeper insights into tumor biology.

Material and method

Blood samples from 157 prostate cancer patients were analyzed, including LPCa ($n = 73$) and mCRPC ($n = 84$). Three key ctDNA methylation markers (C2orf, APC, HAPLN3) were quantified, alongside CTC enumeration and transcriptional profiling of AR, AR-V7, PSA, and PSMA. Correlations between ctDNA methylation burden and CTC transcriptomic activity were examined. Kaplan-Meier survival analysis was used to evaluate the prognostic value of ctDNA methylation in predicting BCR in localized disease and OS in mCRPC patients.

Result and discussion

ctDNA methylation was detected in 61.7% of LPCa and 96.8 % of mCRPC patients, showing a stepwise increase with disease progression ($P < 0.05$). Both CTC counts and CTC-derived gene expression (AR, AR-V7, PSA, PSMA) increased with advancing disease stage ($P < 0.05$). Notably, elevated ctDNA methylation levels were significantly associated with higher CTC transcriptional activity, suggesting its role as a surrogate marker of tumor aggression. In LPCa, APC methylation independently predicted BCR (HR, 2.6, 95% CI 1.05-6.45; $P = 0.039$). In mCRPC, elevated ctDNA methylation levels correlated with worse OS (HR, 3.63, 95% CI 1.84-7.18; $P < 0.001$).

Conclusion

Our findings support ctDNA methylation as a dynamic prognostic biomarker that reflects CTC transcriptional activity and tumor progression. Its ability to predict biochemical recurrence in localized disease and survival outcomes in mCRPC patients underscores its clinical relevance. Integrating ctDNA methylation profiling into liquid biopsy strategies could refine risk stratification and treatment decision-making in prostate cancer management.

EACR25-0699

Identification of Histopathological and Molecular alterations associated with High-risk Oral leukoplakia: A prospective study

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Introduction

Oral leukoplakia (OL) is the most common oral potentially malignant disorder with 30% to 50% lifetime risk to develop oral squamous cell carcinoma (OSCC). In clinic, histological grading remains the gold standard to identify lesions at risk of progression into OSCC but is subjective with unreliable outcome prediction. Moreover, the significance of individual histological characteristics

and underlying molecular alterations associated with progressive OL remains unexplored. Additionally, prospective studies with comprehensive understanding are lacking.

Material and method

The Institutional Ethics Committee approved the study protocol and signed informed consent was obtained from all the participants. This study included 498 OL tissue and blood samples. Pathologists SR and NM designed an assessment form featuring the architectural and cellular changes in OL cases. Haematological parameters were noted at the time of enrolment. DNA was extracted from OL tissues, and the presence of HPV was detected. The copy number was evaluated for 15 genes using DD-PCR. Follow-up at regular intervals of 6 months was done, and clinical outcomes were recorded as free of disease, persistence of lesion, recurrence of lesion, and malignant transformation (MT).

Result and discussion

The mean follow-up period was 31.73 months. In the histopathological assessment of 25 cellular and architectural features, we observed a significant association of dyskeratosis, nuclear hyperchromatism, enlarged nucleoli, and irregular epithelial stratification in both recurrence of lesion and MT. Additionally, an abnormal form of mitosis was observed in MT. Interestingly, inflammation occurrence and intensity (mild, moderate and severe) in tissue and low platelet to lymphocyte ratio in blood were strongly associated with MT. All cases were negative for HPV. Using DD-PCR, we identified novel prognostic biomarkers to stratify high-risk leukoplakia patients. Through this information, we provide a model to identify progressive lesions.

Conclusion

Collectively, this prospective study provides a comprehensive understanding of individual histopathological features, along with CNAs associated with the disease progression. Our findings may aid to stratify high-risk OL patients and determine the prognosis of precancerous lesions, thus helping in clinical management.

EACR25-0761

Tumor educated platelets in diagnostics and monitoring of childhood brain tumors

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Introduction

The most common solid tumors in pediatric patients are brain tumors, which also have the highest mortality rate

among childhood malignancies. Brain tumor diagnosis highly relies on molecular profiling of the tumor tissue supported with radiological imaging. However, biopsy is not always feasible due to the location of the tumor and radiological imaging is not a precise diagnostic method limiting the follow-up of minimal residual disease. To enable safer and repeatable follow-ups while capturing tumor heterogeneity, analyzing tumor-induced alterations in blood could provide cost-effective and precise monitoring, with the potential for microscopic disease detection. Previous research has shown that platelet RNA expression is altered in cancer patients. Moreover, transcriptomic changes in platelets have been used to distinguish different tumor types and monitor tumor progression. In this study, we investigated platelet RNA expression to assess its potential in pediatric brain tumor diagnostics and follow-up.

Material and method

Blood was collected into EDTA tubes from both pediatric brain tumor patients ($n = 24$), and healthy controls without known history of tumor ($n = 27$), all aged between 0–18 years old, at the New Children's Hospital in Helsinki, Finland. All samples were processed within 2 h from collection with platelet isolation by double centrifugation and CD45+ leukocyte depletion. RNA was extracted from platelets, and its integrity and quantity was determined. RNA sequencing (RNA-seq) libraries were constructed from 1 ng of total RNA. Rsubread was used to assign RNA-seq reads to human reference genome, NCBI nuclear rRNA gene models, and to determine read counts per genes. Differentially expressed genes (DEGs) were analyzed using DESeq2 and pathway analysis was made using Enrichr.

Result and discussion

Two high-grade gliomas, five low-grade gliomas, one atypical teratoid rhabdoid tumor, and eight healthy controls (HC) were included in the pilot analysis. We observed differential expression of 42 genes (FDR < 0.1) in platelet RNA (brain tumors vs. HC), of which 37 were upregulated and five downregulated. The majority of DEGs were associated with regulation of immune system. Furthermore, our results indicated that gene expression signature of TEPs may discriminate the aggressive pediatric CNS malignancies from low grade tumors. Confirmatory analyses with expanded cohort are currently ongoing.

Conclusion

The results of pilot analysis demonstrated that pediatric brain tumor patients have altered TEP transcriptome which could be utilized in diagnosing brain tumors and monitoring disease. Overall, TEPs are a new promising diagnostic approach which could potentially be used in combination with other approaches to enable more precise and less riskful diagnostics in the future.

EACR25-0765

Extracellular nicotinamide phosphoribosyltransferase (eNAMPT) in ascitic fluid as a potential biomarker for Ovarian Cancer development and recurrence

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Introduction

Ovarian cancer (OC) is one of the most lethal cancers worldwide. Currently, treatment options are limited to surgery and systemic chemotherapy, but around 80% of patients experience disease relapse and treatment resistance. Despite research advancements, the mechanisms underlying OC recurrence and chemotherapy resistance remain poorly understood. Thus, new insights are needed to identify patients at higher risk of recurrence and to develop more personalized therapeutic strategies. Nicotinamide phosphoribosyltransferase (NAMPT) plays a crucial role in the biosynthesis of NAD from nicotinamide. Extracellular NAMPT (eNAMPT), secreted outside the cell, exerts cytokine-like functions and mediates pro-inflammatory conditions by activating signaling pathways. eNAMPT is involved in various tumorigenic processes, including DNA repair, gene expression regulation, proliferation, invasion, stemness, metastasis, angiogenesis, immune modulation, and drug resistance, making it a promising target for anti-cancer strategies. In this context, the present project aims to evaluate the presence of eNAMPT in the ascitic fluid of OC patients in order to analyze its potential role in OC development and recurrence.

Material and method

eNAMPT levels were evaluated in the ascitic fluid of 42 OC patients treated at the Gynecologic Oncology Unit of the IRCCS in Bologna, at the time of diagnostic laparoscopy and prior to any treatment, using a commercially available sandwich enzyme-linked immunosorbent assay (ELISA) for human eNAMPT (AdipoGen), starting from 100 µl of samples. The amounts of eNAMPT were analyzed and their association with clinical features, prognosis, and therapy response was evaluated.

Result and discussion

Of the 42 samples, 25 showed values higher than 4 ng/ml on a range between 0 and 8 ng/ml (as maximum value), with 19 patients with an expression higher than 6. At the moment, follow-up is too short to have enough events to determine an effect of eNAMPT level on Progression Free Survival. However, eNAMPT level inversely correlates with CA125, a protein often elevated in blood of OC patients and used as biomarker ($p = 0.02$).

Conclusion

High levels of eNAMPT were observed in ascitic fluid samples suggesting that ascites - which is produced in large volumes in OC - is particularly enriched in eNAMPT, and that it is an excellent biological source to elucidate the role of eNAMPT.

EACR25-0804

A novel therapeutic approach for metastatic medulloblastoma

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Introduction

Medulloblastoma (MB) is the most common malignant brain tumor in children. It can disseminate within the central nervous system, particularly in Group 3 tumors, significantly worsening patient prognosis. While current therapies are effective for treating primary MB tumors, disseminated disease remains incurable, underscoring the urgent need for novel and more effective treatments. Recent findings suggest a potential association between a particular axis and MB progression. Based on these findings, we hypothesize that a molecule associated with this axis can be a prognostic marker in MB and its targeting can be a promising strategy to treat metastatic MB.

Material and method

To test our hypothesis, we analyzed the clinical relevance of the expression of a specific gene in MB patients using the R2: Genomics Analysis and Visualization Platform, specifically investigating its association with metastasis and patient survival. Additionally, we overexpressed this particular gene in ONS76 MB cells to evaluate its functional role in cell proliferation and migration. Using these cells, we developed an orthotopic mouse model to study the effects of gene overexpression in tumor invasion, dissemination, and survival. Moreover, we performed an in-silico drug screen to identify potential inhibitors for this molecule and validated their efficacy through both in vitro and in vivo assays.

Result and discussion

Our analysis revealed that elevated expression of this gene is significantly associated with dissemination and poorer survival outcomes in Group 3 MB patients. In vitro, gene overexpression enhanced cell proliferation and migration. In vivo, it resulted in greater MB tumor invasion and dissemination, as well as reduced survival in mice. Through our in-silico drug screen, we identified three inhibitors which were tested. Among these, a lead candidate emerged as the most promising, effectively suppressing the target expression, reducing cell proliferation and migration, and blocking its signaling pathway. Importantly, this compound also significantly reduced tumor burden in treated mice.

Conclusion

Our results suggest this molecule could be a prognostic marker for MB, particularly in patients with Group 3 tumors. Furthermore, the effective inhibition of this molecule in vitro and in vivo, shows potential as a therapeutic strategy for metastatic disease.

EACR25-0808

Changing the paradigm of liquid biopsy storage without cold chain. In-vitro validation of a preservative medium for circulating tumor HPV-DNA stabilization

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Introduction

Human papillomavirus (HPV) has been demonstrated to be released into the bloodstream as circulating tumor DNA (ctHPV-DNA) which may potentially used as prognostic and predictive biomarker for HPV-related malignancies including cervical cancer (CC) and head and neck squamous cell carcinomas (HNSCCs). Despite its potential clinical applications, the analysis of ctDNA for diagnostic procedures remains challenging due to its low concentration, fragmentation into short DNA lengths, blood hemolysis and the rapid degradation within a few hours if not properly preserved. In this study, we evaluated the preservative performance of eNAT® medium (Copan Italia, Brescia) for ctHPV-DNA in artificial human plasma samples under in-vitro setting.

Material and method

Serial dilutions of HeLa cells (ATCC*229) were prepared from a stock suspension (10^{-1} to 10^{-6}) in Dulbecco's phosphate-buffered saline (dPBS). HeLa DNA containing HPV 18 was extracted from 60 μ L of each dilution using NIMBUS platform (Hamilton). DNA quantification was performed with Nanodrop spectrophotometer and Qubit dsDNA High Sensitivity (HS) assay (Thermo Fisher Scientific). A qPCR titration assay (Allplex HPV-HR, Seegene) was subsequently conducted to determine the optimal DNA concentration that best mimicked the characteristics of ctHPV-DNA. HeLa DNA from the 10^{-2} dilution was selected as an inoculum for stability testing. 2:1 dilution ratio of artificial human (ah) plasma (Biochemazon) and eNAT® (ahPlasma:eNAT) was prepared in six replicates by mixing 360 μ L of artificial human plasma with 180 μ L of eNAT®. Into each replicate, 54 μ L of the 10^{-2} HeLa DNA dilution was inoculated, yielding a final dilution of 10^{-3} . DNA extraction, quantification and amplification were performed following the same protocol at baseline (T0) and after two weeks (T2w) of storage at room temperature (RT) of 25°C.

Result and discussion

Regarding ctHPV-DNA stability analysis, Hela DNA samples inoculated in a 2:1 dilution ratio of ahPlasma: eNAT exhibited an average Ct of 30,1 ($\sigma = 0,21$) at T0 and an average Ct of 30,2 ($\sigma = 0,5$) after 2 weeks, indicating consistent ctHPV-DNA preservation at RT (25°C) without the need of cold-chain storage.

Conclusion

Based on preliminary findings, eNAT® medium appears to be a suitable option for long-term storage and transport of human plasma samples for subsequent circulating tumor DNA molecular analysis, potentially facilitating the sample management avoiding the need of cold chain storage and shipment. Further investigations are ongoing to assess the preservation efficiency of eNAT® with additional circulating tumor DNA targets in a clinical setting.

EACR25-0847

Surface plasmon resonance for the detection of EGFRvIII in circulating extracellular vesicles from glioblastoma

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Introduction

Glioblastoma (GBM) is among the most prevalent and aggressive brain tumors, with a poor median survival rate despite advancements in diagnosis. The complexity of this cancer is due to the tumor heterogeneity, which makes patient follow-up and management particularly difficult. To overcome this issue, the identification of circulating biomarkers in liquid biopsies is crucial in GBM patients.

Material and method

A new method of liquid biopsy based on the analysis of the contents of extracellular vesicles (EVs) is currently emerging. Previous studies have shown that the truncated variant of EGFR (EGFRvIII), present in 40% of GBMs, is released in circulating EVs of patients' plasma. As EGFRvIII is constitutively active, it plays a role in tumorigenesis and contributes to chemoresistance. However, early detection of EGFRvIII in EVs derived from liquid biopsy is not suitable with clinical routine applications due to the complexity for isolating EVs from plasma with current technologies. Furthermore, they lack sensitivity for detecting biomarkers at low concentrations in patients' blood. To overcome these challenges, Surface Plasmon Resonance (SPR) is being explored as a real-time detection method. This approach enables the detection of EVs and their surface components without the need for extensive purification steps which makes a promising solution for biomarkers detection in clinical routine.

Result and discussion

Our preliminary technological developments on SPR method have permitted to functionalize the SPR sensor surface to capture EVs. Moreover, our optimized approach enhances the sensitivity of EVs biomarkers detection, such as CD63 and CD81, thereby overcoming

one of the main limitations of liquid biopsy analysis in clinical practice.

Conclusion

Our aim is now to improve the sensitivity of SPR technology for the detection of circulating biomarkers such as EGFRvIII in EVs derived from human glioblastoma. The early detection of very low concentrations of biomarkers in blood will be a major tool for monitoring patients.

EACR25-0849

Advancing Multi-Cancer Early Detection: High-Performance cell-free RNA Profiling with the Flomics Liquid Biopsy Platform

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Introduction

Liquid biopsies are increasingly important diagnostic tools for early cancer detection due to their high sensitivity and minimal invasiveness, with cell-free RNA (cfRNA) emerging as a promising biomarker. At Flomics Biotech we developed a high-quality cfRNA-Seq platform that profiles human plasma cfRNA in a robust and reproducible manner. Our platform combines optimized laboratory protocols for Next-generation Sequencing of plasma RNA with state-of-the-art bio-informatics and machine learning (ML) classification methods to identify cancer-related RNA biomarker signatures. Here we used our cfRNA-Seq platform in the LiquiDx pre-clinical study with the aim of developing a multi-cancer early detection test.

Material and method

We used our cfRNA-seq platform to profile the cfRNA in plasma samples from over 1,000 individuals (median age = 64 years) recruited in collaboration with 9 international clinical partners. The cohort consists of patients with colorectal, lung, breast, pancreatic or prostate cancer, or non-cancer diseases of the same organs, and healthy individuals. Early and late stage patients were recruited for each cancer type, with 22% of patients having stage I cancer. We isolated total RNA from 1 ml of plasma and sequenced at an average depth of 36M reads per sample with Illumina technology. Using the cfRNA-seq data we identified differentially expressed genes (DEGs), performed gene set enrichment analysis (GSEA), and trained an ML classifier to predict patient status and cancer tissue of origin. We employed a stratified 4-fold cross-validation strategy that maintains class balance across training and validation splits, and optimized the model's hyperparameters.

Result and discussion

We identified 114 DEGs ($|\log_{2}FC| > 1$, FDR < 0.05), with GSEA revealing enrichment of metastasis, inflammation, and proliferation-associated genes in cancer patient cfRNA profiles. For cancer vs healthy classification, our ML classifier achieves a mean area

under the ROC curve of 0.92 ± 0.01 , and a sensitivity of 83% at 90% specificity. For classification of different cancer types, at 90% specificity our ML classifier achieves a sensitivity ranging from 69% for breast cancer to 99% for prostate cancer. In contrast to other liquid biopsy-based technologies, our platform maintains a high performance level in detecting stage I cancer patients (80% sensitivity), reinforcing its great potential as a cancer early detection tool.

Conclusion

The Flomics cfRNA-Seq platform delivers high-quality cfRNA data and robust biomarker identification, demonstrating transformative potential in cancer early detection across multiple cancer types and stages, particularly in stage I where cancer detection is more challenging. The platform's high performance and minimal invasiveness position it as a revolutionary tool to improve global cancer diagnostics.

EACR25-0850

Non-Invasive Detection of Circulating mRNA Markers in Colorectal Diseases

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Introduction

Colorectal adenomas (CRA) are abnormal epithelial growths and key precursors to colorectal cancer (CRC). Their timely removal significantly reduces CRC risk, a major global health burden with nearly 1.9 million new cases annually. Early detection strategies, particularly non-invasive biomarkers, are critical for improving patient outcomes. This study aimed to identify and validate mRNA markers for CRA and CRC using a multi-phase approach.

Material and method

In the discovery phase, next-generation sequencing (NGS) was used to analyze transcriptome profiles in CRA (n = 16), incident CRC (n = 10), and cancer-free individuals (CFI, n = 10). We identified 10 genes deregulated between adenomas and adjacent tissues and 8 differentially expressed among tumor tissue, surrounding tissue, and healthy mucosa. For validation, we used RT-qPCR in an expanded cohort (CRA n = 48, CRC, and CFI n = 51). CRC patients were stratified by stage (n = 48 per stage). The most promising markers (CEMIP1, TACSTD2, SP5 and VWA2 specific to CRA, and KRT80, CLDN2, and CEMIP1 to CRC) were then analyzed in liquid biopsies (plasma and stool) from CRA (n = 30), CRC (n = 36), and CFI (n = 51). In CRC patients, plasma markers were assessed at diagnosis and

~10 days post-surgery to evaluate their potential to reflect tumor burden reduction.

Result and discussion

Multi-level validation confirmed differential expression of key mRNA markers in tissues and biofluids, supporting their non-invasive detection potential. Plasma and stool analyses showed significant expression changes, with certain transcripts decreasing post-surgery (CEMIP1), indicating responsiveness to tumor resection. These findings suggest that selected mRNA markers could serve as sensitive and specific biomarkers for early detection of CRA and CRC.

Conclusion

This study demonstrates the feasibility of transcriptomic biomarkers in liquid biopsies for non-invasive CRC screening. The integration of NGS-based discovery with RT-qPCR validation provides a robust framework for identifying clinically relevant markers. Future research should assess their diagnostic utility in larger, independent cohorts.

Supported by GAČR grant 22-05942S and the National Institute for Cancer Research (EXCELES Program, ID: LX22NPO5102) – Funded by the European Union – Next Generation EU.

EACR25-0868

The expression of the tight junctional protein complex Occludin (OCLN) and Zonula Occludens 1 (ZO-1) in human colon cancer and assessment of disease progression and therapeutic responses

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Introduction

Occludin (OCLN) is a membrane integral protein located at the apical membrane region of epithelial and endothelial cells. Together with its anchorage membrane subcoat protein, Zonula Occludens (ZO-1), it contributes to the formation and function of the tight junction and is key to the regulation of paracellular permeability and cell-cell adhesion. The role of the tight junction and its constitutive molecules in cancer are under intense investigation, with Occludin being postulated to have a possible role in colon cancer by defining a subclass of patients. In the present study, we investigated the expression pattern of Occludin together with ZO-1 and attempted to establish relevance in clinical colon cancer.

Material and method

A cohort of colon cancer tissues with matched normal (control) tissues were assessed for transcript levels of Occludin and Zonula Occludens-1 (ZO-1). Expression levels in relation with patient's pathological factors, tumour staging, prognosis and clinical outcome were evaluated.

Result and discussion

Colon cancer tissues exhibited an aberrant expression pattern for both Occludin and ZO-1, compared with normal tissues ($p < 0.001$ by Mann-Whitney U test). High levels of Occludin in colon tumours were associated with a shorter overall survival (OS), distant metastasis

free survival (DMFS) and disease-free survival (DFS) of the patients. Similarly, high levels of ZO-1 also showed a connection with survival outcomes (OS, DFS and DMFS). There was little association with local recurrence. Owing to their biological roles in maintaining the integrity of tight junctions, the expression of Occludin and ZO-1 was also considered in combination, it was found that patients with high expression of both Occludin and ZO-1 had a much poorer survival (OS 58.8%) than those without high expression (OS 85.7%) and those with only one of the proteins highly expressed (OS 70.8%). Further stratification of the patients by EGFR and Her2 status revealed that whilst EGFR status did not contribute to prediction, high expression of Her2 and Occludin identified the group with poorest overall survival (25%).

Conclusion

Expression of the Occludin/ZO1 complex is aberrant in human colon cancer with this aberrant pattern linked to clinical outcome. Moreover, Her2 status contributes to the survival model, implicating this complex as a putative prognostic in colon cancer.

EACR25-0887

Bridging Redox Biology and Immune Infiltrates for a Better Understanding of HER2+ Breast Cancer Outcomes

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Introduction

Understanding the factors that influence therapeutic response and prognosis is crucial for improving the management of HER2-positive breast cancer (HER2+ BC). Despite the success of neoadjuvant therapy (NAT), predicting pathological response and long-term outcomes remains a major challenge. Tumor-infiltrating lymphocytes (TILs) have emerged as immune biomarkers associated with response to NAT and prognosis. At the same time, redox biology plays a fundamental role in shaping the cancer cell phenotype and the tumor microenvironment. However, the extent of their impact on HER2+BC progression and treatment outcomes is still unclear. This study aims to investigate the interplay between immune infiltrates and oxidative stress in HER2+BC, integrating bioinformatics to identify redox-related genes linked to prognosis and immune modulation.

Material and method

Surgically resected specimens from 30 patients with early HER2+ BC who underwent neoadjuvant chemotherapy with trastuzumab and pertuzumab were analysed for TILs (hematoxylin-eosin) and the oxidative damage biomarker 3-nitrotyrosine (3-NT, immunohistochemistry). To explore the associations between the expression of redox-related genes with tumor microenvironment cell populations and patient survival, a bioinformatics study using the TCGA dataset and the GEPIA and TIMER2.0 platforms was performed.

Result and discussion

TILs score correlated positively with tumor size, both at diagnosis and after NAT. 3-NT scores correlated with tumor size at diagnosis and TILs score. Based on the associations identified between TILs, clinical features and oxidative damage, a bioinformatics analysis was conducted to explore redox-related genes that could be critical for HER2+BC prognosis. We identified 53 redox-related genes for which dysregulated expression is associated with survival, with 43 genes associated with higher hazard ratios (HR) and 10 with lower HR. Some of these genes are associated with a higher abundance of specific immune cell populations infiltration. Genes are mostly related to oxidative phosphorylation, response to oxidative stress, and regulation of transcription. Four main pathways are shared by some of the genes identified: HMOX1, TP53-mediated translational control, regulation of PTEN transcription, and VEGFA-VEGFR2 signalling.

Conclusion

This study provides novel insights into the interplay between redox biology and tumor immunity in HER2+ BC. The identification of redox-related genes associated with survival and immune modulation suggests potential prognostic biomarkers. Further research should validate these findings, paving the way for more precise and personalized therapies. Acknowledgements: Fundação para a Ciência e a Tecnologia (FCT) through projects DOI 10.54499/UIDP/04567/2020 and DOI 10.54499/UIDB/04567/2020 to CBIOS. B Inov Grant from SRSRA-OF 2024.

EACR25-0899

Age and Microenvironment Matter: When Survivin Becomes a Predictor of Poor Outcomes in Neuroblastoma

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Introduction

Survivin is a key protein involved in tumor progression and therapy resistance, yet its prognostic significance in neuroblastoma remains underexplored. This study aimed to evaluate the association between survivin expression and clinical outcomes in a cohort of neuroblastoma patients, with detailed analyses focusing on a subset of cases with malignant tumors.

Material and method

Clinical and pathological data from 66 patients diagnosed with neuroblastoma and treated at Pequeno Príncipe and Erasto Gaertner hospitals, Curitiba, Brazil, between 1990 and 2016 were collected. Immunohistochemical staining on tissue microarray slides was used to assess survivin expression in the nucleus and extracellular matrix/cytoplasm. Expression was quantified through color morphometry or hotspot scoring.

Result and discussion

In the total cohort, the median age at diagnosis was 17.5 months. Abdominal mass or pain (35.9%) was the most frequent clinical manifestation. Tumors were most commonly located in the adrenal gland (50.8%), with 42.3% classified as INSS stage 4. Bone marrow infiltration occurred in 27% of cases, residual recurrence

in 21.3%, and 30.3% of patients died from the disease. Survivin expression was observed in benign and malignant tumors, with significantly higher levels in malignant cases ($p < 0.001$). In the subset of 45 malignant tumors, high survivin expression was associated with progression (HR = 7.74, $p < 0.001$) and mortality (HR = 5.83, $p < 0.001$). Kaplan-Meier analyses demonstrated reduced progression-free and overall survival in patients with high survivin expression, particularly in those with metastatic disease or bone marrow involvement. Stratified analyses revealed distinct patterns of survivin expression and clinical impact across different patient subgroups. High survivin (score >2) was paradoxically associated with lower progression rates ($p = 0.043$) in patients <18 months, whereas in older patients, high survivin correlated with significantly higher progression ($p = 0.001$) and overall worse outcomes ($p = 0.003$). In patients with bone marrow infiltration, high survivin expression (>2) was associated with significantly worse survival outcomes ($p = 0.048$) and increased risk of progression ($p = 0.012$). These findings highlight survivin as a prognostic biomarker with context-dependent effects, emphasizing the need for tailored therapeutic strategies based on patient-specific tumor characteristics.

Conclusion

Survivin expression serves as a significant biomarker for neuroblastoma, correlating with aggressive clinical features and poor outcomes. However, its prognostic impact varies by age, metastatic status, and bone marrow involvement. These findings underscore the complexity of survivin's role in neuroblastoma and the necessity for further research to refine its application as a prognostic and potential therapeutic target.

EACR25-0972

Prognostic and therapeutic implications of tumor heterogeneity captured by longitudinal ctDNA in advanced high-grade serous ovarian carcinoma

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Introduction

Circulating tumor DNA (ctDNA) offers advantages in clinical trials and cancer treatment monitoring due to its minimally invasive nature and sampling accessibility. Beyond these benefits, ctDNA can provide a snapshot of tumor heterogeneity, and longitudinal measures can guide therapeutic strategies. While these benefits are evident in many cancer types, they are not well characterized for high-grade serous carcinoma (HGSC), the most lethal subtype of ovarian cancer. This study leverages real-world data from the longitudinal, multi-region, observational, prospective DECIDER trial to investigate the potential of longitudinal ctDNA analysis in advanced-stage HGSC.

Material and method

We collected 35 treatment-naïve plasma samples and 80 matched patient tissue biopsies from 35 patients. Plasma samples were sequenced using either whole-exome sequencing (WES) at 500x coverage or a 1000x targeted panel of over 500 cancer-related genes, while tissue samples were sequenced using either whole-genome sequencing (WGS) at 45x coverage or a 200x targeted panel. For longitudinal analysis, 150 plasma samples collected during primary treatment were sequenced with shallow whole-genome sequencing (sWGS) at a coverage of 0.4x from 30 patients. Mutational profiles were called using GATK4, and ichorCNA was used for longitudinal ctDNA analysis, providing ctDNA fractions and copy number profiles.

Result and discussion

Our results demonstrate that ctDNA captures tissue heterogeneity, revealing distinct clonal populations within individual tumors. Furthermore, ctDNA contains specific tissue mutational profiles, suggesting a complementary benefit to tissue biopsies. During primary treatment response monitoring, patients with decreasing ctDNA levels had better prognoses, while those with stable ctDNA fractions experienced shorter progression-free intervals. Notably, ctDNA levels indicated a greater reduction in patients who received chemotherapy plus bevacizumab compared to chemotherapy alone; however, this observation is limited by the small cohort size.

Conclusion

The ctDNA biopsy technique demonstrated advantages in both diagnosis and treatment monitoring for advanced HGSC patients, particularly in detecting genomic variants missed by tumor biopsies due to heterogeneity. Longitudinal ctDNA monitoring highlighted therapeutic responses, suggesting its potential as a dynamic biomarker for treatment efficacy. This study provides a foundation for larger investigations. We are expanding the cohort to further investigate the potential of ctDNA as a predictive biomarker for treatment response, and to explore its role in guiding personalized treatment strategies in HGSC. This expanded study will provide greater statistical power to validate the trends observed in this small cohort and enable more definitive conclusions regarding the clinical utility of ctDNA in managing HGSC.

EACR25-1009

Biomarkers for neo-adjuvant treatment in locally advanced rectal cancer: preliminary results from a comprehensive characterization of the biological features of good and poor response

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Introduction

Locally advanced rectal cancer (LARC) is treated with neoadjuvant therapy (NAT) followed by surgery. Watch & wait (w&w) strategies pursuing organ preservation are starting to be adopted for patients responding to NAT. Magnetic Resonance Imaging (MRI) plays a pivotal role in the response assessment. However, this approach alone is not optimal for identifying responding patients and novel biomarkers to complement clinical response evaluation are needed. We report preliminary results from a larger study proposing a comprehensive characterization of LARC patients to describe biomarker signatures of response to NAT that could help to select patient who could benefit from w&w strategies.

Material and method

Upon signature of the informed consent, biological specimens and clinical data were collected before and after NAT. Blood circulating immune cells, MRI diffusion weighed imaging (DWI) parameters and the gut microbiome were evaluated. Patients were grouped according to pathological Mandard Tumor Regression Grade (TRG) as responders (R; TRG 1-2) and non-responders (NR; TRG 3-5). Differences between sexes were assessed.

Result and discussion

By February 2025, 55 patients were included, 29 had TRG assessment. The immune cell evaluation in R showed an increase of the early myeloid suppressor (CD11b+, CD14-, CD66b-, CD33+, HLADR-) and Fc ϵ RI α expressing cells from basal to post-NAT sample, while they remained unchanged in NR. PDL1 expressing myeloid cells did not change in R but decreased in NR. Regarding lymphoid cells, R showed higher levels of CD4+PD1+ cells after NAT. Additionally, NR showed a significant increase in CD8+CD4- cells and KLRL1+ CD28- subpopulation from basal to after NAT, which was not observed in R. MRI-based assessment showed high sensitivity but low specificity to detect complete responses. In DWI parameters, the change in the skewness of the signal from basal to post-NAT MRI was higher in R. The basal microbiome profile and diversity indexes were similar in R and NR, while particular genus, such as the butyrate producing bacteria genus Blautia was increased in R. Sex related differences were assessed. Basal CD3+, CD4+ and KLRL1-CD28+ expressing CD8+ cells were elevated in females, while total myeloid cells (CD11b+) were decreased. Basal MRI skewness and kurtosis were lower in females. Finally, female feces were enriched for particular bacteria genus such as Eisenbergiella and Frisingicoccus.

Conclusion

These preliminary results suggest distinctive biological and radiological characteristics of NAT-responders LARC patients, as well as sex-related differences. This study is ongoing and further analyses will clarify the importance of these biomarkers.

EACR25-1069

Sex-Based Differences in Colorectal Cancer Patients: a Multi-Omics Perspective on Primary and Surrogate Tissues

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Introduction

Colorectal cancer (CRC) stands out as a global health challenge, ranking as the third most common cancer and the second in mortality worldwide. Studies on microRNA (miRNA) and metabolite profiles in human feces and plasma offer promising insights into physiological and pathological conditions and some of them show sex-dependent expression patterns. However, an extensive evaluation of such patterns in relation to CRC is lacking. In addition, the emerging concept of a 'sex hormone-gut microbiome axis' further suggests that sex-specific variations in gut microbiota composition may contribute to differential cancer susceptibility. This study investigates the sex-specific variations between CRC patients and healthy individuals by integrative analysis of fecal miRNA and metagenome profiles and plasma metabolome.

Material and method

Multi-omic profiles (transcriptome and metagenome) from fecal samples of 335 healthy individuals ($F = 213$, $M = 142$) and 332 CRC/adenoma patients ($F = 132$, $M = 200$) from four different European cohorts were analyzed. Plasma metabolomic data from two of these cohorts were also available. In addition, miRNA and gene expression data from tumor/adenoma tissues matched with adjacent mucosa ($n=154$) were integrated. Public data from Roadmap Epigenome Project, GTEx, curated MetagenomicData, and GEO were also integrated.

Result and discussion

Significant sex-based differences in fecal miRNA expression and microbiome composition were observed in healthy subjects and CRC patients. Distinct patterns were also identified in circulating metabolites that correlated with sex-specific disease manifestation. Specifically, 7 fecal miRNAs, 15 microbial species, and 11 circulating metabolites showed significantly different levels among healthy men and women under 50 years (adj. $p < 0.05$). Moreover, 37 miRNAs, 8 bacteria and one circulating metabolite were observed to be

differential expressed between healthy women stratified by age, including *Phocaeicola massiliensis*, acylcarnitine 13:0 and miR-200b-3p, a well-known CRC-related miRNA, whose levels were increased in young women with respect to both men and postmenopausal women. In CRC patients, 43 fecal miRNAs, 15 microbial species, and 4 circulating metabolites were associated with different levels in men compared to women (adj. $p < 0.05$). Of interest, miR-200b-3p was downregulated in CRC females compared to CRC males though without any significant evidence.

Conclusion

This study provides evidence of putative sex-based differences in molecular and microbial profiles associated with CRC. Further investigation is currently ongoing to fully elucidate the interaction between specific miRNAs, bacterial communities and metabolites, particularly focusing on the role of estrogens in female subjects. Understanding these sex-specific differences could have important implications for early cancer detection and personalized treatment approaches.

EACR25-1089

Expression of the BMP antagonist Noggin in gastric cancer clinical cohort and its association with drug treatment

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Introduction

Noggin is a secreted antagonist that regulates bone morphogenetic protein (BMP) signalling. Our recent study showed that Noggin promoted the proliferation of gastric cancer cells through upregulation of EGFR. Together with the profound role played by BMPs in stomach development and gastric cancer, further study of their therapeutic potential is provoked. In the present study, we aimed to explore the impact of Noggin on patients' responses to neoadjuvant chemotherapy and to investigate cancer cells' responses to chemotherapy drugs *in vitro*.

Material and method

The transcript level of Noggin was examined in a clinical cohort comprising tumour tissue and paired adjacent normal gastric tissue from patients who underwent neoadjuvant chemotherapy, utilising real-time PCR. Overexpression and knockdown of NOG in gastric cancer cell lines were conducted using lentiviral vectors. The expression of the NOG gene was confirmed at the mRNA and protein levels. Different chemotherapy drugs, including 5-fluorouracil, Oxaliplatin, Docetaxel and Ebirubucin, were tested to examine Noggin's impact on the drug response.

Result and discussion

Noggin transcript levels were higher in normal adjacent tissues ($n = 86$, median = 162.2), $p < 0.001$ compared to its expression (median = 21.9) in tumour tissues ($n = 87$). Higher levels of Noggin expression appeared to be associated with short overall survival (OS), with a log rank $p = 0.095$. A multivariate analysis revealed that Noggin, EGFR and HER4 were associated with poor response to the neoadjuvant chemotherapy. Interestingly,

further analysis showed that higher Noggin expression was significantly associated with poor OS in those patients with HER2 low expression tumours ($p = 0.039$). Overexpression of Noggin in AGS cells presented a good response to Docetaxel and Oxaliplatin similar to the control, but remarkably less responsive to the treatment with 5-Fu.

Conclusion

Higher expression of Noggin in gastric cancer was associated with poor response to neoadjuvant chemotherapy and also shorter OS in those patients with HER2-negative tumours. Overexpression of Noggin resulted in a resistance to 5-Fu.

EACR25-1091

AKT3 as a candidate biomarker of platinum response in epithelial ovarian cancers

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Introduction

Globally, ovarian cancer represents the 8th most common cancer in women. The standard treatment involves surgical intervention, followed by platinum- and taxane-based chemotherapy. However, patients typically relapse and succumb to the disease within 5 years from diagnosis. A significant advancement in patients' survival has been observed with the integration of PARPi into clinical practice. However, the absence of biomarkers to predict platinum response remains a limitation, as platinum sensitivity is a crucial criterion for determining patients' eligibility for PARP maintenance therapy. AKT isoforms (AKT1, AKT2, AKT3) are emerging as interesting prognostic markers and potential targets in EOC due to their distinct roles in cancer biology. In this study we examined the expression of these isoforms at the mRNA level in relation to cisplatin resistance in 11 EOC cell lines of different histotypes.

Material and method

EOC cell lines were cultured in 96 well plates and exposed to cisplatin (range 5–160 μ M) for 24 hours. Thereafter, the cell viability was evaluated by the Presto Blue method, and the IC₅₀ was subsequently determined. From each cell line, RNA was extracted, and the expression levels of AKT isoforms were evaluated through RT-ddPCR. Cell lines genomic data were retrieved from the CCLE database and HRD status determined based on the presence of mutations in genes that confer a Genomic Instability Score (GIS) > 42 (Barnicle A. et al. *Genome Med.* 2024 Dec 18;16:145).

Result and discussion

The results demonstrated that AKT1 and AKT2 exhibited no correlation with cisplatin response, while AKT3 exhibited a strong linear correlation with IC₅₀ values ($R^2 = 0.72$; $p = 0.001$), suggesting an isoform-specific role for AKT3 in mediating cisplatin resistance. AKT1 and AKT2 were overexpressed in HGSC cell lines, while AKT3 expression remained consistent across different histotypes, showcasing its potential as a biomarker for

platinum response in all EOC. Furthermore, AKT3 was significantly under-expressed in HRD cell lines compared to HRP ($p = 0.01$), suggesting its potential as a biomarker not only for platinum resistance but also as a surrogate for HR status.

Conclusion

This study has identified AKT3 as a promising biomarker capable of predicting response to platinum and PARPi therapy. It may also represent a new and targetable molecule for patients resistant to chemotherapy, who have limited treatment options and a poor prognosis.

EACR25-1131

Development of tumor-specific EV-miRNA signatures to improve lung cancer early detection (EV-miR-Test)

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Introduction

Lung cancer (LC) is the deadliest cancer worldwide, causing ~2 million deaths annually. The two primary subtypes are non-small-cell lung cancer (NSCLC, ~85%) and small-cell lung cancer (SCLC, ~15%). Due to late-stage diagnoses and the lack of effective early screening, LC has a low 5-year survival rate of less than 15%. Liquid biopsies have emerged as a promising, non-invasive alternative to tissue biopsies, analyzing circulating tumor cells (CTCs), cell-free DNA (cfDNA), and extracellular vesicles (EVs) in bodily fluids. EVs, abundant and stable carriers of molecular cargos like microRNAs (miRNAs), are critical for understanding cancer biology as they regulate cancer development, progression, and resistance to therapies. Additionally, cell-free miRNAs (cf-miRNAs) are highly stable and tissue-specific, making them promising candidates for LC diagnosis and prognosis.

Material and method

To identify a tumor-specific EV-miRNA signature, a global miRNA expression profiling was performed on early-stage NSCLC patients' plasma samples compared to healthy controls. EVs were isolated using ultracentrifugation and immunoaffinity beads, followed by characterization via Nanoparticle Tracking Analysis and Immunoblotting. Epithelial cell-specific EVs (EP-EVs), enriched for EpCAM+ markers, were captured using an EpCAM antibody. This profiling method was extended to compare miRNA signatures in tumor and normal lung cells, further distinguishing epithelial-origin (EP-EV-miRNAs) from inflammatory-origin (TME-EV-miRNAs). Tumor-specific EV-miR signature was validated through Calibration, Validation, and Clinical groups, while functional assays investigated EV-miRNAs roles in NSCLC cell proliferation, migration and invasion.

Result and discussion

A new EV-miRNA signature was identified, distinguishing LC patients from healthy individuals. The origin of LC-derived EV-miRs (epithelial vs. inflammatory) was elucidated. The analysis of the functional role of EP-EV-miRs and TME-EV-miRs in NSCLC provided insights into their involvement in cancer progression, revealing that dysregulated EV-miRNAs enhanced cell proliferation and invasion in NSCLC cell lines compared to controls.

Conclusion

EV-miRNA could serve as non-invasive biomarkers for monitoring LC progression over time. Developing an accurate, tumor-specific EV-miR Test may reduce false positives in NSCLC screening and refine risk assessment, especially for individuals selected for LDCT screening. Clarifying EV-miRNA origins and functions could lead to an effective early detection test for NSCLC, improving diagnosis, survival rates, and treatment outcomes while potentially lowering healthcare costs.

EACR25-1189

Unraveling the Impact of Genetic Ancestry on Breast Cancer Neoadjuvant Chemotherapy Response: A Comprehensive Analysis

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Introduction

Breast cancer (BC) presents a public health challenge, particularly with low response rates to neoadjuvant chemotherapy (NAC). Colombia's diverse lineage includes indigenous American (IA), European, and African American (AA) ancestries. Genetic ancestry influences gene expression related to tumor risk, progression and drug metabolism. However, the impact of ancestry on NAC response is unclear. This study aimed to predict NAC effectiveness by exploring gene expression patterns, genetic ancestry, and survival factors linked to NAC non-response in Colombian women with invasive BC.

Material and method

An integrative transcriptome, and survival analysis was conducted on 144 samples of BC female patients belonging to Colombian NCI population and candidates for NAC. Patients either responded or did not respond to NAC. To estimate genetic ancestry, genotyping of 106 ancestry informative markers was performed from non-tumoral DNA. Molecular intrinsic subtypes were classified into Luminal A, LuminalB/Her2+, LuminalB/

Her2+, HER2 enriched, and TNBC. Further prognostic validation was conducted in other cohorts. Analyses were performed comparing ancestry as a covariate in differential expression model.

Result and discussion

Non-responders had a higher proportion of advanced tumors and significantly lower survival rates than responders. Subtype-specific variations in European and Indigenous American ancestry proportions were associated with treatment response. Non-responders in Luminal B displayed higher IA ancestry. Subtype grouping with ancestry as a covariate revealed distinct transcriptional profiles and pathway activation patterns, particularly in HER2-enriched and Luminal A subtypes, suggesting the molecular mechanisms of chemotherapy resistance. Key oncogenic differentially expressed genes (DEGs) were identified, including FGF10 and WT1 in Luminal A and HLF in Luminal B/HER2-positive patients. Prognostic validation confirmed the relevance of DEGs, such as WT1, CLEC3A, PRRC1, and TPD52, in BC. Higher levels of IA ancestry were associated with varying survival trends across subtypes. This highlights the prognostic significance of tumor stage, identifies distinct pathway activation patterns associated with chemotherapy resistance and genetic ancestry, and identifies subtype-specific oncogenic DEGs.

Conclusion

This study provides valuable insights into chemotherapy response in Colombian BC patients by integrating clinical, genetic ancestry, and molecular data. This study underscores the importance of examining diverse populations to gain new insights and reduce disparities in BC treatment outcomes. This study stresses the importance of tailored treatment approaches and calls for additional validation through larger studies involving multiple ancestries as well as functional research to enhance precision oncology efforts across diverse groups.

EACR25-1212

Predicting Second-Line Chemotherapy Outcome in Advanced Pancreatic Cancer: A Machine Learning Approach Using PBMC-Based Biomarkers - Translational Findings from the PREDICT Trial

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Introduction

Pancreatic ductal adenocarcinoma (PDAC) remains one of the deadliest cancers, especially in advanced stages. The PREDICT trial (NCT03468335), a phase IIIb/IV study, aimed to predict second-line treatment success for advanced PDAC (aPDAC) using clinical data profiles. This translational research explored how first-line chemotherapy (CTX) response could predict second-line CTX outcome, using PBMC-based liquid biomarkers and machine learning (ML).

Material and method

The study stratified patients into two groups based on short or long second-line CTX (Nal-IRI/5-FU/FA) time to treatment failure (S-/L-TTF2; n = 10 each) after Gem-nabPac first-line CTX. RNA profiling of microdissected PDAC tissue used NanoString™ PanCancer IO360. Differentially regulated genes guided PBMC screening from second-line CTX-naïve patients at protein (flow cytometry, FC) or RNA (RT-qPCR) levels. FC data underwent R-based single-cell clustering with ML-based back-gating of most differential clusters (HyperGates, HGs). Classical ManualGates (MGs) were also created. Feature selection used Weka-based WrapperSubsetEval with eight classifiers. The best-performing classifier/ subset was used for binary classification (S-/L-TTF2 and S-/L-OS) on training (n = 66) and validation (n = 16) PREDICT datasets. This subset was further validated in an external (n = 30) cohort with different first-line and second-line treatment regimens.

Result and discussion

Transcriptome analysis revealed increased inflammation, immune cell activation/infiltration (e.g. activated CD8 T cells), and immune exhaustion in L-TTF2 compared to S-TTF2 tumors. Therefore, circulating PBMCs from second-line CTX-naïve aPDAC patients were screened. Analysis of eight FC panels (19 candidates) revealed 1198 differential clusters with corresponding HGs and 881 MGs. Feature selection, combining FC with RT-qPCR results and clinical parameters, identified a best performing minimal predictive signature (TTF2Pred) of 5 HGs and 2 MGs for 7 PBMC surface protein markers (CXCR4, CD8, CD4, CD62P, CD307b, CD45, CD121b). ML using kernel logistic regression successfully predicted S- and L-TTF2 binary groups (ROC-AUC > 0.90 and accuracy > 87% for training/validation). Further, TTF2Pred showed prognostic value for S- and L-OS binary class prediction (ROC-AUC = 0.80, accuracy = 75%). External validation confirmed performance, revealing highest specificity (Score = 0.96) for patients receiving first-line Gem-nabPac CTX (ROC-AUC = 0.86, Accuracy = 90%).

Conclusion

This study identified a favorable tumor immune micro-environment in L-TTF2 aPDAC patients, characterized by CD8 T cell-inflamed ("hot") tumor tissues prior to second-line CTX. A 7-marker circulating PBMC-based liquid biomarker panel was developed for early prediction of second-line CTX success after first-line Gem-nabPac CTX in aPDAC patients. These findings aim to advance personalized treatment strategies for aPDAC patients.

EACR25-1235

Exploring cfDNA copy number profiles to characterize disease stages in pancreatic adenocarcinoma

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Introduction

Pancreatic ductal adenocarcinoma (PDAC) is a leading cause of cancer-related mortality due to its aggressive biology and late-stage diagnosis. Surgery offers the only chance of cure, yet early recurrence by undetected systemic disease often undermines its benefit. Additional biomarkers are needed to improve prognostication and guide treatment strategies. Circulating tumor DNA (ctDNA) offers a non-invasive method of disease characterization. This study investigates circulating free DNA (cfDNA) copy number profiles across PDAC stages using shallow whole genome sequencing (sWGS).

Material and method

We analyzed 121 cfDNA samples from 54 PDAC patients using sWGS. Copy number profile abnormality (CPA) scores were determined using WISECONDOR, and tumor fractions (TF) were assessed with ichorCNA. We evaluated the ability of these markers to distinguish between resectable, borderline/locally advanced, and metastatic PDAC, comparing their performance to CA19-9 levels and imaging.

Result and discussion

Patients with resectable tumors (n = 27) exhibited significantly lower CPA scores (0.65 ± 0.35 vs 3.47 ± 1.7 ; $p < 0.0001$) and TF ($3.6 \pm 1.9\%$ vs $15.1 \pm 8.0\%$; $p = 0.0002$) compared to those with metastatic disease (n = 8). Patients with borderline/locally advanced tumors (n = 13) and those found to have early metastatic disease diagnosed during exploratory surgery (n = 6) had CPA (0.61 ± 0.21) and TF ($3.3 \pm 1.1\%$) values similar to resectable cases. Both markers showed no clear correlation with CA19-9 but, in some patients, discriminated better between disease stages than CA19-9.

Conclusion

sWGS of cfDNA offers a cost-effective, non-invasive tool that identifies patients with metastasized PDAC and may serve as a complement to CA19-9 and imaging for disease characterization. In this small cohort, markers could not predict unresectability. Further investigations are underway to determine whether specific genomic alterations correlate with prognosis and could aid in detecting early metastatic disease.

EACR25-1236**Elevated monocyte-lymphocyte ratio as a prognostic and predictive biomarker of poor response to radio-immunotherapy in advanced cancer: correlation with immune and metabolic dysregulation**

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Introduction

Growing evidence indicates that the myeloid/lymphoid imbalance plays a pivotal role in the polarization (pro- or anti-tumor) of the immune response following radiation therapy (RT). The monocyte-lymphocyte ratio (MLR) – determined from routine blood count – is a readily accessible inflammatory biomarker that has exhibited promising efficacy in predicting the response to immunotherapy, but its clinical adoption is hindered by its apparent lack of specificity.

Material and method

We prospectively collected sequential paired tumor and blood samples from pan-cancer patients treated in two clinical trials (SABR-PDL1 NCT02992912; ABIMMUNE NCT03212469) that evaluated combinations of immunotherapy with concomitant RT. Samples were obtained i) at baseline, ii) after the start of immunotherapy, before RT, and iii) after RT. Patients from the SABR-PDL1 trial were divided into two cohorts: Discovery and Validation #1. ABIMMUNE was used as a second, independent, Validation cohort (#2). The Discovery cohort was subjected to a comprehensive integrative analysis that encompassed RNA sequencing of biopsies, as well as proteomic and metabolomic analysis of paired serums.

Result and discussion

We collected sequential blood and/or tumor data from N = 182 patients (Discovery N = 55; #1 N = 80; #2 N = 47). We confirmed that the baseline MLR was predictive of treatment response in the Discovery cohort with an AUC = 0.68 (P = 0.0029). A baseline MLR threshold of 0.75 effectively identified patients at risk for reduced PFS (log-rank Hazard Ratio (HR) = 2.6, 95% CI [1.1-6.2]; Pval = 0.0014) and OS (HR 4.2, 95% CI [1.3-13.0]; Pval < 0.0001). Unresponsive patients had a significant increase of MLR after radiotherapy (p = 0.0009, compared to baseline). Using the 0.75 threshold in Validation cohorts #1 and #2, we confirmed the predictive value of baseline MLR for PFS (#1: HR 1.9, 95% CI [1.1-3.4]; Pval = 0.0054; #2: HR 2.7, 95% CI [1.2-6.1]; Pval = 0.0017) and OS (#1: HR 2.9, 95% CI [1.5-5.7]; Pval < 0.0001; #2: HR 3.1, 95% CI [1.5-6.7]; Pval = 0.0004). An elevated MLR was linked to tumor dysregulations in pathways involved in inflammatory response, myeloid differentiation, lymphocyte activation and metabolism, including bile acid and fatty acid metabolisms. The proteomic analysis of serums showed that the MLR correlated with >30 inflammation-related chemokines, among which CXCL8, TGFA, TGFB1, FSTL3 and CCL23 (all p < 0.0001). The MLR strongly correlated with circulating IL6 (p < 0.0001) and CSF1 (p < 0.0001). Last, the MLR reliably reflected the

significant dysregulations characterizing unresponsive patients regarding circulating metabolites: tryptophan derivatives, bile acids and carnitine metabolites.

Conclusion

In advanced cancer, the MLR is strongly linked to a detrimental inflammation-based loop that feeds a tumorigenic myelopoiesis with the sustained production of IL6, CSF1 and suppressive metabolites. A baseline MLR > 0.75 is predictive of pan-cancer poor prognosis.

EACR25-1274**Characterization of miRNA profiles in different subtypes of colorectal adenomas in tissue and fecal samples**

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Introduction

Colorectal cancer (CRC) develops over a long time, often arising from precursor lesions which can progress to malignancy. While genetic aberrations driving to CRC are well-known, alterations in microRNA (miRNA) expression within these precursor lesions are less explored, despite their potential as biomarkers. Using transcriptomic data, we aimed to provide a comprehensive characterization of the miRNA profiles in tissue from non-advanced (nAA) and advanced (AA) adenoma and matched adjacent mucosa samples. We also investigated whether the identified tissue altered profiles are reflected in stool miRNA levels, which can represent non-invasive biomarkers for cancer prevention.

Material and method

RNA-seq and sRNA-seq were performed in adenomas tissues (nAA = 20, AA = 30) and adjacent mucosa collected at colonoscopy in a cross-sectional study. sRNA-seq was also performed in stool from the same patients and from colonoscopy-negative controls (n = 87). Paired differential expression (DE) analyses were performed comparing nAA and AA to their adjacent mucosa to identify subtype-specific alterations at miRNA (DEMs) and gene (DEGs) level. Correlations analyses were performed between the expression levels of DEMs and their validated targets, as well as functional enrichment analyses. Samples from an additional cohort recruited in the CRC screening program (nAA = 29, AA = 80 and controls = 54) will be analyzed to validate findings.

Result and discussion

In adenoma tissues, 647 miRNAs were detected, while 345 were identified in stool samples, with 116 of them detected in both biospecimens. DE analyses revealed 112 miRNAs and 1,803 genes commonly dysregulated across adenoma subtypes. Compared to nAA, which showed 33 DEMs and 547 DEGs, AA showed a higher

dysregulation, with 162 DEMs and 1,950 DEGs, mainly upregulated. Functional miRNA enrichment analyses highlighted dysregulation in several pathways, including inflammatory response, G2M checkpoint inhibition and EMT programs. A set of genes related to these pathways and targeted by upregulated miRNAs was found to be downregulated in AA tissues. In stool samples 60 miRNAs were uniquely DE in AA, while 47 were specific to nAA. However, 28 miRNAs were commonly altered in both subtypes with seven showing a progressive dysregulation from healthy to nAA and AA. Hierarchical clustering on nAA-DEMs in stool identified two distinct clusters of patients reflecting the adenoma subtype. Finally, in AA, four miRNAs were dysregulated in both tissue and stool samples, particularly miR-10a-3p resulted constantly upregulated, respectively compared to adjacent mucosa and stool of healthy individuals.

Conclusion

The miRNA landscape of precancerous lesions reflects the heterogeneity between nAA and AA, both in tissues and stool samples. Further analyses will refine our characterization, focusing on other features of adenomas, such as villosity and dysplasia.

EACR25-1284

Differential protein expression in platelets collected from glioma patients and healthy controls: A Comparative Study

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Introduction

Glioblastoma (GBM) is the most aggressive and most common type of primary brain tumor in adults. Standard multimodal treatment fosters event free survival for approximately 7 months and extends overall survival (OS) to only 15 months. Due to the risk of biopsy complications, the histology verification of the tumor is not always possible. Therefore, in many cases the treatment is based on MRI findings and exclusion of other malignancy in CAT. A confirmative liquid biopsy would provide additional and useful support for clinical decision making and patient counselling. To date no liquid biopsy for GBM is available. Platelets interact with tumor cells in multiple ways, including direct contact, release of cytokines, and uptake of tumor-derived extracellular vesicles (EVs). These interactions cause modifications in platelet RNA and protein profiles, yielding tumor educated platelets (TEPs) that reflect specific tumor characteristics. Our study aim to use proteomic analysis to identify differential protein expression in platelets collected from glioma patients

compared to healthy controls, identifying potential biomarkers for glioma diagnosis and therapeutic monitoring

Material and method

Platelet samples were collected from 14 glioma patients and 14 matching healthy controls. Platelets were isolated using differential centrifugation to minimize contamination with other blood components. Protein extraction and quantification were performed, followed by analysis using mass spectrometry-based proteomics. Protein identification and quantification were conducted with Label-Free Quantification (LFQ-DIA) techniques, and differentially expressed proteins were statistically validated. Bioinformatic tools were employed to analyze pathways and functional annotations related to identified proteins.

Result and discussion

Out of the 3,444 proteins identified and quantified, 463 were differentially expressed between glioma patient platelets and those from healthy controls. Reactome Pathway analysis, gene ontology enrichment, and protein-protein interaction studies revealed that these proteins are involved in key pathways, including vesicle-mediated transport, Cyclin B: Cdk1 complex inactivation, apoptosis, lipid metabolism, cell cycle regulation, glutathione synthesis and recycling, platelet activation, signaling, aggregation, and overall signal transduction.

Conclusion

The study identifies a distinct proteomic profile in platelets from glioma patients compared to healthy individuals, suggesting that platelets in glioma may harbor unique proteins characteristics related to tumor biology. These findings support the potential of platelets as a source of biomarkers for glioma detection, confirmation of diagnosis and monitoring. Future studies with larger cohorts are warranted to validate these proteins as diagnostic markers and explore their mechanistic roles in glioma progression.

EACR25-1357

Investigation of miRNAs expression in Bulgarian patients with early-stage and advanced laryngeal cancer as a prognostic biomarker

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Introduction

Laryngeal squamous cell carcinoma (LSCC) is an aggressive malignancy with poor prognosis, which despite modern treatment protocols, novel molecular markers are required to improve survival. miRNAs, small

non-coding RNAs, are involved in various pathological processes, as well as hypoxia. The aim of the study was to investigate the expression levels, discriminative pattern and correlation between hypoxic miRNAs as a biomarker in plasma distinguishing early stage from advanced laryngeal carcinoma.

Material and method

The expression of miR-144-3p, a hypoxia-related miRNA, was evaluated in 20 early-stage tumours and corresponding normal laryngeal samples, whereas miR-31-3p and miR-196a were evaluated in 20 early-stage plasma samples compared with plasma samples from healthy persons by quantitative real-time polymerase chain reaction (qRT-PCR) analysis. Total RNA isolation, reverse transcription and real-time qPCR were performed with miRNeasy kit, TaqMan™ MicroRNA Reverse Transcription Kit, and TaqMan™ Gene Expression Assay (Applied Biosystems). SPSS v19 (IBM) was used in statistical analysis, and p-value less than 0.05 was taken as significant.

Result and discussion

In the current study, we observed increased expression levels of tumor suppressor gene miR-144-3p (44%, p=0.01) in tumor tissue in comparison with paired normal tissue. In advanced laryngeal tumours, data showed in previous study, miR-144-3p was significantly dysregulated in peritumor mucosa with a pattern of expression consistent with paired tumor samples thus revealing a signature of field cancerization. In early-stage laryngeal tumours (T1, T2) miR-144-3p expression did not reveal such association. Investigation of miR-31-3p and miR-196a expression levels in plasma samples revealed a significant dysregulation of miR-31-3p (69.1% overexpressed, p=0.01) compared to levels of healthy peoples. The ROC curve analysis showed that miR-31-3p can distinguish group of patients with laryngeal tumor from healthy peoples with sensitivity of 66.7% and specificity of 72.2% (AUC = 0.706; 95% CI: 0.523-0.888; p = 0.045) whereas miR-196a did not.

Conclusion

The current findings suggest that miR-31-3p potentially could take a key role as a noninvasive biomarker not only in advanced but in early-stage laryngeal cancer as well. These results may contribute to future studies on elucidating the mechanisms of contribution of hypoxic miRNAs to LSCC.

Project BG-RRP-2.004-0004-C01, group 3.1.5, "Strategic research and innovation program for development of Medical University—Sofia"; SMN Grants: D-190/03.08.2023; D-302/18.12.2023

EACR25-1380

Unraveling Tumor Genetics: The Role of cfDNA Whole-Exome Sequencing for Tracking Tumor Evolution and Treatment Response

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Introduction

Cancer remains the second leading cause of mortality worldwide, emphasizing the urgent need for non-invasive methods to rapidly characterize tumor profiles and monitor treatment responses. Whole-exome sequencing (WES) of cell-free DNA (cfDNA) via liquid biopsy has emerged as a powerful approach to detecting clinically relevant genetic alterations and identifying novel biomarkers associated with therapeutic resistance. This study leverages WES of cfDNA in patients with advanced metastatic cancers to explore its potential in personalized oncology.

Material and method

cfDNA was extracted from 72 samples of 53 patients diagnosed with metastatic cancers across 11 distinct histological subtypes. The samples were prepared using the Illumina® Cell-Free DNA Prep Kit and sequenced on the NovaSeq Xplus platform, achieving sequencing coverage between 163X and 485X. Secondary analysis was conducted using Illumina Connected Analytics®, while variant annotation was performed using Illumina Connected Insights® software. Only clonal mutations with a variant allele frequency (VAF) above 1% were considered, and germline variants (MAF > 1%) were excluded. Variants with allele frequencies between 40-60% or > 95% were classified as "putative germline." To evaluate treatment response over time, longitudinal sampling was conducted in selected patients.

Result and discussion

cfDNA extraction yielded an average input of 119 ng (range: 7.26-792 ng). To date, 35 samples, at different timepoints, from 20 patients have undergone WES analysis, revealing 330 likely pathogenic/pathogenic variants across 230 genes, with an average of nine mutations per sample. The identified mutations provided insights into the aggressive progression observed in these patients. Notably, 14 patients were longitudinally monitored, enabling the correlation of cfDNA-derived mutation profiles with imaging-based assessments of treatment response. In these cases, liquid biopsy results demonstrated evolving mutational landscapes that aligned with either tumor progression or regression. The ability to track these genetic changes in real-time underscores the value of cfDNA WES in precision oncology.

Conclusion

This study highlights the potential of WES of cfDNA as a robust tool for detecting key genetic alterations in metastatic cancers and dynamically monitoring tumor evolution. The identification of driver mutations and resistance-associated alterations provides critical insights that can guide therapeutic decision-making. By facilitating a deeper understanding of cancer biology, this approach supports the development of more targeted and

effective treatments for metastatic patients, ultimately advancing personalized oncology strategies.

EACR25-1390

Establishing a plasma protein signature for clear cell renal cell carcinoma

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Introduction

Clear cell renal cell carcinoma (ccRCC) is the most lethal urological malignancy, with 30% of patients presenting with de novo metastasis in the clinic and an additional 30% further developing recurrent metastasis during disease progression. Current diagnostic and prognostic methods rely primarily on histopathological characterization, which limits early detection and intervention for recurrent metastasis.

Material and method

This study employs liquid biopsy to enhance prognostication and clinical management of ccRCC patients across two sub-cohorts to identify biomarkers for localized and metastatic ccRCC, utilizing longitudinal plasma proteomics. We collected plasma samples from ccRCC patients before surgery (PRE-OP), after surgery (POST-OP), and at longitudinal follow-up time points. Using data-independent mass spectrometry, we analyzed the depleted plasma samples to identify biomarker candidates. Next, we employed network analysis to identify protein modules potentially contributing to the disease phenotype. Finally, we assessed the utility of our candidate proteins as biomarkers.

Result and discussion

Our network analysis approach identified protein modules enriched in matrix remodeling and metabolic dysregulation pathways, perpetuating the ccRCC phenotype. First, we identified five proteins involved in angiogenic and immune responses as biomarker candidates for localized ccRCC. These proteins demonstrated high diagnostic performance, discriminating pre-operative samples from post-operative samples in our localized cohort. Next, we identified six proteins previously implicated in cancer metastasis as biomarker candidates for metastatic ccRCC. These candidates effectively distinguished metastatic from high-grade non-metastatic ccRCC patient plasma samples in our cohort.

Conclusion

These findings underscore the utility of plasma proteomics in biomarker discovery. Further validation of these signatures could inform clinical decision-making, enabling early detection and real-time longitudinal monitoring for ccRCC patients.

EACR25-1397

Novel Blood-Based mRNA Biomarkers: A Promising Approach for Non-Invasive Colorectal Cancer Screening

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Introduction

Colorectal cancer (CRC) is the third most common cancer globally and the second leading cause of cancer-related deaths. Despite advancements in screening, the preferred non-invasive method, the Fecal Immunochemical Test (FIT), has significant limitations, particularly its low sensitivity for detecting pre-cancerous lesions and high false-positive rates. Colonoscopy, while the gold standard for diagnosis, is invasive, costly, and often associated with low patient adherence. Blood-based biomarkers offer a promising alternative, as they provide a minimally invasive approach that could enhance patient compliance and improve early CRC detection. Therefore, there is an urgent need for more accurate and reliable blood-based biomarkers to optimize screening strategies and reduce CRC-related mortality. This study evaluates the clinical potential of mRNA biomarkers in blood-based samples, including plasma and peripheral blood mononuclear cells (PBMCs), for enhancing CRC screening.

Material and method

Following RNA-sequencing analysis and literature review, three mRNA targets – CEACAM6, PRDX6, and NAA60 – were selected for evaluation. PRDX6 and NAA60 were assessed in RNA extracted from PBMCs of 100 individuals, while all three genes were analyzed in plasma RNA samples from 80 individuals who underwent screening colonoscopy. Expression levels were measured using real-time PCR with TaqMan probes.

Result and discussion

All three biomarkers exhibited differential expression across clinical groups. In plasma, CEACAM6 and PRDX6 effectively discriminated CRC lesions, with CEACAM6 achieving an AUC of 0.82 (sensitivity: 89%, specificity: 75%) and PRDX6 an AUC of 0.76 (sensitivity: 63%, specificity: 93%). NAA60 demonstrated strong performance in detecting high-grade dysplasia (HGD) lesions (AUC: 0.73, sensitivity: 81%, specificity: 67%). Notably, CEACAM6 outperformed FIT in CRC detection, while NAA60 surpassed FIT in detecting advanced adenomas. Similarly, in PBMCs, NAA60 distinguished HGD from non-lesion (NL) samples (AUC: 0.83, sensitivity: 88%, specificity: 70%) and exhibited strong discrimination between samples from individuals where non-relevant findings were present in the colonoscopy (no lesions and low-grade dysplasia) and advanced lesions (HGD and CRC) (AUC: 0.78, sensitivity: 92%, specificity: 57%).

Conclusion

These preliminary findings highlight the potential of CEACAM6, PRDX6, and NAA60 as promising blood-based biomarkers for CRC screening. NAA60, in particular, demonstrated a strong performance in

distinguishing advanced precancerous lesions and cancer, making a good candidate for CRC screening. However, further validation in larger, independent cohorts is essential to confirm these findings. Additionally, future studies should explore the combination of these biomarkers to improve overall diagnostic accuracy and optimize non-invasive CRC screening strategies.

EACR25-1404

Aberrant miRNA expression in papillary thyroid carcinoma— clinical applications

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Introduction

MicroRNAs (miRNAs) are single-stranded RNA molecules that act as global regulators of gene expression and thus appear as valuable molecules for early diagnosis, monitoring and prognosis in cancer. Thyroid cancer (THCA) are common malignant diseases with increased incidence in the last decade. The aim of the present study is to establish a personalized transcriptional profile of miRNAs in biological material obtained via fine-needle aspiration biopsy (FNAB) from normal, benign, malignant, and metastatic tissues of patients with papillary thyroid carcinoma (PTC).

Material and method

Bioinformatics Analysis: Based on data from The Cancer Genome Atlas (TCGA), a panel of 10 miRNAs was identified as potentially clinically relevant for PTC.

Molecular Biology Methods: miRNA expression was analyzed in tissue samples obtained via ultrasound-guided FNAB from benign, malignant, and metastatic thyroid nodules of patients with PTC ($n = 38$), and the results were compared with normal tissue.

Result and discussion

Our findings demonstrate that let-7b-5p, miR-146b-5p, miR-182-5p, miR-339-3p, miR-423-5p, miR-450b-5p, miR-484, miR-874-3p, and miR-142-3p exhibit altered expression between normal and tumor tissue.

Additionally, miR-142-3p, miR-146b-5p, let-7b-5p, miR-182-5p, and miR-484 show significant expression differences between benign and malignant nodules. The cumulative expression of the 10-miRNA panel demonstrates diagnostic potential and can be used as a biomarker for distinguishing tumor from normal tissue with $AUC = 0.984$, sensitivity = 92%, and specificity = 97%.

Conclusion

The investigated miRNA panel represents a reliable diagnostic tool for PTC and could facilitate a personalized clinical and therapeutic approach. The identification of a novel, specific miRNA profile in PTC

could provide valuable insights into the malignant and invasive potential of the tumor, as well as its histological characteristics, thereby complementing routine diagnostics. Furthermore, the correlation between an individual's transcriptional profile and their clinical outcome may offer a predictive value for treatment response and disease prognosis.

EACR25-1417

What Drives the Expansion of Mutant Clones in Non-Malignant Gastrointestinal Tissues, and How Is This Related to Cancer Initiation?

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Introduction

Throughout life, adult stem and progenitor cells accumulate genetic alterations that can enhance cellular fitness. These alterations lead to the clonal expansion of their progeny and the progressive populating of the tissue. Mutant cells may expand into clonal populations without necessarily progressing to cancer, even when harbouring mutations in well-known cancer-related genes (cancer drivers). This study aims to identify mutant genes that drive clonal expansion in non-cancerous tissues (somatic drivers) and understand their roles in tissue homeostasis and disease initiation, including cancer. The gastrointestinal (GI) system, frequently exposed to endogenous and exogenous mutagenic factors, accumulates somatic mutations at a high rate due to its rapid cell turnover, making it an ideal model for studying somatic evolution. Our preliminary analysis focused on compiling a comprehensive dataset of somatic mutations in non-cancerous GI tissues, identifying damaging exonic and splicing mutations, and mapping them to known somatic and cancer drivers.

Material and method

By integrating and curating multiple literature sources, we compiled whole-genome and whole-exome sequencing data from 4,205 samples across 12 GI tissue types (appendix, caecum, colon, duodenum, ileum, jejunum, liver, oesophagus, pancreas, rectum, small intestine, and stomach) from 297 individuals. We observed substantial variability in mutation burden across tissues, with the appendix, ileum, and small intestine exhibiting the highest mutation loads, particularly in samples from diseased donors. This variability persisted when considering gene-level mutations, with the median number of mutated genes ranging from 5 to 35, and when focusing on damaging mutations, where the median number of affected genes ranged from 2 to 10.

Result and discussion

Notably, over 70% of samples lacked mutations in known somatic drivers, and nearly 50% lacked mutations in known cancer drivers, underscoring the need for new approaches to identify genetic alterations that drive clonal expansion in non-cancerous tissues. The candidate

driver RHPN2 was the most frequently mutated gene across eight GI organ sites, while mutations in known false positives such as TTN, CSMD1, and DNAH5 were also prevalent, likely due to their length and sequence composition biases. Among somatic drivers, NOTCH1 was the most frequently mutated, consistent with recent literature.

Conclusion

In conclusion, our analysis of non-cancerous GI tissues provides valuable insights into the landscape of somatic mutations and their potential role in clonal expansion. While the low prevalence of mutated somatic drivers is notable, it also highlights the need for further research to identify novel drivers of clonal expansion in non-malignant tissues.

EACR25-1423

Investigating the sheddase (s) responsible for HAI-1 ectodomain release in bladder cancer cell lines

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Introduction

Hepatocyte growth factor activator inhibitor-1 (HAI-1) is a transmembrane protein with a fundamental role in regulating serine protease activity in the tumour microenvironment through inhibitory Kunitz domains in its extracellular domain (ECD). We have previously demonstrated that high-risk non-muscle invasive bladder cancer (NMIBC) patients with high urinary levels of HAI-1 ECD have worse outcomes. However, the mechanism of HAI-1 ECD shedding in bladder cancer (BC) is unknown. The aim of this study is to explore the role of 4 metalloproteinases in HAI-1 shedding, proliferation and migration in BC cell lines

Material and method

HAI-1 shedding was measured in three cell lines (T24, VM-CUB-1, and 5637) in non-stimulated and phorbol ester-stimulated cells in the presence of matrix metalloproteinase (MMP) inhibitors or siRNA knockdown of ADAM 10, ADAM 17, MMP 7, and MMP 14. The effects of MMP inhibitors and knockdowns on HAI-1 shedding, migration, and proliferation were investigated.

Result and discussion

T24 showed the lowest HAI-1 expression and shedding, VM-CUB-1 intermediate, and 5637 had the highest expression as well as shedding. Marimastat and ADAM 10/17 inhibitors significantly suppressed HAI-1 shedding ($p < 0.01$). Among the MMP knockdowns, ADAM 17 exhibited the most significant decrease in HAI-1 shedding, followed by MMP 7. Cellular migration was decreased by ADAM 17 knockdown, whereas the proliferation was reduced by both MMP 14 and ADAM 17 knockdown ($p < 0.05$). Similarly, Marimastat and ADAM 10/17 inhibitors reduced the proliferation capacity of VM-CUB-1 ($p < 0.05$).

Conclusion

Across BC cell lines, higher cytoplasmic HAI-1 expression correlates with higher ECD shedding. MMP inhibitors block shedding, and inhibitor selectivity implicates ADAM17. ADAM 17 sheds HAI-1 ECD and is also closely associated with cellular migration and proliferation.

EACR25-1443

Osteosarcoma biomarker discovery in plasma extracellular vesicles using a targeted proteomics approach

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Introduction

Osteosarcoma (OS) is an aggressive cancer of the bone, predominantly affecting adolescents and older adults. Generally, OS is diagnosed based on imaging tests and confirmed by biopsy, which is often invasive and painful. Till date, there are no established biomarkers for OS. Plasma-derived extracellular vesicles (EVs) are a promising form of liquid biopsy and involve a less invasive procedure for patients. Here, we used a targeted proteomics approach to screen plasma EVs for the presence of potential protein biomarkers of OS and therefore evaluate the diagnostic utility of plasma EVs. Additionally, we tested two commonly used EV isolation methods, ultracentrifugation (UC) and size exclusion chromatography (SEC), to determine which one is better for proteomic analysis.

Material and method

OS patient ($n = 15$) and healthy control ($n = 24$) plasma samples were obtained from the Helsinki biobank. OS cell lines ($n = 7$) were derived from patient biopsies or commercially sourced. EVs were isolated from plasma and from cell culture conditioned media by UC or SEC. The suitability of both methods for plasma EV isolation were assessed in terms of purity and yield. EVs were characterised by nanoparticle tracking analysis, electron microscopy, and Western blotting for CD9, CD63 and Hsp70 expression. Plasma EVs were analysed by a proximity extension assay (Olink) for the absolute quantitation of selected cytokines and immune surveillance-related molecules ($n = 89$). Proteomics results will be further validated with plasma EVs and OS cell line EVs using Western blotting.

Result and discussion

Both UC and SEC yielded an adequate amount of EVs from plasma. In SEC, plasma EV particles did not elute at the expected fractions and EV markers were present throughout all fractions. UC-isolated EVs were richer in protein than SEC-isolated EVs and therefore we chose them for further analyses. Based on proteomics, 18 out of 89 proteins were differentially expressed in OS-derived plasma EVs compared to controls. MMP1, CCL25 and TREM1 expression was significantly higher in OS-EVs than in controls.

Conclusion

UC was a more reliable method than SEC for the isolation of EVs from plasma. Several proteins were differentially expressed in OS compared to control plasma. Based on these findings, plasma EVs may have a diagnostic role in OS.

EACR25-1481

Exosomal miRNAs in Breast Cancer cancerogenesis: the EsomiR Study Update

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Introduction

One of the key challenges in Breast Cancer (BC) prevention is identifying women who are at a higher risk of developing the disease, who might benefit from intensive monitoring and/or chemoprevention. This retrospective, single-center, case-control, study seeks to explore the potential of miRNAs derived from extracellular vesicles (EV-miRNAs) as early biomarkers for detecting women at an increased risk of developing BC. To achieve this purpose, EV-miRNAs from cyst fluid of women with Gross Cyst Disease of the Breast (GCDB) – a benign condition linked to a 2-4 times greater likelihood of developing BC – were analyzed.

Material and method

Cyst fluid samples were chosen from a cohort of 600 patients diagnosed with GCDB between 1985 and 1993, some of whom subsequently developed BC. EV-miRNAs were extracted using the Serum/Plasma exoRNeasy Midi Kit (Qiagen) and quantified by QubitTM using the microRNA Assay Kit (Thermo Fisher Scientific). The EV-miRNome was evaluated by microarrays on the Agilent platform using the SurePrint Human miR Microarrays 8×60 K. MiRNA validation was conducted by RT-qPCR using the miRCURY LNA miRNA SYBR Green qPCR Kit (Qiagen) in the Mastercycler ep realplex.

Result and discussion

A total of 118 samples (58 cases/60 controls) were analysed. Class comparison (Cases vs Controls) was performed to identify differentially expressed EV-miRNAs and subsequently a Logistic regression with LASSO penalization, combined with k-fold cross-validation ($k = 10$), identified 9 miRNAs (miR-3194-5p, miR-4713-3p, miR-6763-5p, miR-4443, miR-6872-3p, miR-769-3p, miR-5195-3p, miR202-3p, miR-6076) and two clinical variables (family history and menopausal status) associated with the risk of developing BC. RT-PCR confirmed the expression of 6 miRNAs out of those (miR-4443, miR-6763-5p, miR-3194-5p, miR202-3p, miR-6076 and miR-6872-3p). The best multivariate model built on these data included clinical variables, age

and three miRNAs (miR-4443, miR-3194-5p, miR-6763-5p) and demonstrated good predictive power [AUC 0.65 (95% CI: 0.54–0.75)]. Further analyses are ongoing to improve the results.

Conclusion

To the best of our knowledge, this study represents the first attempt to identify an EV-miRNA-based BC risk signature using liquid biopsy from cyst fluid. The ultimate goal of this project is to implement this signature into clinical use by prospectively testing it on plasma samples from breast cancer patients.

EACR25-1507

Prognostic value of tumour-infiltrating immune cells in patients with nasopharyngeal carcinoma

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Introduction

Nasopharyngeal carcinoma is of low incidence rate worldwide but is endemic in East Asia, Southeast Asia and Northern Africa. Majority of the endemic cases are associated with infection by Epstein-Barr Virus (EBV), implying the interaction between genetic and environmental factors, viral infection and host immune system in the pathophysiology of NPC. Patients with primary NPC respond well to radiotherapy with or without chemotherapy. However, some patients, especially those with locally advanced NPC are at significant risk for disease recurrence. For this group of patients, addition of immunotherapy may bring clinical benefit as was demonstrated in several clinical trials. It is therefore important to identify biomarkers that prognosticate disease relapse and overall survival.

Material and method

In this study, we curated a panel of 16 markers for characterisation of TME of NPC. This panel includes 6 immune cell markers: T helper cells (CD4), cytotoxic T cells (CD8), regulatory T cells (FOXP3), B cells (CD20), NK cells (CD56), tumour associated macrophages (CD163); epithelial cell marker EpCAM; immune checkpoint molecules: PD1, PDL1, PDL2; other prognostic or functional markers: EBER, CD38, CD39, CD103, CD137, Ki67. With multiplex immunohistochemistry (IHC) staining and subsequent quantification of a cohort of 50 patients with treatment-naïve primary NPC, we quantified the immune cell populations in different stages of the disease, compared the expression of biomarkers in patients with and without recurrent diseases, and examined their prognostic value in overall survival (OS) and time-to-progression (TTP).

Result and discussion

We noted that NPC samples are heavily infiltrated by T cells, B cells, NK cells, and myeloid cells and the immunophenotypes vary with disease status. Moreover, expression of certain markers, e.g., CD163, CD8, and CD103 significantly differs between patients who developed recurrent diseases and those who did not, suggesting the prognostic value of these molecules. Indeed, we noted prolonged overall survival in patients with low proportions of CD163+ or PD1+ CD8+ cells, and high proportion of CD103+, HLA-DR+ or Ki67+

cells. We also observed delayed disease progression in patients with increased infiltration of CD4+, CD8+, CD103+ or Ki67+ cells. Notably, we identified CD103 as an independent prognostic marker for improved overall survival.

Conclusion

We depicted the TME of primary NPC by IHC staining with a curated panel of antibodies. We demonstrated that CD103 is a significant prognostic marker for OS. Patients with low CD103 expression may benefit from upfront treatment intensification.

EACR25-1529

The potential diagnostic value of a circulating serum-derived miRNA-based model in patients with colorectal cancer

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Introduction

Colorectal cancer (CRC) is one of the most prevalent malignancies of the gastrointestinal tract worldwide, with nearly 2 million new cases diagnosed annually. Colonoscopy remains the gold standard for CRC diagnosis; however, its invasiveness, associated discomfort, and lack of routine implementation often lead to late-stage detection. Therefore, there is an urgent need for non-invasive, sensitive, and specific biomarkers for early diagnosis. MicroRNAs (miRNAs), small non-coding RNA molecules, play a crucial role in gene expression regulation and can act as oncogenes or tumor suppressors. MiRNAs are present in a variety of biological fluids, further highlighting their potential as minimally invasive biomarkers in diagnostics. The study analysed the miRNA profile in the serum of CRC patients compared to healthy controls (HC) to identify new diagnostic biomarkers for this cancer.

Material and method

We obtained serum samples from 100 CRC and 100 HC patients. A miRNA was isolated from the serum, then levels of 798 miRNAs were analysed using NanoString nCounter technology. The Ingenuity Pathway Analysis software was used to select potential targets. The STRING database and the Cytoscape programme were used to generate protein-protein interaction networks and to identify hub genes. Functional analysis of target genes was performed using the online databases (KEGG, g:Profiler, and Enrichr). Receiver Operating Characteristic (ROC) analysis was used to assess the

diagnostic value of miRNAs. A logistic regression model for the combined miRNAs in the panel was created using Weka software.

Result and discussion

Our data showed 11 miRNAs with significantly changed expression in CRC group compared to HC ($|FC| \geq 1.5$, FDR ≤ 0.05). The logistic regression model for the three miRNAs combined showed significantly greater diagnostic utility (AUC = 0.958, with 90.7% specificity and 95.6% sensitivity) than each miRNA analysed separately. The indicated target transcripts are involved in pathways leading to tumorigenesis. The results obtained showed serum miRNA profile which is characteristic for patients with CRC. Novel model with three miRNAs that distinguish CRC patients from HC patients with high sensitivity and specificity. The results of this research will be used to prepare a patent application.

Conclusion

Our findings suggest that circulating miRNAs might have a pivotal role in diagnosis of CRC. The development of an IVDR test based on serum miRNA expression could significantly enhance and facilitate the diagnosis of this cancer.

EACR25-1532

Patient-specific cell-free RNA alterations enable cancer classification

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Introduction

Circulating nucleic acids in blood plasma offer a promising approach for minimally invasive cancer detection. While most research has focused on cell-free DNA, the broader range and dynamic, on-demand production of RNA make cell-free RNA an interesting cancer biomarker resource as well.

Material and method

We applied mRNA capture sequencing to analyze blood plasma cfRNA from 266 cancer patients and cancer-free controls (discovery n = 208, 25 locally advanced to metastatic cancer types; replication n = 58, 3 cancer types). We identified fusion transcripts and performed cellular deconvolution, differential abundance, and gene set enrichment in cancer compared to control groups. We

also developed a new case-specific approach that compares an individual patient's cfRNA profile to a reference control population to identify biomarker tail genes - transcripts that deviate by more than three standard deviations from the reference distribution and show some recurrence among patients. The number of biomarker tail genes per sample was used for binary cancer/control classification and performance was assessed by 10x 5-fold cross-validation.

Result and discussion

Our findings revealed both cancer type-specific and pan-cancer cfRNA alterations, but high heterogeneity among patients and cohorts limited the identification of robust cancer biomarkers at the group level. Our biomarker tail gene concept addresses this challenge by comparing individual profiles to a set of reference profiles. The number of biomarker tail genes in a plasma sample effectively distinguished ovarian and prostate cancer patient samples from controls (mean AUC of 0.901 and 0.964, resp.). We also validated this concept in independent cohorts of 65 plasma donors (two lymphoma types) and 24 urine donors (bladder cancer), confirming its potential of leveraging individual patient alterations for cancer detection. To further evaluate the cancer specificity of these signals, we are currently expanding our analyses to include a cohort of non-malignant diseases (n = 150).

Conclusion

Our findings highlight the heterogeneity of cfRNA alterations among cancer patients and suggest that focusing on case-specific changes could facilitate cancer detection.

EACR25-1533

Feasibility of tracking multiple primary cancers (MPCs) using tumor-informed circulating tumor DNA (ctDNA) assays

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Introduction:

Approximately 10-25% of cancer patients (pts) develop MPCs, often metachronous, resulting from environmental exposures, prior therapies, and inherited genetic factors. Monitoring individual tumor progression is challenging due to the lack of sensitive biomarkers and tumor-specific imaging characteristics that can distinguish between recurrences and MPCs. This study demonstrates the feasibility of individually monitoring MPCs using a tumor-informed ctDNA assay.

Material and method

This retrospective analysis used real-world data from commercial ctDNA testing in pts with MPCs who had a personalized, tumor-informed, mPCR-NGS ctDNA assay (SignateraTM, Natera, Inc.) designed for each primary tumor. Pt treatment and the collection of longitudinal blood samples were at the treating physician's discretion. Clinicopathologic information for ctDNA analyses was collected retrospectively from Natera's commercial database. The patterns of tumor-specific ctDNA results for each pt were summarized.

Result and discussion

In total, 41 pts with MPCs underwent ctDNA monitoring for two (40 pts) or three (1 pt) primary tumors. Tumor A included breast (21.9%, n = 9), gastrointestinal (GI) (31.7%, n = 13), genitourinary (GU) (14.6%, n = 6), gynecologic (gyn) (4.9%, n = 2), skin (14.6%, n = 6), sarcoma (2.4%, n = 1), lung cancer (7.3%, n = 3), and unknown histology (2.4%, n = 1). Stage distribution for Tumor A was: stage I: 5 (12.2%), stage II: 8 (19.5%), stage III: 20 (48.8%), stage IV: 6 (14.6%), and unknown: 2 (4.9%). Tumors B and C comprised of GI (38.6%, n = 17), gyn (20.5%, n = 9), breast (13.6%, n = 6), skin (13.6%, n = 6), GU (4.5%, n = 2), lung (4.5%, n = 2), hematological cancer (2.3%, n = 1), and unknown histology (2.3%, n = 1). Stage distribution for Tumor B was: stage I: 8 (18.2%), stage II: 5 (11.4%), stage III: 16 (36.4%), stage IV: 10 (22.7%), and unknown: 5 (11.4%). Among these, 17 pts had multiple primary tumors originating in the same organ. Analysis of clinical data was performed for 33 pts, including one with three primary tumors. The median follow-up was 14.5 months for Tumor A and 2.7 months for Tumor B/C. A total of 302 ctDNA tests were performed: 241 for Tumor A and 61 for Tumor B. Among 33 pts, one tested ctDNA negative in both tumors, while 4 pts tested positive for both tumors at some point. 12 pts tested positive for Tumor A but were consistently negative for tumor B. Lastly, 16 pts were positive for Tumor B or C but serially negative for Tumor A.

Conclusion

This study demonstrates the feasibility of real-time tracking of MPCs using tumor-informed ctDNA assays. Reliable assessment of tumor-specific molecular disease burden during surveillance and treatment monitoring can be critical for timely and informed clinical management decisions and personalized treatment plans.

EACR25-1548

ddPCR as molecular approach to assess MSI-H status in PDAC EUS-FNB samples

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Introduction

Pancreatic ductal adenocarcinoma (PDAC) is an aggressive disease with poor prognosis. Biopsy evaluation is crucial to determine surgical and treatment options. Endoscopic Ultrasound Fine Needle Biopsy (EUS-FNB) can collect adequate tissue for diagnostic purposes, which could be suitable to perform molecular analysis to improve new personalized treatments. The effectiveness of immunotherapy in treating tumors with high microsatellite instability (MSI-H) has been demonstrated in various cancer types. In PDAC, the prevalence of MSI-H is estimated to be around 1–2% of

patients by using immunohistochemistry (IHC), different types of PCR based methods or NGS. Aiming to improve MSI-H detection in PDAC EUS-FNB, droplet digital PCR (ddPCR) technology was used to better stratify patients that could be eligible for immunotherapy.

Material and method

Formalin-fixed-paraffin-embedded (FFPE)-DNA extracted from 43 PDAC EUS-FNB were evaluated for their adequacy in terms of quantity and quality by using the Qubit dsDNA High Sensitivity Assay Kit and the Agilent TapeStation 4200 respectively. MSI-H status was assessed by ddPCR which is based on the analysis of five microsatellites marker loci (BAT-25, BAT-26, NR-21, NR-24 and MONO-27). Samples were also evaluated for the mismatch repair system deficiency (dMMR) analyzing MLH1, MSH2, MSH6 and PMS2 proteins by IHC.

Result and discussion

FFPE-DNA amount, obtained from PDAC EUS-FNB samples, ranged between 35 and 1955 ng in 25 µL of final elution volume. The median concentration was 11.3 ng/µL (range 1.4–78.2). DNA Integrity Number (DIN) median value was 3.1 (range 1–5.9). The fragments average length, defined at the main peak by TapeStation analysis, was 1694 bp (range 384–10,445). The quantity and quality of the DNA was adequate to perform molecular analysis. MSI-H/dMMR status was first assessed using IHC staining, a widely used method for detecting the dMMR phenotype in various types of tumors, and all samples resulted to be microsatellite stable (MSS). However, one of the IHC's drawbacks is that it is restricted in detecting the absence/presence of the four traditional MMR proteins, but not the functional consequences of aberrations at the genomic level, then may not accurately reflect the MMR system status. To overcome the IHC limitations, MSI-H status was also evaluated at the molecular level by using the highly sensitive ddPCR method, and has been detected in 16.28% of cases, compared to the 1–2% frequency detected by traditional techniques.

Conclusion

This study suggests that ddPCR can enhance the detection of MSI-H phenotype in PDAC, resulting in a better selection of patients who might be eligible for immunotherapy and may be excluded by conventional diagnostic methods. This finding is crucial in patients with advanced disease, in which the pancreatic biopsy is the only tissue source to perform diagnostic evaluation.

EACR25-1591

Computational screening for promising protein biomarkers and predictive performance evaluation for lung cancer

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Introduction

Lung cancer is a leading cause of cancer-related mortality worldwide, with early detection being critical for improving survival rates. Protein biomarkers offer a promising alternative for lung cancer screening due to their potential for non-invasive detection and ability to

reflect disease progression. However, identifying reliable protein biomarkers remains a significant challenge due to the uncertainty of tumour biology and the need for robust validation. Currently, there are no universally accepted protein biomarkers for lung cancer screening and most biomarkers that have been assessed to date are not measured in the pre-diagnostic setting, not allowing for evaluating early detection performance. Furthermore, the benefits of incorporating long screening history are unclear and require further investigation.

Material and method

In this study, we analysed a unique population screening data from 248 participants (98 cases and 150 controls) with 94 protein biomarkers, the samples of which go as far as 5 years before final diagnosis. Therefore, the dataset is pre-diagnostic as well as longitudinal. To address the limitations of dataset size, we implemented a novel repeated splitting strategy using LASSO to systematically identify promising biomarkers. As a proof of concept, we then used the selected biomarkers to train the longitudinal machine learning models and evaluate the value of incorporating long screening history.

Result and discussion

CEACAM5, MUC-16, CXL17, WFDC2, hK14 and CPE were identified as promising biomarkers for lung cancer screening. We also showed that the longitudinal analysis using LSTM neural networks gave rise to an ROC AUC score of approximately 0.886 and sensitivity (0.8) of 0.774 based on the current data. However, the real benefit of conducting longitudinal analysis still needs to be further investigated.

Conclusion

In this work, we presented a novel computational methodology for biomarker screening and identified 6 promising protein biomarkers for lung cancer based on a unique long time-span screening dataset. The longitudinal analysis using LSTM neural networks achieves a desirable predictive performance in terms of ROC AUC and sensitivity (0.8).

EACR25-1609

Identification of novel diagnostic biomarkers as therapeutic targets in prostate cancer

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Introduction

Prostate cancer (PCa) is the most common cancer and the second-leading cause of cancer-associated death in males. PCa is considered one of the most prevalent malignancies globally, and metastasis is a major cause of death. Despite advances in screening for early detection of PCa, a large portion of men continue to present with advanced PCa. In general, prostate-specific antigen (PSA) is a protein produced in the prostate gland. The PSA test measures the level of PSA in the blood as a gold standard for screening tools for prostate cancer; however, PSA has low efficiency and usually results in a false-positive test. Other methods to screen PCa, such as MRI scans, phenotypic and molecular tests, such as Formalin-fixed

paraffin-embedded (FFPE) tissues, are not good enough for detection because of late-stage diagnosis, high invasiveness, and expense. Due to many limitations, a novel of PCa screening for diagnosis is required. Peripheral blood mononuclear cells (PBMCs) can provide an inexpensive, less invasive, and highly accurate method. This study aims to discover a potential novel biomarker for PCa screening, utilizing the gene expression of the PBMCs.

Material and method

Data have been searched from the NCBI, GEO database of gene expression in PCa patients and healthy donors' PBMCs were collected. As a result, GSE153514, GSE16120, and GSE46602 were found. All genes from the three GSE groups were intersected with upregulated genes that have been identified. A statistical significance test was conducted in the gene expression of each data set and p-values < 0.001 have been selected. Then, PBMC from a total of 45 healthy donors, and 45 PCa cancer donors were collected. Quantitative RT-PCR (qRT-PCR) was performed in cDNA from all blood samples.

Result and discussion

The relative mRNA expression value of CU1 gene of the PCa group was significantly higher than the healthy group with a significance value. The sensitivity and specificity of this gene as a screening tool were 77.1% and 81.8% respectively. Moreover, the expression of CU1 significantly impacts the cancer progression of prostate cancer cells. The high expression of CU1 was correlated with the cell proliferation and metastatic behavior in PC-3 prostate cancer cell lines.

Conclusion

In conclusion, a novel biomarker CU1 has high potential as a candidate upregulated gene, which could be further investigated as biomarkers and the molecular mechanism to develop as a therapeutic target for PCa.

EACR25-1611

Multi-omics Approaches to Uncover Liquid-Based Cancer-Predicting Biomarkers in Lynch Syndrome

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Introduction

Lynch Syndrome (LS) is an inherited cancer predisposition syndrome caused by a pathogenic mutation in one of the DNA mismatch repair genes (path_MMR): MLH1, MSH2, MSH6, or PMS2. It causes a lifetime elevated cancer risk for various cancers. The cancer risk

depends on the path_MMR gene where MLH1 is known to cause the highest cancer risk. Even with high cancer risk, all carriers don't develop cancer, highlighting the possibility that the cancer risk can be impacted via lifestyle choices. Current risk assessment methods consider sex, age, and path_MMR gene variant but lack individual systems biology markers, which lifestyle choices may influence. Vigilant surveillance and innovative strategies for early detection and precise risk stratification are pivotal for effective risk mitigation in individuals with LS. Our study examined the systemic biology of currently healthy LS carriers by harnessing the multi-omics integration of circulating microRNAs (cmiRs) and circulating metabolites (cMets) to assess their potential in predicting future cancer risk. Both cmiR and cMet levels can be influenced by various factors, including lifestyle choices.

Material and method

Multi-omics data was combined with information on whether the study subject developed cancer (n = 17) or not (n = 99) during a 5.8-year surveillance. Single- and multi-omics data analysis approaches were employed to identify systemic cancer-predicting biomarkers, including Principal Coordinate Analysis, Sparse Partial Least Squares, Weighted Correlation Network Analysis, and the Lasso Cox regression survival model.

Result and discussion

Our study revealed a cmiR cluster associated with upcoming cancer. These cmiRs regulate key signaling pathways related to cancer progression, like PI3-Akt. Notably, the levels of certain metabolites, such as low-density lipoprotein (LDL), and very low-density lipoprotein (VLDL) were elevated in carriers of the MLH1 gene variant compared to carriers of the MSH2 and MSH6 variants. Lipid metabolism dysfunction is connected to a higher risk of tumorigenesis. Additionally, we built a survival model, where three biomarkers hsa-miR-101-3p, hsa-miR-183-5p, and triglycerides in high-density lipoproteins (HDL_TG) were indicators of increased cancer risk.

Conclusion

This study provided insights into systems biology in LS and identified promising cancer biomarkers for evaluation of early-stage cancer risk detection, an alternative tool besides current risk assessment methods.

EACR25-1654

Immune-Related Gene Signature in Melanoma Lymph Node Metastasis: A 6-Gene Predictive Model

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Introduction

Lymph node metastasis is a pivotal event in melanoma progression, directly impacting prognosis and therapeutic decision. Despite advances in molecular profiling, predictive biomarkers for nodal involvement remain scarce. Unraveling the transcriptional differences between metastatic and non-metastatic tumors may illuminate key molecular drivers and enhance risk stratification. This study aimed to identify differentially expressed genes (DEGs) associated with lymph node metastasis and to develop a predictive model for metastatic risk assessment in melanoma.

Material and method

Thirty-five melanoma patients with formalin-fixed paraffin-embedded (FFPE) tumor samples containing at least 60% neoplastic cells were analyzed. Total RNA was extracted using the RNeasy Mini Kit and profiled using the NanoString nCounter® Human Immunology v2 Panel. Data normalization and differential expression analysis were conducted using the nSolver Advanced Analysis module, with subsequent statistical validation in R (v4.3.1). DEGs were identified based on a significance threshold of $p < 0.01$ and a \log_2 fold change ≥ 2 . A logistic regression model incorporating key DEGs was developed, and its predictive performance was assessed using the area under the receiver operating characteristic (ROC) curve (AUC).

Result and discussion

Lymph node metastases were present in 60% of cases. The cohort had a mean age of 57.9 years, with 62.9% male patients. The most common primary tumor site was the lower limb (34.3%), with nodular melanoma (34.3%) and superficial spreading melanoma (31.4%) being the predominant histological subtypes. The median Breslow thickness was 9.3 mm, and most tumors were classified as Clark IV (62.9%), with ulceration present in 60.0% of cases. Six DEGs were identified as significantly associated with lymph node metastasis. CR2 and NCR1 were upregulated in metastatic cases, while FAS, DEFB103A, AHR, and CFI were upregulated in non-metastatic cases. A predictive model incorporating these genes achieved an AUC of 0.79, with a sensitivity of 71.4% and specificity of 78.6%, demonstrating strong discriminative power. The upregulation of CR2 and NCR1 in metastatic tumors suggests their involvement in lymphatic dissemination, whereas the increased expression of FAS, DEFB103A, AHR, and CFI in non-metastatic tumors may reflect immune-mediated resistance to metastasis.

Conclusion

This study identifies a robust immune-related gene signature distinguishing metastatic from non-metastatic melanoma cases and introduces a predictive model with high diagnostic accuracy. These findings underscore the potential of transcriptomic profiling for improving risk assessment and patient stratification in melanoma prognosis. Further validation in larger cohorts and functional studies will be critical for translating these molecular insights into clinical applications.

EACR25-1720

Improving Cancer Screening and Monitoring: The Potential of Oncofetal Chondroitin Sulfate Proteoglycans in Liquid Biopsies

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Introduction

Cancer remains a leading cause of death worldwide, with survival rates heavily dependent on early detection. Screening tests can help detect cancer at an early, more treatable stage before symptoms appear. For example, the fecal immunochemical test (FIT) is widely adopted in many EU countries for colorectal screening (CRC). However, FIT suffers from low sensitivity for early-stage disease and poor screening compliance, and around 30% of CRC patients relapse within 5 years of disease. Furthermore, for other cancers such as bladder cancer (BC), no non-invasive, highly sensitive and specific screening tests are available. Alternative biomarkers that can identify early-stage disease and relapses are sorely needed, especially those that can easily integrate into clinical workflows.

Material and method

Oncofetal chondroitin sulfate proteoglycans (ofCSPGs) are promising pan-cancer biomarkers due to their uniquely modified glycosylation patterns and involvement in tumor progression. The ofCSPGs are shed from tumor tissue into circulation, allowing for non-invasive cancer detection. We validated the expression of ofCSPGs in patient liquid biopsies and tissue using mass spectrometry and immunohistochemistry, respectively. To test their diagnostic potential, we developed two novel diagnostic assays using proprietary antibodies to detect ofCS. These assays were optimized to detect and quantify ofCSPG in both plasma and urine samples from cancer patients.

Result and discussion

ofCSPGs were widely expressed in CRC and BC tumors, with minimal or no detection in matched healthy tissue, and proteomic analysis identified a panel of ofCSPGs in urine and plasma. Drawing from this, preliminary data in a clinical discovery cohort demonstrate high specificity and sensitivity for ofCSPGs in plasma, with excellent AUC scores, underscoring their potential as robust biomarkers. Notably, ofCSPGs were detectable in plasma from adenoma patients, suggesting promise for early-stage CRC detection. Furthermore, longitudinal

monitoring of CRC patients undergoing surgical interventions revealed significant declines in ofCSPG levels after tumor resection, supporting their utility in assessing treatment response and disease progression. Beyond CRC plasma samples, ofCSPGs were consistently detected in urine from BC patients, with background levels in age-matched controls including hematuria and non-symptomatic individuals.

Conclusion

These findings underscore the potential of ofCSPG as minimal invasive biomarkers for cancer screening, patient stratification, and disease monitoring across multiple malignancies. We are now conducting a large-scale study to assess their efficacy in early multi-cancer detection and disease monitoring. Our goal is to develop a non-invasive, highly accurate diagnostic tool to address critical unmet clinical needs and improved outcomes for CRC and BC patients.

EACR25-1769

Virome-based Biomarkers for Non-Invasive Colorectal Cancer Screening

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Introduction

Colorectal cancer (CRC) screening relies on the fecal immunochemical test (FIT), which has limited sensitivity for detecting premalignant advanced adenomas (AA) and proximal tumors. Therefore, more sensitive and specific non-invasive biomarkers are needed. The gut microbiome, including bacteria, archaea, fungi, and viruses, plays a key role in health and disease. While bacterial alterations have been linked to CRC, contributing to inflammation, immune dysregulation, and a pro-tumorigenic microenvironment, the gut virome remains largely unexplored. Phages, the most abundant viruses in the gut, influence bacterial composition and may impact CRC onset and progression. In this study, we explored the stool virome to identify potential viral biomarkers for CRC screening.

Material and method

We analyzed the virome in stool samples from 45 CRC patients, 60 AA patients, and 59 individuals without neoplasia. We performed viral shotgun metagenomics using a custom protocol that included viral enrichment (low-speed centrifugations, filtration, ultracentrifugation,

nuclease digestion), DNA/RNA extraction, retro-transcription, random priming PCR, library preparation and sequencing (150 PE reads). Reads were quality-checked, preprocessed, and taxonomically classified with SeqScreen (Balaji et al., 2022). Differential abundance analysis was conducted using coda4microbiome (Calle et al., 2023).

Result and discussion

The proportion of reads assigned to viruses varied significantly among samples (0.19–96.3%; mean 20.52%). 178 viral families were identified, with Microviridae being the most abundant, followed by Inoviridae and Picornaviridae. Variable selection using penalized regression to differentiate individuals with advanced neoplasia (CRC and AA) from those without neoplasia identified a signature composed of 11 viral families, with Betaflexiviridae emerging as most relevant. The differential abundance analysis at the genus level high-lighted Ravavirus, Chordovirus, and Sustivirus as promising biomarkers.

Conclusion

Our preliminary findings suggest a potential association between Betaflexiviridae viruses and advanced neoplasia, indicating that members from this family may serve as potential biomarkers for AA and CRC detection.

Funding: “Fundación Científica de la Asociación Española contra el Cáncer” (PRYGN211425POSA).

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EACR25-1794

Evaluation of PTK7-ADC in Patient-Derived Explants (BC-PDEs): Investigating Multimodal Data for Personalised Medicine

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Introduction

The recurrence of breast cancer (BC) emphasises the need for novel treatment approaches. PTK7 is an inactive Receptor Tyrosine Kinase highly expressed in BC. The present study leverages breast cancer patient-derived explants (BC-PDEs) to assess the therapeutic impact of a Pfizer PTK7-targeted Antibody Drug Conjugate (PTK7-ADC). By correlating ex-vivo PTK7-ADC responses with clinicopathological, PTK7 expression and genomics data, the aim is to direct individualised treatments and aid the design of future clinical trials with the PTK7-ADC

Material and method

The recurrence of breast cancer (BC) emphasises the need for novel treatment approaches. PTK7 is an inactive Receptor Tyrosine Kinase highly expressed in breast

cancer. The present study leverages breast cancer patient-derived explants (BC-PDEs) to assess the therapeutic impact of a Pfizer PTK7-targeted Antibody Drug Conjugate (PTK7-ADC). By correlating ex-vivo PTK7-ADC responses with clinicopathological, PTK7 expression and genomics data, the aim is to direct individualised treatments and aid the design of future clinical trials with the PTK7-ADC. Methods: BC-PDEs were subjected to PTK7-ADC treatment and a multi-modal dataset was generated including viability data (proliferation/apoptosis/necrosis changes), intrinsic PTK7 expression, clinicopathological and genomics data. The dataset was analysed and correlations were performed to identify significant associations using R programming.

Result and discussion

Tumour areas displayed higher intrinsic PTK7 expression than stroma areas, with higher expression in higher-grade but lower-stage cancers. The PTK7-ADC induced a range of viability changes in BC-PDEs with ~16% of tumours showing a strong cell death response. However, this cell death responses did not correlate with any clinicopathological parameters or intrinsic levels of PTK7 expression. PTK7 genomic mutations were detected in ~16% of samples but did not correlate with PTK7-ADC cell death responses. Hierarchical k-means clustering of viability data identified two distinct clusters, labelled as “Resistant (R) & Sensitive (S)” /RS phenotypes, used to categorise the data for further analysis. Whole exome sequencing data was used to identify mutant genes that were significantly enriched in the RS phenotypes using Fisher's exact test, which resulted in 264 significantly enriched CNV-mutated genes associated with RS phenotype. Pathway enrichment analysis highlighted the Breast Cancer, Wnt and Hippo signalling as top enriched KEGG-pathways.

Conclusion

In BC, while PTK7 expression correlates with more advanced disease grade, PTK7 expression is not a predictive biomarker for PTK7-ADC-mediated cell death response. This suggests that the ADC operates through bystander mechanisms, which require further investigation. Further analysis of 264 genes, may hold promise for the development of a predictive biomarker panel for PTK7-ADC responses in BC patients.

EACR25-1801

Genomic diversity and BCL9L mutational status in CTC pools predict overall survival in metastatic colorectal cancer

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Introduction

Given that cancer dissemination primarily occurs via the bloodstream – where tumor cells detach from the primary tumor, enter circulation, and migrate to distant tissues – there is growing interest in circulating tumor cells (CTCs) due to their purported role in metastasis and potential as a liquid biopsy tool for cancer diagnosis and management. Although the clinical significance of CTCs has already been established, - with elevated CTC counts correlating with aggressive disease in several cancer types, including colorectal cancer (CRC) – early sequencing studies have revealed that CTCs can exhibit considerable genomic and phenotypic heterogeneity within patients. Importantly, increasing evidence suggests that intratumoral genetic heterogeneity is a critical factor influencing clinical outcomes in CRC. We therefore hypothesize that higher genetic variation within the CTC population may contribute to increased disease aggressiveness and reduced overall survival.

Material and method

As such, to investigate the biomarker potential of CTC genomics in predicting survival outcomes, we collected and performed whole-exome sequencing on CTC pools from 30 metastatic CRC patients.

Result and discussion

Our findings reveal substantial variation in mutational burden across patients, with all CTC pools harboring non-silent mutations in key CRC driver genes. Importantly, higher genomic diversity in CTC pools was significantly associated with reduced overall survival. Moreover, non-silent mutations in BCL9L also emerged as a strong predictor of survival. To facilitate clinical translation, we additionally designed a 55-gene panel based on overall mutational load, and demonstrated that it could accurately replicate key WES findings, including the association between CTC genomic diversity and survival.

Conclusion

These findings underscore the relevance of CTC-derived genomic information as a robust prognostic biomarker in metastatic CRC and highlight the potential of liquid biopsies in personalized cancer management.

EACR25-1802

Fecal miRNA profiles and gut metagenome composition in Lynch syndrome: results from a study on human subjects and a mouse model

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Introduction

Lynch Syndrome (LS) is an inherited disorder defined by heterozygous germline mutations at DNA mismatch repair (MMR) genes, leading to a dysfunctional post-replication repair system. LS patients have an increased lifetime risk of developing many types of cancers, mainly colorectal cancer (CRC), which occurs at an earlier age compared to sporadic forms. Despite regular colonoscopies as a preventive tool, a relevant percentage of LS patients still develop CRC. Gut microbiome, environmental factors, and epigenetic factors are involved in CRC development. In this sense, the concomitant analysis of fecal microRNA (miRNA) profiles of the host and microbiome composition may allow the identification of specific fecal markers in LS that could reflect the gut alterations associated with the development of precancer/cancer lesions in these patients. The aim of this study is to analyze the fecal miRNome and the microbial composition alterations in LS patients with a precancer/cancer lesion with respect to LS subjects without lesions. A similar analysis was also performed between all LS patients and a set of healthy. The obtained results were compared with data from an in vivo model using an MMR-deficient mouse model, with a tissue-specific inactivation of Msh2 in the intestinal mucosa (VCMshloxP).

Material and method

We performed small RNA-sequencing (sRNA-seq) and shotgun metagenomics sequencing analyses in fecal samples of two cohorts of LS patients (Dutch, n = 84 and Italian, n = 105) and matched healthy subjects (n = 20 and n = 64, respectively) used as controls for both cohorts. An additional set of 41 Dutch LS patients and their healthy relatives (n = 26) with no MMR mutations was used as a validation cohort. sRNA-seq and shotgun metagenomics sequencing was also performed in the mouse model: in this case, stools were collected in a time progression at five different time points in VCMshloxP mice and animals without the Msh2 mutation that were used as control group.

Result and discussion

Preliminary results from human subjects identified 38 fecal miRNAs whose levels were significantly increased or decreased in all cohorts in LS patients when compared to healthy controls. In addition, levels of 23 miRNAs were significantly altered in LS who presented a lesion at sampling compared to those with negative colonoscopy. In the mouse model, sRNA-seq and metagenomic analyses showed several dysregulated miRNAs and differential microbial relative abundances, either between VCMsh2loxP and controls, or at different time points.

Conclusion

The fecal biomarkers identified may serve as useful diagnostic and prognostic tools for clinical management of LS patients, improving cancer prevention, early diagnosis, and monitoring disease progression.

EACR25-1828

Monitoring Cystatin F Dynamics in Melanoma Patients Undergoing Checkpoint Inhibitor Therapy

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Introduction

Despite the success of Immune checkpoint inhibitor (ICI) therapy in melanoma, patient outcomes can be improved. Some patients develop resistance, and reliable predictive biomarkers are lacking, as PD-L1 and Tumour Mutational Burden (TMB) do not accurately predict outcomes. ICI therapy can also cause autoimmune side effects, making patient selection crucial. Our group identified cystatin F as a negative regulator of cytotoxicity. We showed that increased cystatin F expression correlates with reduced CTL functionality and exhaustion. Anti-PD-1 antibodies further increase cystatin F in CTLs, suggesting it elevates during ICI therapy and contributes to reduced cytotoxicity, potentially serving as a biomarker for ICI response.

Material and method

Peripheral blood and histological tumour samples were collected before immunotherapy. Cystatin F expression was evaluated in CTLs from PBMCs and tumour tissue, and cystatin F-expressing cells in tumours were assessed for functional status to compare blood and tissue-infiltrating CTLs. Public datasets were analysed for cystatin F expression, prognosis, and correlations with immune cell infiltration. Blood was collected from melanoma patients receiving systemic immunotherapy with ICIs (nivolumab, ipilimumab/nivolumab, pembrolizumab) as first-line treatment at the Institute of Oncology Ljubljana, Slovenia at three time points (4 weeks before, 12 (+2), and 28 (+2) weeks after therapy initiation). Cystatin F expression in CTLs was evaluated at each timepoint. Clinical data were tracked for 12 months, and patients were stratified (e.g., responders vs. non-responders). PBMCs were isolated, frozen, thawed, and MACS sorted to obtain CD8+ lymphocytes for flow cytometry. Gene expression for cystatin F and effector molecules of the perforin/granzyme pathway was determined by real-time PCR, and activation status of cystatin F and granzyme activity were assessed using SDS-PAGE and western blot.

Result and discussion

Our analysis revealed that cystatin F expression decreases with treatment duration in patients who respond to ICI therapy. Notably, a sex-based difference in cystatin F dynamics during the course of treatment was also observed, suggesting that cystatin F could serve as a potential biomarker for distinguishing between responders and non-responders.

Conclusion

These findings highlight the importance of monitoring cystatin F levels in melanoma patients undergoing immunotherapy, as it may provide valuable insights into treatment efficacy and help personalize therapy for better patient outcomes. Further studies are needed to explore the mechanisms behind the observed sex differences and to confirm the utility of cystatin F as a predictive biomarker across larger patient cohorts.

EACR25-1834

Defining blood-based immune biomarkers to improve early lung cancer detection during community lung health checks

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Introduction

Lung cancer causes more deaths than any other cancer in the world due to late diagnosis. However, if detected early, curative surgery can be offered. In the last decade, low dose CT scans, delivered through lung health checks, have been instrumental in the lung cancer early detection process for under 75s with approximately 8000 lung cancer diagnoses to date in the UK. Demographic and lifestyle factors are used to stratify patient by risk of lung cancer for CT screening through the PLCO score.

However, in Manchester these scores underestimate lung cancer risk. Furthermore, only 40% of people diagnosed with lung cancer in unscreened populations would have met the criteria for screening in the time preceding diagnosis. We have reasoned that a pre-CT screening test would reduce the number of CT scans needed per positive diagnosis, thereby reducing the cost and broadening the reach. This study aims to develop immune biomarkers which can be used in community screening to provide improved personalised risk profiles to allow clinicians to identify at-risk individuals outside the current cutoffs used with PLCO and LLP scores.

Material and method

Using a CyTOF-based pipeline we have immunoprofiled peripheral whole blood from patients at community lung health checks and lung cancer clinics. Based on cancer-associated cell populations identified in this pipeline, we then utilised preliminary scRNA-seq and multiplex ELISA's to explore chemokine communication between tumour and circulating immune cells in early cancer.

Result and discussion

Our results reveal several potential candidates for improving lung cancer screening including, lung cancer-associated depletion of CD161+ T cells and altered classical monocyte and NAIVE CD8+ T cell phenotype.

Furthermore, we describe several cancer-associated serum chemokines including the promising CXCL9-CXCR3 signalling pathway as a potentially key biomarker of early lung cancer.

Conclusion

This work may constitute the first step towards the integration of blood-based immune biomarkers with existing risk scores which could significantly improve access to lung cancer screening and improve early detection while reducing unnecessary low dose CT scans during community screening.

EACR25-1841

Xenograft derived from circulating tumor cells as a model for preclinical research

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Introduction

New preclinical models are urgently needed to understand the biology of circulating tumor cells (CTCs), their role in dissemination, and to identify potential drugs targeting CTCs. These models include both *in vivo* CTC-derived xenografts (CDX) and *in vitro* cell cultures. In this work we present the derivation and characterization of CTCs-derived model from the blood of a patient with invasive ductal carcinoma of the right breast.

Material and method

The CTCs-enriched fraction was obtained from 9 ml of whole blood by depletion of CD45+ cells and implanted under the renal capsule of female NRG mouse. *In vitro* cell cultures were established by sorting CD298+ human cells from a single cell suspension prepared from xenograft. Selected drugs were tested *in vitro* using 3D cell culture. To test the drugs *in vivo*, tumor fragments were implanted in mammary fat pad of NRG female mice and once growing, mice were given the drugs intra-peritoneally or via oral gavage. A manual caliper was used to monitor the tumor growth.

Result and discussion

The CTCs-enriched fraction formed a tumor (CDX_IBP_01) under the renal capsule within 6 months and its metastatic potential was confirmed *in vivo*. Both 2D and 3D *in vitro* cell cultures were derived from generated CDX. RNA-Seq analysis comparing the primary tumor and CDX_IBP_01 revealed substantial differences associated with cell growth, metabolism, and extra-cellular signaling. The potential of established CDX as a

model for preclinical drug testing was investigated both in vivo and in vitro. Here, we compared the effect of therapeutics previously administered to the patient (gemcitabine and carboplatin) with potentially effective drugs selected based on the results of transcriptomic profiling.

Conclusion

In summary, we present a novel preclinical model of progressive breast cancer derived from CTCs that may serve as a useful model for studying and understanding the plasticity and behaviour of CTCs during tumor progression and for testing therapeutics potentially targeting CTCs.

This work was supported by grants from the Czech Health Research Council (NU21-08-00023, NW24-03-00265), from the Czech Science Foundation (grant no. 24-11793S), and by the project National Institute for Cancer Research (Programme EXCELES, ID Project No. LX22NPO5102) - Funded by the European Union - Next Generation EU.

EACR25-1857

Non-invasive bladder cancer detection: identification of a urinary volatile biomarker panel using GC-MS metabolomics and machine learning

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Introduction

Bladder cancer (BC) remains a major public health concern, for which early detection is essential for improving patient prognosis. Emerging metabolomics research has highlighted the potential of urinary volatile organic compounds (VOCs) as non-invasive biomarkers for the early detection of BC. Cancer-related metabolic alterations give rise to distinct VOC profiles, facilitating the differentiation between malignant and non-malignant conditions. Advanced analytical techniques, such as gas chromatography-mass spectrometry (GC-MS), in combination with machine learning (ML) algorithms, offer a powerful approach for biomarker discovery and validation. This study aimed to leverage ML algorithms applied to GC-MS-based metabolomics data to discover a panel of volatile biomarkers able to accurately discriminate BC patients from cancer-free controls.

Material and method

Urine samples were collected from 177 participants, including 87 patients diagnosed with BC and 90 cancer-free controls [1]. Urine sample analysis was conducted using headspace solid-phase micro-extraction coupled with GC-MS. Five distinct ML algorithms were employed for data analysis: support vector machine (SVM), random forest (RF), partial least squares-discriminant analysis (PLS-DA), extreme gradient boosting (XGBoost), and k-nearest neighbors (k-NN). Model performance was evaluated using receiver operating characteristics (ROC) analysis, with key metrics including area under the curve (AUC), sensitivity, specificity, and accuracy.

Result and discussion

Among the various ML algorithms tested, the RF model demonstrated the highest performance in distinguishing BC patients from cancer-free controls. A panel of eight potential biomarkers was identified through RF for the overall detection of BC, achieving 89% sensitivity, 92% specificity, 91% accuracy, with an AUC of 0.872. This biomarker panel consisted of three ketones, three aldehydes, one fatty alcohol, and one phenolic compound. Of these eight compounds, seven were found at higher levels in the urine of BC patients compared to controls, while one compound was found at lower levels.

Conclusion

Our study identified a panel of eight volatile biomarkers that accurately detect BC. These findings underscore the potential of metabolomics, combined with ML techniques in cancer biomarker discovery.

Implementation of this urinary biomarker panel in clinical practice may revolutionize BC detection by offering a non-invasive and rapid screening method.

[1] This study was approved by both the Data Protection Officer and the Ethics Committee of the Portuguese Institute of Oncology of Porto (CES 82/022) and the Ethics Committee of the Faculty of Pharmacy of the University of Porto (CEFFUP)

EACR25-1903

Photodynamic laser therapy decreases inflammation in oral lichen planus patients

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Introduction

Oral lichen planus (OLP) is an auto-immuno-inflammatory disease with unknown etiology. Chronic inflammation, resulting from basal membrane disruption plays a critical role in the pathogenesis of OLP, therefore WHO defined it as a precancerous condition. Although, topical corticosteroids are the first choice of treatment, their prolonged use may lead to systemic complications. Recently, the use of photodynamic laser therapy (PDLT) has been introduced in the treatment of oral diseases. This study aims to determine and compare the expression of markers associated with epithelial disruption and pro-inflammatory cytokines in patient samples before and after PDL treatment.

Material and method

11 OLP patients (before and after therapy) and 8 healthy controls, all signed an informed consent, were included in this study. The patients were treated with laser 810 nm (CW; 0.50 W; 30 s; 1.2 J/cm²) three times weekly for a month. The samples were taken before and after therapy. The pellets of saliva were used for RNA isolation. The gene expression and secretion of MMP-9 and VEGF were determined by qPCR. The supernatants were analyzed for inflammatory cytokines IL-1 β , IL-6 and TNF- α by ELISA.

Result and discussion

Our study shows that the VEGF level was higher in patients with oral lichen planus compared with the healthy controls despite the lack of statistical difference. After therapy, the expression profile was similar to the control group. We expected MMP9 expression to be increased in the patient group and decreased after PDT but the results did not show a significant difference. The levels of the salivary cytokines IL-1 β (80.86 ± 34.3 pg/mL), IL-6 (26.89 ± 10.34 pg/mL), and TNF- α (11.48 ± 6.58 pg/mL) in OLP patients were significantly higher compared to those in healthy controls (30 ± 10.8 pg/mL, 14.06 ± 6.22 pg/mL, and 5.55 ± 3.65 pg/mL) and decreased after therapy (57.26 ± 24.8 pg/mL; 18.64 ± 6.53 pg/mL and 7.31 ± 4.30 pg/mL).

Conclusion

In conclusion, the decrease in cytokine levels at the end of the treatment course proves the ability of the laser therapy to reduce inflammation in OLP patients but further analyses are necessary to find the pathological mechanism and connection with molecules responsible for tissue degradation.

Acknowledgements: This research was funded by the European Union-Next Generation EU, through the National Recovery and Resilience Plan of the Republic of Bulgaria, project № BG-RRP-2.004-0007-C01

EACR25-1947

Expression of GSK3 β and S6K1 mRNA in triple negative breast cancer

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Introduction

Breast cancer is the most common type of malignancy and the leading cancer-related cause of death in women worldwide. Triple negative breast cancer (TNBC) is characterized by absence of ER, PR and HER-2 receptors and it accounts for 10-15% of all breast cancer cases. In comparison to other types of breast cancer, TNBC is more aggressive, has higher recurrence, metastasis and mortality rates. The only available therapy for TNBC is conventional chemotherapy, to which it readily develops resistance. Aberrant signaling in both PI3K/AKT/mTOR (PAM) pathway and Wnt/ β -catenin pathway is proved to contribute to acquiring resistance to therapy. These signaling pathways are interconnected by glycogen synthase kinase-3 β (GSK3 β) which is a key kinase in regulating β -catenin degradation, while its own activity is regulated by kinases from PAM pathway: S6 kinase 1 (S6K1) and AKT. GSK3 β also suppresses the activity of PTEN, a PAM pathway suppressor. The interrelation between PAM and Wnt pathways provided by GSK3 β could enable the tumor cells to simultaneously activate both pathways in order to survive. Therefore, GSK3 β and S6K1 may be implicated in progression and development of drug resistance.

Material and method

Total RNA was extracted from FFPE samples of paired tumor and normal tissues of 119 patients diagnosed with TNBC. RNA was reverse transcribed and obtained cDNA was used for real-time PCR expression analysis of GSK3 β and S6K1 genes. Obtained results were analysed with clinical, pathohistological and immunohistochemistry data of Ki-67, EGFR, PD-L1 and androgen receptor (AR) proteins.

Result and discussion

The analysis of mRNA expression shows that it significantly changes in 45.4% patients for GSK3 β and in 25.2% for S6K1. In majority of those patients the mRNA level is decreased both for GSK3 β (90.7%) and S6K1 (56.7%). There is a positive correlation between expression of GSK3 β and S6K1 mRNA ($p < 0.0001$, $r = 0.3511$). We further analysed association of GSK3 β and S6K1 expression with pathohistological parameters as well as protein expression of Ki-67, EGFR, PD-L1 and AR, assessed by immunohistochemistry. The expression of S6K1 is negatively correlated with EGFR protein level ($p = 0.004$, $r = -0.2618$), while there is also association between change in S6K1 expression and low level of AR ($p = 0.0238$). The analysis in simultaneous changes in GSK3 β and S6K1 mRNA levels also shows association

of decreased levels of both mRNA with low AR and high EGFR protein expression ($p = 0.0083$ and $p = 0.0385$ respectively). We previously established that high expression of EGFR combined with low expression of both PD-L1 and AR represent a “high-risk” phenotype of TNBC.

Conclusion

Our findings show that GSK3 β and S6K1 mRNA levels are positively correlated and that the decrease in expression of both genes could be considered as a contributing factor in “high-risk” profile of TNBC. Therefore we suggest EGFR+, PD-L1-, AR-, GSK3 β - and S6K1- as up to date high risk profile for TNBC patients.

EACR25-1955

Mutational enrichment of CTCs and CTC clusters from breast cancer xenografts

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Introduction

Preclinical and clinical studies indicate that circulating tumor cells (CTCs) are crucial in the metastatic cascade. CTC clusters, aggregates of tumor cells that detach from primary tumors, are often associated with poor clinical outcomes. While it is believed that cells within these clusters cooperate during dispersal, dissemination, and colonization, the extent of genomic heterogeneity within CTC clusters and their relationship to primary tumor and metastatic clones remains largely unexplored.

Material and method

To investigate these questions, we established mouse xenografts using the triple-negative breast cancer cell line MDA-MB-231. We collected tissue samples from primary tumors and metastases and blood samples to isolate both single CTCs and CTC clusters. Whole-exome sequencing was performed on these samples, enabling the identification of single-nucleotide and copy-number variants through a custom bioinformatic pipeline.

Result and discussion

Our analysis revealed ample genomic heterogeneity among CTCs and CTC clusters at the single nucleotide variant level. We identified a set of genes more frequently mutated in CTC and CTC clusters than in primary tumors and metastases, suggesting that they might play a role in the formation and survival of CTCs and CTC clusters.

Conclusion

This study offers new insights into the genomic landscape of CTCs and CTC clusters, identifying novel genes that might play a significant role in CTC biology.

EACR25-1958

Inertial Microfluidics for Circulating Tumor Cell (CTC) Separation with Serpentine Channels: A Numerical and Experimental Study

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Introduction

Circulating Tumor Cells (CTCs), which are rare cancer cells that detach from a primary malignancy and infiltrate the circulatory system, are instrumental in the advancement of metastatic cancer. One of the most effective techniques for detecting and isolating CTCs from the bloodstream is inertial focusing through microfluidic devices. Because inertial microfluidics is a passive technique, no markers are required. In order to separate CTC from other blood cells, this technique makes use of the dimensional differences between the cells. In this work, CTCs are isolated from other blood cells using hydrodynamic force-based inertial microfluidics systems.

Material and method

The current study assesses the effects of drag and inertial forces on cells of different sizes in serpentine microchannels with an internal radius of 800 μm and a curvature angle of 280°. The important feature of the microchannel structures is the different depths (60 μm , 73 μm , 90 μm and 105 μm) and different angles between the outlet channels (73 and 90 degrees). In the presented system, the best results were obtained by selecting a channel depth of 105 μm and an outlet channel angle of 73 degrees. These special microchannel geometries facilitate parallelization with optical detection and provide effective size-based separation of CTCs. The COMSOL Multiphysics 6.2 software was used to perform three-dimensional numerical simulations and estimated the paths taken by cells of different types in order to verify the performance of the microchannel designs. The simulation results were subsequently supported by experimental investigations employing polydimethylsiloxane (PDMS) microchannels with different configurations and sizes of cells. In relation to this study, the HeLa cell line was used as a CTC model, while the Jurkat and K-562 cell lines were used to mimic blood cells.

Result and discussion

The results demonstrated that by employing hydrodynamic forces, the developed microfluidic device can isolate CTCs with high sensitivity. In accordance with the simulation results, an effective separation on the microfluidic chip led to the collection the CTCs with a success rate of over 90%. The performance of this system, which does not require external force and is label-free, is better and more effective than the majority of systems found in the literature.

Conclusion

This label-free approach provides a cost-effective way to monitor and detect early cancer, which will be helpful in clinical trials. Future research will focus on the improvement of microchannel design. Its suitability for different cancer types and clinical environments will be also explored.

EACR25-1961

Activated CD4+T cells as prognostic and predictive biomarkers in cutaneous melanoma

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Introduction

Due to its rising incidence, cutaneous melanoma (CM) has emerged as a significant public health concern. Despite the remarkable success of immune-checkpoint blockade therapies in improving overall survival of advanced CM patients, mortality rates remain high at advanced stages – nearly 60,000 patients die every year due to CM, worldwide. The main challenge lies in primary and secondary resistance to immunotherapy. Thus, there is an urgent need to uncover new biomarkers and understand the mechanisms of immunotherapy resistance to improve advanced CM patient management.

Material and method

A comprehensive immune subset characterization was conducted using flow cytometry and RT-qPCR on surgical specimens and peripheral blood samples from 50 stage III/IV CM patients who underwent resection surgery or immunotherapy at our host institute (adjuvant and palliative settings). Peripheral blood from 15 healthy donors was also analysed. Flow cytometry analysis focused on CD8+/CD4+ T cell activation markers, regulatory T cells and associated immunosuppression markers, and monocytic subpopulations. Plasma cytokines were measured via ELISA.

Result and discussion

Stage IV CM patients exhibited reduced levels of activated CD4+ T cells compared to healthy controls, indicating a weakened anti-tumour response. High expression of CD4+ T cell activation markers was associated with longer overall survival (OS) and progression-free survival (PFS) (OS: $p = 0.009$, HR = 0.811; PFS: $p = 0.032$, HR = 0.912), independent of other clinical variables. ROC curve analysis revealed superior predictive accuracy for OS over PFS (AUC = 0.795 vs. 0.728). These findings identify activated CD4+ T cells as a potential prognostic biomarker in CM. Additionally, higher levels of these cells were linked to good immunotherapy response (AUC = 0.729), whereas lower levels were found in patients who relapsed during or after adjuvant immunotherapy. Interestingly, preliminary

results show that higher expression of CD4+ T cell activation markers, combined with increased co-stimulatory receptor expression on monocytes, correlated with extended relapse-free survival in the adjuvant setting ($p = 0.006$, HR = 0.827). This combined biomarker profile exhibited strong predictive accuracy for therapeutic benefit in both palliative and adjuvant contexts (AUC = 0.866), supporting its potential as a predictive biomarker for immunotherapy response in CM.

Conclusion

Thus far, our results highlight the potential of activated CD4+ T cells as a prognostic and predictive tool in CM. Identification of novel biomarkers can improve patient stratification, preventing unnecessary exposure to costly treatments and immune-related toxicity in non-responders. Ongoing analysis aim to further characterize these populations along with their impact on clinical outcomes and immunotherapy response.

EACR25-1963

Characterization and evaluation of CD24 and NPY as biomarkers for metastatic castration-resistant prostate cancer (mCRPC)

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Introduction

In 2022, prostate cancer was the most diagnosed cancer, and the third most deadly cancer, among men in Europe. The current standard of care includes androgen ablation therapy, which reduces androgen receptor (AR) signaling. However, prostate cancers often develop resistance to this therapy and recur in an incurable form called metastatic castration-resistant prostate cancer (mCRPC). Previous whole-genome RNAseq results indicated a possible role for various proteins in mCRPC, including cluster of differentiation 24 (CD24) and neuropeptide Y (NPY).

Material and method

In the current study, we analyzed tissue microarrays representing 127 primary prostate cancers (with matched normal adjacent tissue) and 124 metastases (from 34 patients) using immunohistochemistry to detect CD24 or NPY.

Result and discussion

CD24 was more highly expressed in cancer tissue than in adjacent normal tissue. Compared to the normal tissues, mean scores were nearly double in cancer tissues for nuclear ($p < 0.001$), cytoplasmic ($p < 0.001$), and

membranous staining ($p = 0.012$). In metastases, Nuclear CD24 was expressed less in bone than in liver or lymph nodes ($p = 0.053$ and 0.027, respectively). CD24 in metastases correlated weakly with final serum PSA ($r = 0.305$, $p = 0.084$) but not with other clinical factors. Cytoplasmic expression of NPY was lower in prostate cancers that later recurred (6.7, 95% CI 2.6–10.9) compared with cancers that did not recur (14.6, 95% CI 5.2–23.9; $p = 0.111$). Cytoplasmic expression of NPY was weakly correlated with time between androgen ablation and death ($r = 0.335$, $p = 0.053$). A very weak positive correlation was found between cellular PSA expression and nuclear CD24 ($r = 0.205$, $p = 0.023$) or cytoplasmic NPY ($r = 0.190$, $p = 0.041$). No correlation was found with AR, CHGA, or SYP.

Conclusion

The data suggest a potential role in cancer transformation for CD24. Bone metastases showed lower expression of CD24 compared to visceral sites, also suggesting a possible role for this protein in the metastatic niche. NPY showed some ability to predict prostate cancer recurrence. Cytoplasmic expression was less than half in cancers that later recurred, suggesting a metastasis suppressor role for this protein. This agrees with recently published evidence that serum NPY levels are negatively correlated with mCRPC progression.

EACR25-1978

Capturing the Full Spectrum: High Dynamic Range Biomarker Detection with Photon Upconverting Nanoparticles

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Introduction

Advances in targeted cancer therapies have shown an increasing need for quantitative detection of ultra-low expressing biomarkers. In breast cancer diagnostics, for example the distinction between HER2-0 and HER2-low patients has gained significant attention over recent years. Tissue microarrays (TMA) are an excellent tool for high throughput analysis of various specimens under similar conditions. In many cases, the amount of protein expression varies significantly, and gold standard HRP/DAB-based labelling tends to saturate quickly. To address the needs for ultra-low detection without saturation at high expression levels, we have developed a novel, highly sensitive IHC method based on photon-upconverting nanoparticles (UCNP). UCNPs (30–40 nm) are excited in the near-infrared and emit visible light, eliminating tissue autofluorescence and allowing for background-free imaging.

Material and method

- Sensitivity was demonstrated by titrating the primary anti-HER2 antibody A0485 (Dako) on HER2 over-expressing cells and comparing it with the traditional HRP/DAB labelling.
- Limit of detection was assessed using IHC calibrator beads from Boston Cell Standards.
- Dynamic range and sensitivity were further demonstrated by analyzing TMA cores with ultra-low and high HER2 expression located on the same slide.

- UCNP photostability was demonstrated by rescanning HER2-labelled cells over 500 times and measuring the emission signal intensity.

Result and discussion

- Under the same experimental conditions, UCNP showed 10x higher sensitivity compared to HRP/DAB.
- Limit of detection (LOD) of 4000 HER2 molecules/bead was calculated from the analysis of Boston Cell Standards IHC calibrator beads.
- UCNP labelling of HER2 in a TMA showed high sensitivity and discrimination of high HER2-expressing samples that appeared saturated using a reference method.
- UCNPs had constant emission signal over more than 500 scan cycles.

Conclusion

UCNP technology demonstrated high sensitivity and dynamic range compared to other traditional IHC methods. Enhanced sensitivity and robustness could lead to more accurate diagnoses and improve the development of targeted therapies for patients with cancer where quantitation and/or ultra-low detection of biomarkers are important.

EACR25-1993

Tissue Factor (TF) and Factor VII/TF Ratio (fVIIa:TF) as Potential Indicators of Malignancy in Pancreatic Cystic Lesions

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Introduction

The premalignant pancreatic cellular genotype can remain stable for years before undergoing rapid malignant transformation, often associated with inflammation. Tissue factor (TF) is an inflammatory modulator regulated by factor VIIa (fVIIa) which regulates its levels and activity. The presence of TF in pancreatic ductal adenocarcinoma (PDAC) and its role in cell proliferation, angiogenesis, and metastasis suggest that TF may serve as a marker of the inflammatory microenvironment that drives precursor lesions of pancreatic cancer. This study examined the clinical value of measuring TF levels within pancreatic cyst fluid (PCyF) for detecting malignant transformation.

Material and method

PCyF from 50 patients with pancreatic cystic lesions (TEM-PAC, REC 18/LO/0736) was analysed in a blinded

manner. TF and fVIIa proteins were measured using ELISA. A cut-off value for TF concentration was determined using ROC analysis and compared to conventional assessment parameters (radiological features, carcinoembryonic antigen (CEA), and amylase).

Result and discussion

Patients were categorised into four groups based on histology. Significant histological stage-dependent increases in TF levels were observed, which correlated with the progression from normal ductal epithelium to invasive adenocarcinoma. The cyst cohort was stratified into two risk groups based on the pathological cyst type. The low-risk 'non-resective' group included patients with benign cysts and low-grade dysplasia, and the high-risk 'resective' group included patients with high-grade dysplasia and invasive/malignant cysts. The mean TF concentration was significantly elevated ($p < 0.0001$) in the resective group (2.06 ng/ml, 95% CI 1.38, 2.73) compared to the non-resective group (0.58 ng/ml, 95% CI 0.34, 0.82). A strong positive correlation between TF concentration and the resective group was observed ($\chi^2 = 18.1$, $r = 0.603$, $p < 0.001$) at a TF cut-off value of 1.599 ng/ml, AUC 0.867, $p < 0.001$; odds ratio 20.0 (4.2, 94.9), $p < 0.001$). Additionally, the fVIIa:TF ratio was significantly lower ($p = 0.011$) in the resective group (mean = 101 [95% CI 0.63, 201.4]) compared to the non-resective group (mean = 897.8 [95% CI 298.6, 1497]).

Conclusion

Cyst-associated TF levels appear to correlate with cytological progression towards the malignant phenotype, while the fVIIa:TF ratio indicates a decline in the body's ability to contain the disease. Such indicators may allow for better discrimination (specificity 90%) of the 'resective' lesion, reduce healthcare costs, and provide a more nuanced tool for monitoring indeterminate cystic lesions.

EACR25-2029

Morphometric analysis of neoplastic cell clusters in high-grade serous ovarian cancer ascites may prove to be a novel prognostic factor: a retrospective study

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Introduction

High-grade serous carcinoma of the ovary is the most frequent intraperitoneal malignancy in women. It is associated with a poor prognostic outcome owing to the late appearance of clinical signs leading to a delayed diagnosis, and with resistance to platinum-based chemotherapy. One of the clinical signs is the development of ascites. The detection of neoplastic cells in ascites fluid is important as it indicates tumor progression and is associated with shorter survival. Microscopic cytopsin analysis of this fluid reveals the cytological and architectural features of the neoplastic cells, allowing the pathologist to identify rapidly the malignancy and the

histologic type. In association with immunocytochemistry, this process ensures a definite diagnosis and provides a specific etiology. Our objective was to provide proof-of-principle that the automatized analysis of general cytomorphological criteria, such as carcinomatous cell clustering, in malignant ascites fluid is of prognostic value in high-grade serous carcinoma.

Material and method

We performed a retrospective analysis of the ascites fluid of 24 advanced-stage high-grade serous ovarian cancer patients naïve of treatment and collected their biological, clinical and cytomorphological (number and size of neoplastic clusters) data. Neoplastic cells were detected using BerEP4 staining. Overall and progression-free survival were used as clinical outcomes and were adjusted with biological and clinical covariates using Cox regression models.

Result and discussion

After adjustment with different covariates (age, body mass index, sugarbaker score, WHO performance status) we found that the low number of neoplastic cell clusters and percentage of neoplastic cells in fluid was significantly associated with shorter overall and progression-free survival. These results were independent of the peritoneal implantation of neoplastic cells.

Conclusion

We propose a novel strategy to improve high-grade ovarian serous carcinoma diagnostics using a more informative but simple analysis of ascites tumor cell morphology. The number of neoplastic clusters may prove to be a new prognosis factor.

EACR25-2032

Optical Genome Mapping for detecting Homologous Recombination Deficiency in breast and ovarian cancers

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Introduction

Homologous recombination deficiency (HRD) leads to genomic instability resulting in specific genomic alterations (genomic scar). One strong component of this scar is structural variants (SV), best called by high coverage whole genome sequencing (WGS). The potential of Optical Genome Mapping (OGM) technology in calling SVs suggested a cost-effective and computationally efficient model for HRD testing, as compared with WGS.

Material and method

We analyzed the OGM SV output from a training set of 40 samples of triple-negative breast cancer (TNBC, n = 25) and high-grade ovarian cancer (HGOC, n = 15) with known HRD status coming from shallowHRDv2 (Callens et al. *Oncogene* 2023) and MyChoiceHRD from Myriad®. SV features associated with HRD or nonHRD status were selected to create a lasso logistic regression model able to determine the HRD status of novel tumors. This model was validated on an independent cohort of 30 TNBC and HGOC.

Result and discussion

Based on a training set, novel and previously described features associated with HRD or nonHRD tumors were identified. Isolated and balanced translocations were found strongly associated with HRD, while two novel features were enriched in nonHRD cases. The linear HRD classifier using these features showed minimal accuracy of 97% in the validation series. This HRD classifier corrected at least one case of the validation series to a subsequently confirmed HRD status.

Consistent with previous studies, duplications and deletions smaller than 25kb were found enriched in BRCA1- and BRCA2-inactivated tumors, respectively.

Conclusion

In conclusion, we demonstrate the promising value of OGM as a fast, cost-effective and accurate technique to determine HRD status in TNBC and HGOC, tolerating low tumor content due to its ultra-high coverage, which also allowed to revisit genomic instability features associated with nonHRD tumors.

EACR25-2044

Combined microfluidic isolation and immunofluorescence staining of circulating tumour cells for the assessment of Androgen Receptor expression in metastatic Prostate cancer blood samples

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Introduction

Background. Measurement of Androgen receptor (AR) expression can be integral to determining treatment response of metastatic Prostate cancer (PCa) patients. The ANGLE Portrait® AR Assay (Research Use Only (RUO)) allows direct interrogation of AR activity; combining identification and phenotyping of CTCs isolated through the epitope-independent Parsortix® instrument with assessment of AR expression levels and intracellular localisation, a combination which may facilitate minimally invasive longitudinal monitoring of patients throughout treatment.

Material and method

Methods. Peripheral blood from 10 healthy volunteers was drawn into Streck Cell-Free DNA tubes and spiked with LNCaP cells pretreated with ascending concentrations of an AR inhibitor, or untreated PC-3 cells as a negative control. Spiked samples were processed with the Parsortix® instrument (RUO) within 144 hours

of collection. Harvests were cytocentrifuged onto ANGLE CellKeep™ slides then stained using ANGLE's Portrait® AR IF assay, an antibody panel comprising a nuclear dye (Hoechst), antibodies against epithelial markers (FITC), AR (Cy3), mesenchymal markers (Cy7), and antigens expressed by white blood cells (Cy5). Stained slides were imaged using a BioView DeNovo imaging system. The workflow was then repeated using peripheral blood drawn from 20 castration-resistant PCa patients (unspiked).

Result and discussion

Analytical Results. A statistically significant negative correlation ($p < 0.0001$) was observed between AR inhibitor treatment concentration and AR expression detected in the Cy3 channel. In vehicle-treated LNCaP, AR positivity was 83.6%, whilst in LNCaP treated with 10nM or 1000nM inhibitor concentration AR positivity was 62.4% and 15.2% respectively. The dynamic range of mean signal intensity between vehicle-treated LNCaP and PC-3 was 3.9:1. **Patient Results.** ≥ 1 CTCs were identified in 13 (65%) of patients, with a median of 6 and mean of 121 CTCs identified per patient. CTC clusters harvested by the Parsortix® instrument were observed in 77% of the CTC+ patients. Cluster size ranged from 2–105 CTCs per cluster, and the number of clusters per patient ranged from 1–181. Of the CTC+ patients, 54% (7/13) presented AR+ CTCs, 3/7 showed CTCs with cytoplasmic AR positivity only. The remaining 4/7 showed a mix of nuclear and cytoplasmic positivity across detected CTCs. 58% (50/86) of detected EMT CTCs were AR+, compared to 32% (466/1475) of mesenchymal and 23% (4/17) of epithelial, suggesting that AR signalling may promote an EMT phenotype in CTCs.

Conclusion

Conclusions. The Portrait AR workflow was able to accurately assess AR expression and localisation in CTCs isolated from PCa patients. This demonstrates that combining epitope-independent CTC isolation with immunofluorescence staining for detection of AR in CTCs has the capability to enable dynamic, easy monitoring of AR status, with potential utility for longitudinal monitoring of patient outcomes.

EACR25-2073

miRNAs as biomarkers of PARP inhibitors resistance in ovarian Patient-Derived Tumor Organoids

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Introduction

Ovarian cancers are the second leading cause of death from gynaecological cancers worldwide, primarily due to late diagnosis combined with the development of resistance to platinum-based chemotherapy. The difficulty in predicting response to conventional as well as innovative treatments as PARP inhibitors (PARPi) highlights the need to identify new biomarkers to guide therapeutic decisions. However, access to biological samples from PARPi-resistant patients is complex. It is therefore essential to develop complementary approaches to identify relevant biomarkers.

Material and method

Recently, we have demonstrated the interest of patient-derived tumour organoids (PDTO) in identifying microRNAs (miRNAs) predictive of platinum response after developing a robust methodology to study miRNAs expression in the cellular and extracellular fractions of PDTO. In this study, we attempted to establish PARPi-resistant PDTOs, with the aim of assessing changes in the expression of cellular and extracellular (culture supernatant) miRNAs in relation to the acquisition of acquired PARPi-resistance. Five PDTO lines, sensitive to PARPi, will be treated for one week with a PARPi (niraparib or olaparib), at a dose corresponding to their IC₅₀, for 6 cycles. PDTO will be harvested at the end of each cycle to identify potential variations in miRNAs expression using an omic approach, which will then be validated individually by RTqPCR.

Result and discussion

Preliminary results show a threefold shift in the IC₅₀ of one PDTO line after six cycles of Olaparib or niraparib. It is therefore necessary to expose PDTOs to a threefold higher dose of PARPi to kill 50% of our PDTO. This work is being carried out in parallel on two other lines. It will then be necessary to validate these results on a larger number of PDTO lines and on biological samples (tumor/serum) from cohorts of patients with acquired resistance to PARPi with a view to clinical application.

Conclusion

This kinetic approach will make it possible to identify miRNAs whose expression evolves during the acquisition of resistance to PARPi, thus offering new avenues for identifying biomarkers for therapeutic monitoring.

EACR25-2095

Development of novel biomarker-based methods for early detection of cancer aggressiveness and cancer treatment stratification in Head and neck squamous cell carcinoma

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Introduction

Head and neck squamous cell carcinoma (HNSCC) is highly invasive and metastatic cancer. A major clinical challenge in HNSCC is lack of diagnostic methods to predict and target metastatic behavior of the cancer. The most effective solution to this challenge is the development of a biomarkers that could identify aggressive, i.e. metastatic, cancer at an early stage. Biomarkers of this type would enable the use of efficient and multimodal treatment methods in individualized cancer treatment. Based on our recent research LIM domain and actin binding 1 (LIMA1) isoforms could potentially be used as a marker to identify patients having increased risk of metastasis during follow-up. LIMA1 signaling in HNSCC is also associated with metastatic pathways such as EMT (Endothelial Mesenchymal Transition) by expression of markers like Vimentin and CD44, increasing the importance of studying LIMA1 in cancers and enabling its use in designing targeted treatment options. The expression of LIMA1 in relation to cancer metastasis has been studied recently in other cancer types as well, however specific roles of LIMA1 isoforms in the HNSCC progression, metastases formation, and in cancer therapy targeting are largely unknown.

Material and method

Mass spectrometry of patient derived tumor samples. Flag tag pull down mass spectrometry of LIMA1 isoforms, Western Blots, Mass spectrometry of HNSCC cell lines where LIMA1 isoforms were silenced ie., comparison between SCR vs siLIMA1. Drug screen with LIMA1 knockout vs wild type cell lines. HNSCC cell lines with different LIMA1 isoform profiles are chosen and knock out models has been created for drug screening and other invitro experiments.

Result and discussion

A more detailed understanding of LIMA1-related signaling would seem to create entirely new diagnostic possibilities for cancer treatment stratification. In our ongoing studies, we focus on revealing the following clinical questions important for both LIMA1 and HNSCC. The mechanistic role of LIMA1 isoforms related CD44 cancer metastasis. The protein landscape of the LIMA1 interaction and regulation is studied in detail. The HNSCC patient samples stratified for aggressive metastasis are screened for drug sensitivity to plan the patient specific treatment choices. There is a very clear evidence of specific LIMA1 isoform interaction and regulation with CD44 which has been studied using the above mentioned methods.

Conclusion

The regulation of these aggressive HNSCC markers LIMA1 and CD44 can serve as a potential compound biomarker for identifying a novel HNSCC subtype who are responders of specific targeted treatment.

EACR25-2143**Unveiling the Role of EVs-derived miR-15b-5p, -1293, -376a-3p in Clear Cell Renal Cell Carcinoma Progression**

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Introduction

Clear cell renal cell carcinoma (ccRCC) is the most aggressive subtype of RCC, characterized by high metastatic potential and limited treatment options. Due to its asymptomatic nature, there is an urgent need for reliable biomarkers and novel therapeutic targets. miRNAs have emerged as promising biomarkers for predicting pathological grade, recurrence, and survival, while also serving as key regulators of signaling pathways that drive ccRCC aggressiveness and progression. Extracellular vesicles (EVs) play a crucial role in modulating the tumor microenvironment and serve as key carriers of miRNAs, positioning EV-derived miRNAs as potential prognostic biomarkers. The present study investigates the role of EV-derived miR-15b-5p, -1293, and -376a-3p and their gene targets in 3D spheroids to assess their prognostic and therapeutic relevance.

Material and method

In silico analysis was conducted to identify the miRNA target genes. 3D spheroids were generated using agarose-based micro-molds from a normal renal cell line (HCK-8) and primary ccRCC cell lines (786-O and Caki-2). Spheroids were collected, and EVs were isolated from the spheroid culture medium using the Total Exosomes Isolation Reagent (Invitrogen®). Spheroids were characterized through luminescence (CellTiter-Glo® 3D Cell Viability Assay), morphological assessment (size and shape), and immunofluorescence (DAPI + CellMask Orange staining). EVs were characterized via transmission electron microscopy (TEM), nanoparticle tracking analysis (NTA), and ELISA (CD63 evaluation). The miRNAs' expression profiles and target genes were analyzed using qRT-PCR.

Result and discussion

miRNA expression analysis in spheroid cells revealed that all three miRNAs were expressed across all cell lines, with distinct expression patterns among different clones, suggesting their role in tumor biology and reflecting ccRCC heterogeneity. In EVs, 786-O cells did not secrete any of the three miRNAs, indicating a possible alternative mechanism for tumor-micro-environment interactions and progression. Notably, EV-derived miR-1293 and -376a effectively distinguished tumor from normal renal cells, supporting their potential as circulating prognostic biomarkers. Regarding target gene expression, TGF-β1 mRNA was significantly upregulated in both cells and EVs from 786-O and Caki-2 compared to HCK-8. While SMURF1 was elevated in 786-O and Caki-2 cells, as well as in Caki-2 EVs, SMAD7 was undetectable, suggesting a disrupted

negative feedback control. These findings indicate hyperactivation of the TGF-β/Smad signaling pathway, potentially driving a more aggressive and metastatic tumor phenotype.

Conclusion

Our findings highlight the TGF-β/Smad pathway as a key driver of ccRCC aggressiveness while reinforcing the relevance of EV-derived miRNAs as prognostic biomarkers. Moreover, they provide valuable insights into ccRCC biology and underscore potential targets for future research.

EACR25-2161**Advanced detection of targetable gene-drivers: tracking ctDNA changes using digital PCR in liquid biopsies of advanced non-small cell lung cancer (NSCLC)**

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Introduction

Acquired resistance remains a major challenge in the treatment of advanced stage NSCLC, limiting long-term efficacy of targeted therapies. As the number of targeted agents increases, the ability to dynamically monitor tumour evolution is critical for optimising patient management. Liquid biopsies provide a minimally invasive approach to assess treatment response and detect markers of resistance in real time.

Material and method

Using digital PCR (dPCR), we conducted a retrospective analysis of ctDNA, aiming to:

- (i) compare oncogene driver variant allele frequency (VAF) at baseline and disease progression, and
- (ii) evaluate dPCR as a tool for real-time assessment of treatment response dynamics.

Samples were collected prior to first-line advanced or adjuvant treatment for NSCLC, and at subsequent disease progression/relapse. Analysis was conducted on these paired samples from NSCLCs known to harbour EGFR or KRAS mutations ($n=18$ and 5 respectively).

Mutational status was determined via National Health Service (NHS) molecular testing, using an NGS panel on tissue. ctDNA was extracted (QIAamp MinElute cfDNA kit) and analysed by digital PCR (dPCR) (QIAcuityDx), reporting wild-type and mutant allele abundance

Result and discussion

Testing by dPCR detected mutations in 21/23 cases, offering 100% concordance with the NHS molecular results for KRAS (G12C 3, G12D 1, and G12V 1) and 88% EGFR (ex19 deletion 5, L858R 6 and T790M 5). This included detection in baseline samples with EGFR (13/16) and KRAS (5/5) mutations, as well as progression/relapse samples (15/16 and 5/5, respectively). EGFR ctDNA VAF increased at progression relative to baseline in 11 of 16 cases (69%), including all cases tested for EGFR T790M (n = 5), and most EGFR ex19 cases (4/5). VAF of L858R was more commonly reduced at disease progression (4/6, 67%), though this group was most heterogeneous with respect to treatments received (Gefitinib 3, Erlotinib 1, Osimertinib 2). Overall, this analysis revealed a mixed response in KRAS ctDNA dynamics where 3 cases showed increase at disease progression (G12D, and 2 G12C), and 2 decreasing (G12V and G12C). Ongoing clinical correlation will be reported, detailing these differences in the context of overall tumour burden.

Conclusion

Our findings confirm the feasibility of ctDNA monitoring in advanced-stage NSCLC and demonstrate strong concordance between dPCR and NHS molecular testing by NGS. The high accuracy of dPCR supports its potential as a complementary tool for detecting resistance mechanisms and refining treatment strategies. Crucially, ctDNA analysis is derived from minimally invasive liquid biopsies, enabling frequent monitoring at a low patient burden but with potentially high clinical benefit. A prospective study is planned to further investigate ctDNA dynamics stratified by mutation and treatment type.

EACR25-2168

Unveiling the Role of Extracellular Vesicle miRNAs in HCC Pathogenesis and Therapy

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Introduction

Hepatocellular cancer (HCC) is the sixth most common tumor worldwide and the third leading cause of cancer-related death. The main etiologies include Hepatitis virus B (HBV), Hepatitis virus C (HCV), metabolic dysfunction associated fatty liver disease (MASH) and excessive alcohol consumption. Although several biomarkers have already been proposed for the diagnosis of hepatocellular carcinoma (HCC), the search for novel, more effective biomarkers remains a priority to improve both diagnosis and prognosis. In this context, circulating extracellular vesicles (EVs) are now recognized as critical mediators of intercellular communication.

Biomarkers such as microRNAs (miRNAs) represent a promising tool to gain new insights into the molecular pathogenesis of HCC. They hold great potential in

facilitating individualized clinical decisions, including early detection, treatment prediction, and prognostic assessment.

Material and method

Extracellular vesicles (EVs) were isolated from the serum of cirrhotic patients with and without HCC. Patients in each category were further stratified into four groups based on their underlying etiology: HBV, HCV, MASH, and alcohol-related (ALC). EVs were analyzed via nanoparticle tracking analysis and characterized by immunoblotting. A multiplex bead-based approach coupled with flow cytometry was then used to assess the immunological profile of serum-derived EVs. Next, EV-miRNAs were extracted and sequenced to identify differentially expressed miRNAs between HCC and non-HCC samples. Bioinformatic analyses were performed to identify key dysregulated miRNAs. Candidate EV-miRNAs were validated by digital PCR (dPCR).

Result and discussion

Our results revealed a distinct EV-miRNA signature differentiating HCC patients from non-HCC individuals. Functional studies demonstrated that altered miRNAs influence key oncogenic pathways, including proliferation, invasion, and inflammatory response, highlighting their active role in tumor progression. Notably, specific EV-miRNAs were associated with critical molecular mechanisms underlying HCC pathogenesis, reinforcing their potential as both diagnostic biomarkers and therapeutic targets.

Conclusion

This study highlights the potential of EV-derived miRNAs as non-invasive biomarkers for HCC diagnosis and monitoring, as well as promising therapeutic targets. Given their stability in circulation and detectability in biological fluids, these miRNAs may play a crucial role in modulating key pathways involved in hepatocarcinogenesis. Further validation and functional study will be essential to establish their clinical utility, paving the way for more precise and personalized approaches in HCC prevention and treatment.

EACR25-2198

Comparative molecular analysis of CTCs and cfDNA Detection using Parsortix® System

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Introduction

Liquid biopsies offer a minimally invasive approach for cancer monitoring through the analysis of circulating tumour cells (CTCs) and circulating tumor DNA (ctDNA). The ability to detect tumor mutations in liquid biopsies may provide a powerful, non-invasive tool for real-time monitoring of tumor dynamics, which may enable rapid adjustments to clinical strategies. This study investigates the detection of seven clinically relevant oncogenic mutations in spike-in experiments as well as the concordance of genomic alterations between CTC and ctDNA in cancer patient samples.

Material and method

Blood from three healthy volunteers was collected in K2EDTA tubes and processed using the Parsortix®

system (ANGLE plc). Cancer cell lines (H1975 and SK-MEL-28) were added to background cells harvested with the Parsortix® system at five different levels (0, 2, 5, 10, and 20 cells) to evaluate detection sensitivity across seven clinically relevant mutations (BRAF V600E, CDK4 R24C, PTEN T167A, EGFR L858R, EGFR T790M, PIK3CA G118D, and TP53 R273H).

Additionally, blood samples from 27 lung cancer patients were collected in K2EDTA tubes and processed using the Parsortix® system. gDNA and ctDNA was then isolated from CTCs and blood plasma. Multiple blood draws were processed for some patients: five patients had multiple blood samples collected and analysed (between 2 and 4 draws, with few months delay between blood collection). Genomic analysis was performed using Illumina Cell-Free DNA prep with enrichment combined with a 79-gene custom panel targeting Lung Cancer and sequenced on Illumina NextSeq2000.

Result and discussion

In contrived experiments with varying cell numbers, BRAF V600E, EGFR mutations (L858R and T790M), and TP53 R273H were consistently detected (100%) at both 10 and 20 cells. In contrast, other mutations, including CDK4 R24C, PTEN T167A, and PIK3CA G118D, exhibited inconsistent detection at 10 cells but showed significant improvement at 20 cells. Analysis of clinical samples revealed that the majority of clinical samples (30/39) displayed unique variants between CTC gDNA and ctDNA from the same patient at the same timepoint. Specifically, 971 variants were detected in CTCs gDNA only, 661 variants were detected in ctDNA only and 204 variants were detected in both analytes.

Conclusion

This study demonstrates that CTCs and ctDNA provide complementary insights in liquid biopsy analysis. The observed variation in concordance suggests that analyzing both CTCs and ctDNA offers a more comprehensive assessment of tumor heterogeneity than either analyte alone. Integrating Parsortix® CTC isolation with Illumina-based ctDNA analysis has the potential to improve the detection of clinically relevant mutations, thereby enhancing patient monitoring and informing treatment selection.

EACR25-2206

Machine Learning Analysis of cfDNA Fragment Patterns from Minimal Coverage Whole Genome Sequencing Data for Cancer Detection

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Introduction

Prostate cancer is a prevalent health concern in Slovakia, with incidence rates exceeding the EU average. Therefore, the development of robust tools, particularly for early detection, is essential for improving patient outcomes and reducing mortality rates. Emerging diagnostic tools based on detection of aberrant fragmentation patterns in circulating tumor DNA (ctDNA) hold considerable promise to detect and characterize cancer. However, analyzing ctDNA within typical whole genome sequencing set-up with ultra-low genome coverage around 1x presents significant challenges for reliable anomaly detection. We propose a fragmentomics-based model utilizing various characteristics of ctDNA fragments. We also analyzed the impact of emerging end-motif patterns as a biomarker to evaluate the association of fragment sizes and 4-mer end-motifs in cancer and healthy samples.

Material and method

We obtained blood plasma samples of 71 prostate cancer patients with presence of primary tumor and 366 cancer-free control samples. We performed ultra low-coverage whole genome sequencing (WGS) and divided samples into training, validation and testing sets considering the age balance of the subgroups to minimize the potential confounding effects of age-related variations. We utilize a combination of state-of-the-art tools in the domain, public databases and own designed tools for extraction of a variety of fragmentomic metrics from short sequenced reads, incorporating them into an in-house pipeline. Then we employed advanced statistical and machine learning models for classification of samples into categories of healthy or oncological patients. The proportion of specific motifs was then evaluated against the characteristics of the studied oncological disease, individually for each motif, as well as for possibly biologically relevant groups of motifs.

Result and discussion

We propose a ctDNA extraction workflow along with a machine learning model to classify blood samples sequenced by ultra low-coverage WGS data, which demonstrates a high prediction rate to distinguish between prostate cancer patients and healthy individuals. We demonstrate that combining various fragmentomics-based characteristics with other genomic variabilities improves the prediction accuracy of cancer screening approaches.

Conclusion

Thorough analysis of ctDNA fragments presents a promising screening method for the detection of prostate cancer. Combining a wide range of measured ctDNA characteristics leads to incremental improvements in predictions. Due to the predominant representation of patients with early-stage cancer, its potential lies in early diagnosis and its application in preventive settings.

This research was supported by grants APVV-21-0296 (INCAM), APVV-23-0611 (SkeletIT), APVV-23-0601 (DoHumNA), VV-MVP-24-0290 (GESTALT) and APVV-23-0520 (INHALE).

EACR25-2209

Performance of a novel NGS-based technology for mutation profiling in liquid

biopsies derived from metastatic colorectal cancer patients

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Introduction

Colorectal cancer (CRC) is the most prevalent gastrointestinal malignancy and the second leading cause of cancer-related deaths. Symptoms of CRC often manifest only in the advanced stages. Circulating tumor DNA (ctDNA), tumor-released DNA in the bloodstream, may display cancer-related mutations originating from all metastatic lesions of the patient. Liquid biopsy in forms of ctDNA analysis provides a non-invasive method for monitoring treatment response, detecting minimal residual disease (MRD), and identifying resistance-associated mutations. In this study, we used serial plasma samples from metastatic colorectal cancer (mCRC) patients to assess a novel next generation sequencing (NGS)-based Bridge Capture technology for mutation profiling and MRD detection. We also evaluated the scalability of the technology using synthetic DNA targets.

Material and method

This study included 80 plasma samples collected from ten mCRC patients participating in the AXOAXI phase II trial. Written informed consent was obtained from all patients. Each patient had a codon 12 or 13 KRAS mutation in the primary tumor specimen, enabling a tumor-informed approach to ctDNA analysis. Cell-free DNA (cfDNA) was extracted from 2 ml plasma using QiaSymphony DSP Circulating DNA Kit (QIAGEN). The performance of a novel Bridge Capture™ technology was compared to those of droplet digital PCR (ddPCR; Bio-Rad) and Ion AmpliSeq™ Cancer Hotspot Panel v2 (Thermo Fisher Scientific).

Result and discussion

Bridge Capture 282-probe panel and ddPCR detected KRAS mutation in 35 of the 80 ctDNA samples (44%), while 33 samples (41%) were negative with both methods. Twelve samples showed discrepant results, with

eight samples being positive with Bridge Capture but negative with ddPCR and four samples being positive with ddPCR but negative with Bridge Capture. The KRAS variant allele frequency (VAF) values showed a very strong correlation (Spearman $\rho = 0.86$). In a subset of ten samples analyzed with NGS-based AmpliSeq, both Bridge Capture and AmpliSeq identified 15 CRC driver mutations with strong correlation in VAF values ($\rho = 0.74$). Bridge Capture further detected several additional oncogenic mutations. Scalability of Bridge Capture was confirmed using an expanded 851-probe panel and synthetic DNA targets, with dilutions from 0.03% to 1% VAF showing a very strong linear correlation between the observed and expected total VAF signals for all probes (Pearson $r = 0.99$).

Conclusion

This study demonstrates the potential of Bridge Capture to improve mutation profiling and MRD detection in mCRC plasma samples. It shows substantial agreement with other technologies in clinical sample validation, and the VAF values strongly correlate with those from ddPCR and the NGS-based Ion AmpliSeq. The high scalability of the probe panel enables comprehensive ctDNA analysis. Further validation in larger and diverse cohorts is necessary to establish its clinical utility.

EACR25-2216

MicroRNAs for the Detection of Basal Breast Tumor Subtype

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Introduction

Breast cancer (BC) is currently the most common cancer affecting females worldwide [1]. The heterogeneous nature of breast tumors is addressed histologically to encompass multiple subtypes based on the expression of hormone receptors [2]. Advanced screening techniques fail to detect the most aggressive form, the basal tumor subtype, due to an early incidence, tissue density, and lack of specific receptor biomarkers [3]. Molecular markers such as microRNAs (miRNAs) known to regulate post-transcriptional gene expression are reported to be aberrantly expressed in a tumor tissue-specific manner and are detectable in biofluids [4]. Aim:

Identification of microRNAs correlating with the presence of basal tumors in patient-matched plasma as liquid biopsy biomarkers of basal subtype of breast cancer.

Material and method

In silico screening of miRNA-sequencing datasets from The Cancer Genome Atlas (TCGA) and SCAN-B led to the selection of a panel of significantly deregulated miRNAs [$p\text{-value} < 0.05$; fold change > 2.0]. Candidate miRNAs were screened across clinically classified basal tumors [$n = 6$] and matched plasma [$n = 6$] retrospectively, stored at the Cancer Biobank of Lambe Institute for Translational Research, Galway. Ethical approval was obtained from the CREC at University Hospital Galway (Galway C.A. 2073). RNA extraction from tissues and plasma was completed using specific Qiagen kits, reverse transcribed with TaqMan™ cDNA synthesis kit, and expression quantitated by real-time

PCR using TaqMan probes. Data normalization using the 2- $\Delta\Delta CT$ method [5] and expression significance were analysed using XLSTAT (version 2023.3.1).

Result and discussion

miRNAs hsa-miR-125b-5p, hsa-miR-135b-5p, hsa-miR-195-5p, hsa-miR-21-3p, and hsa-miR-31-3p showed significant expression difference between basal tumors and matched plasma [p -value < 0.05], and in basal tumors when compared to normal plasma, indicating the disease-specific expression status. The expression significance of these miRNAs needs to be confirmed in a large sample cohort to validate its diagnostic potential.

Conclusion

Liquid biopsy-based testing for basal breast cancer will enable the development of a specific diagnostic test to conduct multiple minimally invasive screenings to detect an early onset or relapse of breast cancer post-therapy.
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EACR25-2231

Machine learning-driven Integration of bulk transcriptomics and spatial analysis for prognostic stratification in unresectable high-grade serous tubo-ovarian cancer

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Introduction

Despite advances in molecular profiling, the prognosis for many high-grade serous tubo-ovarian cancer (HGSC) patients remains poor, with conventional biomarkers failing to clarify disease progression fully. We leverage machine learning to uncover transcriptomic signatures that enable early risk stratification of unresectable HGSC patients by integrating bulk and spatial sequencing data.

Material and method

An unselected cohort of 67 HGSC patients enrolled in an institutional genomic program (NCT06020625) at Fondazione Policlinico Gemelli IRCCS Rome was analysed. All patients underwent platinum-based neo-adjuvant chemotherapy (NACT), followed by interval

debulking surgery (IDS), if feasible. DNA and RNA from chemo-naïve tissue blocks were subjected to targeted and transcriptome sequencing. Transcriptomic signatures were identified using unsupervised k-means clustering and were integrated with somatic alterations. Random forest models (RFM) were trained on differentially expressed genes. The intra-tumoral distribution of these signatures and markers was assessed using Visium spatial transcriptomics on publicly available HGSC samples collected at IDS. The prognostic relevance was evaluated by event-free survival (EFS).

Result and discussion

We identified four transcriptomic profiles: energetic, proliferative, mesenchymal, and immunoreactive; partially refining the clusters proposed by The Cancer Genome Atlas. The energetic-related profile's cumulative gene expression correlated with low chemotherapy response score (CRS1, $p \leq 0.001$), IDS' failure ($p < 0.05$), short platinum-free interval (PFI < 12 months, $p < 0.01$), and high genomic instability (copy number alterations counts > 30 , $p < 0.05$). We integrated those profiles using a generalised linear model (GLM) to classify chemosensitivity (CRS3 vs. non-CRS3) and survival (PFI $> = 12$ vs. < 12 months). The energetic and proliferative profiles emerged as key drivers of poor response and early relapse. We applied RFM within each profile, identifying molecular markers critical for patients' stratification. At a spatial level, the energetic profile remained dominant after NACT; CRS2 samples exhibited divergent spatial patterns, either resembling CRS1 or CRS3, underscoring the intratumoral heterogeneity of chemosensitivity.

Conclusion

The energetic and proliferative profiles emerged as key determinants of poor platinum response and early relapse. The RFM identified markers which might represent the basis for an innovative, time- and cost-effective multi-gene panel for early risk stratification. Spatial analysis revealed the persistence of the energetic profile after NACT. The heterogeneity within CRS2 samples, rather than being considered a challenging 'gray zone', may represent a group that requires better stratification to distinguish patients who truly respond to platinum-based chemotherapy.

EACR25-2234

Systemic dissemination in glioblastoma multiforme patients

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Introduction

Glioblastoma multiforme (GBM) is a highly invasive subtype of the central nervous system malignant tumors. Circulating tumor cells (CTCs) are tumor cells released into the peripheral blood and migrating to distant organs.

CTC presence is related to tumor spread and recurrence. In GBM, factors like short patient survival, the blood-brain barrier, and lack of lymphatic drainage in brain tissue make systemic dissemination rare but possible. The liquid biopsy (LB) concept has gained significant attention in the last decade and has the potential to revolutionize cancer care. Thus, noninvasive CTC detection can hold potential for diagnostic, predictive, and prognostic applications.

Material and method

After validating the CTC detection procedure by spiking experiments of the U87-MG cell line, the peripheral blood samples from curatively resected GBM patients were collected preoperatively in Cell-Free DNA BCT® tubes (Streck, Inc.). CTC presence was analyzed by CytoTrack CT11TM (2/C), a semi-automated immunofluorescence microscopy using glial fibrillary acidic protein, vimentin, and CD45 immunostaining, and DAPI counterstaining. The preliminary Kaplan-Maier survival analysis based on CTC positivity was performed in 39 patients with a follow-up longer than two years.

Result and discussion

We analyzed the CTC presence in 58 peripheral blood samples of GBM patients who underwent their first surgery. The CTCs or cancer cell clusters were found in 35% of cases. We also analyzed 19 blood samples of relapsing patients with a positivity rate of 57.9%. The Kaplan-Maier survival analysis did not show a correlation between CTC presence and survival. This phenomenon may be associated with the generally short survival of these patients, which is expected to increase with advances in oncology and palliative care and/or a possible suppression of tumor cell growth outside the CNS. The patient enrolment and clinical data recording are still ongoing.

Conclusion

We have observed the CTCs in the peripheral blood of GBM patients. The patient enrolment and clinical data recording are still ongoing.

EACR25-2237

Detection of Aggressive Prostate Cancer in Urine by Fragment-Level DNA

Methylation

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Introduction

Prostate cancer (PCa) is the second most commonly diagnosed cancer in men representing 1.46 million new cases and 396,000 deaths worldwide in 2022. By 2040 these numbers are projected to double. An earlier diagnosis of less advanced disease provides most men the best chance of curative treatment. However, screening with PSA may lead to the diagnosis of more indolent, low risk tumors and subsequent overtreatment. We've previously shown that methylated DNA markers

(MDMs) for PCa can be detected in urine using next generation sequencing (NGS) and fragment-level analysis. Here, we address the specific detection of aggressive PCa (Gleason 7-9) using targeted NGS on urine cfDNA samples.

Material and method

Buffered urines were collected from 15 patients with biopsy confirmed PCa (Gleason score 7-9). The control arm consisted of 8 G-6 PCa patients and 22 otherwise healthy age matched cancer-free men. cfDNA was isolated and enzymatic methyl-seq libraries were prepared and enriched by hybrid capture using synthetic probes mapping to 190 differentially methylated regions (DMRs) selected from an earlier PCa/normal tissue marker discovery study in our lab. These tissue DMRs were tested against a methylome-wide dataset of 36 clean and disease control urines to ensure low non-cancer background signal (<1%). Indexed libraries were sequenced at 560X deduplicated coverage and fragment-level CpG methylation information was extracted from individual sample .bam files. Mapped sequencing reads exhibiting concordant methylation were used to derive a quantitative α -score. Reads had to contain at minimum 5 CpGs and have an α -value (% 5mC per read) > 60%. Positivity for any of the 190 markers required an α -score greater than 4 sigma (σ) above the mean of the control arm score. At least two tumor-derived fragments for a given DMR had to be present for a positive call. Values were variance-adjusted to ensure equal weighting.

Result and discussion

Of the 190 markers, 30 were positive for 4 or more aggressive PCa patients. Marker positivity per sample ranged from 1 to 98. At 90% specificity, tumor-derived fragments were detected in 13 of 15 cases (87%), which included 10 G-7, 2 G-8, and 3 G-9 patients. As was expected, the number of tumor-derived fragments was proportional to the grading of the cancers. Two G-7s were missed, though the sequencing coverage for one of the missed samples was significantly lower than the others due to poor library enrichment. At 100% specificity, the sensitivity dropped to 60%. 4 of the 8 G-6 and 17 of the 22 cancer-free controls were not positive for any of the 190 markers.

Conclusion

By employing DNA fragment-level methylation analysis of well-vetted PCa marker candidates, we were able to detect the presence of intermediate and high-grade cancers in the urine of patients in this retrospective case-control study. These observational results are encouraging and warrant further studies.

EACR25-2266

Elevated miR-21-5p Expression as a Prognostic Biomarker in Cervical Cancer: Insights from the Indian Patient Cohort

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Introduction

MicroRNAs (miRNAs) are small, non-coding RNA molecules that play a pivotal role in the regulation of gene expression. MiR-21-5p is a well-characterized

oncogenic miRNA implicated in the pathogenesis of various cancers, including cervical cancer.

Material and method

The purpose of this study was to determine the expression levels of miR-21-5p in Indian cervical cancer patients and to estimate the potential of this biomarker as a blood-based diagnostic tool. Total RNA was extracted from blood sample of 50 cervical cancer patients and 35 age-matched healthy individuals. Real-time quantitative PCR is done using the kit Mir-X miRNA First-Strand Synthesis and TB Green qRT-PCR (Takara Bio USA, Inc.), miR-21-5p expression was quantified in blood samples and U6 as the internal reference gene.

Result and discussion

The results demonstrated a significant upregulation of miR-21-5p in cervical cancer patients compared to healthy controls, with an average fold change of 1.877 ± 0.174 ($p < 0.001$) relative to the control sample of 0.905 ± 0.173 . This significant rise in miR-21-5p expression suggests that it may play a part in the development of cervical cancer. MiR-21-5p levels and cervical cancer stages were found to be significantly correlated by statistical analysis ($p < 0.05$).

Conclusion

These findings highlight the diagnostic and prognostic potential of miR-21-5p as a blood-based biomarker in cervical cancer. The consistent upregulation of miR-21-5p in blood samples from cervical cancer patients shows its potential for early detection of cervical carcinoma. This study contributes to a better understanding of circulating miRNA biomarkers and emphasizes the importance of population-specific validation.

EACR25-2273

A Novel Multimodal Liquid Biopsy workflow for the characterization of Circulating Tumor Cells in Metastatic Breast Cancer

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Introduction

Circulating Tumor Cells (CTCs) enumeration provides valuable prognostic and treatment-response information, but its clinical utility remains limited due to the lack of comprehensive biological characterization. Despite technological advancements, confirming the tumoral nature of CTCs and assessing their genetic alterations remain challenging. In this study, we present a novel multimodal approach to address these limitations in the context of Metastatic Breast Cancer (MBC). The method employs Tethis' SmartBioSurface® slides, which enable efficient adhesion of CTCs without prior selection, preserving their integrity and allowing direct biomarkers analysis.

Material and method

Blood samples were collected in EDTA tubes from 27 MBC patients enrolled at San Raffaele Hospital (Milan, Italy) and processed within 6 hours of collection. After red blood cell lysis, the entire white blood cell fraction, including rare cells, was adhered on nanostructured titanium dioxide-coated slides (SmartBioSurface® slides). CTCs identification was performed through immunofluorescence staining targeting epithelial (pan-cytokeratins, EpCAM) and tumor-specific markers (ER, HER2), followed by AI-driven detection and imaging analysis. When indicated, CTCs were subsequently analyzed by Hematoxylin/Eosin stain and FISH for ERBB2 amplification.

Result and discussion

The Tethis approach defines CTCs based on the co-expression of epithelial and tumor markers, employing a 0.3 CTCs/ml threshold. Among 20 MBC patients with ER-positive histology, Epithelial+/ER+ CTCs were detected in 10 cases (50%). Similarly, among 9 MBC patients with HER2-positive tumors, 4 (44.4%) showed Epithelial+/HER2+ CTCs. In addition to single CTCs, clusters of CTCs were detected and characterized in 5 MBC patients (18.5%). Furthermore, we demonstrated the feasibility of a multimodal analysis on SmartBio-Surface® slides, with no cell detachment or sample degradation. In a HER2+ MBC case, HER2 status was first assessed in CTCs via immunofluorescence, subsequently confirmed by cytological evaluation in brightfield, and further validated by FISH for ERBB2 amplification.

Conclusion

The liquid biopsy workflow using SmartBioSurface® slides – combining immunofluorescence, cytological staining, and FISH – proves to be a powerful tool for single-cell characterization of ER and HER2 expression, as well as genetic alterations, in CTCs from MBC patients. These promising findings highlight its potential for tracking metastatic cancer progression and detecting biomarker-related changes, paving the way for improved disease monitoring and personalized treatment strategies.

EACR25-2275

Transcriptional profiling of patients with metastatic hormone-sensitive prostate cancer to uncover specific signatures linked to the transition from androgen-dependent to androgen-independent phenotypes

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Introduction

Metastatic hormone-sensitive prostate cancers (mHSPC) initially respond to androgen deprivation therapy (ADT), but resistance often develops, leading to disease progression and poor outcomes. Understanding the transcriptomic changes that facilitate the shift from androgen dependence to independence is crucial for developing targeted therapies.

Material and method

Sixty patients with mHSPC undergoing ADT plus androgen receptor pathway inhibitors (ARPI) were enrolled. We defined responder patients (R) as those who achieved complete/partial response or stable disease within 6 months of the first treatment, while non-responder (NR) patients were those with biochemical or radiological progression within 6 months of treatment. Total RNA was extracted from Formalin-Fixed Paraffin-Embedded (FFPE) tissue samples using Maxwell® RSC RNA FFPE Kit, and RNA was profiled using NanoString® Tumor Signaling 360 Panel.

Result and discussion

To identify gene signatures associated with response to ADT + ARPI, we analyzed differentially expressed genes between NR and R patients. Notably, NR patients exhibited significant upregulation of genes linked to cell cycle progression and DNA replication. The mitotic kinases AURKB and PLK1 were the most markedly upregulated genes in NR patients, indicating unchecked cell proliferation. The hyper-expression of KIF23 and CDC20 in NR vs R further highlighted aberrant regulation of cell cycle progression. Importantly, NR patients' biopsies were characterised also by increased expression of metastasis-promoting genes, such as HGF, which may influence therapy response. Collectively, these findings delineate deregulated genes that may sustain the transition to androgen independence inducing ADT+ARPI unresponsiveness. Kaplan-Meier survival analysis reinforced the clinical significance of these findings, revealing that patients with hyper-expression of AURKB, KIF23, and CDC20 had significantly shorter progression-free survival (PFS) compared to those with lower expression levels (p-values of 0.0037, 0.0007, and 0.0289, respectively). These results underscore the potential of these molecular markers as predictive tools for resistance to ADT + ARPI and as potential targets for future therapeutic interventions.

Conclusion

This study offers novel insights into the transcriptomic profile of mHSPC and uncovers key gene signatures associated with the shift from androgen dependence to independence, which contributes to resistance against standard-of-care therapies. Validation in larger cohorts is crucial to establish the predictive value of these genes and to identify patients who could benefit from new combinatory treatments designed to delay this critical transition.

EACR25-2281

Identification of outcome-associated tumor perivascular features in renal cell carcinoma

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Introduction

One third of patients with clear cell renal cell carcinoma (CCRCC) eventually develop metastasis. The introduction of anti-angiogenic therapy and immuno-therapy

in metastatic CCRCC (mCCRCC) has significantly improved outcomes for many patients. Among vascular endothelial growth factor tyrosine kinase inhibitors (TKIs), sunitinib is the most commonly used first-line drug. However, treatment is administered without specific patient selection criteria and relapse occurs in approximately 40% of cases. Given the lack of clinical stratification of these patients, predictive and prognostic biomarkers are needed to improve their clinical outcome.

Material and method

This work explored the clinical impact of perivascular (PV) characteristics in RCC by performing multiplex-based tissue profiling in a population-based cohort (N = 70) and a sunitinib-treated mCCRCC cohort (N = 61). Vessel features and tumor PV cell subsets were characterized based on multimarker (CD34, PDGFRα, PDGFRβ, αSMA and pSMAD2) expression combinations. In both cohorts, patients were categorized using a tertile-based dichotomization of the variables, with the upper tertile compared against the combined lower and middle tertiles to explore associations.

Result and discussion

Two PV cell subsets, PER4 (PDGFRB+/ASMA+/PDGFRα-/pSMASD2-) and PER5 (PDGFRB+/ASMA-/PDGFRα-/pSMASD2-), showed statistically significant survival associations using multiple endpoints in both cohorts. In the sunitinib-treated cohort (OS from treatment), PER4 (log-rank p < 0.001) and PER5 (log-rank p = 0.002) were associated with poor survival. Similarly, in the population-based cohort (OS), PER4 (log-rank p = 0.043) and PER5 (log-rank p = 0.001) were also linked to worse survival. Univariate and multivariate analyses confirmed robust survival associations for the identified PV cell subsets PER4 and PER5.

Conclusion

Collectively, this study identified candidate prognostic or TKI predictive response markers in RCC. These data provided in-depth characterization of PV cell subsets associated with clinical outcome in RCC. These data could be further explored to find clinically useful patient selection to improve the accuracy of anti-angiogenic therapy administration in RCC.

EACR25-2308

miRNA profiling in bile extracellular vesicles from cholangiocarcinoma patients

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Introduction

Cholangiocarcinoma (CCA) is a highly aggressive form of bile duct cancer accounting for approximately 15% of liver tumors. Although considered a rare cancer in Europe, its incidence has been rising over the past 20 years. The lack of reliable biomarkers for the early diagnosis of CCA significantly limits effective treatments and worsens patient survival. In this context, extracellular vesicles (EVs) have emerged as promising sources of biomarkers for disease, as their molecular cargo reflects the pathological state of their cells of origin. In this study, miRNA contained in EVs isolated from CCA patients were analyzed to explore potential biomarkers for this disease.

Material and method

EVs were isolated from biliary aspirates of CCA patients and control patients (with lithiasis or benign stenosis) through ultracentrifugation and characterized by nanoparticle tracking analysis, transmission electron microscopy and atomic force microscopy, as well as by the detection of CD9 and CD63. RNA was isolated by TRI Reagent® LS and analyzed with the Affymetrix miRNA 4.0 array. DEmiRNA were identified considering a fold change of 1.5 and p-value < 0.05. Functional analysis of differentially expressed miRNAs (DEmiRNA) was performed using the TAM 2.0 tool. Additionally, the targets of the DEmiRNA were predicted, and an over-representation analysis was conducted with the validated targets to identify the enriched Reactome pathways.

Result and discussion

A total of 53 DEmiRNA were identified, all of which were upregulated in CCA patients compared to controls. Functional analysis revealed that the DEmiRNA were involved in processes such as Cell differentiation, Cell division, Cholesterol Metabolism, Epithelial-to-Mesenchymal Transition, and Glucose Metabolism. The transcription factors identified involved in the expression of these DEmiRNA were LIN28, MYC, and ZEB1, among others. Finally, the functional analysis of the downregulated targets by the DEmiRNA showed the most affected Reactome pathways were the RHO GTPase cycle, Transcriptional regulation by TP53, DNA repair, and Cell cycle checkpoints.

Conclusion

These findings suggest that miRNA contained in EVs from biliary aspirates could be evaluated as potential biomarkers to improve CCA diagnosis. Furthermore, the identified DEmiRNA may provide key insights into the underlying mechanisms of disease progression, contributing to a better understanding of CCA.

Acknowledgements: Project IMP22/08, funded by the Health Research Institute of the Balearic Islands (IdISBa) and co-funded by the European Union.

EACR25-2368

Detection of circulating metastasis-initiating cells predicts therapy efficacy and prognosis in lung cancer patients treated with neoadjuvant chemotherapy

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Introduction

Platinum-based neoadjuvant chemotherapy (pb-NACT) is the primary approach for locally advanced non-small cell lung cancer (LA-NSCLC). In preclinical models, cisplatin-induced damage activates CXCL12/CXCR4 axis, paradoxically leading to the dissemination of CD133+/CXCR4+ metastasis-initiating cells (MICs). Our aim was to assess the prognostic significance of CTCs number/phenotype in LA-NSCLC patients undergoing pb-NACT

Material and method

A total of 72 patients were enrolled and blood samples were collected at baseline (T1), post-pb-NACT (T2), and post-surgery (T3). CTC analyses have been completed for 65 patients at T1 and T2 and for n=22 at T3, exploiting cryopreserved PBMCs and a marker-independent strategy combining Parsortix® pre-enrichment step and DEPArrayTM technology. Single CTCs were identified as cells negative for a cocktail of immune cells markers and characterized for CXCR4, CD133 and PDL-1 expression. Isolated single cells were subjected to LowPass sequencing, and the CNA profiles of different CTC subsets were obtained.

Result and discussion

At T1, a higher number of CTCs and in particular of CXCR4+CTCs (>5/10 ml blood) was associated with a lower Disease Control Rate (DCR) ($p < 0.0001$; AUC adjusted [CI 95%]: 0.945 (0.766 - 0.992)), shorter EFS (HR = 6.8, $p = 0.0001$) and OS (HR = 3.6, $p = 0.001$). At T2, we observed that subset of the circulating MIC (CD133+CXCR4+) (>1/10 ml blood) was associated with an inferior overall response rate (ORR) ($p = 0.007$, AUC adjusted [CI 95%]: 0.836 [0.699 - 0.912]) and represented a negative prognostic biomarker for OS (HR = 8.2, $p < 0.0001$), whereas total number of CTC was not associated with patients outcome. Finally, detection of more than one MIC post-surgery showed a trend toward reduced DFS ($n = 20$ patients, 8.15 vs 29.8 months, $p = 0.000$). No correlation in PD-L1 expression was found between tissue biopsies and CTCs, but interestingly an higher frequency of PD-L1+CTCs at T1 was associated with inferior DCR ($p < 0.0001$) and could potentially identify a subgroup of patients who could benefit most from neo-adjuvant immunotherapy. Class comparison analysis of CNA profile of CTC isolated from chemotherapy responder and non-responder patients, identified specific chromosomal regions linked to treatment response. Moreover, CTCs expressing CXCR4 and CD133 markers showed distinctive CNA patterns and higher genomic instability, suggestive of a greater metastatic potential.

Conclusion

Specific subsets of CTCs could serve as novel biomarkers for assessing the effectiveness of pb-NACT and outcome in LA-NSCLC. Specifically, elevated levels of CXCR4+CTC at baseline along with increased MIC post-pb-NACT and post-surgery indicate a suboptimal

response to treatment and unfavorable prognostic factors for survival, suggesting the potential need for alternative treatment/therapy intensification.

EACR25-2369

UHRF1 role in the repression of Embryonic Morphogenesis genes predicts better prognosis and improves clinical and molecular classification in gastric cancer

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Introduction

The key epigenetic regulator UHRF1 recruits DNA methyltransferases and chromatin modifiers to maintain genome stability, DNA methylation, chromatin structure, gene expression, and stem cell homeostasis. In cancer, UHRF1 overexpression can drive tumorigenesis by disrupting epigenetic stability, causing dysregulation of DNA methylation, altered histone marks, and onco-suppressor silencing. To further investigate the UHRF1 oncogenic mechanisms, we analysed its role in clinicopathological and molecular features using The Cancer Genome Atlas (TCGA) and the Asian Cancer Research Group (ACRG) datasets.

Material and method

Omics and clinical data were retrieved from TCGA and GEO portals and were analysed using R packages. Chromatin immunoprecipitation (ChIP) was performed on published patient-derived xenografts (PDX).

Result and discussion

Stratifying patients based on UHRF1 expression (UH/UL) and analysing survival data and clinicopathological parameters revealed that high UHRF1 expression defines better prognosis in gastric cancer (GC) (UH-BP, pVal = 0.0049), but poorer prognosis in renal and adrenocortical (ACC) cancers (UH-WP). UHRF1 acts as an independent prognostic marker (pVal=8.69e-03). Gene ontology analysis identified Embryonic Morphogenesis (EM) as the only category that classifies better and poorer prognoses based on UHRF1 expression. As predicted, UH-EML and UL-EMH categories in GC and UL-EML and UH-EMH in renal and ACC cancers showed the lowest and highest EM gene expression, respectively (pVal < 0.0001), with a >35% difference in 5- and 10-year survival rates.

Promoter DNA methylation of EM genes in GC was higher in UH-EML (pVal < 0.0001), inversely correlated with gene expression ($r = -0.52$; pVal = 3.09e-12), and linked to better prognosis (pVal = 0.009). ChIP showed that selected EM gene promoters in GC PDX cells exhibit closed and open chromatin patterns in UH-EML and UL-EMH. In GC, the expression patterns and clinical outcome of oncofoetal genes largely overlapped (>85%) with UHRF1-EM-based patient stratification. UHRF1 interacting epigenetic regulators (EZH2, SUV39H1/2,

DNMT1) negatively correlated with EM and oncofoetal gene expression, defining a favourable prognosis UL-EMH GC patients relapse twice as much as UH-EML ones (58% vs 27%, pVal = 0.003). UH-EML category showed an immune and stromal infiltration correlating with better prognosis in GC, whereas UL-EMH with poorer prognosis (pVal ≤ 0.001) Receiver Operating Characteristic curves revealed that UHRF1-EM stratification outperformed the current molecular classifications (AUC = 0.56 and 0.64 compared to 0.46 and 0.48, for TCGA and ACRG), but was slightly lower than pathological stage (AUC = 0.62 and 0.74).

Conclusion

These findings underscore the diagnostic and prognostic value of the combined UHRF1-EM-oncofoetal categorisation in GC, providing an enhanced molecular classification that could improve patient-specific treatment strategies.

EACR25-2389

Pre-cancer MicroRNA Profile of Normal Tissues Adjacent to Tumor (NAT) in HER2-positive Subtype of Breast Cancer

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Introduction

The Human Epidermal Growth Factor Receptor 2 (HER2) is an important predictive and prognostic marker of the aggressive HER2-enriched subtype of breast cancer [1, 2]. With HER2-targeted therapy, pathological complete response (pCR) is achievable and the precise surgical excision of tumor cells creates a ‘clear’ margin of ‘normal’ cells ensuring loco-regional disease control [3]. Recurrence post treatments are primarily due to residual infiltration of tumor cells in normal tissues beyond the assessed surgical margin [3] or the early onset of carcinogenesis events in normal tissues adjacent to the tumor (NATs) driven by field cancerization [4]. MicroRNAs are regulatory transcripts produced early on during gene expression and may provide insights into the malignant transformation in the tumor microenvironment. Aim: Comparative profiling of microRNAs as traceable molecular markers of field cancerization in normal adjacent to tumor (NAT) tissues in HER2+ Breast Cancer.

Material and method

A panel of miRNAs chosen from RNA-seq dataset [GSE131599] were screened in HER2+ tumors [n = 14], and matched NATs [n = 14]. Normal mammary tissues were used as healthy controls [n = 10]. The study received ethical approval from CREC (Galway C.A. 2073) and samples from the Biobank of Lambe Institute for Translational Research, Galway were used. Wet lab experiments included RNA extraction from tissues, cDNA synthesis, real-time PCR on Quant Studio 6, data normalization in the 2-ΔΔCT method, and statistical analysis [Mann Whitney t-test] using XLSTAT (version 2023.3.1).

Result and discussion

A distinct panel of miRNAs from the discovery panel was found upregulated specifically in HER2+ tumor tissue and a second set in Normal controls [p-value <

0.05; fold change > 2.0]. A shared expression profile of miRNAs across NATs and healthy tissues highlighted tissues of a common origin and were predominantly tumor suppressor miRNAs. A loss of expression of these miRNAs may correspond to malignant transformation.

Conclusion

MicroRNAs upregulated in NATs are potential traceable biomarkers of relapse and field cancerization in the HER2+ breast cancer subtype.

EACR25-2392

Leveraging MicroRNA Biomarkers to Dissect PD-L1 Regulation and Improve Immunotherapy Response in Lung Cancer

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Introduction

Non-small-cell lung cancer (NSCLC) remains a leading cause of cancer-related deaths, due to late diagnosis and therapy resistance. Immune checkpoint inhibitors (ICIs), either alone or in combinations with chemotherapy (CT), achieve durable responses in approximately 10–15% of patients with advanced disease. However, many patients do not respond and remain at risk for adverse effects. PD-L1 expression, the only FDA-approved biomarker for ICI selection, has limitations due to technical discrepancies and incomplete knowledge about its regulation.

Emerging evidence suggests that microRNAs (miRNAs) influence PD-L1 expression, either by direct binding or through the modulation of regulatory genes. This study aims to elucidate miRNA-mediated PD-L1 regulation in NSCLC.

Material and method

To identify miRNAs involved in PD-L1 regulation in lung cancer cells, we utilized a pooled lentiviral library to overexpress the entire human miRNome ($N = 2,580$). Transduced cells were sorted based on PD-L1 expression (PD-L1High & PD-L1Low), followed by next-generation sequencing (NGS) of genomic DNA to identify miRNAs enriched or depleted in each population. To explore the association between miRNA expression and PD-L1 abundance in patient tumor cells, we analyzed miRNA and PD-L1 expression (mRNA & protein) from the TCGA-LUAD dataset ($N = 507$). Additionally, we performed whole miRNome profiling (miRNA-seq) on a cohort of stage IV NSCLC patients ($N = 35$) treated with ICIs or ICI+CT as first-line therapy, categorized by PD-L1 expression levels (TPS: <1%, 1–49%, and >50%).

Result and discussion

The in vitro screening identified 550 candidate miRNAs significantly associated with PD-L1 expression ($p \leq 0.05$), with 458 miRNAs enriched/depleted in PD-L1High cells and 92 miRNAs enriched/depleted in PD-

L1Low cells. Of these, 21 miRNAs exhibited differential expression in the TCGA-LUAD dataset when comparing PD-L1High and PD-L1Low patient tumors (based on either PD-L1 mRNA or protein levels). These candidates were further validated in an independent NSCLC cohort receiving ICI-based therapy. More importantly, computational target prediction using the TargetScan algorithm identified 7 miRNAs with putative binding sites in the PD-L1 3'UTR, suggesting direct regulatory interactions. These findings highlight a subset of miRNAs as key regulators of PD-L1, with potential implications for refining patient selection for ICIs. Ongoing functional studies aim to validate their impact on PD-L1 expression and assess their predictive value for immunotherapy response.

Conclusion

This study aims to identify miRNA-based biomarkers which are superior in terms of their stability in FFPE samples, capable of predicting ICI response in NSCLC patients; while unveiling novel mechanisms involved in immunotherapy response in order to enhance patient stratification and guide the development of more effective immunotherapy strategies.

EACR25-2431

Genome-wide DNA methylation profiling reveals a novel DNA methylation signature for early detection of oral cancer using saliva based liquid biopsy

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Introduction

Early detection of oral cancer (OC) remains to be a critical challenge due to the lack of reliable and accessible tools for early detection and screening. Unfortunately, 60% of patients are diagnosed at advanced stages which are associated with a poor prognosis, highlighting the urgent need to develop non-invasive and effective molecular strategies to improve early diagnosis and survival rates. Herein, we performed a global DNA methylation profiling to identify novel DNA methylation markers for early and non-invasive detection of OC.

Material and method

To discover novel DNA methylation markers for the diagnosis of OC, genome-wide analysis using the Infinium HumanMethylationEPIC array was carried out in 19 OC patients including matched tumor tissue, adjacent non-tumor tissue and saliva samples. Differentially methylated CpGs (DMCs) were selected for validation in an independent cohort of saliva samples from 123 OC patients and 79 healthy controls by quantitative-methylation specific PCR. Receiver operating characteristic (ROC) curves were constructed to evaluate the diagnostic performance of individual CpG sites and models.

Result and discussion

DNA methylome analysis revealed 2752 and 17612 DMCs in tumor and saliva, respectively. Focusing on shared DMCs at gene promoter region, we identified a set of shared hypermethylated DMCs in tumor and saliva that could effectively discriminate OC. Of these, five selected CpGs were further confirmed hypermethylated in OC in the saliva independent validation cohort, yielding to AUC values >0.80. A three-CpG methylation signature showed the best diagnostic performance for discriminating OC and controls: sensitivity: 91.9%, specificity: 89.9%, positive predictive value: 93.4%, negative predictive value: 87.7% and accuracy: 0.911.

Conclusion

Our study identifies a DNA methylation signature with high diagnostic accuracy for OC detection, offering a non-invasive and efficient tool for developing a molecular screening program through a saliva-based epigenetic test.

EACR25-2437

Influence of opioid analgesia, opioid and cannabinoid receptors expression in tumor tissue on survival of patients with pancreatic cancer

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Introduction

Pancreatic cancer (PDAC) is one of the most common causes of cancer-related death in the world. PDAC patients are often treated with opioid analgesia after surgery. These drugs act through opioid and cannabinoid receptors, which pathways are involved in tumor progression and metastases and can negatively affect the survival of patients. In a previous study, we determined the effect of morphine analgesia and gene expression of cannabinoid receptor 2, opioid growth factor receptor and cannabinoid receptor delta on survival of pancreatic cancer patients. In this study, we associate our gene expression results with protein expression in selected patients.

Material and method

Protein expression of opioid receptors mu, delta, nociceptin and cannabinoid receptor 2 was analyzed immuno-histochemically in FFPE tissue samples in 30 pancreatic cancer patients. Statistical analysis was performed using R software. Relationship between opioid/cannabinoid receptors expression in tumor tissue and patients survival was analysed using COX regression, Kruskal-Wallis/ANOVA test and Kaplan-Meier method.

Result and discussion

Using immunohistochemistry, we analyzed the protein expression of opioid receptor mu, delta, nociceptin and cannabinoid receptor 2 in tissues of 30 patients with pancreatic cancer. One patient was excluded from the analysis. Membrane, nuclear, and cytoplasmic positivity and positivity in the stroma of tumor tissue were assessed. And membrane, nuclear and cytoplasmic positivity in ductal and acinar cells was also assessed. We detected no or only weak positivity of mu, delta and nociceptin receptor expression in tumor, ductal or acinar cells at the protein level. Conversely, at the gene level, high positivity of expression of the opioid receptor delta was detected in approximately 50% of patients and was associated with shorter overall survival. High positivity of protein expression was detected in the case of cannabinoid receptor 2 in the cytoplasm of tumor and ductal cells. High CB2 protein positivity was observed in approximately 50% of patients as well as high gene expression positivity. High expression of CB2 receptor in tumor tissue was associated with significantly longer overall survival in patients with pancreatic cancer.

Conclusion

Cannabinoid receptor 2 is highly expressed at the gene and protein level in pancreatic cancer tissue and its high expression improves OS.

Acknowledgement: This study was supported by European Union - Next Generation EU (LX22NPO5102) and Palacky University Olomouc (IGA LF UP 2025_006).

EACR25-2453

Exploiting the heterogeneity of Tumour-derived Extracellular Vesicles in breast cancer to establish proteomic signatures as prognostic biomarkers in liquid biopsies

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Introduction

Breast cancer is the most frequent cancer type among women worldwide and cancer metastasis is still the major cause of cancer related death. Predicting the risk of metastasis and therapy resistance remains an obstacle to improved treatment. Extracellular vesicles (EVs) are known to mediate cell-to-cell interaction within the tumour microenvironment (TME), influencing therapeutic resistance, and metastasis formation.

Tumour-derived EVs (tEVs) comprise a mixture of EVs secreted from malignant and non-malignant cells of the tumour, which are released into biological fluids such as blood. Hence, (tEV)-associated cargo proteins could serve as valuable biomarkers in liquid biopsies. This study aims to identify EV-based molecular signatures that could be used in liquid biopsies to predict the tumour microenvironment composition and response to treatment.

Material and method

Using the MMTV-PyMT transgenic mouse model of breast cancer, we isolated tEVs secreted from distinct TME cells using an immunoprecipitation-based approach following ultracentrifugation of total tEVs. The tEVs were then analyzed for their proteomic content using nano-liquid chromatography-mass spectrometry/mass spectrometry (Nano LC-MS/MS). Total EVs isolated from the plasma of non-tumour bearing and tumour bearing mice were also included in our analyses. EVs isolated from in vitro cultures of corresponding distinct cell lines were used as control. All EV samples were further characterized using transmission electron microscopy (TEM), scanning electron microscopy (SEM) and western blotting for EV-specific markers. Proteomic analysis was performed to identify unique protein signatures within the EV subsets.

Result and discussion

We isolated CD31+ endothelial cell-derived EVs, CD140a+ cancer-associated fibroblast EVs, CD11b+ myeloid cell-derived EVs and enriched cancer cell derived EVs (negative selection). By investigating their physical properties, we confirmed that the purified particles are small EVs. Proteomic analysis revealed distinct protein signatures in tEVs across the different tEV subpopulations. In plasma samples, specific proteins were uniquely detected while others were significantly enriched in tumour-bearing mice compared to tumour-free animals. Similarly, immunoprecipitated EVs (IP-EVs) contained uniquely abundant proteins in each subpopulation. These results were compared to cell line-derived EVs thus validating cell-specific proteomic signatures.

Conclusion

Our findings suggest that different tEV subpopulations carry unique molecular signatures, highlighting their potential to be used as predictive biomarkers for treatment response in liquid biopsies. Exploiting the heterogeneity of circulating tEVs to predict response to therapy could be an exciting route towards improved patient stratification and treatment of choice.

EACR25-2467

Deciphering the Potential of Liquid Biopsies Based on Circulating Cell-Free DNA as a Biomarker for Head and Neck Cancer Management

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Introduction

Head and neck squamous cell carcinoma (HNSCC), particularly the human papillomavirus (HPV)-negative subtype, represents a significant clinical challenge due to late diagnoses, limited biomarkers, and poor survival outcomes. This study investigates the potential of circulating cell-free DNA (ccfDNA) as a minimally invasive biomarker for diagnosis, prognosis, and disease monitoring in HNSCC, aiming to improve early detection and personalized treatment strategies.

Material and method

A multicenter, prospective case-control study was conducted, enrolling 85 treatment-naïve and recurrent HPV-negative HNSCC patients and 28 healthy controls. Blood samples were collected at baseline and multiple follow-up timepoints. Plasma ccfDNA was isolated and quantified using two methods: fluorometry (Qubit) and quantitative real-time polymerase chain reaction (qPCR). Diagnostic accuracy was assessed using receiver operating characteristic (ROC) curve analysis, while prognostic value was evaluated through Kaplan-Meier survival analysis. Longitudinal ccfDNA kinetics were analyzed to monitor disease progression and treatment response.

Result and discussion

Baseline ccfDNA levels were significantly elevated in HNSCC patients compared to healthy controls (2147 ± 1617 GE/mL vs. 1235 ± 345.1 GE/mL, $p < 0.001$), with a diagnostic accuracy of 70.5% (AUC = 0.705). Notably, higher ccfDNA levels were observed even in early-stage HNSCC patients, suggesting its potential for early detection. While ccfDNA levels correlated with patient age ($p = 0.024$), no significant associations were found with tumor stage, size, or location. In locally advanced HNSCC (LA-HNSCC), patients with lower post-treatment ccfDNA levels at the 10-week follow-up demonstrated significantly longer progression-free survival (PFS) compared to those with higher levels (16.37 months vs. 9.63 months, $p < 0.05$). Longitudinal analysis revealed dynamic ccfDNA changes during treatment, with inter-patient variability in ccfDNA kinetics. However, no significant correlation was observed between ccfDNA levels and imaging-based treatment response. Strong agreement was found between Qubit and qPCR quantification methods ($r = 0.8987$, $p < 0.001$), though Qubit consistently yielded higher ccfDNA concentrations.

Conclusion

This study highlights the potential of ccfDNA as a diagnostic, prognostic, and monitoring biomarker in HNSCC, particularly for early detection and risk stratification. Fluorometric quantification offers a practical, cost-effective, and minimally invasive

approach for integration into clinical practice. However, further well-designed studies with larger cohorts are needed to validate these findings, optimize ccfDNA-based strategies, and explore its combined use with other biomarkers to enhance diagnostic and prognostic accuracy in HNSCC management.

EACR25-2478

Novel blood-based protein biomarker signature for early detection of colorectal cancer and advanced adenomas

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Introduction

Colorectal cancer (CRC) is the second-leading cause of cancer-related mortality in Europe and the United States, but early detection boosts the 5-year relative survival rate to 91%. While colonoscopy remains the gold standard for screening, its invasiveness reduces adherence. Although less invasive stool-based and blood-based biomarker tests have advanced screening efforts, additional strategies are needed to enhance accuracy, particularly for early-stage CRC and advanced adenomas (AA). In this study, part of the Horizon-funded DIOPTRA project for Mission Cancer, a blood-based protein biomarker approach to improve early detection was investigated.

Material and method

A prospective clinical study was conducted to collect blood samples from individuals undergoing colonoscopy (N=260). Participants were categorized into four groups: Healthy, Non-AA (NAA), AA, and CRC based on the colonoscopy findings and subsequent histopathological diagnosis. Each sample was analyzed using Olink's next-generation proteomics platform, based on the proximity extension assay technology, measuring 3,072 proteins in blood. Bioinformatic analysis, utilizing machine learning algorithms and differential expression methods, identified a 22-protein signature capable of distinguishing the four study groups.

Result and discussion

In a 5-fold cross-validation scheme, the linear combination of these proteins achieved a sensitivity of 90.7% (95% CI: [79.7%, 96.9%]) for CRC detection and a specificity of 90.0% (95% CI: [73.5%, 97.9%]) for advanced neoplasia (calculated by combining the Healthy and NAA groups). Notably, the model's sensitivity for stage I/II CRC was 95.8% (95% CI: [78.9%, 99.9%]) and for AA was 53.7% (95% CI: [47.8%, 59.4%]), demonstrating the potential of protein signatures in blood to detect early stage cancer and advanced precancerous lesions.

Conclusion

Future work is focused on developing multiplex immunoassays for these biomarkers to orthogonally

validate the findings and assess the signature's analytical and clinical performance in a large participant cohort from the DIOPTRA validation studies ($N = 1600$ participants). This protein biomarker signature could pave the way for more accurate, patient-friendly CRC screening methods that detect both cancer and its precursors at earlier, more treatable stages.

EACR25-2479

Unraveling Extracellular Vesicle MicroRNA Signatures as Novel Biomarkers and Therapeutic Targets in Sarcopenic Hepatocellular Carcinoma

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Introduction

Hepatocellular carcinoma (HCC) is the predominant form of liver cancer, with a high mortality rate often exacerbated by sarcopenia, a condition characterized by severe muscle wasting. Sarcopenia significantly worsens patient prognosis and quality of life, yet its underlying molecular mechanisms remain poorly understood. Emerging evidence suggests that extracellular vesicle (EV)-derived microRNAs (miRNAs) play a critical role in cancer progression and intercellular communication. This study aims to identify and validate novel EV-miRNA signatures specifically associated with sarcopenic HCC, thereby advancing diagnostic, prognostic, and therapeutic strategies for affected patients.

Material and method

The study involves a multi-tiered approach integrating molecular biology techniques, high-throughput sequencing, and bioinformatics analysis. Serum samples from patients with HCC (both sarcopenic and non-sarcopenic) and healthy controls will be collected and processed for EV isolation. EVs will be characterized using nanoparticle tracking analysis (NTA), western blotting, and transmission electron microscopy. High-throughput sequencing will be employed to profile miRNA content, followed by bioinformatics analysis to identify differentially expressed miRNAs linked to sarcopenic HCC. The identified miRNA candidates will be validated using droplet digital PCR (ddPCR) in an independent patient cohort. Additionally, functional assays using HCC-derived EVs will be conducted on muscle cells to evaluate their role in metabolic dysregulation and muscle wasting.

Result and discussion

Preliminary data suggest that a subset of EV-miRNAs exhibits distinct expression patterns in sarcopenic HCC patients compared to non-sarcopenic counterparts and healthy controls. These miRNAs appear to be involved in pathways regulating muscle metabolism, inflammation, and cancer progression. Functional consequences of EV-mediated miRNA transfer to skeletal muscle cells will be further explored, revealing potential molecular

mechanisms contributing to sarcopenia. Artificial intelligence-assisted meta-analysis will enhance the prognostic accuracy of these biomarkers. The integration of these findings could pave the way for novel RNA-based therapeutic interventions aimed at mitigating muscle loss in HCC patients.

Conclusion

This study represents a groundbreaking step in understanding the role of EV-miRNAs in sarcopenic HCC. By identifying specific miRNA signatures, this project seeks to improve early diagnosis, refine prognostic models, and develop innovative therapeutic strategies. The findings have the potential to revolutionize HCC management by offering a non-invasive, liquid biopsy-based approach for monitoring disease progression and response to therapy. Ultimately, the project applies precision medicine to improve therapies for liver cancer and metabolic dysfunctions.

EACR25-2504

Extracellular Vesicles in Stool: Unlocking New Protein Biomarkers for Colorectal Cancer and Premalignant Lesions

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Introduction

Colorectal cancer (CRC) is the fourth most common cancer worldwide, with a higher incidence in developed countries. Despite the implementation of screening programs based on fecal occult blood (FOB) tests, which have contributed to a reduction in mortality rates, CRC remains a significant public health concern. Extracellular vesicles (EVs) are lipid-based vesicles released by cells that carry various macromolecules, making them a promising source for discovering new biomarkers. Since FOB testing has a high rate of false positives, often leading to unnecessary exploratory colonoscopies, this study aims to identify more specific CRC biomarkers within fecal EVs from patients.

Material and method

20 stool samples from healthy individuals ($N = 5$) and patients with different grades of colonic lesions (including cancer) ($N = 15$) were analyzed. EVs were isolated using size-exclusion chromatography, followed by characterization through nanoparticle tracking analysis, transmission electron and atomic force microscopy, and the detection of tetraspanins CD9 and CD63. The protein content of the EVs was then analyzed through proteomics using Liquid Chromatography - Mass Spectrometry to identify differential expressed proteins (DEP) between healthy individuals and patients with

colonic lesions. Also, an Over Representation Analysis (ORA) was conducted on the DEP using the WebGestalt tool. Finally, a study of the Protein-Protein Interaction Networks (PPIN) of the DEP was performed using the STRING database.

Result and discussion

EVs isolation method was successfully optimized, ensuring effective separation of EVs from free proteins in stool samples. A total of 65 upregulated and 142 downregulated DEP (out of 484 detected) were identified in the fecal EVs of patients with premalignant lesions or CRC (with a Fold Change > 2 and p-value < 0.05), highlighting potential biomarkers for disease detection. Moreover, the ORA revealed significant enrichment ratios in multiple pathways for both upregulated (mainly related to immune response and antioxidant activity) and downregulated (principally linked to metabolic reprogramming, cell-cell interactions and cell death) proteins in patient with colonic lesions-derived EVs. Lastly, PPIN analysis revealed connections between the DEP found in the stool EVs.

Conclusion

This study underscores the feasibility of using fecal EVs as a non-invasive approach for early CRC detection, including the detection of colonic lesions that are highly likely to develop into CRC in the short to medium term. This method could enhance diagnostic accuracy while reducing the need for unnecessary colonoscopies. Future research should focus on validating the identified biomarkers in larger and independent patient cohorts.

Acknowledgements: Project PROSALUT2023-4, from the Balearic Islands Government, and CINUIB Fundraising Project (FUEIB).

EACR25-2523

Comparative Analysis of Technologies for Circulating Tumor Cell Capture, Purity, and Survival: A Comprehensive Evaluation

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Introduction

Circulating tumor cells (CTCs) are crucial for understanding cancer metastasis and prognosis, offering valuable insights into tumor dissemination and therapeutic resistance. However, their detection remains a significant challenge due to their low abundance in blood and considerable heterogeneity across different tumor types. To address these challenges, reliable and sensitive CTC capture and analysis technologies are essential. Despite many existing CTC technologies, the cross comparison among multiple platforms is rarely done. This study aims to compare various CTC isolation methods and evaluate capture efficiency, purity score, and culture viability.

Material and method

Luminal breast cancer patient-derived CTC lines were spiked into human blood (100 CTCs/ml) and mouse

blood (400 CTCs/animal). The technologies tested included the TellDx CTC System, the Genesis System with Celselect Slides, and non-equipment-based methods, such as the RosetteSep™ Human CD45 Depletion Cocktail and the EasySep™ Mouse/Human Chimera Isolation Kit. Flow cytometry (FC) was also used for comparison. Capture efficiency, purity score, and survival in culture were evaluated. Capture efficiency was calculated as the percentage of recovered CTCs from the total spiked cells. Purity score was determined as $\log[(\text{CTC}_\text{out}/\text{PBMC}_\text{out}) / (\text{CTC}_\text{in}/\text{PBMC}_\text{in})]$. Survival was assessed by measuring viable cells via fluorescence, normalized to day 1.

Result and discussion

TellDx demonstrated the highest capture efficiency for human CTCs ($88.1 \pm 3.7\%$), followed by Genesis ($40.6 \pm 12.1\%$), RosetteSep™ ($36.5 \pm 9.0\%$), and FC ($7.6 \pm 4.5\%$). In the mouse model, Genesis exhibited the highest efficiency ($62.2 \pm 4.6\%$), followed by EasySep™ Chimera ($13.7 \pm 5.0\%$). The TellDx system achieved the highest purity score (3.8 ± 0.2), with no significant differences between Genesis and RosetteSep for human samples ($p > 0.05$, Tukey). Ongoing experiments have assessed the impact of each method on cell viability and heterogeneity over extended periods.

Conclusion

This study provides the first comprehensive evaluation of both equipment-based and non-equipment-based technologies for CTC isolation, considering capture efficiency, purity score, and post-capture survival. These findings are critical for advancing reliable and versatile CTC detection methods with potential applications in cancer diagnostics and therapy.

EACR25-2555

Proteomic quantification of receptor tyrosine kinases in small intestine, colon, appendix and peritoneum tumours

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Introduction

Alterations in the abundance and activity of receptor tyrosine kinases (RTKs) are associated with cancer progression, which makes them important disease biomarkers and pharmacological targets for cancer treatment. Appendix tumors are rare entities that usually

metastasize to the peritoneum, for which there is no targeted treatment. Small intestine (SI) cancer is also rare but rising in the last decades. Colorectal cancer is a leading cause of death. Although RTKs are promising targets in oncology, their abundance has not been studied thoroughly in the above cancer types. This study aimed to quantify, for the first time, RTKs in patients with these cancer types, as well as healthy subjects for comparison.

Material and method

Tissues were homogenized, and epithelial cells were extracted from small intestine (5 healthy, 7 non-tumor peri-carcinomatous, 8 tumor), colon (5 healthy, 2 non-tumor peri-carcinomatous, 3 tumor), appendix (5 healthy, 16 non-tumor peri-carcinomatous, 15 tumor) and peritoneum (44 non-tumor peri-carcinomatous, 63 tumor) tissues. Exosomes from plasma (6 healthy subjects, 19 patients with appendix tumors) were prepared (liquid biopsy). Filter-aided sample preparation was used for protein digestion, and global liquid chromatography–mass spectrometry for quantification of all the identified proteins, including RTKs.

Result and discussion

RTKs had low abundance in SI and colon tissues, with only 3 of them being quantifiable. Similar trends were observed in both cancer types. EGFR was 7-fold lower in non-tumor compared with healthy SI and not detected in SI tumors. ERBB2 and INSR were 4- and 2-fold lower, respectively, in SI tumors compared with healthy SI. Similarly, EGFR, ERBB2 and INSR were 10-, 13- and 5-fold lower in colon tumors compared with healthy colons. Higher number of RTKs were quantifiable in appendix and peritoneum tissues with several of them being impacted in tumors. More profound differences were observed in appendix cancer. Interestingly, CSF1R, EPHA2, and IGF1R were only identified in tumor appendix. UFO and KIT were only identified in non-tumor and tumor appendix, at similar levels, but not in healthy appendix. INSR was 2-fold lower in non-tumor and tumor compared with healthy appendix, while ERBB2 was not detected in tumor appendix. In peritoneum tumors, PGFRA was 2-fold higher compared with non-tumor peritoneum. Additionally, NTRK2 and MET were not detected in tumor in contrast to non-tumor peritoneum. Lastly, in exosomes isolated from plasma of AT patients, only PGFRA and VGFR3 were identifiable and quantifiable, with VGFR3 being exclusively identifiable in tumor.

Conclusion

Overall, our study provides, for the first time, absolute quantification of RTKs in SI, colon, appendix and peritoneum cancer, reflecting significant perturbations of several RTKs in tumors. Our findings could be used as potential markers for diagnosis, prognosis and treatment, after further investigation and validation.

EACR25-2577

Unraveling Novel Metastasis Biomarkers: Liquid Biopsy to Guide Treatment Decision in Colorectal Cancer

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Introduction

The incidence of Colorectal Cancer (CRC) in young patients is increasing each year and near half of these cases are diagnosed at advanced stages. We have previously identified a copy-number variant (CNV) molecular signature in primary CRCs (some at an early age of onset) significantly associated with synchronous distant metastases. Mimicking the identified CNV signature in a 3D CRC cell model led to the acquisition of a Circulating Tumor Cells-like molecular profile with a gene expression signature associated with hybrid Epithelial-to-Mesenchymal Transition and increased stemness/chemoresistance. Phenotypically, it promoted the acquisition of a pro-metastatic phenotype in vitro, facilitating early budding of small cell clusters from the tumor aggregate, thus mimicking synchronous distant metastasis in vitro.

Material and method

To evaluate if these CNVs could be detected in circulation and to mitigate the need for new biomarkers for longitudinal disease monitoring and predictive of therapy response, we conducted a non-interventive trial by following 19 CRC patients (<65 years) collecting >200 liquid biopsies of peripheral blood collected at different treatment stages. Cell-free DNA (cfDNA) was isolated from plasma and CNV analysis was performed by ddPCR.

Result and discussion

Three CNVs were detected in cfDNA and shown to be candidate circulating biomarkers (B1, B2 and B4). Loss of B2 was identified in all patients along treatment and associated with disease progression especially when maintained over time (either metastases dimension/number and/or spread to distant organs), being indicative of disease progression up to 6 months before radiological methods. Similarly, loss of B1 was also associated with disease progression in 13/19 cases 1-3 months before radiological progression. Gain of B4 and persistence of this profile was also associated with disease progression 1-5 months before radiological progression. These circulating biomarkers have demonstrated predictive potential for local or distant disease recurrence in response to treatments and in the postoperative context. CNVs normalization often coincided with post-surgery moments and/or response to therapy with disease regression by radiological methods. These CNVs were

not detected on genomic DNA of lymphocytes from the respective blood samples. These markers anticipated disease progression earlier and appeared to be more specific than conventional biochemical markers, sometimes dissociated from the radiological response. The persistence of this profile over several liquid biopsy collections has been shown to anticipate periods of disease escalation, particularly in M1 stages.

Conclusion

The modulation of these biomarkers demonstrated predictive potential for therapeutic response and disease progression, and could potentially be used as circulating biomarkers for disease monitoring and personalized clinical management throughout patient follow-up.

Cancer Cell Biology

EACR25-0021

The role of SLC7A9 in inducing ferroptosis resistance in colorectal cancer

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Introduction

Ferroptosis is a ferro-dependent programmed cell death characterized by lethally accumulation of lipid hydroperoxides, closely related to extensive cystine transport and glutathione (GSH) metabolism. Solute Carrier Family 7 Member 9 (SLC7A9), a functional subunit of the cystine transporter System Xc-, belongs to the same family as the classical ferroptosis inhibitor SLC7A11 which has been deeply investigated. However, SLC7A9 expression in colorectal cancer (CRC) and mechanism in regulating ferroptosis remain elusive.

Material and method

The clinical implications of SLC7A9 were explored with TCGA CRC cohort. Effects of SLC7A9 expression on proliferation and death of CRC cell lines were investigated with SLC7A9 shRNA and overexpression system. Transcriptome and non-target metabolome sequencing were introduced to screen the related pathways and metabolites. Lipid and iron metabolism were characterized with western blot and total iron content assay. LDH cytotoxicity assay, GSH/GSSG assay, MDA and C11 BODIPY assays were used to explore cell ferroptosis and oxidation-reduction (REDOX) state. Cystine tracer experiments (FITC-L-cystine and 13C2-L-cystine), flow cytometry and LC-MS assay were introduced to examine the cystine transportation via SLC7A9.

Result and discussion

The expression of SLC7A9 in CRC was significantly higher compared to adjacent normal tissues. Moreover, CRC patients with higher SLC7A9 expression depicted worse survival outcomes. We validated that over-expression of SLC7A9 promotes CRC proliferation. Further transcriptomic and non-target metabolomics analyses indicated that higher expression of SLC7A9 was

related to upregulated GSH metabolism, amino acid metabolism, and cystine metabolism. Western blotting and total iron content assay revealed similar iron state and identical phospholipid metabolism as well as ALOX and NOX family. Exploration of intracellular REDOX state showed that higher expression of SLC7A9 depicted reduced state, lower level of MDA and oxidized lipid peroxides, and a more potent resistance to ferroptosis. Cystine tracer experiments confirmed that CRC with higher level of SLC7A9 can efficiently transport cystine intracellularly and participate in GSH synthesis to counteract ferroptosis.

Conclusion

Accordingly, our study has unveiled the role of SLC7A9 in CRC ferroptosis resistance by transporting cystine intracellularly, promoting the synthesis of GSH and eliminating oxidized lipid peroxides.

EACR25-0023

Innovation and Optimization of Surgical Treatment Strategies for Gliomas

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Introduction

Gliomas, the most common primary brain tumors, present a significant therapeutic challenge due to their infiltrative nature and proximity to critical brain structures. Surgical resection remains the cornerstone of treatment for these tumors, aiming for maximal safe resection while minimizing neurological deficits.

Material and method

This review explores the innovative and optimized surgical strategies for glioma management, focusing on recent advancements in intraoperative imaging, neuro-navigation, and minimally invasive techniques. Advanced intraoperative imaging techniques like 5-ALA fluorescence-guided surgery and intraoperative MRI have greatly enhanced tumor resection and patient outcomes. These technologies offer real-time visualization of tumor margins, enabling more precise and aggressive removal while protecting critical brain areas. Neuronavigation systems have evolved to offer high precision in glioma surgery, with the incorporation of diffusion tensor imaging (DTI) and functional MRI (fMRI) data. These systems enable surgeons to navigate around critical brain structures and plan resection paths that minimize post-operative neurological deficits. Minimally invasive surgical techniques, such as endoscopic procedures and laser interstitial thermal therapy (LITT), have emerged as promising strategies to mitigate surgical morbidity and enhance the quality of life in glioma patients. These methodologies facilitate the precise ablation of tumor tissue while minimizing disruption to the adjacent normal brain tissue.

Result and discussion

Furthermore, the role of awake craniotomy in glioma surgery has been redefined, providing a unique opportunity for intraoperative neurological assessment and functional mapping, thus ensuring maximal tumor resection with preservation of neurological function. In conclusion, the innovation and optimization of surgical strategies for gliomas involve a multidisciplinary

approach, incorporating cutting-edge technology and refined surgical techniques. These advancements not only enhance the extent of tumor resection but also improve patient safety and long-term outcomes.

Conclusion

Future directions in glioma surgery will likely focus on further personalizing treatment strategies, integrating molecular and genetic tumor characteristics, and exploring the synergistic effects of surgery with adjuvant therapies.

EACR25-0056

Downregulation of MicroRNA-497-5p Promotes Tumor Cell Malignancy and Contributes to Hepatitis B Virus-Related Hepatocellular Carcinoma Recurrence

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Introduction

High hepatocellular carcinoma (HCC) recurrence after curative surgical resection leads to poor patient prognosis, highlighting an urgent need for valuable prognostic biomarkers and potential therapeutic targets.

Material and method

This study evaluated the clinical association of microRNAs (miRNAs) with post-resection hepatitis B virus (HBV)-related HCC recurrence and explored their tumorigenic functions in human HCC cell lines.

Result and discussion

The results showed that miR-497-5p had a decreased tumor-to-non-tumor (T/NT) expression ratio in patients with HCC recurrence compared with patients without HCC recurrence after curative surgical resection. The decreased T/NT expression ratio of miR-497-5p independently predicted a higher risk of post-resection HCC recurrence. Moreover, overexpression of miR-497-5p exhibited inhibitory effects on proliferation, anchorage-independent growth, migration, and invasion abilities of human HCC cell lines.

Conclusion

Collectively, this study suggested miR-497-5p as a promising prognostic biomarker and therapeutic target for HBV-related HCC recurrence after curative surgical resection.

EACR25-0063

Comprehensive bioinformatics analysis DNA damage repair gene with prognosis and immune response in Lung adenocarcinoma

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Introduction

Abnormal expression of DNA damage repair (DDR) genes is observed in Lung adenocarcinoma (LUAD). However, there are few studies exploring the correlation between DDR gene expression levels and prognosis, as well as immune responses in patients with LUAD.

Material and method

All data obtained from The Cancer Genome Atlas (TCGA) database. Differentially expressed genes (DEGs) were identified using the Wilcoxon rank-sum test. Univariate and multivariate Cox regression analyses were performed for DEGs, and after optimization based on AIC values, six DDR genes were sorted as prognostic genes for patients with LUAD to calculate the risk score. Simultaneously, a nomogram was conducted and validated by calibration curve and C-index. Single-sample gene set enrichment analysis (ssGSEA), CIBERSORT algorithms, and ESTIMATE scores were applied to evaluate the immune filtration of tumor samples. Subsequently, anticarcinogen sensitivity analysis and unsupervised clustering was used to excavate the correlation between the prognostic-significant DDR genes and clinical features.

Result and discussion

Six DDR genes (RPA 3, ENDOV, DDB 2, CCNO, FAAP20 and BLM) were preserved as prognostic genes to estimate risk scores, which was applied to establish the prognostic model and stratified LUAD patients into high or low risk groups. Immune filtration analysis, anticarcinogen sensitivity analysis, and unsupervised clustering were applied to reveal the character of prognosis-significant DDR genes between low- and high-risk groups.

Conclusion

We identified 6 prognosis-significant DNA repair genes to establish prediction models and immune responses in LUAD patients, which may could serve as a novel prognostic biomarker and therapeutic target for LUAD.

EACR25-0112

The integrated stress response drives MET oncogene overexpression in cancers

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Introduction

Cancer cells rely on invasive growth to survive in a hostile tumour microenvironment; this growth is characterised by interconnected processes such as epithelial-to-mesenchymal transition and migration. A master regulator of these events is the MET oncogene, which is overexpressed in the vast majority of cancers. However, since genetic alterations in the MET oncogene are relatively infrequent, the mechanisms that drive this pervasive MET overexpression remain obscure. Here, we demonstrate that the 5' untranslated region (5'UTR) of MET mRNA harbours two functional stress-responsive elements, conferring translational regulation by the integrated stress response (ISR), which is mediated by

phosphorylation of eukaryotic translation initiation factor 2 alpha (eIF2 α) at serine 52.

Material and method

To investigate MET regulation under stress, we exposed cancer cell models to stressors mimicking the tumour microenvironment: serum starvation, leucine deprivation, hypoxia, irradiation, and chemotherapeutic agents. Functional reporter assays were used to analyze the MET 5'UTR, focusing on the role of its upstream open reading frames (uORFs). We applied CRISPR/Cas9 and Prime Editing to introduce uORF or eIF2 α (S52A) mutations and used ISR pathway inhibitors to evaluate their effects on MET translation. Western blotting was performed to assess MET protein expression, and migration and invasion assays were used to evaluate MET-dependent invasive growth after ISR activation.

Result and discussion

We identified two stress-responsive elements in the MET 5'UTR that regulate translation via ISR activation. Stress conditions induced MET protein overexpression through eIF2 α phosphorylation. Disruption of this pathway – either by mutating the uORFs, generating eIF2 α (S52A) mutations, or applying ISR inhibitors – markedly reduced stress-induced MET translation. Functionally, this reduction blunted MET-driven invasive growth, highlighting the essential role of ISR in facilitating MET-mediated invasive growth.

Conclusion

These findings indicate that upregulation of the MET oncogene is a functional requirement linking the integrated stress response to cancer progression. Moreover, they highlight the concept of adaptive translational control of an oncogene as a critical player in invasive growth, providing a new perspective on the role of stress-responsive mechanisms in driving tumor progression.

EACR25-0117

How to kill your fibroblasts and save the cancer stem cells in your culture

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Introduction

Cancer stem cells (CSCs) are a small but critical subpopulation within tumors that drive progression, metastasis, and resistance to conventional therapies. The purpose of this study was to develop a reliable and cost-effective method to isolate and characterize CSCs from metastasized tissues, overcoming the limitations of traditional cell culture methods that fail to sustain CSCs while promoting the growth of non-cancerous cells such as tumor-associated fibroblasts and macrophages.

Material and method

We utilized a selective culture system designed to deplete benign stromal cells while supporting the growth of CSCs in a defined environment.

Result and discussion

Using this approach, CSCs were successfully isolated from a gastrointestinal stromal tumor (GIST) liver metastasis. The CSCs exhibited distinct stem cell properties, confirmed by fluorescence-activated cell sorting (FACS) analysis of stem cell markers SOX2 and OCT4. These cells were maintained in culture for up to 49 days, enabling extended studies of their biological properties. Next-generation sequencing (NGS) of the isolated CSCs further validated the retention of proto-oncogene KIT mutations, characteristic of the patient's tumor.

Conclusion

In conclusion, this method offers a cost-effective and resource-sensitive platform for isolating and cultivating CSCs from patient-derived tumor samples, facilitating advanced studies of their biology and therapeutic vulnerabilities.

EACR25-0122

Effects of the anti-diabetic dipeptidyl-peptidase 4 inhibitor on radiation response in breast carcinoma cells with different metastatic potential

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Introduction

Diabetes mellitus and cancer are two of the leading causes of illness and death globally. Patients diagnosed with both breast cancer and diabetes often face poorer clinical outcomes compared to those without diabetes. This may be due to the complex interactions between diabetes, its treatments, and cancer therapies. In recent years, incretin-based therapies, particularly dipeptidyl-peptidase 4 (DPP4) inhibitors, have become a mainstay in diabetes treatment. However, it remains unclear how these drugs may influence the effects of radiation therapy, which is a key treatment for breast cancer. The primary aim of this study was to investigate how the DPP4 inhibitor sitagliptin might affect the radiation response of breast carcinoma cells with differing metastatic capacities. The study focused on both triple-negative breast cancer (TNBC) and hormone-dependent breast cancer cells.

Material and method

Invasive (INV) breast cancer cells were derived from the parental TNBC (MDA-MB-231) and hormone-dependent (T47D) cell lines. Both parental and INV cells were treated with sitagliptin (4 μ M), radiation (2 or 8 Gy), and their combination. The study assessed changes in cell cycle, cell death, and molecular mechanisms triggered by these treatments. In order to determine whether sitagliptin

can change the survival of the irradiated cells, the clonogenic assay was additionally performed.

Result and discussion

The results demonstrated that INV breast carcinoma cells exhibited elevated DPP4/CD26 expression, though sitagliptin treatment did not induce apoptosis or cell cycle arrest in either parental or INV cells. In parental MDA-MB-231 cells, radiation primarily induced cellular senescence, whereas in INV MDA-MB-231 cells, autophagy was the predominant response. Although sitagliptin did not significantly reduce these radiation-induced effects, a slight decrease was noticed. In contrast, T47D cells did not display senescence following any treatment, although a marked downregulation was noted in INV T47D cells. Parental T47D cells exhibited increased expression of autophagy markers in response to higher radiation doses, an effect further enhanced by sitagliptin. INV T47D cells showed significantly increased autophagy marker expression following combined treatment with sitagliptin and radiation.

Conclusion

The findings indicate that sitagliptin does not substantially alter radiation-induced cell cycle arrest or cell death in breast carcinoma cells. Nevertheless, radiation triggers senescence and autophagy in TNBC cells, with sitagliptin potentially modulating these responses, thereby conferring some protection against radiation-induced cellular damage. Hormone-dependent breast carcinoma cells appear less responsive to these protective mechanisms, instead undergoing autophagy-mediated cell death. Future studies will focus on characterizing the molecular and metabolic differences.

EACR25-0124

Unique lipidomic signature in colorectal adenocarcinoma

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Introduction

Colorectal cancer (CRC) has a poor prognosis when diagnosed at the advanced stage, but early diagnosis is difficult due to non-specific symptoms. The situation strongly calls for novel non-invasive biomarkers for early diagnosis. Many cancers present with lipid dysbiosis profiles, but studies on lipidomic changes in CRC are lacking. Hypoxia stabilizes the hypoxia-inducible factor (HIF-1) protein which alters the expression of genes involved in lipid metabolism. Here, we investigated the lipidome of CRC in hypoxia to deduce potential biomarkers.

Material and method

The colorectal adenocarcinoma (COAD) cell lines, HCT116, HT29, SW480, were cultured in normoxia (21% O₂) or hypoxia (0.1% O₂) for 72 h. A western blot was performed to quantify HIF-1 expression. Lipids were extracted using a modified Bligh and Dyer method, with water/ice-cold methanol/chloroform (2:2:1, v/v/v).

Untargeted lipidome analysis was performed using positive and negative ion mode liquid chromatography-mass spectrometry. Peaks were annotated based on the NIST17 library, and were analyzed by principal

component analysis and orthogonal projections to latent structures discriminant analysis, with species with a variable importance projection score >1.0 being considered relevant to group separation.

Result and discussion

HIF-1α was constitutively expressed in hypoxia in all cell lines, with limited expression in normoxia. In hypoxia, relative abundance of saturated fatty acid (SFA) levels increased ($p < 0.05$) and polyunsaturated fatty acids (PUFA) decreased ($p < 0.05$) compared to normoxia in all cell lines, with monounsaturated fatty acids (MUFA) not showing any trends. This is consistent with the literature. Specifically, the SFA stearic acid (18:0), the MUFAAs phosphatidylcholine (18:0/18:1) and hexosylceramide (d18:1/16:0) were increased in hypoxia ($p < 0.001$). Interestingly, stearic acid is known to increase in hypoxia, but some studies have suggested it as an anti-tumorigenesis agent by inhibiting tumor proliferation. Elevated levels of phosphatidylcholine (18:0/18:1) and hexosylceramide (d18:1/16:0) have been reported in tissues from prostate cancer, breast cancer, and in the plasma of CRC patients. Our results provide further evidence for these as biomarkers in COAD and highlight the potential underlying mechanism for the increased levels of these lipids.

Conclusion

This study identified 3 key lipidomic changes in COAD due to hypoxia. These targets have been reported in COAD or other cancers, and here we evidence the role of hypoxia in this dysbiosis. Further mechanistic studies are required to target these lipids as biomarkers as well as delineate their potential in therapeutics.

EACR25-0136

Characterizing Bladder Cancer Cells: Morphological and Biophysical Signatures for Advanced Label-Free Detection

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Introduction

Bladder cancer (BC) is a highly heterogeneous disease, marked by considerable variation in cellular morphology and biophysical signatures. As BC progresses, cells transition from epithelial to mesenchymal (EMT) phenotypes, complicating diagnosis and treatment. Traditional diagnostic methods, such as urine cytology, fail to detect low-grade tumors due to their low sensitivity, particularly in the face of sparse cellular content (<5 cells/mL), further diminishing their diagnostic reliability. While molecular techniques have advanced, there is an urgent need for non-invasive, label-

free methods that can address these limitations. This gap in clinical practice leaves us without a reliable tool for accurately detecting BC, especially in its early stages when intervention is most critical. New research frontiers in cell biophysics offer new ways for label-free BC detection, addressing these challenges. Aiming to identify key biophysical biomarkers and improve clinical outcomes, we profiled morphological, light-metrics and clustering features of BC cell lines and clinical samples using imaging flow cytometry.

Material and method

BC cell lines ($n = 15$), controls ($n = 6$) and clinical samples ($n = 2$) were cultured and subsequently fixed for ImageStream analysis. Morphological criteria such as diameter, area, perimeter, elongation, compactness, and circularity and light-based metrics, e.g., brightfield and light-side scatter contrast were quantified. Self-clustering frequency was also evaluated. Data analysed using two-way ANOVA and multiple comparisons with significance set at $p < 0.001$.

Result and discussion

Significant morphological and light-based differences were observed between BC cells and controls, including clinical cases, and these differences correlated with the EMT scale. Cells with a high EMT score (more mesenchymal) exhibited increased elongation and reduced compactness and circularity, traits linked to higher metastatic potential. Light-based metrics revealed greater contrast and side-scattering intensity in cells with a low EMT score (more epithelial), suggesting a potential marker for early-stage diagnosis. Additionally, self-clustering frequency was inversely correlated with EMT score, highlighting its potential as a diagnostic risk stratification tool.

Conclusion

The study shows that biophysical parameters can serve as effective, label-free biomarkers for BC diagnosis and risk, while offering a non-invasive method for early detection. Ultimately, the findings are crucial for clinicians and researchers aiming to establish a foundation for validating biophysical signatures as cancer biomarkers, with the potential to transform clinical practice and enhance survival rates.

EACR25-0138

Studying Stem Cell Fate & Plasticity in Pre-cancerous Gastric Organoids

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Introduction

Gastric stem cells (GSCs) sustain the gastric epithelium, ensuring its health and function. A single GSC will proliferate and differentiate to form a clonal gland. However, aberrations in genetic and epigenetic landscapes can derail this process, setting the stage for the pre-malignant phenotype, gastric intestinal metaplasia (GIM), to take over. Through staining of gastric gland

serial sections, we demonstrated the sudden moment that an individual GSC deviates from their native gastric lineage to assume an intestinal-like identity, before then taking over the whole gland. Now, the question remains: How does that GSC become set up to fall to an intestinal lineage?

Material and method

Leveraging a robust translational pipeline, we have cultivated patient-matched normal, GIM and duodenal organoids from clinical biopsies, culminating in a repertoire of 22 organoid lines from 6 patients. Through interrogation of these organoid lines, we aim to understand the processes pushing GSC to develop into GIM.

Result and discussion

Utilising rt-PCR and confocal microscopy, we have demonstrated the nuanced expression patterns of gastric and intestinal markers within these organoids. Furthermore, our ongoing methylation analyses has demonstrated the epigenetic forces governing the transition from normalcy to pathology. We have shown distinct methylation signatures associated with each phenotype. Next, we hope to uncover specific differentially methylated gene sets that demonstrate the processes altered as GIM takes hold. Alongside the methylome work, sc-RNA sequencing stands to be as a powerful tool to decode the transcriptional dynamics orchestrating GSC lineage decisions hinted at in earlier analyses. Additionally, this sequencing will allow us to interrogate how the transcriptional dynamics within GSC affect the transcriptional activities of the whole cellular population. Moreover, employing novel techniques such as thiol-reactive organoid barcoding *in situ* (TOBis) and cytometry by time-of-flight (CyTOF), we aim to decipher the intricate post-translational signalling cascades underlying the acquisition of a cancerous phenotype in GIM.

Conclusion

In summation, our comprehensive investigation should underscore the transformative potential of GIM organoids in unravelling the intricacies of metaplastic evolution. These multidimensional analyses will not only illuminate the pathophysiological underpinnings of GIM but also intimate pivotal evidence for the development of prognostic models in gastric cancer risk stratification.

EACR25-0150

Increasing autophagy activity suppresses *Helicobacter pylori* related gastric cancer tumorigenesis

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Introduction

Helicobacter pylori (*H. pylori*) infection induces autophagy in human gastric cancer (GC) cells. *H. pylori* infection of GC cells increase the secretion of hepatocyte derived growth factor (HDGF). HDGF, a heparin-binding protein, plays a crucial role in gastric carcinogenesis. We speculate that secretory autophagy participates in *H. pylori* infection-induced HDGF secretion to affect GC tumorigenesis. *H. pylori* alters mesenchymal stem cells to differentiate into tumor-associated fibroblasts and affects mitochondrial metabolism of the cancer cells and the surrounding cells in the microenvironment. This study

explored the role of autophagy in GC under *H. pylori* infection conditions.

Material and method

GES-1, AGS, and MKN45 GC cells were used for immunoblotting, immunofluorescent staining, lentiviral shRNA, CRISP-Cas9 system, MTT, focus formation, wound healing, and Transwell™ assays. Clinical GC specimens were analyzed using tissue microarray (IR) and TCGA database. Xenograft GC nude mouse with *H. pylori* infection was established.

Result and discussion

Clinical GC specimens and TCGA STAD data analysis reveal that the level of the autophagy marker protein LC3 is high in tumor tissues compared to non-tumor parts, which is correlated with low overall survival rates of GC patients. *H. pylori* 49503 strain infection of GC cells robustly increased autophagy activity accompanied by decreased cell proliferation, focus and tumor-sphere formation, as well as cell migration. Notably, increasing autophagy by inducers further promotes the suppression of cell proliferation, motility as well as focus and tumor-sphere formation. Investigation of *H. pylori* infected AGS cells under TEM reveals that *H. pylori* is detected in autophagic like vesicles and the outer membrane of the cell. In contrast, fewer *H. pylori* was detected in the Atg5 gene knockout cells, indicating the importance of autophagy. RNA-Seq and pathway analysis disclosed that autophagy participates in diverse GC-relevant pathways, including PI3K-Akt, MAPK, and JAK-STAT pathways. To validate the in vitro findings, the xenograft GC mouse model demonstrated that increasing autophagy activity by the inducer [amiodarone] significantly reduces GC cell proliferation and tumor growth.

Conclusion

This study provides evidence that increasing autophagy activity may suppress *H. pylori*-related GC tumor development. Competing interests The authors declare that they have no competing interests.

EACR25-0159

Novel applications of senolytics to prevent treatment resistance in cervical cancer

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Introduction

Cervical cancer remains a significant global health concern, ranking as the fourth most common malignancy among women. Over 90% of cases are linked to high-risk subtypes of the human papillomavirus (HPV). Despite advancements in prevention, treatment resistance continues to hinder improved patient outcomes. One major contributor to this resistance is senescence induction, particularly the senescence-associated secretory phenotype (SASP), which promotes the tumour microenvironment. Targeting senescent cells with senolytic agents presents a promising therapeutic approach to overcome these challenges. Navitoclax, a senolytic under investigation, has shown considerable potential in inducing apoptosis in senescent cells. However, its synergistic effects with carboplatin and its

influence on treatment resistance in cervical cancer remain unexplored. This study investigates the mechanisms underlying the combination treatment of navitoclax and carboplatin, evaluating its impact on treatment resistance through a personalized medicine approach, with comparisons between 2D and 3D models using immortalized cervical cancer cell lines. Improve treatment effectiveness, reduce side effects and healthcare expenses, and lower the risk of recurrence.

Material and method

Ethical approval was granted by Stellenbosch University. Following patient consent, cervical tumor biopsies were collected, dissociated, and cultured to confluence before being treated with low-dose chemotherapies. A retrospective analysis examined correlations between senescence markers and treatment responses. Cytotoxicity was assessed via MTT assays, while senescence was evaluated using SA-β-gal staining and western blot analyses for markers such as p16, p21, p53, and Rb. Further, treatment resistance markers including ABC and MDR1 were measured. Select biopsies were cultured in 3D using Clinostar bioreactors. Experiments on HeLa, SiHa, and CaSki cell lines in 2D and 3D employed assays such as MTT, Annexin V, and confocal microscopy

Result and discussion

Patient-derived data revealed an increase in senescence and treatment resistance markers post-chemotherapy, though individual responses varied. Variations between 2D and 3D cultures further highlighted the need for advanced models to study drug responses. Co-treatment with carboplatin and navitoclax in both 2D and 3D cell lines led to enhanced apoptosis and reduced resistance marker expression, confirmed by western blot and confocal microscopy analyses.

Conclusion

These findings demonstrate a clear link between chemotherapy-induced senescence and treatment resistance, supporting the therapeutic potential of senolytics in overcoming drug resistance. This study underscores the importance of integrating senolytics into conventional treatment regimens, advancing personalized therapeutic strategies to improve outcomes for cervical cancer patients.

EACR25-0161

Carboplatin-induced transcriptional landscape and growth morphology of ovarian cancer cell models growing in 3D stem-cells supporting environment

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Introduction

Ovarian cancer is the most lethal cancer of the female reproductive system with a significantly increasing incidence and mortality rate. Three-dimensional (3D) cancer models are changing the way research is conducted as they allow replication in a more complex and physiologically accurate in vitro system compared to the traditional two-dimensional (2D) model.

Material and method

In this study, three ovarian cancer cell lines (MES-OV, OVCAR-3 and SK-OV-3) and their carboplatin (CBP)-resistant sublines (MES-OV CBP, OVCAR-3 CBP and SK-OV-3 CBP) were cultured in specialized media that promote stem cell proliferation. Immunohistochemistry, confocal microscopy and live cell imaging techniques were used to detect morphology and the formation of 3D structures. RNA-Seq and gene ontology analyses were performed to investigate the differences in the transcriptional landscape between the parental and CBP-resistant sublines.

Result and discussion

In 2D monolayers, all three CBP-resistant sublines showed a mesenchymal phenotype compared to their respective epithelial parental cells. However, under 3D conditions, the parental cell lines developed predominantly spherical structures, whereas the resistant cell lines developed predominantly vessel-like structures. A hollow lumen was observed within the spheres. It was also observed that the formation of the spheres begins with a single-cell division. Diversity of gene expression related to reorganization and regulation of the extracellular matrix, apoptosis and proliferation, transport of ions and binding of substrates was noted.

Conclusion

Our data highlight the importance of accurately characterizing the specific structures developed by resistant cell lines in a 3D environment and discovering the genes responsible for their formation.

EACR25-0172

Intracellular Ca²⁺ depletion induces FOXM1 SUMOylation and accumulation on the inner nuclear membrane resulting in accelerated G2/M cell cycle transition

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Introduction

Intracellular Ca²⁺ have important functions in regulating the activity of numerous transcription factors and cancer development. FOXM1 is a key transcription factor and an important oncogenic protein involved in tumorigenesis, but whether Ca²⁺ regulates the function of FOXM1 remains unclear.

Material and method

BAPTA-AM was used to cause intracellular Ca²⁺ depletion, and immunofluorescence staining was used to observe the distribution of FOXM1 in cells.

Result and discussion

Here, we found that Ca²⁺ promotes the entry of FOXM1 into the nucleus in many cancer cell lines, but Ca²⁺ depletion causes FOXM1 to accumulate at the inner nuclear membrane (INM). Further experiments revealed that sequestered FOXM1 colocalized with lamin B in the INM and was influenced by the activity of nuclear exportin 1 (XPO1). To investigate the mechanism by which intracellular Ca²⁺ affects FOXM1 sequestration, we found that among posttranslational modifications, only FOXM1 SUMOylation showed a significant increase under conditions of Ca²⁺ depletion, while

inhibition of FOXM1 SUMOylation using siRNAs or inhibitors rescued FOXM1 sequestration. Furthermore, Ca²⁺-dependent SUMOylation of FOXM1 appears to enhance the G2/M transition of the cell cycle and reduce apoptosis.

Conclusion

In conclusion, our results provide a molecular basis for the regulatory relationship between Ca²⁺ signaling and FOXM1, and we look forward to elucidating the biological functions associated with Ca²⁺-dependent FOXM1 SUMOylation in the future.

EACR25-0195

Progesterone receptor plays pivotal roles in regulating mitochondria function and mitochondria mediated caspase-independent apoptosis in breast cancer cells

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Introduction

There have been conflicting reports of effects of progestin and progesterone receptor (PR) on breast cancer from both clinical and laboratory studies. Although progestin in hormone replacement therapy (HRT) is associated with an increased risk of breast cancer, its use in clinical trials as a second line of endocrine therapy have reported clinical benefits. There have been suggestions that the cancer promoting effect of progestins in HRT is due to progestin activation of androgen receptor. To clarify the role of PR in breast cancer, it is important to have a whole proteomic understanding of PR activities in response to pure agonist.

Material and method

Tandem mass tag (TMT) proteomic analysis of protein changes induced by agonist-activated PR was conducted in MCF-7 cells, in which PR levels were increased by transfection to facilitate unambiguous detection of PR activity.

Result and discussion

The analysis identified reproducibly 4915 PR regulated proteins and 678 phosphorylated peptides of diverse functions. It revealed that PR induced broad down-regulation of cell cycle regulators for G1-S progression and mitosis, including CDK1/2/4, CCNB1/B2, proteins for chromatin condensation, Ndc80 complex and chromosomal passenger complex. PR also regulated numerous proteins to drive mitochondria mediated apoptosis through hypoxia and p53 pathways including the activation of HIF1A-BNIP/BNIP3L axis and upregulation of death receptors. Importantly, PR significantly regulated 200 mitochondria proteins involved in metabolism, ATP generation and apoptosis. AIF/AIFM1, AIFM2, ENDOG and HtrA2/Omi are among the well-known mitochondrial proteins that are released during apoptosis to cleave apoptosis inhibitors and break DNA. Although PR also upregulated some oncogenic proteins such as EGFR, IRS2 and RUNX1 and suppressors of ferroptosis such as NUPR1, LCN2 and SLC7A11, these upregulations were functionally ineffective.

Conclusion

In conclusion, the study provides a whole proteomic view of intrinsic effects of agonist activated PR that is broadly growth suppressive and proapoptotic through mitochondrial mechanisms independent of caspases. The data provide conclusive evidence that high levels of PR activated by pure agonist exert strong anti-tumoral effect on breast cancer. The proteomic data set is valuable for research and development of PR targeted therapy in breast cancer.

EACR25-0200

Using organoids and Drosophila to explore the role of protein kinase N as an intestinal tumour suppressor

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Introduction

Gastrointestinal tract cancers represent a significant global health challenge, with colorectal cancer (CRC) ranking as the second leading cause of cancer-related mortality in the United Kingdom. Previous investigations within the lab, using bioinformatics and mouse models, have identified the Rho effector kinase protein kinase N2 (PKN2) as a candidate CRC tumour suppressor. PKN2 expression is lost as tumours progress, with low expression associated with poor outcomes. Furthermore, the PKN2 gene is situated within the 1p22 locus, which is subject to shallow deletions in approximately 30% of CRC cases. Importantly, conditional PKN2KO in mice led to increased colon tumour development in a colitis-associated cancer model. PKN2KO mouse intestines were also found to be sensitive to inflammatory insults, with evidence that the epithelial barrier may be compromised. We hypothesise that PKN2 plays a key role in maintaining the epithelial barrier and the prevention of tissue inflammation and damage, which are precursors to tumour development.

Material and method

This study aims to elucidate the tumour suppressor functions of PKN2 using CRC cell lines, colon-derived organoids and Drosophila as models. PKN2KO CRC cell lines were generated utilizing CRISPR-lentiviral technology, to investigate the role of PKN2 in the integrity of cell junctions. To explore a role in a non-cancer epithelium, we successfully derived colon organoids from WT and conditional PKN2KO mice. These organoids will be used to assess the impact of PKN2 loss on organoid development, cell junction integrity and associated signalling pathways. Additionally, RNAseq transcriptomics will explore the global impact of PKN2KO on organoid phenotypes. To investigate the evolutionary conservation of PKN2 function, we are studying the impact of RNAi depletion of Drosophila PKN (dPKN) on gut morphology, cell junctions and response to inflammatory injury.

Result and discussion

Here, we show that PKN2 localises to cell junctions and plays a role in maintaining junction integrity in CRC cell lines. FITC-Dextran diffusion assays using PKN2 CRISPR KO cell monolayers indicated evidence of

impaired epithelial barrier. Initial organoid imaging experiments corroborate the PKN2 localisation to the cell junctions. Furthermore, preliminary imaging of Drosophila utilizing GFP-tagged dPKN has shown conserved localisation to epithelial junctions in the Drosophila midgut.

Conclusion

Collectively, our data strongly suggest that PKN2 plays a critical role in stabilizing cell junctions, thereby elucidating its function as a tumour suppressor in the context of colorectal cancer.

EACR25-0237

Short-term fasting and zinc ionophores cooperate to promote ferroptosis in colorectal cancer

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Introduction

Short-term fasting (STF) induces metabolic rewiring in cancer cells, exposing novel therapeutic vulnerabilities, as we previously reported for cholesterol biosynthesis inhibitors. In these agents, we found that reductions in circulating insulin, IGF1 and leptin play a key role in the sensitizing effect of STF by blunting cholesterol production and promoting cholesterol efflux in cancer cells.

Material and method

To identify additional agents that could be repurposed for oncologic applications in colorectal cancer (CRC) treatment, we screened commercially available drug libraries in HT29 CRC cells to identify compounds that acquire anticancer effects under fasting-mimicking culture conditions (FMCC; 1% FBS and 50 mg/dL glucose). Zinc ionophores evaluated in this study included the antiparasitic drug clioquinol, the investigational agent, PBT2, and chloroquine. In vitro studies were conducted using CRC cell lines HT29, HCT116, and CT26. For our *in vivo* experiments, we generated HCT116 xenografts (*in* athymic nude mice) and CT26 allografts (*in* BALB/c mice) and treated them with zinc ionophores, weekly 48h water-only STF or their combination with or without concomitant administration of coenzyme Q10 or zinc chloride (ZnCl₂). We monitored the expression levels of solute carrier family 7 member 11 (SLC7A11) and glutathione peroxidase 4 (GPX4), key regulators of ferroptosis, using RT-qPCR and Western blotting. In addition we quantified zinc levels, glutathione (GSH and GSSG), malondialdehyde, 4-hydroxynonenal, ATP and AMP in cultured cells and tumors using commercially available kits.

Result and discussion

FMCC and STF cooperated to enhance the antitumor activity of zinc ionophores in CRC models both *in vitro* and *in vivo*. In tumor-bearing mice, this antitumor effect was further compounded by ZnCl₂ administration. STF alone reduced GSH/GSSG levels in the tumors while

increasing lipid peroxides and other oxidative stress markers. Combining STF with zinc ionophores resulted in zinc accumulation, compounded GSH depletion, and elevated lipid peroxide formation within tumors. The ferroptosis inhibitors coenzyme Q10 and ferrostatin-1 abrogated the anticancer effects of combined STF and zinc-ionophores. STF combined with zinc ionophores reduced the expression of GPX4 and of the cystine transporter SLC7A11, leading to disruption of the SLC7A11/GSH/GPX4 axis. Overall, these data support the notion that ferroptosis is the primary mechanism of GSH depletion-induced cell death mediated by STF and zinc ionophores in CRC tumors.

Conclusion

Our data demonstrate that STF predisposes CRC to zinc-induced ferroptosis by depleting tumor GSH levels and downregulating GPX4 and SLC7A11.

EACR25-0242

Repurposing piroxicam enhances the antineoplastic effects of docetaxel and enzalutamide in prostate cancer cells using two-dimensional and three-dimensional *in vitro* culture models

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Introduction

Drug repurposing is gaining consideration in cancer due to the challenges of poor outcomes and resistance associated with the current conventional modalities. Inflammation plays a critical role in cancer progression, making it a promising therapeutic target. Non-steroidal anti-inflammatory drugs (NSAIDs), widely used for treating inflammation, are being explored for their potential efficacy in cancer treatment, including prostate cancer (PCa). This study aims to evaluate the efficacy of Piroxicam (PXM), an NSAID, in enhancing the sensitivity of PCa cells to chemotherapeutic and hormonal drugs.

Material and method

Differentially expressed genes between murine PCa models PLum AD (androgen-dependent) and PLum AI (androgen-independent) were identified via computational analysis to pinpoint therapeutic targets. In two-dimensional (2D) cell culture, cell proliferation, viability, and migration assays were performed on PLum-AD and PLum-AI cells treated with PXM, alone or in combination with docetaxel (Doc) or enzalutamide (Enz). Additionally, the impact of these treatments on stem-like progenitor cells was assessed using three-dimensional (3D)-Matrigel™-based sphere-forming and organoid formation assays.

Result and discussion

Transcriptomic analysis revealed that inflammatory pathways are enriched during PCa progression, making them viable targets for NSAID-based interventions. Single treatment of PXM demonstrated significant anti-

cancer effects on PLum-AD and PLum-AI cells, evidenced by reduced cell proliferation, viability, migration, sphere growth, and organoid growth. Interestingly, PXM treatment in combination with Doc or Enz resulted in more pronounced anti-neoplastic effects compared to single-drug exposure.

Conclusion

Our work suggests PXM as a potential adjunctive therapy to enhance the efficacy of conventional treatments in PCa, paving the way for potential clinical applications.

EACR25-0248

ADAR2-dependent editing activity induces the differentiation of osteosarcoma cells by targeting IGFBP7

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Introduction

Osteosarcoma (OS) is the most common primary bone tumor affecting the juvenile population, with a high rate of recurrence and metastasis. RNA editing was described to play a main role in cancer development and in this study we investigated the role of ADAR2 (Adenosine Deaminase Acting on RNA 2, AD2) editing enzyme in OS with *in vitro* and *in vivo* analysis.

Result and discussion

Gene and protein analyses evidenced that AD2 expression is lower in Saos2 and 143B cells compared to osteoblasts; AD2 level inversely correlated to the aggressiveness of tumor cells. To confirm this possible link, microarray dataset downloaded from the NCBI GEO database (GSE85537) showed that intratibially injected OS Well5 cells with increased ability to metastasize to the lung had lower level of AD2 compared to the same cells that remained into the bone. To study the role of AD2 in OS cells, Saos2 and 143B cell lines were transfected with Empty plasmid (E) or vectors overexpressing AD2 or the Inactive form of AD2 (IN). The overexpression of ADAR2 in Saos2 cells reduced the proliferation rate, as confirmed by G1 arrest of cell cycle [G0/G1 cell population (%), E: 46.8±3.0, AD2: 64.9±4.3%, IN: 43.5±2.1. *p<0.01 vs. E; #p<0.01 vs. IN] and migration ability [Wound repair (μm), E: 161.8±47.4, AD2: 62.7±34.3%, IN: 146.2±63.7. *p<0.01 vs. E; #p<0.01 vs. IN] compared to E- and IN-transfected cells. An enhanced ability to form mineralized nodules was observed in AD2-overexpressing Saos2 as shown by Alizarin Red (Arbitrary Unit, E: 1.0±0.1, AD2: 13.7±2.0%, IN: 1.3±0.2. *p=0.0003 vs. E; #p=0.0015 vs. IN) and Von Kossa stainings. Real-Time expression and western blot analysis revealed high levels of Osterix and ALP in AD2-Saos2. The overexpression of AD2 in 143B cells also led to reduced proliferation ability, G1 arrest of

cell cycle and increase of osteoblast markers expression. Intratibial injection of transfected Saos2 cells in NSG mice led to the formation of bone tumor reduced in size in AD2-cells injected mice [Tumor size (xy2/2), E: 1.56 ± 0.51 , AD: $0.47 \pm 0.44^{*#}$, IN: 1.25 ± 0.16 . * $p < 0.01$ vs. E; # $p < 0.01$ vs. IN] with reduced number of liver, kidney and lung metastases. Moreover, the deep-seq analysis revealed a significant editing level of IGFBP7 transcripts in AD2 cells compared to E- and IN-transfected cells. Particularly, the K95R edited form of IGFBP7 fails to stimulate the IGF1R pathway. These results were also confirmed by the treatment of Saos2 cells with human recombinant K95R-IGFBP7 [pIgflr/Igflr/β-Actin. Untreated cells (NT): 1.00 ± 0.01 ; WT: $2.36 \pm 0.70^{*#}$; K95R: 1.02 ± 0.65 . * $p < 0.01$ vs. NT; # $p < 0.05$ vs. K95R] compared to WT-IGFBP7 or untreated cells. Moreover, K95R-IGFBP7 is able to increase the expression of Runx2 compared to WT-IGFBP7, suggesting a role in the induction of OS cell differentiation.

Conclusion

Our results showed that ADAR2 acts as tumor suppressor in OS cells editing IGFBP7 and may represent a novel therapeutic target for this rare pediatric tumor.

EACR25-0286

Exploring LDH inhibitors with chemotherapeutic agents as dual therapy for breast cancer

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Introduction

Breast cancer is the most prevalent malignancy among women, with cases projected to exceed 2 million by 2030. Chemotherapy remains a key treatment, but prolonged use leads to severe side effects, resistance, and recurrence, necessitating novel strategies. Cancer cells undergo metabolic reprogramming, favoring aerobic glycolysis over oxidative phosphorylation – a phenomenon known as the Warburg effect. Lactate dehydrogenase (LDH) plays a crucial role in this shift, promoting tumor progression and resistance. Elevated LDH levels correlate with poor prognosis, making it a promising therapeutic target. This study evaluates natural and synthetic LDH inhibitors in combination with chemotherapeutic agents in MCF-7 (hormone-positive) and MDA-MB-231 (metastatic) cells. In silico docking with LDH-A (PDB ID: 1I10) was conducted to assess binding interactions, followed by in vitro cell-based analysis in breast cancer cell lines. Findings aim to reveal synergistic effects, offering insights into metabolic disruption as a strategy to enhance breast cancer treatment efficacy.

Material and method

All drugs (5 chemotherapeutic agents and 6 LDH inhibitors) were purchased from Sigma, TCI and SRL Chemicals. Cell culture reagents were obtained from HiMedia, and assay kits from Thermo Scientific, Invitrogen, and Genetix. MCF-7 and MDA-MB-231 cells (NCCS, Pune) were maintained in DMEM with 10% FBS at 37°C, 5% CO₂. Hoechst and DHE staining assessed DNA damage and ROS. ELISA was performed

per the manufacturer's protocol. Docking studies were conducted using Schrödinger (PDB: 1I10), including ligand preparation, minimization, and XP docking. MD simulations followed system setup, minimization, and dynamics. Cytotoxicity was evaluated using MTT assay in MDA-MB-231 and MCF-7 cells with monotherapy and combination therapy by measuring absorbance at 595 nm. Scratch assay was performed on MDA-MB-231 cells and % cell migration was indicated by the ability of the cells to migrate into a scratch wound of the confluent cell monolayer.

Result and discussion

LDH inhibitors significantly enhanced the cytotoxic potential of chemotherapy in combination therapy compared to monotherapy. Paclitaxel, Methotrexate, and 5-Fluorouracil showed superior cytotoxicity and were further analyzed. The increased efficacy in combination therapy suggests that LDH inhibition can enhance chemotherapy response, potentially overcoming resistance and improving treatment outcomes.

Conclusion

The findings highlight the therapeutic potential of LDH inhibition in breast cancer treatment. By disrupting cancer cell metabolism, LDH inhibitors can enhance chemotherapy efficacy, reduce resistance, and improve overall therapeutic response, supporting their role as adjuvant agents in combination therapies.

EACR25-0318

Constituent compounds from Bazi Bushen regulate autophagy and improve the senescence feature of vascular endothelial cells

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Introduction

Bazi Bushen (BZBS), a formulated Chinese medicine, has been shown to exert potential protective effect on vascular endothelial cells and improve senescence-related cells decline. Cellular senescence is a hallmark of ageing, contributing to endothelial dysfunction and the development of age-related vascular diseases. Autophagy plays a crucial role in regulating cellular repair and its dysregulation has been implicated in ageing-related deterioration. Given the importance of autophagy in vascular health, the present study investigated the effects of BZBS and its leading constituent compounds on the function and autophagy of human vascular endothelial cells. We further explored the molecular mechanism by which the compounds operated in cells, with a focus on key autophagy regulators, including sequestosome-1 (SQSTM1) and autophagy-related protein LC3.

Material and method

Human vascular endothelial cells derived from peripheral and cerebral origins were used in this study. Cell proliferations were measured between the wild-type (WT) and BZBS-treated cells. To evaluate autophagic flux,

mRFP-EGFP dual fluorescent LC3 plasmid was transfected into the endothelial cells. Autophagic activity was assessed by quantifying the autophagosomes and autolysosomes puncta. To validate the autophagic response, Western blot analysis was conducted to measure the expression levels of LC3-I, LC3-II, and SQSTM1/p62. Changes in the ratio of LC3-II to LC3-I and the degradation of SQSTM1 were assessed to determine autophagic activity following treatment. Mass spectrometry-based proteomics and protein blotting were further employed to analyse changes in autophagy associated signalling pathways.

Result and discussion

BZBS treatment significantly enhanced endothelial cell proliferation and induced a marked increase in the number of autophagosomes ($p < 0.01$, at 20 µg/ml) compared with the WT cells without signs of cytotoxicity. In double-blinded tests on multiple compounds separated from BZBS, three of these purified compounds, namely Compound-5 (schisandrin A or deoxyschizandrin), Compound-10 (Azelaic acid), and Compound-13 (gomisin D), significantly increased autophagosome formation in endothelial cells.

Comprehensive proteomics analysis revealed that BZBS and these lead compounds led to marked dephosphorylation of SQSTM1 at S28, S272 and T269; the phosphorylation of these residues was known to inhibit the autophagy process in cells.

Conclusion

Our findings indicate that BZBS enhances autophagy in vascular endothelial cells, potentially reversing endothelial cell ageing. This effect is mediated, at least in part, through the dephosphorylation of SQSTM1, which alleviates its inhibitory effect on autophagy. These results suggest that BZBS may offer therapeutic potential in preventing age-related vascular dysfunction. Further investigations are warranted to explore its clinical applications in ageing-related diseases.

EACR25-0330

Targeting the DNA damage response prevents regrowth of colorectal peritoneal metastasis-derived organoids following treatment with mitomycin C

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Introduction

A minority of Colorectal Cancer (CRC) patients with peritoneal metastases is eligible for cytoreductive surgery (CRS) followed by hyperthermic intraperitoneal chemotherapy (HIPEC). However, recurrence rates are high (~80%). We tested whether inhibitors of the DNA damage response (DDR) could prevent recurrence in an *in vitro* HIPEC model.

Material and method

Peritoneal metastasis-derived organoids (PMDOs; n=10) were treated with inhibitors of ATR (berzosertib, ceralasertib, elimusertib), CHK1 (rabusertib), and WEE1

(adavosertib) alone, and in combination with MMC, oxaliplatin, or irinotecan. Western blotting was used to determine Chk1 phosphorylation and markers of DNA damage. Microscopy and ATP quantification were used to measure the effects of (combination) treatments on cell viability and recurrence/regrowth.

Result and discussion

All PMDOs displayed rapid regrowth (recurrence) following single-drug chemotherapy treatment. Berzosertib inhibited chemotherapy-induced Chk1 phosphorylation, augmented DNA damage, and abrogated recurrence in all 10 MMC-treated PMDOs. The combination with oxaliplatin and irinotecan was less effective. *In vitro* HIPEC with MMC followed by 'adjuvant' treatment with any of the DDR inhibitors for 3 days completely prevented PMDO recurrence.

Conclusion

PMDOs can be completely eradicated if MMC treatment is followed by inhibition of ATR or other DDR kinases. DDR inhibitors may, therefore, have value in the adjuvant treatment of peritoneal metastases following CRS-HIPEC.

EACR25-0334

Exploring the Tumor-Suppressive Role of MLN51 (Metastatic Lymph Node 51) in Lung Adenocarcinoma

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Introduction

MLN51 (Metastatic Lymph Node 51), initially identified from metastatic lymph node, is now also known as a core component of the exon junction complex (EJC). It is a protein participating in fundamental RNA processing mechanisms including splicing, localization and degradation. Despite its known role in RNA metabolism, its functional significance in tumorigenesis, particularly in lung adenocarcinoma (LUAD), remains poorly characterized.

Material and method

Clinical relevance of MLN51 was investigated through immunohistochemistry on lung cancer tissues and integrated analysis of TCGA datasets, correlating expression patterns with clinical staging and survival outcomes in LUAD. Lentiviral-mediated knockdown and overexpression models were established in human lung adenocarcinoma A549 and PC9 cell lines. Cellular assays employed included cell growth, Transwell invasion, adhesion assays, and wound healing migration analyses. Mechanistic explorations were carried out via quantitative proteomics by way of mass spectrometry to identify MLN51 protein interaction networks and pathway associations.

Result and discussion

Immunohistochemical analysis revealed that the MLN51 protein staining, mainly in the cytoplasmic region of the cells, is seen more prominently in normal lung tissues than in lung adenocarcinoma. In the TCGA database, the expression of MLN51 transcript was found to be low in

cancer tissues, both in LUAD ($p < 0.001$) and squamous cell carcinoma, when compared with normal tissues. Survival analysis demonstrated improved overall survival in patients with higher MLN51 expression (HR = 0.62, 95% CI 0.47–0.82, $p = 0.003$). Functional studies showed MLN51 overexpression suppressed malignant phenotypes, reducing the rate of cell growth ($p < 0.0001$), invasion capacity ($p < 0.001$), and migration rate compared with control cells. Proteomic analysis identified the top 200 upregulated and downregulated genes, which were subjected to GO analysis. The results indicate that these genes are mainly involved in protein translation and RNA splicing, cell adhesion molecule binding and ECM receptor interactions, processes that play key roles in tumour progression and metastasis.

Conclusion

Our multi-dimensional analyses disclose a pivotal role of MLN51 in lung adenocarcinoma. The inverse correlation between MLN51 expression and disease progression indicates its potential as both a prognostic biomarker and therapeutic target. Proteomic analyses indicate that MLN51 regulates tumour progression via multiple pathways related to protein translation, RNA splicing, cell adhesion and ECM receptor interactions, processes crucial for cancer cell survival, metastasis, and drug resistance. The study thus collectively suggests that MLN51 acts as a tumour suppressor in lung adenocarcinoma with prognostic and therapeutic value.

EACR25-0335

Can transient inactivation replace loss of the wildtype tumor suppressor as the second hit for colorectal cancer initiation?

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Introduction

To address the phenomena of breast cancer initiation in cases with just one germline mutation and no apparent loss of the wildtype allele, a recent study has proposed transient inactivation of the wildtype BRCA2 protein by glycolytic metabolite methylglyoxal (MGO) as the second ‘hit’ of the ‘Knudsen’s two-hit’ mechanism of cancer initiation. We have previously identified NR0B2, an orphan nuclear receptor, as a candidate tumor suppressor in microsatellite-stable, APC mutation-negative familial colorectal cancer (CRC) patients. However, germline truncation of NR0B2 was not accompanied by loss of the wildtype allele in the tumors.

Material and method

In the colon, normal mucosa cells obtain energy mainly by oxidative phosphorylation rather than glycolysis. It is thus unlikely that MGO could be present in large enough quantities to initiate tumorigenesis. Ethanol is naturally present at low concentration in the human gut via fermentation activity of the intestinal flora. Common ‘A’ variants in rs1229984 and rs671 of the alcohol dehydrogenase (ADH1B) and aldehyde dehydrogenase 2 (ALDH2) genes respectively in East Asians could perturb the ethanol detoxification pathway leading to accumulation of the acetaldehyde metabolite. We screened for the genotypes of these two enzymes in the

germline of these patients by Sanger sequencing and assessed the expression of the wildtype NR0B2 peptide in their matched mucosa-tumor samples using semi-quantitative near infrared Western Blots.

Result and discussion

Substantial downregulation (63%) of the wildtype NR0B2 peptide was found in the tumor of a case with one ‘A’ allele each in rs1229984 and rs671 compared to its matched mucosa. Excess acetaldehyde could form adducts with the wildtype NR0B2 peptide, leading to its downregulation. Nevertheless, this phenomenon is not replicated in the tumors of three other patients indicating that the downregulation is probably transient and dynamic and hence not captured in tumor samples of these other patients. In CRC, cancer initiation occurs at the aberrant crypt foci or early adenoma stage which progresses to tumor formation only after many years.

Conclusion

Accumulation of the acetaldehyde metabolite could serve as the endogenous toxin and the second hit for the initiation of CRC tumorigenesis in cases with just one germline NR0B2 mutation and no loss of the wildtype allele.

EACR25-0337

PTX3 Promotes Tumor Cell Migration Through the miR-1255b/CYP2J2 Axis in Oral Cancer

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Introduction

Oral cancer poses major clinical challenges because of its invasiveness and poor prognosis. Pentraxin 3 (PTX3) is an immune-modulating protein implicated in various types of cancer, although its role in oral cancer remains unclear. In this study, we examined the expression patterns, functional roles, and underlying molecular mechanisms of PTX3 in oral cancer.

Material and method

The clinical significance of PTX3 in patients with oral cancer was investigated using data from the Gene Expression Omnibus (GEO) and TCGA datasets. Cell migration and invasion were evaluated using Transwell assays to assess the roles of PTX3 in oral cancer metastasis. Luciferase reporter assays, western blotting, and real-time reverse-transcription quantitative polymerase chain reaction (RT-qPCR) were used to elucidate the underlying mechanisms of PTX3.

Result and discussion

Bioinformatics and clinical data analyses revealed high PTX3 levels in oral cancer tissues, associated with reduced patient survival. Functional assays revealed that PTX3 promotes tumor cell migration and invasion in patients with oral cancer although it has no effect on cell proliferation. Transcriptomic profiling identified cytochrome P450 family 2 subfamily J member 2 (CYP2J2) as a downstream target of PTX3.

Mechanistically, PTX3 inhibits the expression of CYP2J2 by modulating the focal adhesion kinase/Src/extracellular signal-regulated kinase signaling pathway and upregulating miR-1255b, which directly interacts with the 3'-untranslated region of CYP2J2. Additionally, CYP2J2 downregulation mediates the promigratory effects of PTX3 on tumor cells in oral cancer.

Conclusion

Overall, our findings elucidate a novel regulatory axis that involves PTX3, miR-1255b, and CYP2J2 and drives the progression of oral cancer, highlighting PTX3 as a potential therapeutic target and prognostic biomarker in oral cancer management.

EACR25-0340

PFKFB4 mediates cell migration through regulating MX1 and MX2 expression in oral squamous cell carcinoma

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Introduction

PFKFB4 (6-phosphofructo-2-kinase/ fructose-2,6-biphosphatase 4), an isoform of the glycolytic enzyme phosphofructokinase 2 (PFK2), is a crucial regulator of glucose catabolism and broadly implicated in other cellular mechanisms/processes, such as transcriptional regulation, cell cycle, and autophagy in a non-glycolysis-dependent manner. Nevertheless, its impacts on the disease progression of oral squamous cell carcinoma (OSCC) remain largely unknown.

Material and method

Cell viability and flow cytometry assays were used to determine proliferation of OSCC cells. Boyden chamber assays were used to detect migration and invasion of OSCC cells. The regulatory mechanisms of PFKFB4 in the cell migration of OSCC were elucidated by RNA sequencing, western blotting, and real-time reverse-transcription quantitative polymerase chain reaction (real time RT-PCR).

Result and discussion

In our survey, upregulation of PFKFB4 was observed in a majority of cancer types, including head and neck cancer, and high levels of PFKFB4 were associated with advanced tumor stage and poor survival in OSCC patients. In vitro experiments, alterations in PFKFB4 levels affected cell migration and invasion but not proliferation in OSCC. Furthermore, we identified a list of differentially expressed genes in PFKFB4-silencing OSCC cells, among which MX1 and MX2 acted as a negative regulator of OSCC migration. In addition, PFKFB4 knockdown dampened the phosphorylation of src and p38 in OSCC cells, implicating a role of PFKFB4 in signal transduction.

Conclusion

Taken together, these results indicate that MX1 and MX2 negatively regulated OSCC migration downstream of

PFKFB4, which may offer potential avenues for controlling metastasis of oral malignancies.

EACR25-0353

Sequential Targeting of IAP and BCL-2 Family Proteins May Enhance Apoptotic Sensitivity in Non-Small Cell Lung Cancer

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Introduction

Non-small cell lung cancer (NSCLC) remains a major challenge due to its resistance to apoptosis, limiting therapeutic success. Targeting survival pathways with Smac mimetics (e.g., Birinapant, Compound A) and BH3 mimetics (e.g., S63845, ABT-263) offers a promising approach. This study examines whether pre-treatment strategies enhance apoptosis compared to direct drug combination in NSCLC cells.

Material and method

NSCLC cell lines were categorized into two groups based on apoptotic sensitivity. Group 1 (H727, LXF289, H358, CORL23, LCLC103H, SKMES1, H1299) responded to Smac mimetics alone, while Group 2 (A549, CALU1, COLO, H441, H2009, SKLU1) required additional BH3 mimetics. Group 1 cells were treated with Compound A, TNF- α , and caspase inhibitor QVD, and apoptosis was quantified via Annexin V/PI staining using flow cytometry. Necroptosis was assessed using RIPK1 and RIPK3 inhibitors (Necrostatin-1, GSK872) but did not significantly contribute to cell death. Group 2 cells underwent additional treatment with BH3 mimetics (S63845, ABT-199, WEHI-539, ABT-263). In one of sequential treatments, Birinapant was added first, followed by a 2-hour incubation before Smac mimetics, then TNF- α was introduced 30 minutes later. Group 1 cells were incubated for 24 hours, whereas Group 2 cells underwent 24-hour and 48-hour treatments to assess time-dependent effects.

Result and discussion

Group 1 cells exhibited significant apoptosis with Smac mimetics alone, demonstrating reliance on IAP-mediated survival. Group 2 cells required BH3 mimetics for apoptosis, highlighting dependence on MCL-1/BCL-2 proteins. Sequential drug administration enhanced apoptosis, particularly in Group 2 cells. The sequential combination of Birinapant + S63845 + TNF- α was one of the most effective. Necroptosis inhibition did not alter cell death rates, confirming apoptosis as the dominant pathway. Extended 48-hour incubation further increased apoptosis in resistant cells.

Conclusion

Our findings highlight the importance of sequential drug administration in apoptosis induction. Smac mimetics effectively induced apoptosis in sensitive NSCLC cells, while resistant cells may require BH3 mimetics. The lack of necroptotic response confirms apoptosis as the primary mechanism. The common and possible genetic factors in the literature (e.g., mutant K-Ras, loss of p53, FHIT deletions) may contribute to therapy resistance. Optimizing pre-treatment regimens could enhance therapeutic efficacy in NSCLC patients.

EACR25-0362

Epigenetic regulation by histone modification in the epithelial-to-mesenchymal transition of lung cancer brain metastasis

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Introduction

The epithelial-to-mesenchymal transition (EMT) is an essential step for cancer metastasis to the brain. The transcription factors (TFs), which are associated with EMT, plays a key role during EMT. The objective of this study was to investigate the epigenetic role of histone lysine methylation/demethylation on the expression of EMT associated TFs during the metastasis of lung adenocarcinoma to the brain.

Material and method

Paired samples of lung adenocarcinoma and brain metastasis (BM) were analyzed in 46 individual patients. Both samples were obtained by surgical resection or biopsy of the lung and brain. The paraffin fixed formalin-embedded samples were obtained from the pathology archives in our institute. In samples of lung adenocarcinoma and BM, immunohistochemical staining was performed for epithelial markers, mesenchymal markers, EMT-TFs, histone lysine methyltransferase and demethylase.

Result and discussion

Several EMT-TFs such as Slug, Twist, ZEB1, and FOXC2 had higher immunoreactivity in brain metastasis than lung cancer. The immunoreactivity of EMT-TFs such as Slug (15.6% vs. 42.6%, $p=0.005$), Twist (23.6% vs. 45.9%, $p = 0.010$) and ZEB1 (15.0% vs. 55.9%, $p = 0.002$) was increased in BM compared with that in lung adenocarcinoma. Epigenetic inducers such as H3K4 methyltransferase (MLL4, $p = 0.018$) and H3K36me3 demethylase (UTX, $p = 0.003$) were statistically increased, and epigenetic repressors such as EZH2 (H3K27 methyltransferase, $p = 0.046$) were significantly decreased in BM compared with those in lung adenocarcinoma. The expression of UTX-ZEB1 (R^2 linear = 1.204) and MLL4-Slug (R^2 linear = 0.987) was increased in direct proportion, and EZH2-Twist (R^2 linear = -2.723) decreased in reverse proportion.

Conclusion

The results suggest that certain histone lysine methyltransferase/demethylase, such as MLL4, UTX, and EZH2, regulate the expression of EMT-TFs such as Slug, ZEB1, and Twist epigenetically, which may thereby influence cancer metastasis from the lung to the brain.

EACR25-0366

A multi-omics approach to dissect cell-intrinsic functions of IDO1 in ovarian cancer

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Introduction

Indoleamine 2,3-dioxygenase 1 (IDO1) is an enzyme that oxidizes tryptophan to start the first step of kynurenine pathway product generation. IDO1 is implicated in cancer pathophysiology because of its potential to deplete tryptophan (TRP) in local tumor microenvironments (TME), thereby impairing immune cell functions. Thus, IDO1 inhibitors were developed to “reactivate” T cell proliferation and function in the TME. So far, these drugs collectively failed to show efficacy in multiple clinical trials including ovarian cancer, suggesting a more in-depth study of the molecular function of IDO1 in cancer cells is warranted.

Material and method

We used single-cell RNA sequencing (scRNASeq) and deep-visual-proteomics (DVP) to study the IDO1+ tumor fraction in ovarian cancer patient tissues. We also employed a wide range of ovarian cancer cell lines to reconstitute key features of the clinical system where IDO1 expression was exogenously modulated with IFN-γ in control or IDO1-deficient backgrounds. Then, stimulated cells were analyzed using life-cell imaging to determine cell death and immunoblotting to characterize the activated molecular pathways.

Result and discussion

The results from the scRNASeq and DVP suggested that IDO1 expression is heterogeneous and is specifically linked to IFN-γ secreted by T cells in the ovarian TME. Subsequently, we used the beforementioned in-vitro system and investigated the response of ovarian cancer cells to IFN-γ stimulation. We discovered that IDO1 induction with IFN-γ results in an IDO1-dependent cell death caused by TRP starvation via activation of the GCN2 integrated stress response pathway and not by accumulation of kynurenine. The phenotype is completely abolished whenever IDO1 is knock-down or if an IDO1 inhibitor is added to the stimulated cells. To highlight the clinical relevance of our study, we detected a correlation between IDO1+ and the starvation signature in patient data, confirming that IDO1 expressing cells undergo TRP starvation *in vivo*.

Conclusion

In summary, our study provides evidence supporting a link between IFN-γ and IDO1 expression in ovarian cancer patients and suggests a crucial role of TRP in promoting cancer survival. These findings may offer new insights into the molecular mechanisms underlying the detrimental effects of IDO1 inhibition in ovarian cancer. On one hand, IDO1 inhibition might reinvigorate the immune response against the tumor; on the other, IDO1 inhibition might protect the tumor cells from TRP starvation and therefore, it might undermine its therapeutic potential.

EACR25-0373**Treatment with 3-bromopyruvate boosts the effect of chemotherapy in acute myeloid leukemia**

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Introduction

Most cancer cells rely on glycolysis for energy production, even in the presence of oxygen, a phenomenon known as the Warburg effect. This metabolic reprogramming results in increased glucose consumption, lactate production, and upregulation of glycolysis-related enzymes, supporting rapid proliferation and tumor progression. In Acute Myeloid Leukemia (AML), a highly aggressive blood cancer, altered glucose metabolism has been identified as a prognostic marker, and was also associated with treatment response, including resistance to chemotherapy. Given the reliance of AML cells on glucose metabolism, targeting this pathway presents a promising therapeutic strategy. 3-Bromopyruvate (3-BP), a pyruvate derivative, has demonstrated anti-cancer activity in solid tumors. However, its potential as a chemo-sensitizing agent in AML remains largely unexplored.

Material and method

This study explored glucose metabolism in AML as a therapeutic target by evaluating the effect of combination of 3-BP with standard chemotherapeutic agents daunorubicin (DNR) and cytarabine (Ara-C). Additionally, we developed two AML cell line models resistant to Ara-C (KG-1 Ara-R, MOLM13 Ara-R), which exhibited metabolic adaptations and varying sensitivity to metabolic inhibitors. The effects of 3-BP on chemotherapy response were assessed on cell viability (trypan blue staining), metabolic profiling (Western blot and colorimetric kits), mitochondrial activity (flow cytometry and Seahorse), and antioxidant capacity (colorimetric kit). Furthermore, we established an *in vivo* AML model using the chicken embryo to validate the therapeutic effect of 3-BP in combination with chemotherapy.

Result and discussion

Our findings demonstrated that 3-BP enhanced the cytotoxic effects of chemotherapy in AML cell models, even the context of acquired resistance (Ara-C resistant cells). Pre-treatment with 3-BP, in particular,

significantly reduced the EC50 values of DNR and Ara-C, supporting the chemo-sensitization. Mechanistically, 3-BP pre-treatment led to elevated ROS levels and decreased the levels of reduced glutathione, which likely contributed to the enhanced efficacy of chemotherapy. Moreover, the results obtained with *in vivo* chicken embryo AML model supported the *in vitro* results, reinforcing the potential of targeting glucose metabolism to improve AML treatment outcomes.

Conclusion

This study highlights the therapeutic potential of modulating glucose metabolism in AML. Combining 3-BP with standard chemotherapy effectively enhanced drug response. These findings support the exploration of metabolic inhibitors as adjuvants in AML therapy to improve treatment efficacy while potentially reducing drug toxicity. Further clinical investigations are warranted to validate these findings and optimize therapeutic strategies for AML patients.

EACR25-0426**Deciphering the Breast Cancer Metastasis Transcriptome: A Deep Dive into the Enigmatic Role of Circular RNAs**

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Introduction

Breast cancer metastasis remains a significant challenge in modern medicine and science. The increasing incidence of breast cancer, coupled with the high mortality linked to metastasis, underscores the urgent need for innovative therapeutic strategies. While research on protein-coding genes brought only incremental progress in anticancer therapies, increasing attention is now directed toward understanding the roles of non-coding RNAs – particularly the recently discovered circular RNAs (circRNAs).

Material and method

To investigate transcriptomic changes during metastasis, we analyzed cells from primary breast tumors ($n = 3$), pleural effusions ($n = 3$), and breast-to-brain metastases ($n = 3$), with normal epithelial cells as non-malignant control. Using Arraystar Inc. platforms, we profiled mRNAs ($n = 20,424$), long non-coding RNAs ($n = 37,823$), and circRNAs ($n = 12,828$), selecting transcripts with a fold-change ≥ 2 and p-value < 0.05 for bioinformatics analyses. We examined their molecular functions, interactions, correlations of circRNAs with linear counterparts, and the influence of culture conditions on the circRNA landscape.

Result and discussion

Hierarchical clustering of significantly deregulated transcripts classified cells by their origin. Notably, the circRNA-based analysis provided a clearer separation of

cells based on their origin compared to the analysis of linear transcripts. Among the identified circRNAs, 165 were significantly deregulated in breast cancer metastases relative to primary tumors. The most upregulated circRNA in metastatic samples, circPolar2A, harbored binding sites for tumor-suppressive microRNAs, including miR-7, miR-637, or miR-498. Additionally, the top five upregulated circRNAs contained multiple binding sites for AGO2, a key regulator of gene transcription. Distinct circRNA profiles were observed between different sites of metastases – pleural effusion and brain – where 117 circRNAs were differentially expressed. Importantly, circRNAs were deregulated independently of their linear counterparts. Furthermore, culture conditions favoring stemness and differentiation induced subtle shifts in circRNA expression. Although these changes did not reach statistical significance, they partially blurred the distinction cells based on their site of origin.

Conclusion

The circular RNA landscape reflects cellular status and site of origin, effectively distinguishing cancerous from normal breast epithelial cells. The most deregulated circRNAs have the potential to modulate metastasis by binding to tumor-suppressive microRNAs or altering global gene expression. Additionally, circRNAs represent a unique class of independently regulated transcripts, rather than mere by-products of transcription.

Consequently, circRNAs emerge as valuable biomarkers and promising mechanistic targets whose modulation could pave the way for paradigm-shifting therapeutic strategies.

EACR25-0430

Cyclic increase in the histamine receptor H1-ADAM9-Snail/Slug axis as a potential therapeutic target for EMT-mediated progression of oral squamous cell carcinoma

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Introduction

The intricate involvement of the histaminergic system, encompassing histamine and histamine receptors, in the progression of diverse neoplasias has attracted considerable scrutiny. Histamine receptor H1 (HRH1) was reported to be overexpressed in several cancer types, but its specific functional implications in oral squamous cell carcinoma (OSCC) predominantly remain unexplored.

Material and method

Levels of HRH1 were evaluated in 177 OSCC patients by immunohistochemical (IHC) staining or by an in silico analysis. HRH1 genetic depletion or overexpression was performed in *in vitro* and *in vivo* OSCC models. HRH1-

deficient cells were RNA sequenced, and gene ontology and enrichment analyses were applied to the output data. Protein-based biochemical assays such as Western blotting, coimmunoprecipitation, and a protease array were used to investigate HRH1-regulated pathways. A drug repurposing approach was tested using anti-histamines combined with a signal transducer and activator of transcription 3 (STAT3) inhibitor on different cellular functions.

Result and discussion

Our findings indicate that dysregulated high levels of HRH1 were correlated with lymph node (LN) metastasis and poor prognoses in OSCC patients. We identified a disintegrin and metalloprotease 9 (ADAM9) as a critical downstream target of HRH1, promoting protumorigenic and prometastatic characteristics both *in vitro* and *in vivo*. Molecular investigations revealed that the cyclic increase in the HRH1-ADAM9-Snail/Slug axis promoted progression of the epithelial-to-mesenchymal transition (EMT). Clinical analyses demonstrated significant correlations of HRH1 expression with ADAM9 and with EMT-related markers, with elevated ADAM9 also associated with LN metastasis in OSCC patients. Regarding therapeutic aspects, we discovered that activated STAT3 acts as a compensatory pathway for the long-term HRH1 signaling blockade in OSCC cells. Combining inhibition of HRH1 and STAT3 using their respective inhibitors or short hairpin (sh)RNAs enhanced the tumor-suppressive effects compared to HRH1 inhibition/depletion alone in OSCC cells and a xenograft model.

Conclusion

In summary, HRH1 has emerged as a valuable biomarker for predicting OSCC progression, and combined targeting of HRH1 and STAT3 may represent a promising strategy for preventing OSCC progression.

EACR25-0432

Sesamin inhibits human colorectal cancer HCT-116 cell growth by inducing apoptosis

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Introduction

Colorectal cancer is one of the leading causes of cancer-related deaths worldwide, highlighting the urgent need for natural compounds capable of inhibiting tumor growth and inducing cancer cell death. Sesamin, a bioactive compound derived from sesame (*Sesamum indicum*), is known for its health benefits, including melanin synthesis inhibition and lipid metabolism regulation.

Material and method

This study investigates the potential anti-cancer effects and molecular mechanisms of sesamin in HCT-116 colorectal cancer cells. HCT-116 cells were treated with 0.5, 1, 2, and 3.5 mM sesamin for 48 hours to evaluate changes in cell viability, cell cycle progression, apoptosis, and the expression of related regulatory molecules.

Result and discussion

The results showed that sesamin at concentrations above 0.5 mM significantly reduced cell viability. Treatment with >1 mM sesamin led to a significant increase in the proportion of cells in the Sub-G1 and G0/G1 phases while decreasing those in the S and G2/M phases. Sesamin at concentrations above 0.5 mM significantly increased reactive oxygen species (ROS) levels, while >1 mM sesamin significantly reduced mitochondrial membrane potential in HCT-116 cells. Additionally, sesamin at 2 and 3.5 mM significantly upregulated the mRNA expression of Bcl-2-associated X (Bax), caspase-3, -8, and -9, and enhanced apoptosis. However, there was no significant difference in the mRNA expression of B-cell lymphoma-2 (Bcl-2) and poly (ADP-ribose) polymerase (PARP) between treatment groups.

Conclusion

In conclusion, sesamin, particularly at 2 and 3.5 mM, effectively inhibited HCT-116 cell growth by promoting apoptosis. These findings suggest that sesamin may serve as a promising bioactive compound for colorectal cancer therapy.

EACR25-0433

A novel role of Kinesin Family Member 20A (KIF20A) in Hepatocellular carcinoma cell metabolism and ferroptosis

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Introduction

Hepatocellular carcinoma (HCC) represents a significant form of liver cancer, accounting for the fourth-highest number of cancer-related deaths on a global scale. Ferroptosis, a form of regulated cell death characterised by iron-dependent lipid peroxidation, has been increasingly implicated in HCC cancer progression and the role of Kinesin Family Member 20A (KIF20A), a member of the kinesin superfamily proteins that is vital during mitosis, might contribute to the resistance to ferroptosis of HCC. Here, we identify KIF20A as a novel biomarker of HCC and characterize the underlying mechanisms inducing ferroptosis.

Material and method

The genes that were differentially expressed in HCC and associated with ferroptosis were selected through an analysis of the GEO database and the Ferrdb website. The mRNA and protein levels of KIF20A were examined in HCC cell lines using RT-qPCR and western blotting, respectively. Experiments were conducted on the proliferation of HCC cells, as well as on ferroptosis, using overexpression and knockdown of KIF20A. Assays such as malondialdehyde (MDA) detection and Ferrorange assays were employed. Furthermore, the mechanisms by

which KIF20A regulates ferroptosis in HCC were investigated.

Result and discussion

Among the top 18 differentially expressed genes, we discovered KIF20A most potently related to the overall survival rate. The expression of KIF20A was significantly compared between HCC tissues and adjacent non-cancerous tissues. Our findings revealed that the proliferation of KIF20A-knockdown cells was significantly inhibited in two cell lines, SNU449 and PLC. Furthermore, we observed an increase in the levels of malondialdehyde (MDA), Fe²⁺, and intracellular reactive oxygen species (ROS) in these cells. Conversely, overexpressing this gene yielded opposite results. Knocking down KIF20A activates the pathway of ferroptosis through the KEGG pathway, and HMOX1 was identified by RNA sequencing as a downstream target of KIF20A influencing ferroptosis. Furthermore, our findings indicate that KIF20A inhibits ferroptosis in HCC cells via the HMOX1/SLC7A11/GPX4 pathway.

Conclusion

Collectively, our results uncover a novel role of KIF20A in the survival of HCC cells, their specific metabolic pathways linked to ferroptotic cell death. Our results suggest that KIF20A promotes the proliferation of HCC cells and inhibits ferroptosis by regulating the HMOX1/SLC7A11/GPX4 axis.

EACR25-0434

KSRP-mediated Wnt/β-catenin activation promotes follicular thyroid cancer progression and stemness

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Introduction

The K-homology-type splicing regulatory protein (KSRP) is an RNA-binding protein involved in multiple cellular processes, including mRNA stability and translational regulation through its recognition of adenine–uracil-rich elements in the 3'-untranslated regions of target mRNAs. Despite its ability to regulate approximately 16% of transcript expression, its precise role in cancer remains unclear. This study aims to elucidate the oncogenic role of KSRP in follicular thyroid cancer (FTC) and its potential impact on Wnt/β-catenin signaling.

Material and method

We analyzed KSRP expression in publicly available clinical databases and our own FTC patient cohort. Additionally, we examined KSRP expression in a pair of FTC cell lines with differing metastatic potential. Functional assays, including knockdown and over-expression studies, were conducted to evaluate the effects of KSRP on in vivo metastasis and in vitro tumor-promoting functions such as migration, invasion, and sphere formation. Gene Set Enrichment Analysis (GSEA) and quantitative PCR (Q-PCR) were performed to identify Wnt signaling components affected by KSRP.

Result and discussion

Our findings identify KSRP as an oncogenic factor in FTC, with significantly elevated expression in FTC tissues compared to normal thyroid or adenoma tissues. Metastatic FTC cell lines also exhibited higher KSRP levels. Functional assays revealed that KSRP enhances FTC cell invasiveness and stemness. Mechanistically, KSRP promotes β -catenin nuclear accumulation and transcriptional activity by negatively regulating two Wnt signaling inhibitors, DACT2 and SFRP2. These findings suggest that KSRP plays a crucial role in promoting tumor progression via Wnt/ β -catenin signaling activation.

Conclusion

This study highlights the oncogenic function of KSRP in FTC and its role in modulating Wnt/ β -catenin signaling. Given its impact on tumor progression and metastasis, KSRP may serve as a potential prognostic marker and therapeutic target for FTC patients.

EACR25-0436

The Effect of RG7388 and Etoposide Combination on Cell Death in a Neuroblastoma Cell Line

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Introduction

Neuroblastoma, which originates from neural crest cells, is the most common extracranial solid tumor of childhood and is responsible for 15% of deaths. Despite standard therapies, survival rates in high-risk neuroblastoma cases are low and accelerate the search for new treatment strategies. Although p53 mutations are rare in neuroblastoma compared to other cancer types, they are observed in 15% of patients with neuroblastoma during recurrence. Evidence of p53 inactivation suggests that this may lead to chemotherapy resistance. MDM2 is a protein that regulates p53 and MDM2 inhibition can promote cell death by increasing p53 activation.

Although RG7388, a potent and selective MDM2 inhibitor, has been shown to reduce survival in neuroblastoma cells, the underlying cell death mechanism has not been investigated yet. Etoposide, a part of neuroblastoma treatment, inhibits topoisomerase-II, which is involved in DNA replication and recombination and decreases cell survival. However, alternative combinations are needed due to the resistance mechanism developed by cancer cells against the apoptotic cell death pathway.

Material and method

This study investigates the effect of combined RG7388 and etoposide treatment on cell survival in neuroblastoma cells. SHSY-5Y cells were seeded in 5x10³ cells/well in 96 well plates. After the 24-hour incubation period, etoposide (A), RG7388 (B), and etoposide + RG7388 (C) were added to the growth medium for 24 or 48 hours. After this incubation period, the drug cytotoxicity screening was determined using an MTT colorimetric assay. All statistical analyses were performed using one-way analysis of variance (ANOVA) and followed by

Tukey's multiple comparison tests. A p-value less than 0.05 was considered to be significant.

Result and discussion

IC50 values for etoposide were 64.2 and 4.4 μ M for 24 and 48 hours, respectively. IC50 values of RG7388 were 40 and 7.6 μ M for 24 and 48 hours, respectively. IC50 for combined treatment was determined as 6.09 μ M for 48 hours. The combination index (CI) is above 1 at the combined IC50 dose, and antagonism is achieved, while at the combined dose that kills approximately 80% of the cells, the CI index is below 1 and synergism is achieved.

Conclusion

Combined treatment of MDM2 and topoisomerase-II inhibitors shows a synergistic effect in neuroblastoma and promotes cell survival. Elucidation of the type of cell death induced by these drugs may provide new insights into treating neuroblastoma.

This study was supported Anadolu University within the scope of the project coded BGT-2023-2343.

EACR25-0442

"FastM": a fasting-mimicking cell culture medium for refined cancer research

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Introduction

Studies on fasting have shown promising results in enhancing the efficacy of cancer therapies, however, in vitro procedures published so far have not reflected the physiological composition of the fasted state sufficiently. This arises the need for a specialized cell culture medium that resembles the metabolic environment after fasting. To address this gap, we formulated FastM, a cell culture medium designed to mimic the effects of fasting in vitro.

Material and method

The formulation of FastM is based on PlasmalyteTM, a commercially available cell culture medium that reflects the composition of healthy human plasma. The concentration of each individual component in FastM was determined by comparative analyses of existing literature, open access metabolomics databases, and by NMR measurements of human serum after 12 and 36 hours of fasting. Viability assays were performed to investigate the synergistic effect of certain metabolites and drugs.

Result and discussion

NMR results revealed distinct metabolic differences between serums after 12 hours versus 36 hours of fasting. In concordance with metabolomics data from databases and literature, we determined the metabolites that were most significantly changed upon fasting, namely glucose, insulin, branched-chain amino acids, and several ketone bodies. We formulated FastM to contain these key nutrients at respective concentrations, relative to those in

Plasmax™. Then, we tested the efficacy and cytotoxicity of selected drugs on different breast cancer cell lines cultured in Plasmax™, FastM, and a previously used starvation medium by performing viability assays. Additionally, we analyzed how single key metabolites (e.g., different ketone bodies, glucose, insulin), affect the responsiveness of breast cancer cells to certain drugs. Hereby, we could show a significant potentiation of treatment efficacy in selected cell line-drug combinations upon altering concentrations of glucose and β-hydroxybutyrate, a key ketone body, to resemble the fasting state.

Conclusion

We formulated FastM, a cell culture medium which will allow deeper understanding of how fasting influences cancer cell behavior, metabolic profiles, and, ultimately, treatment efficacy. Compared to currently used cell culture media aiming to induce fasting responses, which simply reduce the amount of nutrients, FastM offers a more complex and realistic fasting environment for cancer cells. This approach has the potential to significantly advance cancer research by offering a method for exploring fasting strategies and their therapeutic implications. Furthermore, this medium can be used for in vitro experiments beyond cancer research.

EACR25-0455

Cingulin expression and clinical/prognostic value in breast cancer

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Introduction

Cingulin is a vital part of the apical junctional complex (AJC) and regulates many pathological processes, such as tumorigenesis and inflammation. This gene has been verified as closely associated to the biological processes of cancer. Cingulin has also been studied in the context of various cancers, including lung cancer, osteosarcoma, and endometrial cancer. Unfortunately, there is limited research in breast cancer. This study aims to address this and investigate the function of Cingulin in breast cancer.

Material and method

Expression levels of Cingulin in human breast tumour and normal tissues were quantitatively determined by way of gene transcript analyses and assessed using clinical pathophysiological parameters. A breast cancer tissue microarray (TMA) containing 136 breast cancer tissues was immunohistochemically (IHC) stained for Cingulin and expression and localisation analysed and scored. In addition, siRNA plasmid was used to construct a Cingulin knockdown cell model which was used for proteomic analysis. Statistical methods utilised included Mann Whitney U test for comparisons, Fisher test was applied for IHC scoring, logistic regression and Kaplan-Meier's methods for survival analyses.

Result and discussion

Cingulin showed low expression in breast tumours compared with normal breast tissues. In predicting overall survival ($P = 0.06$), changes in expression nearly reached significance, even though the number of clinical sample was small. Additionally, the TCGA public

database showed significance which supplemented our results ($HR = 0.67$, $P = 0.0037$). When using grade classification, Cingulin expression showed a declining trend from grade1 to grade3. Staining of the TMA breast cancer tissue revealed high expression of in normal tissue and the expression in tumour tissue was suppressed ($P = 0.0038$). Moreover, the expression of Cingulin was higher in T1+T2 stages compared to T3+T4 stages ($P = 0.0038$). Proteomic analysis indicated that BAX was upregulated after the knockdown of Cingulin, while HLA-B was upregulated.

Conclusion

Cingulin is aberrantly expressed in clinical breast cancer and has significant value in predicting progression and outcome of patients survival. According to IHC score result, Cingulin may suppress the function of breast cancer. It may also indicate that Cingulin, together with BAX, may activate the apoptosis procedure in breast cancer. Moreover, the change for HLA-B may provide a potential immunotherapeutic target in breast cancer.

EACR25-0463

SPINT2 Expression in Cancer: Focus on Its Role and Therapeutic Impact in BRAF-Mutated Melanoma

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Introduction

Serine protease inhibitor Kunitz-type 2 (SPINT2) inhibits serine proteases such as hepsin, matriptase, and hepatocyte growth factor activator (HGFA). These proteases interact with the extracellular matrix and can influence signalling pathways that affect tumor progression. HGFA converts pro-HGF into bioactive HGF, which promotes cancer cell proliferation, migration, invasion, survival, and resistance to therapy. HGF activates its receptor c-Met, which is implicated in resistance to BRAF inhibitors in melanoma. BRAF mutations occur in 50% of melanoma cases, and although BRAF inhibitors improve survival, resistance remains a challenge. Despite SPINT2's potential role in cancer, little is known about its impact. This study aims to investigate the expression of SPINT2 in cancer and its functional and therapeutic impact in BRAF-mutated melanoma.

Material and method

SPINT2 expression and association with survival in tumors were analyzed using bioinformatic tools. SPINT2 protein expression and gene promoter methylation status were evaluated in formalin-fixed paraffin- embedded (FFPE) samples from cancers like cervix, lung, prostate, gastrointestinal, glioma, and melanoma. SPINT2 over-

expressing transfectants were generated in A375 and WM9 melanoma cells, and effects were assessed using 2D and 3D cell culture models (CCM). The impact of SPINT2 on tumor growth and angiogenesis was explored through Chick Chorioallantoic Membrane (CAM) assay. SPINT2's biological role was analyzed using Nanostring's PanCancer Progression panel and Proteome Profiler Human XL Oncology array. SPINT2's effect on melanoma cell sensitivity to BRAF/MEK inhibitors, and cumulative effect on spheroid growth and migration was evaluated. Finally, the influence of SPINT2 on MET inhibitors was examined.

Result and discussion

SPINT2 expression was lower in melanoma, liver, and glioma compared to other cancers. In silico analysis showed that reduced expression of this gene is correlated with increased tumor aggressiveness. In FFPE samples, SPINT2 downregulation in melanoma was linked to promoter hypermethylation. Functional studies in A375 and WM9 cells revealed that overexpression of this molecule decreased cell viability, migration, and proliferation in 2D and 3D CCM. Additionally, SPINT2 inhibited tumor growth and angiogenesis in CAM assay, suggesting a tumor suppressive role. At the molecular level, SPINT2 influenced genes and proteins involved in extracellular matrix remodeling, angiogenesis, and migration. Expression of this gene also increased melanoma cell sensitivity to BRAF and MEK inhibitors, enhancing therapy efficacy in spheroid growth and migration. In contrast, SPINT2 reduced sensitivity to MET inhibitors.

Conclusion

SPINT2 downregulation is linked to increased cancer aggressiveness. In melanoma, this protein functions as a tumor suppressor and holds potential as a therapeutic biomarker for BRAF-mutated melanoma patients.

EACR25-0467

The Role of ADAMTS1 in Oral Squamous Cell Carcinoma: Impact on Invasion, Lymph Node Metastasis, and Prognosis via the ADAMTS1-L1CAM-EGFR Signaling Axis

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Introduction

ADAMTS1, a matrix metalloprotease, is implicated in cancer progression, though its precise function remains unclear. In oral squamous cell carcinoma (OSCC), the role of ADAMTS1 is still under investigation. TCGA

data indicate that ADAMTS1 expression is reduced in head and neck squamous cell carcinoma (HNSCC) tissues compared to normal tissues. However, elevated ADAMTS1 levels are associated with poorer prognosis in patients. This study seeks to elucidate the role of ADAMTS1 in OSCC, focusing on its impact on cell invasion, lymph node metastasis, and its potential as a therapeutic target.

Material and method

The study analyzed TCGA data to assess ADAMTS1 expression in HNSCC and its impact on survival. Four OSCC cell lines were used to examine ADAMTS1's effects on invasion and tumor growth through knockdown and overexpression. OSCC xenografts in mice evaluated ADAMTS1's role in tumor growth and lymph node metastasis. Mechanistic studies investigated the ADAMTS1-L1CAM-EGFR axis, and immunohistochemistry correlated ADAMTS1, L1CAM, and EGFR expression with clinical outcomes. Apigenin (API) was tested for its ability to inhibit the ADAMTS1-L1CAM-EGFR pathway, reducing OSCC invasion and metastasis.

Result and discussion

TCGA data showed that ADAMTS1 expression was lower in HNSCC tissues than in normal tissues, but higher levels were associated with worse prognosis. In vitro, ADAMTS1 correlated with increased invasion in OSCC cell lines, with knockdown reducing invasion and metastasis, and overexpression enhancing these abilities. Mechanistic studies revealed that the ADAMTS1-L1CAM-EGFR axis activates EGFR and induces epithelial-mesenchymal transition (EMT), promoting invasion and metastasis. High expression of ADAMTS1, L1CAM, and EGFR was linked to poor prognosis. Treatment with the API inhibited this pathway, reducing OSCC invasion and metastasis, and extended survival in xenograft models, suggesting API as a potential therapeutic agent for OSCC.

Conclusion

This study shows that ADAMTS1 promotes OSCC progression via the L1CAM-EGFR pathway, enhancing invasion and lymph node metastasis through EMT. High expression of ADAMTS1, L1CAM, and EGFR correlates with poor prognosis in HNSCC, suggesting their potential as biomarkers. API was identified as a therapeutic agent targeting this pathway, reducing OSCC metastasis and improving outcomes.

EACR25-0491

Exploiting Cellular Senescence to Enhance Precision Oncolytic Immunotherapy

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Introduction

Cellular senescence is a complex state characterized by irreversible cell cycle arrest, acquisition of a senescence-associated secretory phenotype (SASP), and alterations in cell surface proteins. Senescent cells upregulate MHC

class I expression, modifying their antigenic profile and surface immunopeptidome, thereby influencing immune recognition and clearance. In cancer therapy, widely used chemotherapeutic agents and radiotherapy can induce senescence, leading to a paradox: while senescent cells may suppress tumor growth, they can also promote tumor progression. Addressing residual senescent cancer cells remains a critical unmet clinical need in oncology.

Material and method

We investigate the potential of senescence-associated cancer (neo)antigens for vaccine development through immunopeptidomic and mRNA sequencing analyses of in vitro senescence models, where senescence is induced via chemotherapeutic treatments. Immunopeptidomics analysis is performed to identify MHC class I-bound peptides uniquely presented by senescent cells. Following antigen identification and selection, we evaluate the immunogenicity of candidate peptides in vivo and incorporate them into SenCRAd, a personalized oncolytic viral platform designed to target senescent cancer cells. As a future step, we aim to expand our analysis to patient tumor samples, stratified based on senescence burden, to validate the clinical relevance of senescence-associated antigen presentation.

Result and discussion

We have established that CT26 colorectal carcinoma cells undergo senescence following doxorubicin treatment and upregulate MHC class I expression, suggesting potential alterations in antigen presentation. To characterize these changes at the peptide level, we performed immunopeptidomic analysis comparing proliferating and senescent CT26 cells. We have identified a set of senescence-associated MHC class I-restricted peptides that are currently being evaluated for immunogenicity in vivo, with ongoing experiments assessing their ability to elicit antigen-specific immune responses. These findings will provide critical insight into whether senescence-associated peptides can serve as viable targets for immunotherapeutic strategies aimed at eliminating senescent cancer cells.

Conclusion

Our study explores the immunogenic potential of senescent cancer cells and aims to develop SenCRAd, an oncolytic viral vaccine targeting these cells. This approach could provide a novel therapeutic strategy to eliminate senescent cancer cells, preventing recurrence and improving cancer treatment outcomes.

EACR25-0510

Investigating COPZ1 dependency in in vitro murine models of Thyroid Cancer

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Introduction

Thyroid cancer (TC) is the most common endocrine malignancy, generally successfully treated with standard therapies. However, aggressive forms remain resistant to current treatments. The concept of non-oncogene addiction (NOA) implies that cancer cells depend on non-mutated genes that are essential for their uncontrolled growth; thus NOA genes may represent potential targets

for anti-cancer treatment. We have previously identified COPZ1, the $\zeta 1$ component of the coatomer complex, as a NOA vulnerability in TC. COPZ1 is involved in intracellular trafficking, endosome maturation, lipid homeostasis and autophagy. Inhibition of COPZ1 induced endoplasmic reticulum (ER) stress, inflammatory responses and immunogenic cell death in TC cells, without affecting normal cells. The translational value of our results requires in vivo experiments in immunocompetent murine models. Preliminary to the generation of in vivo preclinical models of COPZ1 silencing, we have characterized the susceptibility to COPZ1 inhibition of four murine cell lines.

Material and method

COPZ1 was silenced through a siRNA transfection in four murine TC cell lines: T3531L, 3610R, T4888M and 3868. Cell proliferation was evaluated by crystal violet assay. Protein expression was analyzed by Western Blot. Cytokines secretion was quantified in conditioned media by ELISA Array. Gene expression was analyzed by Real Time PCR (RT-PCR), using TaqMan probes. The levels of intracellular oxidative stress were measured using commercial kits (ROS-Glo™ H2O2 Assay and GSH/GSSG-Glo™ Assay).

Result and discussion

After COPZ1 depletion, all four murine TC cell lines underwent a reduction in relative proliferation with a different extent for each one. T3531L and 3610R activated a transcriptional program associated with viral mimicry response and IFN pathway. Moreover, both cell lines released several cytokines associated with immune components recruitment. On the contrary, COPZ1 silencing didn't cause a modulation of inflammatory-related genes expression in T4888M and 3868 cell lines, but ER stress was induced as documented by the up-regulation of Chop and BiP. Emerging evidence suggests that ER stress can be involved in other forms of cell death, such as ferroptosis, a type of regulated cell death driven by oxidative stress and the iron-dependent accumulation of lipid peroxides. Oxidative stress increased in T4888M and 3868 cell lines after COPZ1 silencing, as demonstrated by higher levels of ROS and by lower GSH/GSSG ratio. Additionally, ferroptosis markers such as HO-1 and TFRC were overexpressed in COPZ1-silenced cells. These results suggest that ferroptosis could represent a cell death mechanism activated by COPZ1 depletion.

Conclusion

The described results demonstrate that COPZ1 silencing reduces cell proliferation in murine TC cells and that different cell death mechanisms are activated. Further analysis is still needed to confirm the activation of ferroptosis.

EACR25-0512

Regulation of EMT by the U-BOX Domain of CHIP/STUB1 in Colon Cancer Cells

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Introduction

Colorectal cancer (CRC) remains one of the most prevalent malignant tumors worldwide, with metastasis being the leading cause of mortality. Epithelial-mesenchymal transition (EMT) plays crucial role in facilitating metastasis by enabling epithelial cells to acquire mesenchymal characteristics, thereby enhancing their migratory and invasive capacities. EMT is tightly regulated by transcription factors such as Snail1, Slug, and Twist1, which modulate the expression of epithelial markers (e.g., E-cadherin, Claudin1) and mesenchymal markers (e.g., N-cadherin, Vimentin). This study investigates the role of the U-BOX domain of CHIP/STUB1 in regulating EMT in CRC.

Material and method

We established CRC cell lines (HT-29 and HCT116) with U-BOX domain deletion and CHIP/STUB1 knockout. EMT characteristics were evaluated using cell morphology analysis, wound healing assays, invasion assays, colony formation assays. Expression of EMT related molecules were examined by RT-qPCR and western blotting.

Result and discussion

HT-29 and HCT116 cells with U-BOX domain deletion exhibited more pronounced EMT characteristics compared to their wild-type counterparts, displaying elongated morphology, increased migration, and enhanced invasion capabilities. Molecular analyses revealed a significant increase in Slug expression, a key EMT transcription factor. Additionally, these cells showed downregulation of epithelial markers (E-cadherin and Claudin1) and upregulation of mesenchymal markers (N-cadherin and Vimentin). Interestingly, these EMT characteristics were not observed in HT-29 CHIP/STUB1 knockout cells, suggesting a specific role for the U-BOX domain of CHIP/STUB1 in EMT regulation.

Conclusion

The U-BOX domain of CHIP/STUB1 plays a critical role in EMT regulation in CRC cells. Its deletion enhances EMT characteristics, whereas CHIP/STUB1 knockout does not, highlighting its specific function, which further investigation is necessary to elucidate the precise molecular mechanisms involved.

EACR25-0515

Fuelling the fight: The Impact of a Ketogenic Diet on Chemotherapy-induced DNA damage in Acute Leukemia

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Introduction

Acute Myeloid leukemia (AML) is an aggressive bone marrow malignancy that requires chemotherapy treatment. However, about 50% of patients relapse, due to the ability of leukemic cells to proliferate despite dysfunctional DNA damage repair mechanisms, coupled with resistance to apoptosis and escape from immune surveillance. Additionally, dysfunctional leukemia cells and chemotherapy toxicity compromises the immune system, frequently resulting in life-threatening infections. Preclinical data from multiple cancer models suggest that ketogenic diets (KD) administered as an adjuvant to chemotherapy can reduce cancer cell proliferation, increase apoptosis, and deprive cancer cells of glucose. Aim: (1) To examine the effects of a KD administered during chemotherapy on markers of apoptosis and DNA damage and repair in leukemic cells from patients with AML. (2) To evaluate the impact of a KD on glucose levels and infections during chemotherapy and the following period of neutropenia.

Material and method

Leukemic cells were isolated from the peripheral blood of patients with AML participating in a KD study. Participants were randomized to receive a control diet ($n = 6$) or a KD ($n = 10$) during chemotherapy. Samples were collected before, during, and after treatment. Apoptosis and DNA damage and repair were assessed using flow cytometry. Blood glucose and infections were measured.

Result and discussion

The KD group had stable glucose levels during treatment, while the control group had high and unstable levels. Preliminary data showed a trend toward reduced infection rates during hospitalization with the KD (~20–40% reduction). During chemotherapy, the KD group showed lower levels of DNA repair markers (Chk1 p317, $p < 0.01$ and p-ATM, $p < 0.01$) in leukemic cells, despite similar levels of DNA damage markers (yH2AX, $p = 0.31$) and no significant differences in apoptosis between the groups. The control group showed a decrease in DNA repair markers (p-ATM) in non-malignant T-cells from baseline to post-chemotherapy ($p < 0.01$), while the ketogenic group showed a trend toward an increase ($p = 0.23$).

Conclusion

A KD administered as an adjuvant to chemotherapy in patients with AML may protect against infection and chemotherapy-induced T-cell damage by enhancing DNA repair, while potentially limiting DNA repair in leukemic cells. Further clinical and mechanistic studies in larger cohorts are warranted.

EACR25-0533

High levels of DNA replication initiation factors lead to ATRi sensitivity via excessive origin firing

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Introduction

Inhibitors of ATR, a central kinase controlling DNA replication origin firing and cellular checkpoint activity, are currently in multiple clinical trials, yet mechanisms underpinning sensitivity and robust patient stratification biomarkers are lacking. In our new study we carefully characterized the mechanism underlying sensitivity to the important clinical target, the ATR kinase. Using an unbiased, multimodal approach in multiple cancer cell lines, AML patient samples and wider databases, we discovered an overlooked role for deregulated DNA replication origin firing in ATR inhibitor sensitivity.

Material and method

We used a non-invasive live microscope to monitor cell confluence in a panel of breast cancer cell lines to identify sensitive and resistant cell lines to ATR inhibition. We performed immunofluorescence microscopy to investigate rates of chromosome instability and replication stress. We then performed proteomics, phospho-proteomics and gene expression analysis to discover determinants of ATRi sensitivity. In parallel, we applied a newly developed machine learning coupled with long-read sequencing method to investigate DNA replication dynamics. Then, we used publicly available transcriptomic data from Cancer Cell Line Encyclopaedia and published Acute Myeloid Leukaemia patient data to investigate gene expression signature enrichment in larger datasets and including more cancer types.

Result and discussion

We discovered that all cell lines had increased levels of replication stress and chromosome instability upon ATRi, regardless of sensitivity. Interestingly, sensitive cell lines had higher expression and activity of DNA replication origin firing factors at basal. Moreover, in response to ATR inhibition, they massively increased origin firing, leading to the activation of the DNA damage response pathway and cell death. ATRi sensitivity was partly rescued upon co-treatment with XL-413, a CDC7 inhibitor that decreases origin firing. High expression of DNA replication initiation factors correlated with ATRi sensitivity across multiple cancer types, and in acute myeloid leukaemia patient samples.

Conclusion

Our study reveals that in sensitive cancer cell lines or patient samples, excessive origin firing is a detrimental response that leads to cell death. Moreover, this vulnerable state can be detected in sensitive samples by a higher level of origin firing-related factors, providing potential new and specific biomarkers for ATR inhibitor sensitivity that are compatible with clinical application.

EACR25-0543

LAMP1, LAMP2 and LAMP2A in colorectal cancer: potential prognostic biomarkers?

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Introduction

Lysosomal homeostasis and the dual function of autophagy, either suppressing or facilitating tumor growth, have been shown to play an active role in cancer biology. Dysregulated expression of lysosome-associated membrane proteins (LAMPs) has been demonstrated in various pathologies. Their augmented levels suggest potential involvement in tumor progression. The current study aimed to examine LAMP1, LAMP2 and specifically the LAMP2A isoform and to assess the clinical significance of serum protein and gene levels compared to their tissue expression in primary CRC and healthy individuals.

Material and method

Forty-one CRC patients (tissue as blood samples) and nontumorous colonic tissue and blood samples from healthy donors were examined by ELISA, qPCR, and immunohisto-chemistry.

Result and discussion

It was found that mRNA levels were significantly higher in CRC white blood cells than in healthy individuals. Additionally, the findings were supported by the apparent upregulated LAMP1, LAMP2 and LAMP2A levels in the CRC tissue immunostaining. The three examined proteins were observed within the tumor buds at the invasive front of the tumor in contrast to the feeble signal in tumor parenchyma and noncancerous tissue. However, while plasma protein levels of LAMP1 remained elevated in the patient group, LAMP2 exhibited a contrastingly higher pattern in the control group.

Conclusion

We present novel data for exploring the feasibility of LAMP1, LAMP2 and LAMP2A in liquid and tissue biopsy in CRC that could serve as future prognostic biomarkers for CRC stratification and treatment.

Acknowledgments: This study was supported by the Bulgarian National Science Fund - grant KIT-06-H-63/8 (13.12.2022) and by the European Union-NextGenerationEU, through the National Recovery and Resilience Plan of the Republic of Bulgaria, project N°BG-RRP-2.004-0007-C01.

EACR25-0554

Novel 1-benzhydryl piperazine scaffold-based HDAC inhibitor b7 induces synergistic antiproliferative effect with vemurafenib in colorectal cancer cells harboring BRAF mutation

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Introduction

Colorectal cancer (CRC) is heterogeneous malignancy characterized by variations in molecular profiles and clinical manifestations. Therefore, treatments for CRC are based on histopathological type and clinical stage of disease. However, in patients with metastatic CRC, available therapeutic options have shown limited effect. In recent years, histone deacetylases (HDAC)s have been identified as relevant contributors to pathogenesis and metastatic invasion of CRC. As HDAC6 is often over-expressed in CRC and correlates with poor disease prognosis it represents a good potential therapeutic target. The aim of this study was to evaluate antitumor activity of four novel HDAC inhibitors with 1-benzhydryl piperazine as a surface recognition group, that differ in hydrocarbon linker in HT-29 (with BRAFV600 mutation) and HCT-116 (BRAF wild type) human CRC lines.

Material and method

HT-29 and HCT-116 human CRC cells cultured in RPMI1640 cell culture medium (CM) were treated with b2, b3, b4, and b7 HDAC inhibitors and b3 and b7 in combination with BRAF inhibitor vemurafenib for 72h with at 37°C and 5% CO₂ in humid atmosphere. Cytotoxicity and IC₅₀ values were estimated by MTT assay. Cell cycle analysis and PI/annexinV assay for apoptosis were performed by flow cytometry. Statistical differences between the control (CM) and treatment were estimated by one-way ANOVA and Student's t-test.

Result and discussion

b2 and b4 compounds with low potency for HDAC6 inhibition did not show any cytotoxic activity while b3 compound with higher potency for HDAC6 inhibition showed significant cytotoxic activity on HCT-116 and HT-29 cells with IC₅₀ (μ M) of 54.06 and 71.49, respectively. Furthermore, b7 panHDAC (HDAC1/3/6/8) inhibitor, which is potent in terms of HDAC6 inhibition, induced significant antiproliferative effect with IC₅₀ (μ M) of 22.04 and 58.99 for HCT-116 and HT-29, respectively. Antiproliferative effects of b3 and b7 were more profound on HCT-116 cells and in both cell lines accompanied with increased apoptosis and alterations in cell cycle distribution. Furthermore, it was evaluated on HT-29 cell line whether b3 and b7 compounds could improve the effect of BRAF inhibitor vemurafenib. Cytotoxic effect of combination treatment of b3 and b7 with vemurafenib was evaluated by Chou-Talalay model. Our results showed synergistic effect of b7 and slight synergistic effect for b3 for concentrations lower than IC₅₀ (12.5 μ M and 15 μ M, respectively).

Conclusion

Our findings of synergistic antitumor effect of HDAC and BRAF inhibition may provide rationale for investigations of their joint use in therapy of patients with CRC harboring BRAF mutation and evaluation of the potential of this therapy to increase response rate and overcome acquired resistance to treatment with BRAF inhibitors.

EACR25-0556

LOXL2 Inhibition: A New Strategy for Triple-Negative Breast Cancer

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Introduction

Lysyl oxidase-like 2 (LOXL2) is a member of the lysyl oxidase (LOX) enzyme family. These enzymes play a crucial role in the cross-linking of elastin and collagen, key components of the extracellular matrix. LOXL2 has been implicated in the regulation of tumor progression and angiogenesis, notably in triple-negative breast cancer [1]. This suggests that inhibitors of LOXL2 could offer potential therapeutic benefits in the treatment of this aggressive form of breast cancer [2]

Material and method

Computational docking studies were performed using previously reported inhibitors to explore their potential binding modes to LOXL2. In-house available compounds containing structures aligned with the computational chemistry insights, were screened for anti-LOXL2 activity using the Amplex Ultra Red assay [3]. A novel LOXL2 inhibitor was identified (AK75) that was then assessed in human triple-negative breast cancer cells (MDA-MB-231) and normal-like breast cells (MCF10A). The effects of AK75 on cell viability (MTT assay), migration (wound healing, single-cell tracking, and transwell assays), 2D and 3D invasion, cell morphology, DNA damage (γ -H2AX immunofluorescence), and matrix metalloproteinases (MMP) activity (zymography) were investigated.

Result and discussion

AK75 inhibits LOXL2 with an IC₅₀ value in the very low micromolar range. This compound is not selectively cytotoxic for cancer cells and does not induce DNA damage. In MDA-MB-231 cells, AK75 reduces chemotaxis, 2D and 3D invasion, MMP2 activity, and alters cell morphology.

Conclusion

This study explores the potential of AK75, a novel LOXL2 inhibitor, towards an eventual therapeutic strategy for triple-negative breast cancer treatment.

EACR25-0563

The DC6-KSP signaling Pathway is essentially required for Metabolic Adaptation and Leukemic Progression

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Introduction

Acute Myeloid Leukemia (AML) is a highly aggressive cancer originating from myeloid lineage stem cell precursors, affecting both adults and children. It is notorious for drug resistance and high relapse rates. While DC6 has been implicated in the progression of

various solid tumors and multiple myeloma, its role in AML has yet to be elucidated.

Material and method

We employed models of mixed lineage leukemia-AF9/NRAS-induced AML and blast crisis chronic myeloid leukemia (bcCML) triggered by BCR-ABL/NUP98-HOXA9 to investigate the role of DC6 in aggressive myeloid leukemia.

Result and discussion

Our study demonstrates that DC6 is pivotal in regulating autophagy and mTOR localization under amino acid deprivation in AML cells. Notably, DC6 expression is elevated in AML cells and patient samples, with high levels correlating with poor prognosis. Our findings also show that conditional DC6 deletion impairs leukemogenesis in an MLL-AF9/NRAS mouse model. Further investigation reveals that DC6 interacts with KSP, enhancing its stability by preventing proteasomal degradation. Moreover, DC6 orchestrates the relocation of the KSP-mTOR complex from lysosomes under amino acid scarcity, critically influencing AML cell survival.

Conclusion

Collectively, our data illuminate the indispensable role of the DC6-KSP pathway in AML progression, particularly under conditions of amino acid deprivation.

EACR25-0564

Nutlin-3a Inhibits Lysosomal Fusion by Inducing SNARE Dysfunction in KRAS mutant/p53 Wild-Type Lung Cancer

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Introduction

Nutlin-3a is an MDM2 antagonist that inhibits the interaction between MDM2 and p53, thereby activating p53 and modulating various cellular stress responses. In our previous study, we showed that nutlin-3a suppresses the hexosamine biosynthetic pathway via GFPT2 down-regulation, leading to lysosomal dysfunction and impaired autophagosome–lysosome fusion in KRAS mutant/p53 wild-type lung cancer cells. The SNARE complex – comprising STX17 on autophagosomes, VAMP8 on lysosomes, and SNAP29 bridging the two – mediates the fusion of autophagosomes and lysosomes, the final step of autophagy, known as autophagic flux. Additionally, N-/O-glycosylation of lysosomal proteins has been proposed to potentially influence the stability and interactions of the SNARE complex. Therefore, we investigated the underlying mechanism of nutlin-3a-induced disruption of autophagosome–lysosome fusion.

Material and method

Localization of autophagosome and lysosome was visualized by confocal microscopy after staining with their respective markers such as LC3 and LAMP1. Expression levels of lysosomal and autophagosome-related molecules along with SNARE complex components were measured by RT-qPCR and western blotting. Further analysis of SNARE complex

interactions as well as N-/O-glycosylation of relevant molecules was examined by immunoprecipitation.

Result and discussion

Nutlin-3a treatment markedly reduced SNAP29 expression, as confirmed by RT-qPCR and western blotting. Immunoprecipitation analysis further demonstrated an increase in N-/O-glycosylation levels of LAMP1 and LAMP2, along with a significant reduction in the interactions among SNAP29, STX17, and VAMP8. These findings suggest that SNAP29 reduction and glycosylation-modified lysosomes contribute to the disruption of SNARE complex formation, thereby impairing autophagosome–lysosome fusion. Confocal microscopy corroborated these findings by showing reduced SNAP29–VAMP8 colocalization. Additionally, nutlin-3a treatment induced an increase in Galectin-3, a marker of lysosomal membrane permeabilization, while western blot analysis showed TFEB activation and increased expression of CTSB, LAMP1, and LAMP2. Collectively, these results suggest a compensatory mechanism attempting to counteract nutlin-3a-induced lysosomal dysfunction.

Conclusion

Our results demonstrate that nutlin-3a disrupts autophagosome–lysosome fusion by downregulating SNAP29 and impairing SNARE complex assembly in KRAS mutant/p53 wild-type lung cancer cells.

EACR25-0577

Understanding the interplay of EPHA2 and CD44 in colorectal cancer stemness, EMT and therapy resistance

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Introduction

Receptor tyrosine kinases (RTKs) are one of the crucial signal transduction pathway regulators that support cell growth, survival, and motility. Recent genomic analyses have linked the expression of many Ephrin RTKs with metastasis and anti EGFR therapy resistance in colorectal cancer. The ephrin RTK signaling is quite complex and elaborate in nature. The signaling can happen both in the presence and absence of ephrin ligand. In recent years more and more evidences are coming up showing that ligand independent signaling carried by EPH receptors by physically interacting with other transmembrane or cytoplasmic proteins is involved in some crucial aspects of cancer biology like therapy resistance and metastasis. We hypothesize that EPHA2 and CD44 form a functional complex that enhances CRC drug resistance by regulating stemness and EMT. We aim to determine whether this is a direct molecular mechanism or a downstream signaling consequence.

Material and method

Cell lines used: HCT116 (more metastatic) and SW480 (more epithelial). CD44 knocked down HCT116 and SW480. EPHA2 inhibitor: ALW-II-42-47. Functional assays: cell cycle analysis through FACS, MTT for cell viability and proliferation, qPCR for gene expression analysis, wound healing for migration, FACS for cell surface marker detection. Co-immunoprecipitation to detect protein interaction.

Result and discussion

1. EPHA2 inhibition effectively blocks cell cycle progression in nM ranges in the anti EGFR resistant cells.
2. EPHA2 inhibition reduces the surface expression of stemness markers in cancer cells.
3. The combination of EPHA2 inhibition and CD44 knockdown has increased effectiveness in blocking cell proliferation.
4. qPCR, colony and sphere formation assays show that EPHA2 inhibition leads to reversal of EMT and reduction of stemness respectively.
5. Co-IP confirms the protein interaction between EPHA2 and CD44.

Conclusion

We found that ALW a small molecule inhibitor of tyrosine phosphatase, effectively halts the cell proliferation in nanomolar concentrations. This molecule also reduces the surface expression of stemness markers like CD44 and CD133. Past studies showed that EPHA2 and CD44 levels are correlated in CRC, but is there any functional relation between the two, or just a causative effect. In present study we showed that EPHA2 and CD44 interact physically. CD44 KD cells when treated with ALW show stronger EMT to MET reversal. Knockdown of CD44 also reduces the protein expression of EPHA2 in the CRC cell line. Thus, we have found out that CD44 interacts with EPHA2 possibly regulating cell proliferation, stemness and EMT.

EACR25-0581

Role of mast cells in promoting breast cancer stem-like properties via heparanase and MUC1/estrogen receptor axis

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Introduction

Breast cancer is the most frequent malignancy in women and exhibits significant heterogeneity, leading to variable clinical outcomes. Tumor aggressiveness is heavily influenced by immune infiltration. Among immune cells, mast cells (MCs) have been implicated in breast cancer prognosis; however, their role remains controversial. While MCs are generally associated with a favorable prognosis in basal breast cancer, they exhibit a negative prognostic impact in luminal and HER2-positive subtypes. In this study, we aimed to elucidate the molecular mechanisms by which MCs influence tumor-initiating properties in breast cancer.

Material and method

MCs were co-injected with limiting concentrations of breast cancer cells *in vivo* to analyze tumor engraftment rates. We explored the involvement of heparanase in promoting tumor stem-like feature by pharmacological inhibition. Additionally, we analyzed soluble factors,

including cytokines and proteoglycans, in MC-conditioned media following stimulation with a TLR4 agonist or heparanase inhibitors. We further characterized the extracellular vesicles (EVs) released by MCs under these conditions and examined their impact on cancer stem-like properties.

Result and discussion

Our results demonstrate that MCs enhance tumor-initiating properties in breast cancer cells. Heparanase activity was found to be crucial for this process, as both its enzymatic function and the stimulation of MCs with heparan sulfate (a TLR4 agonist and product of heparanase activity) promoted cancer cell stem-like traits. Pharmacological inhibition of heparanase abolished these effects, confirming its key role. Notably, soluble factors secreted by MCs induced the expression of MUC1, which in turn upregulated estrogen receptor (ER) levels and activity. This MUC1/ER axis was found to be essential for maintaining cancer stem-like properties, particularly in HER2-negative breast cancer cells, thereby enhancing mammosphere formation, stem-related gene expression, and resistance to tamoxifen treatment. Furthermore, our analysis of MC-derived soluble factors revealed that TLR4 stimulation increased the release of EVs, while heparanase inhibition suppressed this effect. Depletion of EVs impaired the ability of MCs to induce cancer stem-like properties, highlighting their crucial role in mediating MC-driven tumor-initiating features.

Conclusion

Our findings unveil a novel mechanism by which MCs contribute to breast cancer aggressiveness through the regulation of tumor-initiating properties. Specifically, MC-derived heparanase activity and extracellular vesicle release play a central role in promoting cancer stem-like traits, with distinct effects depending on breast cancer subtype. Ongoing studies aim to characterize the specific type and composition of EVs responsible for these effects, with potential implications for targeted therapeutic interventions.

EACR25-0584

Heterogeneous and dynamic response of squamous esophageal cancer to CDK4/6 inhibition

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Introduction

Esophageal squamous cell carcinoma (eSCC) is among the deadliest cancers worldwide, with minimal improvement in 5-year survival rates over the past two decades. Beyond its highly heterogeneous oncogenic landscape, the cyclin D-CDK4/6-pRB axis is dysregulated in 98% of cases. CDK4/6 inhibition has been shown to promote T-cell-mediated tumor clearance by fostering a tumor-suppressive immune microenvironment. Our study aimed to investigate the response of eSCC to the FDA-approved CDK4/6 inhibitor, Palbociclib, and explore the possible interplay between cancer cells, the tumor microenvironment, and immune cells upon treatment.

Material and method

A panel of 22 human eSCC cell lines were used to assess the effects of the CDK4/6 inhibitor, Palbociclib. Cell cycle analysis was performed via flow cytometry, and micronuclei formation was evaluated by immunostaining. Activation of the cGAS-STING pathway and inflammatory responses were analyzed using western blot, RNA sequencing, and multiplex cytokine assays. To model the tumor microenvironment, eSCC spheroids were embedded in a microfluidic device, co-cultured with human umbilical vein endothelial cells (HUVECs) and lung fibroblasts (LF) to induce vascularization. Immune cell infiltration was evaluated by introducing human PBMCs into the microfluidic device following Palbociclib treatment, before being stained and imaged by immunofluorescence for quantitative image analysis.

Result and discussion

Palbociclib treatment resulted in reduction of S-phase progression in 90% of eSCC cell lines, with prolonged exposure leading to nuclear abnormalities, including micronuclei formation. Immunostaining revealed that many of these micronuclei lacked Lamin A/C. This loss correlated with the activation of the cGAS-STING pathway, as confirmed by the upregulation of inflammatory and interferon response genes. In a 3D tumor model, Palbociclib treatment led to increased PBMCs infiltration into eSCC spheroids, as visualized by immunofluorescence quantification. These results suggest that Palbociclib promotes immune cell recruitment, potentially enhancing anti-tumor immunity. These findings reveal a dual effect of Palbociclib in eSCC: direct inhibition of tumor cell proliferation on selected cell lines and indirect modulation of immune responses.

Conclusion

Prolonged Palbociclib treatment induces micronuclei formation, cGAS-STING activation, and an inflammatory response. ESCC 3D model reveals indirect immune-modulating effects of CDK4/6 inhibition, supporting its potential for combination with immunotherapy. However, the heterogeneous response among eSCC cell lines highlights the need to further investigate genomic determinants of Palbociclib sensitivity to optimize treatment strategies.

EACR25-0589

TRIB2 overexpression impairs autophagy flux and compromises efficient clearance of cellular cargo

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Introduction

Autophagy is a crucial cellular process that degrades and recycles intracellular components to maintain homeostasis, particularly under stress conditions. TRIB2, a pseudokinase implicated in cancer progression, has been associated with drug resistance and cell survival mechanisms. However, its role in autophagy remains unclear. This study investigates the impact of TRIB2 on

autophagy regulation and its potential mechanisms of action.

Material and method

We investigated TRIB2's role in autophagy using U2OS cells with TRIB2 overexpression and UACC62 cells with TRIB2 knockout. Autophagy was evaluated by measuring LC3B-II and p62 protein levels under various conditions. We employed a tandem mRFP-GFP-LC3 reporter assay and co-immunoprecipitation experiments to further assess TRIB2's impact on autophagy.

Result and discussion

TRIB2 overexpression resulted in decreased LC3B-II levels and increased p62 accumulation, suggesting autophagy suppression. Conversely, TRIB2 KO cells exhibited slightly increased LC3B-II levels, indicating enhanced basal autophagy. Under starvation conditions, TRIB2-expressing cells displayed impaired autophagy flux, evidenced by reduced LC3-II accumulation in CQ-treated samples and compromised p62 degradation. The mRFP-GFP-LC3 assay further confirmed autophagy inhibition, showing increased autophagosome accumulation with reduced autolysosome formation in TRIB2-overexpressing cells. Importantly, co-immunoprecipitation studies revealed that TRIB2 interacts with p62 under nutrient-rich conditions but not during starvation, indicating a role in autophagy suppression. No interaction was observed between TRIB2 and ULK1 or Beclin1, suggesting TRIB2 acts in the later stages of autophagy rather than at the initiation step.

Conclusion

Our findings demonstrate that TRIB2 negatively regulates autophagy by impairing cargo clearance at later stages, likely through its interaction with p62. This highlights TRIB2 as a potential regulator of autophagy-related cellular stress responses and provides new insights into its role in cancer cell survival. Further research is needed to explore TRIB2 as a therapeutic target in diseases where autophagy plays a critical role.

EACR25-0626

TACSTD2 expression is a marker for the early formation of colon adenoma

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Introduction

Colon adenomas constitute preneoplastic lesions that emerge from the intestinal epithelium and can further progress into colon cancer if left untreated. Although early detection of colon cancer and its preneoplastic precursors is decisive for survival and treatment options, no suitable biomarkers are available to detect the transformation of a healthy intestinal epithelium into adenomas. Therefore, we aimed to identify novel drivers associated with the development of precancerous lesions in the colon.

Material and method

We performed whole-transcriptome sequencing of human adenoma samples and matched colon mucosa and performed a validation of the most interesting signals in a separate patient cohort and eight independent public datasets. Genome-wide methylation profiling of additional cases of adenoma tissue pairs was also conducted and confirmed by pyrosequencing. Functional analyses were performed *in vitro* and *in vivo* using organoids and tissue samples derived from Apcmin/+ mice as well as from the inflammation-associated azoxymethane/dextran sodium sulfate (AOM/DSS) and the sporadic colon carcinogenesis (six AOM injections) mouse models. Potential novel candidates were evaluated by immunohistochemical staining in human adenomas of sporadic and familial adenomatous polyposis (FAP) origin as well as in early-stage colon tumors (pT1).

Result and discussion

From a total of 1,917 differentially expressed genes and 148,191 differentially methylated CpG sites detected in adenomas compared with matched mucosa samples, we selected TACSTD2 among other genes including MMP7, MMP1, CLDN2, CLDN1, and ETV4 for further validation. Indeed, TACSTD2 promoter hypomethylation in adenoma tissues was validated in 20 additional tissue pairs and corresponded with increased TACSTD2 expression. Moreover, TACSTD2, which encodes for the protein and cancer driver TROP2, was overexpressed in human adenomas of both sporadic and FAP origin, in pT1 colon tumors as well as in murine adenomas and their organoid derivatives. Using patient- and mouse-derived organoids, we observed differences in the regeneration, colony formation and anoikis evasion potential in a TACSTD2-dependent manner, with high levels of TACSTD2 promoting adenoma progression and features of tumor aggressiveness.

Conclusion

TACSTD2 is overexpressed in mouse and human adenomas of both sporadic and FAP origin compared to normal mucosa and might drive the development of colonic precancerous lesions. Therefore, TACSTD2 could serve as a novel marker for the identification of transforming foci in colon biopsies.

EACR25-0655

Combined oncogene and pyruvate dehydrogenase kinase inhibition enhances tumor response in resistant NSCLC cells

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Introduction

The metabolic reprogramming of tumor cells plays a critical role in cancer progression, contributing to drug resistance and tumor survival. Tyrosine kinase inhibitors (TKIs) have shown promising clinical results by targeting specific signaling pathways involved in cancer cell proliferation, survival, and metastasis. However, the development of resistance to TKIs remains a significant challenge in oncology for the onset of multiple secondary resistance mechanisms. In parallel, dichloroacetate (DCA), a small molecule that alters cancer cell metabolism by inhibiting pyruvate dehydrogenase kinase (PDK), has been investigated for its potential to reverse the Warburg effect and reprogram tumor cell metabolism. Here, we explored the rationale behind combining TKIs with DCA, focusing on the synergistic effects that may arise from the dual inhibition of oncogenic signaling and metabolic reprogramming.

Material and method

We selected three NSCLC cell line models (H1975, H1993, A549) and determined the optimal DCA dose to reverse the Warburg effect. Consequently, EGFR-mutant H1975, c-MET amplified H1993 and KRAS-mutant A549 NSCLC cells, were treated with TKIs (osimertinib, crizotinib and selumetinib, respectively), DCA alone or in combination with TKIs (halved dose). Several assays were performed to evaluate the combination effect on tumor response.

Result and discussion

Our results highlighted that a very low dose of DCA is necessary to restore oxidative phosphorylation. The proposed combined approach, compared to TKI alone, led to a shift from glycolysis to oxidative phosphorylation, data confirmed by input (glucose) and output (ATP) levels and also by a metabolomic analysis. Moreover, a significant decrease in cell proliferation was observed in combo conditions compared to TKI and to untreated cells. On the other hand, a relevant increase of apoptosis, due to a higher BIM expression levels associated to a decrease of mitochondrial membrane

potential, and autophagy was also observed. In addition to these findings, all cell lines treated showed a reduced migration and invasion ability due to the slower wound closure and the reduced protrusion in a 2D and 3D cell culture assay, respectively.

Conclusion

Our findings indicate that the combination of TKIs and DCA, at low doses, represents a promising therapeutic approach to overcome resistance and improve clinical outcomes in target cancer therapy.

EACR25-0682

Beta-catenin differential expression in salivary gland tumors from intercalated duct: an immunohistochemical and molecular study

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Introduction

Salivary gland tumors present a wide morphologic variation, especially among neoplasms originated from intercalated duct and with prominent or partial involvement of myoepithelial component. Currently, there are few studies correlating histological subtypes with genetic alterations in benign and malignant salivary gland tumors, especially the beta-catenin staining and the presence of CTNNB1 mutation in basal cell adenoma. Thus, we analyzed our case series of benign and malignant tumors originating from the intercalated duct to verify whether there is an association between the immunohistochemical staining pattern and mutation among these tumor subtypes and possible differences between them. Therefore, understanding the molecular basis of these lesions would be useful for differential diagnosis and classification by evaluating their mutational profiles.

Material and method

Immunohistochemistry (IHC) and Next Generation Sequencing (NGS) were performed for tumor analysis. IHC was applied to 40 cases, and NGS to 31 cases with available paraffin material. IHC followed the Ventana BenchMark Ultra protocol (Roche), evaluating staining localization and intensity. NGS targeted exon 3 of the CTNNB1 gene using the Ion GeneStudio S5 Plus platform, analyzed with IGV_2.18.4 software, and verified in the Franklin database. True alterations required complete amplicon coverage, sufficient reads, consistency in forward and reverse reads, and an alteration reading frequency of 2% or higher [1].

Result and discussion

Of the 31 samples sequenced in the specific exon, 27 samples also had immunohistochemical analysis that can be compared. The analyses were carried out manually and we can highlight 7 cases that had the mutation validated and confirmed. We observed that 3 out of 6 basal cell adenoma cases, and 1 out of 3 basal cell adenocarcinoma cases showed the p.Ile35Thr mutation, all demonstrating nuclear beta-catenin staining. One carcinoma ex-pleomorphic adenoma case showed a mutation at codon 40, considered a pathogenic variant and the beta-catenin staining was observed as a discontinued membrane pattern. Two out of 8 pleomorphic adenoma cases had a mutation at codon 34, classified as pathogenic mutation, however no IHC information was available. Both mutations have not been previously reported in the tumor types studied. We can observe differences in beta-catenin staining pattern and molecular profile between tumor subtypes from intercalated duct, although a larger casuistry is necessary to confirm these differential patterns.

Conclusion

Few alterations were observed in these tumors, and we can observe differences especially when comparing basal cell adenoma and adenocarcinoma or pleomorphic adenoma. Further studies are necessary to investigate the influence and the role of beta-catenin in these tumors.

[1] Ethics Committee protocol 2706/19

EACR25-0684

Repurposing Antihistamines: Exploring Their Potential as N-WASP Inhibitors in Breast Cancer Metastasis

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Introduction

Breast cancer metastasis is the leading cause of cancer-related mortality, emphasizing the need for novel therapeutic approaches. Neural Wiskott-Aldrich syndrome protein (N-WASP), a crucial regulator of actin polymerization and cytoskeletal dynamics, plays a significant role in cancer cell motility and invasion. Inhibiting N-WASP may suppress the metastatic spread of cancer cells. Drug repurposing provides an efficient pathway to accelerate treatment development, especially when the drugs have well-established safety profiles. This study investigates the potential of two anti-histamines – cyproheptadine (a first-generation compound) and desloratadine (aerius®, a second-generation agent) – as N-WASP inhibitors to hinder breast cancer cell migration and proliferation.

Material and method

MDA-MB-231 and MCF-7 breast cancer cell lines, along with HECV endothelial cells, were utilized. Proliferation was assessed using cytotoxicity assays, while migration and adhesion were evaluated through wound healing and Electric Cell-substrate Impedance Sensing (ECIS) assays. N-WASP expression at the protein and mRNA levels was analysed using immunofluorescence staining, western

blotting, and qPCR. To explore the molecular basis of drug-protein interactions, molecular docking simulations were performed, targeting the VCA domain of N-WASP.

Result and discussion

Both antihistamines significantly inhibited cancer cell proliferation. Desloratadine reduced MDA-MB-231 proliferation by 51.76% ($p < 0.0001$), whereas cyproheptadine decreased it by 15.72% ($p = 0.0007$). In MCF-7 cells, cyproheptadine exhibited a stronger inhibition rate (43.18%) compared to desloratadine (39.23%). Migration assays revealed substantial reductions in cell motility, with cyproheptadine decreasing MCF-7 migration by 35.89% ($p < 0.0001$) and desloratadine by 27.32% ($p = 0.0014$). ECIS assays confirmed that both treatments impaired cell adhesion and migration. Protein analysis indicated a marked downregulation of N-WASP in treated cells, confirmed through immunofluorescence and western blotting. Interestingly, qPCR results revealed no significant changes in N-WASP mRNA levels, suggesting post-transcriptional regulation. Molecular docking simulations demonstrated that both antihistamines interact with key residues within the N-WASP VCA domain. The findings support the hypothesis that both drugs disrupt cytoskeletal dynamics essential for cancer cell migration and adhesion.

Conclusion

This study demonstrates that cyproheptadine and desloratadine inhibit breast cancer cell proliferation, adhesion, and migration by downregulating N-WASP protein expression. Repurposing antihistamines as anti-metastatic agents presents a promising and cost-effective strategy for breast cancer treatment.

EACR25-0690

Insights from Ultrastructural Changes, Histopathology, Gene Expression, and Prognostic Outcomes Between Apoptosis and Ferroptosis in Primary and Chemoreduced Retinoblastoma

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Introduction

Understanding the distinctions between apoptosis and ferroptosis is essential for exploring their roles in different biological processes and their potential prognostic implications in retinoblastoma (Rb). Therefore, this study aims to explore the morphological, pathological and molecular implication of apoptosis and ferroptosis in retinoblastoma patients and their prognostic outcome.

Material and method

A total of 123 prospective cases were included among which 82 cases underwent primary enucleation and 41 cases received chemotherapy/radiotherapy before enucleation (chemoreduced retinoblastoma) for a period of 2 years. Immunohistochemistry was performed for

Transferrin receptor (TFR1) protein and caspases on formalin-fixed paraffin embedded (FFPE) tissues. mRNA expression level was measured for ferroptosis-related genes (SLC7A11, SLC3A2, GPX4) and regulator of apoptosis (Caspases 3, 8 and 9 genes) using qRT-PCR in all Rb cases and normal control. Expression was finally correlated with clinical and histopathological parameters.

Result and discussion

Transmission electron microscopy (TEM) was also performed to observe ultrastructural changes. Kaplan-Meier survival graph was plotted to predict the patient outcome. There was male preponderance with more than 50% of the cases belong to <2 years of age. Cytoplasmic expression of TFR1 protein was found in 78% cases. Upregulation of GPX4 mRNA level was prominent in our cases. SLC7A11 and SLC3A2 was inversely expressed in primary retinoblastoma cases. Scleral invasion was statistically significant with TFR1 expression and unregulated GPX4 gene level. Upregulated mRNA expression of Caspase-3 and -8 was found in 85% and 72.5% cases, respectively. Down-regulation of Caspases-9 was found in 70% of the cases, and this was statistically significant with massive choroidal invasion.

Conclusion

TEM showed morphological features of cell death that corresponds to apoptosis and ferroptosis. This study found altered and differential expression of ferroptosis-related markers and apoptosis regulated genes which could predict the prognosis of retinoblastoma patients.

EACR25-0719

Polycomb Complex Protein PHC3 Coordinates with HOTAIR Pathway to Promote Colorectal Cancer Progression

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Introduction

Colorectal cancer (CRC) is the third most prevalent and fatal malignancy worldwide. Despite advances in surgical and chemotherapeutic interventions, high recurrence rates suggest that the underlying mechanisms of CRC progression remain unclear. Polyhomeotic homolog 3 (PHC3), a transcriptional regulator within the polycomb repressive complex 1 (PRC1), has been implicated in epigenetic gene regulation via monoubiquitylation of histone H2A at lysine 119 (H2AK119ub). Our previous transcriptomic analysis revealed that PHC3 is upregulated in CRC and is associated with poor patient prognosis. However, the precise role of PHC3 in CRC tumorigenesis requires further elucidation.

Material and method

We collected clinical CRC specimens and performed integrated transcriptomic profiling to identify differentially expressed genes. Functional assays, including proliferation, migration, and invasion assays, were conducted in CRC cell lines with PHC3 knockdown

or overexpression. A xenograft mouse model was employed to assess tumorigenic potential *in vivo*. RNA-IP-qPCR analysis was performed to identify the interaction between PHC3 and lncHOTAIR.

Additionally, high-throughput RNA sequencing and Hi-ChIP-seq for H2AK119ub were conducted to investigate PHC3-mediated transcriptional and chromatin modifications.

Result and discussion

We found that PHC3 was upregulated in CRC patients, and high PHC3 expression was correlated with poor prognosis. Knockdown of PHC3 significantly suppressed CRC cell proliferation, migration, and invasion, whereas PHC3 overexpression enhanced these oncogenic properties. Mouse xenograft experiments confirmed that PHC3 promotes tumor growth. Mechanistically, PHC3 depletion led to reduced expression of BMI1, a canonical PRC1 component, and survival-associated signaling molecules. Transcriptomic profiling identified that PHC3 regulates the HOTAIR signaling pathway and influences tumor-related genes such as WNT, FOXM1, and CDKN1A. RNA-IP-qPCR analysis revealed that PHC3 interacted with lncHOTAIR. Furthermore, Hi-ChIP-seq analysis demonstrated that PHC3 depletion alters H2AK119ub occupancy at specific chromatin loci, implicating PHC3 in the epigenetic modulation of gene expression in CRC.

Conclusion

Our findings suggest that PHC3 plays a critical role in CRC tumorigenesis by regulating the HOTAIR signaling pathway and modifying chromatin structure through H2AK119ub. Targeting PHC3-mediated epigenetic mechanisms may provide novel therapeutic strategies for CRC treatment.

EACR25-0731

Identification and Functional Characterization of Theranostic Biomarkers in Pancreatic Ductal Adenocarcinoma

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Introduction

Pancreatic ductal adenocarcinoma (PDAC) is characterized by a poor prognosis and a high recurrence rate following surgical resection. The urgent need for reliable theranostic biomarkers and corresponding therapeutic strategies highlights the importance of ongoing research efforts. This study aims to identify biomarkers that facilitate early detection, enable risk stratification for postoperative recurrence, and inform the development of precision medicine through biomarker-driven therapeutic approaches.

Material and method

Bioinformatic and immunohistochemical analyses were performed on PDAC specimens. Functional studies were conducted by knocking down individual and multiple biomarkers in PDAC cell lines to assess their effects on cellular function [1].

Result and discussion

Bioinformatic analyses identified four biomarkers – ADAM9 (a disintegrin and metalloprotease 9), MTHFD2 (methylenetetrahydrofolate dehydrogenase), RRM2 (ribonucleoside-diphosphate reductase subunit M2), and SLC2A1 (solute carrier family 2 member 1) – whose elevated expression correlated with poor clinical outcomes in PDAC patients. A higher cumulative expression of these biomarkers was associated with an increased hazard ratio, underscoring their prognostic significance. Immunohistochemical analysis of PDAC specimens revealed significantly elevated H-scores for these biomarkers in patients with advanced tumor stages. Functionally, these biomarkers appeared to act synergistically in driving tumor progression. Notably, simultaneous knockdown of all four genes resulted in a more pronounced reduction in cell proliferation compared to the silencing of individual genes.

Furthermore, knockdown of a single gene influenced the expression of the others, suggesting compensatory regulatory mechanisms among these biomarkers. Pharmacological inhibition targeting these biomarkers in combination further enhanced apoptotic markers and effectively reduced PDAC cell viability. *In vivo*, this translated to significant tumor growth suppression, as evidenced by a marked reduction in tumor size.

Conclusion

Targeting the four identified theranostic biomarkers in PDAC cells holds promise for the development of more effective and precision-targeted therapeutic strategies. These findings provide a foundation for improving treatment outcomes and prognostic accuracy in PDAC patients.

[1] All experimental procedures were conducted in accordance with ethical guidelines and were approved by the Institutional Animal Care and Use Committee and the Institutional Review Board at China Medical University Hospital.

EACR25-0736

Western diet-associated colonic insulin resistance boosts DNA error-prone quiescent stem cells and senescence of the homeostatic LGR5+ stem cells in the colon

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Introduction

The increasing incidence of early-onset colorectal cancer (eoCRC) may be a consequence of poor dietary habits, as up to 70% of CRC risk factors are nutrition-related. Therefore, in this study we aimed to elucidate the impact of distinct Western diet (WD)-associated phenotypes, such as obesity and metabolic syndrome (MetS), during early life on colonic epithelial cell (EC) biology in the context of CRC.

Material and method

7-week-old male C57BL/6 mice, recapitulating a human age of 13, were fed a WD or control diet for 6 or 12 weeks. Murine colonic tissues were phenotyped using immunohistochemistry, real-time PCR or Western blot. NMR-metabolomics was used for analysis of serum samples. Mass spectrometry-based lipidomics was used or analysis of fecal samples. To assess relevance to human biology, paired normal and tumoral tissue samples from CRC patients were analyzed. Metabolic effects on intestinal stem cell differentiation were validated in the non-malignant human colonic epithelial cell line HCEC-1CT and ex vivo generated human colon-derived 3D organoids.

Result and discussion

While 6 weeks of WD feeding led to obesity, extending the feeding duration to 12 weeks induced MetS, characterized by obesity, hyperlipidemia, and both systemic and colonic insulin resistance. MetS was accompanied by reduced AKT signaling but marked MAPK pathway activation, promoting hyperproliferation of colonic ECs. Unexpectedly, the colonic expression of the DNA mismatch repair enzyme MSH2 was down-regulated in MetS mice despite elevated cell proliferation. Lipidomic analysis identified the WD-associated polyunsaturated omega-6 fatty acid arachidonic acid (AA) as the most potent trigger of hyperproliferation in colonic ECs. Stimulation of HCEC-1CT cells and human colonic organoids with AA led to cellular overgrowth, boosting the HOPX⁺ quiescent stem cell compartment at the +4 position as well as the REG4⁺ precursor cells of secretory ECs at the expense of the cycling LGR5⁺ stem cell and the OLFM4⁺ precursor cells of absorptive ECs. In silico single-cell analysis revealed high expression of the insulin receptor in the stem cells, and the transit-amplifying cells. However, the quiescent stem cell at +4 position, which is inherently more susceptible to DNA damage due to its quiescent cell state, as well as the REG4⁺ secretory precursors exhibited higher levels of insulin-independent growth factor receptors in contrast to LGR5⁺ or OLFM4⁺ cells.

Conclusion

Our findings suggest that early exposure to colonic WD-related MetS may lead to the senescence of cycling colonic LGR5⁺ stem cells, while boosting DNA error-prone HOPX⁺ quiescent stem cells, thereby altering intestinal EC homeostasis and increasing susceptibility to develop CRC.

EACR25-0760

Low-density lipoprotein receptor-related protein 11 promotes lung adenocarcinoma proliferation by suppressing ubiquitin like modifier activating enzyme 7

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Introduction

Low-density lipoprotein receptor related protein 11 (LRP11) is a transmembrane protein in the low-density lipoprotein receptor family. It is highly expressed in various cancers and linked to poor prognosis. However, its role in tumor progression remains unclear.

Material and method

We analyzed LRP11 expression in lung cancer using the Cancer Cell Line Encyclopedia (CCLE) dataset. Overexpression and knockout experiments were performed in lung adenocarcinoma cell lines PC9, VMRC-LCD, and A549. LRP11 was overexpressed in PC9 and VMRC-LCD cells via plasmid transfection. Knockout was achieved in A549 cells using CRISPR-Cas9. RNA sequencing (RNA-seq) was conducted to identify differentially expressed genes associated with LRP11 expression. Knockdown of ubiquitin-like modifier activating enzyme 7 (UBA7) was performed using siRNA. We also assessed LRP11 expression and its prognostic significance in lung adenocarcinoma patients using the Cancer Genome Atlas (TCGA) dataset.

Result and discussion

CCLE data showed that 30.2% (13/43) of lung cancer cell lines, mainly adenocarcinomas, had high LRP11 expression. Overexpression of LRP11 significantly enhanced cell proliferation in PC9 and VMRC-LCD. LRP11 knockout suppressed proliferation and migration in A549. RNA-seq analysis revealed that LRP11 was negatively correlated with UBA7, an E1 enzyme involved in ISG15-mediated protein modification. UBA7 knockdown restored proliferation in LRP11-knockout A549 cells. It indicates that LRP11 promotes tumor growth by suppressing UBA7. Considering that ISG15 modification is induced by interferons and tumor necrosis factor, LRP11 may regulate cellular responses to these cytokines. Clinically, LRP11 expression was higher in advanced-stage lung adenocarcinoma. Patients with high LRP11 had significantly shorter survival. A negative correlation between LRP11 and UBA7 was also observed in the patient cohort.

Conclusion

This study demonstrates that LRP11 promotes proliferation of lung adenocarcinoma by downregulating

UBA7. Our findings reveal a novel mechanism underlying LRP11's pro-tumor function. Given its clinical relevance, LRP11 could be a potential biomarker and therapeutic target.

EACR25-0767

The protooncogene SETDB1 affects microtubule dynamics through HDAC6

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Introduction

SETDB1 is a methyltransferase responsible for methylation Histone 3 at Lys9 that leads to repression of gene expression. SETDB1 is overexpressed in many cancers and is thought to support tumorigenic transcriptional profiles. As expected from its activity, SETDB1 is mainly a nuclear protein. Still, there is a substantial cytoplasmic fraction of SETDB1 that its role is ambiguous. Here, we found a moonlight function for SETDB1 in regulating microtubule (MT) dynamics by promoting a-tubulin deacetylation by HDAC6.

Material and method

Association with MTs was determined by immunostaining, MT co-sedimentation assay, and co-immunoprecipitation. Mitosis kinetics was measured by timelapse microscopy.

Result and discussion

SETDB1 interacts with MTs and reduces MT polymerization rate in a manner that is independent of its methyltransferase activity. Consequently, altered SETDB1 levels led to slower mitotic kinetics and a higher mitotic catastrophe rate. In search of the mechanism by which SETDB1 affects MT dynamics, we found that SETDB1 interacts with the major a-tubulin deacetylase HDAC6. Tubulin acetylation increases the flexibility of MTs to enhance their stability against mechanical stress, while HDAC6 supports cell proliferation, migration, and chemoresistance in cancer cells. Overexpression of SETDB1 led to reduced tubulin acetylation levels, while SETDB1 knockdown increased tubulin acetylation levels. Thus, suggesting that SETDB1 promotes HDAC6 activity. Furthermore, overexpression of SETDB1 led to an increase in the protein levels of both endogenous and exogenous HDAC6, suggesting that SETDB1 enhances the protein stability of HDAC6. SETDB1 also elevated the association of HDAC6 with MTs but not with tubulin dimers.

Conclusion

Our results support a model in which SETDB1 promotes MT destabilization by stimulating the activity of HDAC6 towards MTs. SETDB1 does so by both stabilizing HDAC6 protein and recruiting HDAC6 to MTs. Through this mechanism, SETDB1 can alter MT dynamics to support tumor progression.

Conflicts of Interest: G Gerlitz has a US Patent App on Setdb1-microtubule interaction and use thereof (18/227,122, 2024).

EACR25-0774

Deciphering how the TRPM7 channel is involved in breast cancer modulation

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Introduction

Transient receptor potential melastatin 7 (TRPM7) is a peculiar channel characterized by both ion channel and enzymatic activity and is, therefore, known as chanzyme. It plays an important role in calcium and magnesium homeostasis. Recent studies suggest that TRPM7 is implicated in tumor modulation, contributing to various cancer hallmarks such as cell proliferation, survival, and metastasis. A comprehensive understanding of the role of TRPM7 in the intricate cancer metabolism, particularly in breast cancer, may offer novel therapeutic strategies. In this study, we explored the expression and function of TRPM7 in different breast cancer cell lines and investigated its potential to modulate tumor through specific activators, inhibitors and small interfering RNA (siRNA).

Material and method

The cell lines employed in this study were MCF-10A, MCF-7, MDA-MB-231 and SK-BR-3. TRPM7 expression was analysed by quantitative PCR (qPCR), while channel activity was assessed by analysing the signal of Fura-2, a calcium-sensitive fluorescent dye, in the presence of specific activators and inhibitors. To investigate the effect of TRPM7 on cancer modulation, cells were infected with siRNA. Cell viability and gene expression were analysed.

Result and discussion

Preliminary results demonstrated that TRPM7 was significantly more expressed in MCF-7 cells compared to other cell lines. Modulation of TRPM7 activity, by specific activators and inhibitors, induced changes in calcium influx, underscoring the channel's functional role in regulating cellular ion homeostasis. Furthermore, silencing of TRPM7 resulted in a marked decrease in both cell viability and mRNA expression, highlighting its crucial involvement in cellular processes. These results suggested a noteworthy impact of TRPM7 on breast cancer progression. Several ongoing experiments aim to investigate the role of TRPM7 in breast cancer metabolism.

Conclusion

The findings highlight the central role of TRPM7 in the modulation of breast cancer. Indeed, inhibition of channel activity or its silencing by siRNA has been shown to reduce calcium influx, impair cell viability and alter gene expression, supporting its involvement in cancer modulation. These observations provide basis for the exploration of targeted therapies. Further studies are essential to fully understand the broader implications of

TRPM7 in cancer metabolism and to evaluate its viability as a target for innovative therapeutic strategies.

EACR25-0796

Investigation of driver genes in cancer-associated copy number alterations

identifies B4GALT5 as a glycooncogene

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Introduction

Cancer emerges from aberrant communication between cells of a multicellular organism. The glycan coat that surrounds the cells is an important player in cellular communication. While altered cell surface glycans are known biomarkers for cancer, glycan biosynthesis itself has not been identified as a potential oncogenic pathway. Moreover, only a handful of inhibitors have been developed against glycan synthesising enzymes in cancer.

Material and method

To understand the oncogenic potential of glycan biosynthetic pathways, we searched for copy number alterations (CNA)-based glycooncogenes. We developed a bioinformatic-experimental pipeline to prioritize driver genes in the copy number altered segments of cancer genomes that correctly identified several well-established oncogenes/TSGs and also discerned novel potential oncogenes and TSGs, including glycooncogenes. We characterise and validate the glycooncogenes using *in vitro* growth, 3D spheroids models and biochemical assays combining genetic and pharmacological perturbations.

Result and discussion

Among the several pathways enriched, glycooncogenes belonging to the glycosphingolipid pathway was found to be oncogenic. We further validate B4GALT5, a key enzyme of the glycosphingolipid pathway as a novel glycooncogene, whose genomic amplification promotes oncogenic features including increased growth, migration, addiction and is associated with worst prognosis in patients. We also find that glycooncogenes cooperate with known oncogene and TSG like KRAS and TP53 respectively, thereby making them cancer vulnerabilities in previously untreatable cancers.

Conclusion

This study provides an important resource of nearly 1500 novel high-confidence CNA-based oncogenes and TSGs [1], and also identifies glycosylation pathways with onco-regulatory properties and opens up a new group of enzymes as potential therapeutic targets for cancer.

[1] URL: frusso.shinyapps.io/CNA_database

EACR25-0806

Role of the antioxidant metabolism (AM) at various EMT/MET states of pancreatic ductal adenocarcinoma (PDAC) cells

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Introduction

The consecutive events of Epithelial-to-mesenchymal transition (EMT) and Mesenchymal-to-epithelial transition (MET) are considered as driving force in the metastasis of PDAC. Recent studies suggest that oxidative stress (OS) plays a crucial role in EMT/MET-plasticity of cancer cells. Nrf2, as a key regulator of cellular antioxidant (AO) response, efficiently protects cells against OS and, along with this, provides multiple growth advantages for cancer cells fostering metastasis. Addressing this issue, we elucidated a crosstalk of TGF- β 1-mediated EMT and IFN γ /TNF α /IL-1 β (IIT) induced MET of PDAC cells with NRF2 driven AM.

Material and method

Basal phenotypes of different Panc-1 cell clones: 1C3, 3D2, 4B9 were determined by studying expression of E/M state markers on RNA and protein levels. The clones were assessed for their responsiveness to TGF- β 1 and IIT in terms of Nrf2 activity and its downstream AO gene expression, as well as for ROS levels, energy metabolism in different E/M states (glycolysis and mitochondrial respiration real-time measurements, Seahorse assay), and glucose metabolism involved in the redox-homeostasis by stable 13C-isotope tracing.

Result and discussion

Substantial differences were identified in the phenotypes of the Panc1-clones: 1C3 has a mesenchymal, 3D2 an epithelial, 4B9 an intermediate phenotype. Nrf2 activity also differed in the clones with the highest in 1C3 and the lowest in 3D2. AO gene expression was highest in 1C3 and lowest in 4B9. ROS levels were highest in 4B9 and lower in 1C3 and 3D2. TGF- β 1 and IIT treatments increased ROS levels in all clones. Seahorse measurements showed that 3D2 has the highest mitochondrial activity and TGF- β 1 decreased this activity in 3D2 and 4B9 but not in 1C3, while IIT increased it in 4B9 and 1C3 but not in 3D2. By contrast, 1C3 is the most glycolytic clone and TGF- β 1 significantly increased glycolytic parameters in 4B9 and 3D2, but only slightly in 1C3. IIT increased glycolysis in 4B9 and 1C3, but not in 3D2. U-13C-Glucose was differentially metabolized in the clones related to AM: 1C3 showed the highest 13C-enrichment in glutamate and glutathione (GSH) but the lowest enrichment in the TCA intermediate 2-oxo-glutarate. By contrast, 3D2 exhibited the greatest 13C-enrichment in citrate and 2-oxoglutarate but lowest in GSH. 1,2-13C-Glucose tracing revealed greater metabolization through the pentose-phosphate-pathway than glycolysis in 1C3 and 4B9 clones.

Conclusion

Our findings reveal a differential correlation between EMT/MET states of the Panc-1 clones and AM controlled by Nrf2. The most mesenchymal phenotype (1C3) exhibits the highest AM and glycolytic activity while the most epithelial clone (3D2) possesses less AM and glycolytic activity. Thus, AM can differentially affect the metastatic process by differentially impacting EMT and MET. More studies on the crosstalk are needed for novel concepts to overcome metastasis and to improve the existing therapy options in PDAC.

EACR25-0810

Targeting KDM4-Driven Metabolic Reprogramming in Castration-Resistant Prostate Cancer

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Introduction

Prostate cancer, particularly in its castration-resistant form (CRPC), poses a significant therapeutic challenge due to its aggressive progression and resistance to conventional treatments. Recent studies have highlighted the KDM4 family of histone demethylases (KDM4A, KDM4B, and KDM4C) as important epigenetic regulators that contribute to the development of prostate cancer. In this study, we focused on the metabolic modulation by KDM4 members and the development of KDM4 inhibitors.

Material and method

CRPC cells (C4-2B or CWR22Rv1) were depleted of KDM4, and a series of analyses were conducted to identify their expression and metabolic profiles. These analyses included cell proliferation studies (both *in vitro* and *in xenografts*), microarray experiments, quantitative reverse transcription PCR, Seahorse Flux assays, and metabolomic evaluations. Immunoprecipitation was performed to determine interaction regions, while reporter activity assays and chromatin immunoprecipitation analyses were used to characterize the mechanistic actions of the complex. Additionally, a structure-based approach was employed to develop KDM4 inhibitors, and these compounds' cytotoxic effects on various cancer cell lines were evaluated.

Result and discussion

We showed that the knockdown of KDM4B impaired the proliferation of CRPC cells, shifted metabolism from the Warburg effect to oxidative phosphorylation (OXPHOS), and suppressed the expression of several genes, including those targeted by c-Myc. Additionally, we demonstrated that KDM4B physically interacts with c-Myc, and both proteins are co-recruited to the c-Myc-binding sequence on the promoters of metabolic genes. Furthermore, a series of KDM4 inhibitors were synthesized, and their inhibitory effects were evaluated.

Conclusion

KDM4B partners with c-Myc and serves as a coactivator of c-Myc to directly enhance c-Myc-mediated metabolism, hence promoting CRPC progression. Targeting KDM4B is thus an alternative therapeutic strategy for advanced prostate cancers.

EACR25-0820

Urea cycle upregulation is a metabolic adaptation driving liver-specific metastatic organotropism

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Introduction

Metastatic cells exhibit preference for colonizing specific tissues, a phenomenon known as metastatic organotropism. Among the most common metastatic sites, lung and liver are primary targets for disseminated cells, suggesting that these organs offer permissive environments for colonization. However, the metabolic adaptations that allow tumor cells to thrive in these tissues remain unclear.

Material and method

To investigate the metabolic adaptations of metastatic cells in the liver and lung environments, we modified A375 and 4T1 cells to express an OMP25-bound HA tag on their outer mitochondrial membrane. These cells were injected into mice to isolate and immunoprecipitate mitochondria from primary tumors, lung and liver metastases, allowing us to dissect the differential proteome using TMT isobaric labelling. Additionally, to assess the functional impact of mitochondrial genes on metastatic organotropism, we conducted an *in vivo* CRISPR screening with a LOF sgRNA library targeting nuclear-encoded mitochondrial genes in MDA-MB-231 cells.

Result and discussion

Our proteomic analysis revealed significant differences between primary tumor, lung and liver metastases in both A375 and 4T1 models. Notably, we identified an increase in urea cycle-related enzymes as a liver-specific metabolic adaptation. This was further corroborated by the selective depletion of sgRNAs targeting the cycle-limiting enzyme CPS1 in liver metastases in the CRISPR screening. Analysis of CPS1 expression at both the mRNA and protein levels showed a significant liver-specific elevation in metastases across various tumor types in mouse models. This upregulation was consistently observed in public databases of breast and colorectal cancer patients, further validating its potential role in liver metastasis. Functionally, CPS1 KO cells exhibited reduced metastatic capacity to the liver, with no impact on their growth in 2D or 3D culture, primary tumor formation, or metastatic burden in the lungs. Since CPS1 is critical for ammonium detoxification, we confirmed that ammonium levels were significantly higher in the liver compared to tumors, plasma, lung, or brain. The addition of ammonium at physiological levels impaired the proliferation of CPS1-depleted cells, which also exhibited a decrease in mitochondrial respiration when cultured with ammonium. These results suggest that CPS1 deficiency sensitises metastatic cells to the high ammonium concentrations present in the liver.

environment, thus affecting their ability to colonise this tissue.

Conclusion

Our findings reveal that metastatic cells undergo distinct metabolic adaptations depending on the secondary tissue, with CPS1 playing a key role in liver-specific colonization. These insights refine our understanding of metastatic organotropism and postulate CPS1 as a potential therapeutic target to disrupt liver metastasis.

EACR25-0845

Impact of the E2F1-BASP1 complex on regulating MYC expression

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Introduction

The gene regulator MYC controls multiple cellular processes including cell proliferation and differentiation, but MYC is also aberrantly activated in multiple human tumours. For this reason, MYC is considered as major cancer driver representing a promising therapeutic target although direct MYC inhibition has remained difficult. Therefore, indirect strategies targeting the MYC network appears to be a more suitable approach. One of the downregulated transcriptional MYC targets is the BASP1 gene encoding a neuronal signalling protein and a transcriptional corepressor. On the other site, ectopic expression of BASP1 suppresses MYC mRNA expression, rendering MYC and BASP1 mutually exclusive. Furthermore, BASP1 overexpression interferes with MYC-induced cell transformation.

Material and method

To investigate the regulation of MYC transcription in the context of BASP1, we performed a reverse chromatin immunoprecipitation (R-ChIP) screen using the colon cancer cell line SW480, and SW480 with a CRISPR-activated BASP1 gene. Using biotin-labelled oligodeoxynucleotides targeting the MYC promoter region, crosslinked proteins were pulled down using streptavidin-coated magnetic beads. Subsequently, proteins were detected by mass spectrometry (MS). Thereafter, candidate proteins interacting with BASP1 were identified by co-immunoprecipitation (Co-IP), and their influence on MYC transcription was tested by luciferase reporter gene assays.

Result and discussion

The R-ChIP screen identified 541 unique proteins binding to the MYC promoter, and 107 thereof were specific for BASP1-expressing SW480. Among these, we have identified proteins that are known to interact with the cell cycle regulator E2F1. By performing Co-IP experiments, we found that BASP1 and E2F1 form a complex. Whereas E2F1 upregulates MYC transcription in SW480, the presence of BASP1 significantly reduces E2F1-mediated MYC activation.

Conclusion

A physical interaction between the cell cycle promoter E2F1 and the potential tumour suppressor BASP1 was discovered. By further investigating the function of the BASP1-E2F1 complex, we expect to gain more insight into the molecular mechanism of oncogenic MYC

regulation, and to develop novel therapeutic strategies to target oncogenic functions of the gene regulators E2F1 or MYC.

EACR25-0854

Restoration of Drug Sensitivity and Apoptosis to Drug-Resistant Oesophageal Cancer Cells by USP18 Knockdown in the Presence of IFN- α or Poly (I:C)

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Introduction

Oesophageal cancer is challenging to treat, often exhibiting resistance to current treatments (chemo- or radiotherapy). The five-year survival rate for patients is less than 20% across Europe. Thus, new treatment approaches are urgently required. Our group has previously reported differential expression of the ISGylation network in cells that respond differently to drug treatment. ISGylation is a mechanism similar to ubiquitination, where ISG15 is conjugated to targets via the sequential cooperation of E1, E2, and E3 enzymes. Ubiquitin-specific peptidase 18 (USP18), an ISG15-specific enzyme, can remove ISG15, reversing this reaction. Additionally, USP18 is a negative regulator of the interferon (IFN) response. We are examining USP18's role in chemosensitivity in oesophageal cancer cells.

Material and method

Knockdown of USP18 was achieved with siRNA and confirmed by western blot. Response to drug treatments and Poly (I:C) was assessed with colony assays and morphological analysis. Apoptosis was evaluated by quantifying active caspase-3 by flow cytometry. Autophagy was assessed by quantifying autophagosomes with Cyto-ID and flow cytometry. The autophagy marker LC3II and the cell signalling effects of Poly (I:C) were quantified by western blot.

Result and discussion

Knockdown of USP18 in the presence of IFN- α significantly increased the chemosensitivity of KYSE450 and KYSE140 cells to 5-fluorouracil (5-FU) and oxaliplatin. In addition, extensive apoptosis was induced in these cell lines which were previously reported as apoptosis-incompetent. Autophagosome formation and expression of LC3II were also increased in the presence of IFN- α +/- 5-FU or oxaliplatin in both cell lines upon USP18 depletion. These effects may be due to hypersensitivity to IFN- α , or key protein targets that become ISGylated in the absence of USP18 may influence survival pathways. Consistent with its role as a deISGylase, knockdown of USP18 in the presence of IFN- α significantly increased the levels of ISG15 conjugates. We are currently conducting mass spectral analysis to identify and functionally categorise these ISGylated proteins. We also assessed whether stimulating Type I IFN signalling with a viral mimetic, Poly (I:C), could synergise with USP18 depletion as a potential alternative to IFN- α . Knockdown of USP18 in the presence of Poly (I:C) induced apoptosis in the KYSE450

cells but not in the KYSE140 cell line. We are exploring the mechanism behind this differential response to Poly (I:C).

Conclusion

These data identify USP18 as a regulator of two pathways (apoptosis/autophagy) that can have a major impact on drug sensitivity. Ultimately, we aim to identify the mechanisms underlying these effects and establish whether inhibition of USP18, either in the presence of IFN- α or synthetic inducers (e.g. Poly (I:C)), offers a promising new approach to chemo-sensitise oesophageal cancer.

EACR25-0855

PARP12 as a Novel Therapeutic Target in Breast Cancer: Integrating ADP-Ribosylation, DNA Damage Response, and Hormone Therapy Resistance

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Introduction

Breast cancer is the leading cause of death among women under 65 in developed countries, largely due to the emergence of multifactorial drug resistance mechanisms. The PI3K/AKT pathway plays a pivotal role in mediating endocrine resistance, and targeting key steps in this cascade represents a promising strategy to counteract therapy resistance. Mono-ADP-ribosylation (MARylation) is a post-translational modification catalyzed by mono-ADP-ribosyltransferases (mARTs) of the PARP family. This process involves transferring a single ADP-ribose unit from NAD to cellular targets, including proteins and nucleic acids, thereby governing essential cellular functions that are often dysregulated in disease. Notably, the mART PARP12 has been implicated in the development of drug resistance in estrogen receptor-positive breast cancer, marking it as a promising therapeutic target.

Material and method

In our study, we explored the role of PARP12 in breast cancer cell survival and its molecular contribution to resistance acquisition using a combination of biochemical and cell biology-based approaches.

Result and discussion

Our data demonstrate that AKT is a novel substrate for PARP12-mediated MARylation, a modification that is critical for AKT activation and cell survival. Consequently, the loss of PARP12 triggers apoptosis in a subset of estrogen receptor-positive breast cancer cells. We further show that inhibiting PARP12 transcription leads to increased DNA damage, accompanied by enhanced nuclear localization of p53 and a strengthened p53-AKT interaction. Under these conditions, AKT loses its capacity to regulate downstream targets such as the FOXO transcription factors, thereby promoting cell death through the upregulation of FOXO1, which activates the apoptotic cascade. Moreover, our findings link PARP12 activity to the DNA damage response, a connection of particular interest for tumors with compromised DNA repair mechanisms.

Conclusion

Overall, our study uncovers a novel regulatory mechanism of AKT activation and apoptosis driven by PARP12-mediated MARylation, highlighting PARP12 as a potential pharmacological target for overcoming endocrine resistance in breast cancer.

EACR25-0858

Epithelial Protein Lost in Neoplasm (EPLIN) in Head and Neck Cancer: A Prospective Oncogenic Molecule Linked with Prognosis

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Introduction

EPLIN (Epithelial Protein Lost in Neoplasm, also known as LIMA1) was initially identified as a protein reduced or lost in malignant cells and tissues, hence was postulated as a putative tumour and metastasis suppressor. However, recent reports have indicated that its role in cancer is more complex and is tumour type dependent. For example, in colorectal, gastric and kidney cancers, it is tumour suppressive whereas it has a contrast role in other cancer types such as pancreatic cancer. Our study aimed to investigate the expression levels of EPLIN in head and neck cancer (HNC) tissues and its correlation with clinical features and the biological impact on HNC cells.

Material and method

In this study, we analysed the expression levels of EPLIN in HNC tissues using immunohistochemistry and correlated the expression with clinical pathological factors. Additionally, using public databases, we assessed the relationship of EPLIN with patient prognosis, lymph node metastasis, cancer grading and gender. In vitro, we created EPLIN knockdown and overexpression cell models using human HNC cancer cell lines and evaluated the impact of EPLIN expression on a range of biological functions of cancer cells. Immunoprecipitation (IP) and immunofluorescence co-localization (IFC) assays confirmed the direct interaction between proteins.

Result and discussion

Compared with normal tissues, EPLIN expression was upregulated in HNC cancer tissues, where higher levels were associated with advanced cancer stages and poorer clinical outcomes. The upregulation was significantly linked with increased tumour grade ($p = 0.003$), T-stage ($p = 0.007$) and tumour TNM staging ($p = 0.002$). Patients with high levels of EPLIN had a significantly reduced overall survival (OS) ($p < 0.00001$). The in vitro experiments demonstrated that knocking down EPLIN in HNC cells significantly impaired their migration and invasion capabilities, while overexpression of EPLIN enhanced these processes. Furthermore, it was identified that a group of metastasis related molecules including paxillin (PXN), moesin (MSN), actinin-1 (ACTN1) and integrin- α 3 (ITGA3) were significantly correlated with the levels of EPLIN in HNC cancer tissues ($r = 0.68$, 0.66 , 0.66 and 0.65 respectively, $p < 0.001$ for all). A genetic signature comprised of EPLIN-Paxillin-Actinin1

had a marked increased pattern in head and neck cancer ($p = 0.00353$). The co-IP assays further confirmed that EPLIN directly interacts with PNX and ACTN1.

Conclusion

The present study is the first to report the potential oncogenic role of EPLIN in head and neck cancer, demonstrating EPLIN as an indicator of poor survival. Additionally, we are the first to propose the potential connection of the EPLIN-Paxillin-Actinin1 network, which may play a role in the tumorigenesis of head and neck cancer. Thus, EPLIN holds significant clinical and therapeutic value, constituting a promising therapeutic target.

EACR25-0864

Discovering Potential Anti-Oral Squamous Cell Carcinoma Mechanisms from Kochiae Fructus Using Network-Based Pharmacology Analysis and Experimental Validation

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Introduction

The natural product Kochiae Fructus (KF) is the ripe fruit of Kochia scoparia (L.) Schrad and is renowned for its anti-inflammatory, anticancer, anti-fungal, and anti-pruritic effects. This study examined the anticancer effect of components of KF to assess its potential as an adjuvant for cancer treatment. Network-based pharmacological and docking analyses of KF found associations with oral squamous cell carcinoma.

Material and method

The molecular docking of oleanolic acid (OA) with LC3 and SQSTM1 had high binding scores, and hydrogen binding with amino acids of the receptors suggests that OA is involved in autophagy, rather than the apoptosis pathway. For experimental validation, we exposed SCC-15 squamous carcinoma cells derived from a human tongue lesion to KF extract (KFE), OA, and cisplatin.

Result and discussion

The KFE caused SCC-15 cell death, and induced an accumulation of the autophagy marker proteins LC3 and p62/SQSTM1. The novelty of this study lies in the discovery that the change in autophagy protein levels can be related to the regulatory death of SCC-15 cells.

Conclusion

These findings suggest that KF is a promising candidate for future studies to provide insight into the role of autophagy in cancer cells and advance our understanding of cancer prevention and treatment.

EACR25-0869

Investigating the Cell of Origin in Merkel Cell Carcinoma Using a Human Pluripotent Stem Cell-derived Model

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Introduction

Merkel cell carcinoma (MCC) is a rare yet highly aggressive neuroendocrine skin cancer with a mortality rate of approximately one in three affected individuals. MCC derives its name from Merkel cells (MCs) since they share histological and morphological similarities. MCs are neuroendocrine cells that closely interact with sensory neurons and regulate touch and tactile sensations. MCC research is limited by the rarity and post-mitotic nature of MCs, making isolation and mechanistic studies challenging. We are establishing the first differentiation protocol from human pluripotent stem cells (hPSCs) to generate human MCs to shed light on their biology in health and cancer. Apart from MCs, other cells of epidermal origin, such as epidermal stem cells, keratinocytes, pre-B cells, or even fibroblasts, have been proposed as plausible cells of origin (COO). Generating COO candidates with the same genetic background under controlled conditions will allow the comparison of their oncogenic potential and the timing of oncogenic transformation, offering crucial insights into the earliest events in MCC development.

Material and method

Our stepwise differentiation protocol allows us to differentiate hPSCs into Merkel cell lineage, passing through a stage of epidermal stem cells. We confirm MC identity by analyzing marker gene expression and evaluating mechanosensory function through interaction studies with hPSC-derived sensory neurons. The transcriptomic profiles of all COOs generated from hPSCs are validated using bulk RNA sequencing. To model oncogenic transformation, the hPSC line is genetically modified using CRISPR/Cas9 to introduce a doxycycline-inducible TP53 and RB1 double knockout.

Result and discussion

Preliminary results show that our protocol can direct hPSCs to cells resembling MCs. Indeed, around day 50 of differentiation, we observed the expression of specific markers (ATOH1, K8, K20, ISL1; which uniquely identifies MCs in the epidermis) through immunofluorescence and qPCR. Quantification of marker expression revealed ~65–70% efficiency. Electron microscopy confirmed MC morphology, desmosomes, and microvilli. We also generated other prospective COOs, including epidermal stem cells, keratinocytes, and fibroblasts of the same genetic lineages. Future experiments will introduce MCC-specific mutations to assess oncogenic competence across various epidermal cell types.

Conclusion

To our knowledge, we have developed the first protocol to differentiate MCs from hPSCs. This technical advantage will allow us to study physiological and pathological events that occur in humans, including the interaction of MCs with sensory neurons in the context of touch and pain responses, the timing of oncogenic

competence acquisition in MCC, and how different putative COOs respond to MCC-specific mutations, offering insights into disease progression.

EACR25-0870

Therapy-induced senescence of bone-marrow mesenchymal stem cells contribute to increased cell invasiveness, stemness, vascular mimicry and macrophages recruitment in TNBC by inducing PD-L1 overexpression

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Introduction

Tumor microenvironment (TME) is a complex ecosystem into which every component plays a precise role in fostering tumor establishment, growth and metastatic spread. Decades of research has proven the crucial involvement of stromal components of the TME in promoting tumour aggressiveness causing increased tumor cell migration, invasiveness, stemness as well as altered immune checkpoint expression as PD-L1, with an overall pro-tumorigenic role that contributes to confer resistance to standard treatments. Moreover, during chemo-radiotherapy, a common off-target effect is represented by Therapy-Induced-Senescence (TIS) of both stromal and tumor components of the TME that, not killed, acquire a senescent phenotype remaining metabolically active. By secreting the Senescence Associated Secretory Phenotype (SASP), senescent cells could further contribute to chemoresistance, immuno-checkpoint blockade and metastatic outspread. In this framework, the role of Doxorubicin (Doxo) in determining a senescent phenotype in human bone-marrow mesenchymal stem cells (BM-MSCs) and the consequent impact on triple-negative breast cancer (TNBC) cell aggressiveness including the recruitment of macrophages is investigated.

Material and method

BM-MSCs were treated with Doxo (10 nM) for 72 h to induce a senescent phenotype and then cultured in complete medium for 48 h. Doxo-induced alterations in BM-MSC morphology, cell cycle and proliferation were investigated. At this time, expression of senescence-associated markers (e.g. β-Galactosidase, Lamin B1) was evaluated by western-blot. TNBC cell lines, BT-549 and MDA-MB231, were grown in the conditioned medium obtained from senescent BM-MSC cells (CM-S-MSC) for 48h and investigated for acquired stemness characteristics (e.g. CD44 expression and spheroid formation), increased migration and matrigel invasion, EMT marker expression, PD-L1 overexpression and vascular mimicry. Furthermore, CM-S-MSCs impact on recruitment of activated macrophages labeled with NIR-probe was evaluated in trans-well chambers (3 μm insert).

Result and discussion

The treatment of BM-MSCs with Doxo caused an increase of β-Galactosidase expression levels and cytosolic deposition as well as a decrease of Lamin B1 expression, known markers of senescence. TNBC cells grown in CM-S-MSC showed increased PD-L1 expression levels, increased migration and matrigel invasion, an enhancement in stemness characteristics as shown by higher spheroid number and size as well as CD44 expression levels and involvement of EMT program. Furthermore, senescent BM-MSCs promoted the activation and recruitment of macrophages.

Conclusion

Our results shed light on the detrimental role played by chemotherapy-induced senescent BM-MSCs in contributing to TNBC aggressiveness.

EACR25-0873

Aldehyde metabolism as a target for metastatic melanoma

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Introduction

Metastasis, the primary cause of mortality in cancer patients, is particularly fatal in melanoma, which is responsible for over 70% of skin cancer-related deaths. Despite its prevalence, the molecular and metabolic mechanisms driving metastasis are not well understood. Hence, our goal is to unravel the mitochondrial metabolic factors that underline the metastatic potential of melanoma cells.

Material and method

We have optimized a metastatic melanoma model, where SK-Mel-147 human melanoma cell line, containing EGFP and luciferase are injected either subcutaneously or via tail vein into immunodeficient NSG mice. Using this model, Mel-147-GFP-LUC cells with HA-tagged mitochondria (3XHA-EGFP-OMP25 gen) primary tumors and metastases were generated. At the end of the experiment, mice were sacrificed, and primary tumors and lungs were dissected for examination of metastases (GFP+) under a fluorescence stereoscope. Then, samples were subjected to immunoprecipitation using anti-HA magnetic beads to immunocapture tagged mitochondria exclusively from human melanoma cells. After elution, mitochondrial samples were analyzed by quantitative proteomics (tandem mass tags (TMT)). Then, a curated list of candidates was generated, in the form of mitochondrial proteins. To validate our hits, we are deleting (by CRISPR/Cas9 technique) and/or over-expressing the genes corresponding to our candidates in different

melanoma cell lines. Later we have assessed the metastatic potential both in vitro (proliferation assays, migration assays...), and in vivo.

Result and discussion

Using our innovative platform, we mapped dynamic changes in the mitochondrial proteome throughout tumor progression and metastasis. Our analysis revealed a significant upregulation of aldehyde dehydrogenase 2 (ALDH2) in lung metastases compared to primary tumors and cultured cells. This heightened ALDH2 expression in metastatic cells was directly driven by the lung microenvironment. To assess its functional relevance, we engineered ALDH2 knock-out (KO) cells, revealing a marked reduction in their metastatic potential while primary tumor growth remained unaffected. This impairment may be related from the inability to detoxify reactive aldehyde species, which are particularly abundant in these organs, thereby compromising the cellular fitness of ALDH2 KO cells. Furthermore, in vivo transcriptomic analysis revealed altered immune-related gene expression profiles in ALDH2 KO cells, an effect absent in vitro. These findings underscore the critical role of the lung microenvironment in regulating ALDH2 expression and shaping immune interactions during metastatic progression.

Conclusion

Together, our findings shed light on the molecular and metabolic adaptations that metastasizing cells establish to overcome natural barriers to organ colonization. These insights reveal potential vulnerabilities that could be exploited for therapeutic intervention.

EACR25-0874

Aged-dependent metabolic reprogramming of microglia as a determinant factor in the development and progression of brain metastases

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Introduction

Brain metastasis occurs when cancer cells spread to the brain, where the immunosuppressive environment and unique metabolic demands complicate treatment. Aging exacerbates these challenges by altering the tumor microenvironment, affecting nutrient availability, inflammation, and immune function. These age-related changes can influence metastatic progression, highlighting aging as a critical but often overlooked factor in brain metastasis. A fundamental question to address is how aging impacts the metabolic fitness of immune cells and how these changes negatively affect the progression of brain metastasis. We hypothesize that the decline in immune activity during aging is caused by dysregulated metabolism. Therefore, restoring the metabolic fitness of immune cells would be highly beneficial in enhancing their ability to suppress brain metastasis.

Material and method

To further investigate this, we developed a model featuring specific ablation of mitochondrial complex III in microglia by crossing Uqcrc floxed and Tmem119-

cre/ERT2 mice (referred to as QPC KO). This model demonstrated that mice with ETC-deficient microglia developed larger brain metastases. We propose to utilize this newly generated mouse model, which mimics the effects of aging through deficient mitochondrial metabolism in microglia, to examine the role of mitochondrial metabolism in the anti-tumor function of microglia. Our aim is to elucidate how and why these processes are impaired with age.

Result and discussion

Our preliminary data, obtained from RNA-seq analysis of tumor-associated and resting microglia in young and old mice, revealed significant differences in microglial activation between the two age groups. Notably, pathways related to mitochondrial oxidative metabolism were particularly impaired with age.

Conclusion

These findings suggest that mitochondrial metabolism is crucial for microglial activation and anti-tumor function, while aging hinders these adaptive mechanisms.

EACR25-0875

The TMBIM4 Golgi Apparatus Ion Channel Supports Cancer Cell Survival by Altering Cysteine Availability

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Introduction

Accumulated evidence implicates the Golgi apparatus (GA) in processes associated with several cancer hallmarks. The Transmembrane BAX Inhibitor Motif Containing 4 (TMBIM4) is a GA-localized calcium ion channel that regulates intracellular Ca^{2+} dynamics, inhibits apoptosis, and promotes cell motility. Although cancer cells can survive and proliferate in adverse conditions, such as nutrient-deficient environments, the contribution of the GA to this process is unclear. Here, we present a novel GA-dependent process driving cancer cell survival under starvation conditions.

Material and method

The impact of TMBIM4 on cell survival was assessed in invasive osteosarcoma U2-OS cells genetically modified to overexpress either the wild-type TMBIM4 or a TMBIM4-null mutant. To mimic a nutrient-deprived environment typical of many tumours, cells were maintained in culture at high confluence for prolonged periods without media replacement. Flow cytometry was used to evaluate intracellular reactive oxygen species levels, cell survival, death, and proliferation, while oxidative damage and lipid peroxidation were analysed via immunofluorescence. A genetically encoded biosensor was employed to measure the intracellular reduced/oxidised glutathione (GSH/GSSG) ratio.

Proteomic analysis under normal and prolonged culture conditions were conducted using untargeted mass spectrometry.

Result and discussion

In starvation conditions, TMBIM4 overexpression strongly enhanced cell survival. This effect was pH-dependent, occurring only at pH 7.4–7.8, aligning with the proposed pH-dependent gating mechanism of TMBIM family proteins. TMBIM4 overexpressing cells presented less lipid peroxidation and DNA damage despite having higher levels of ROS and maintained a higher GSH/GSSG ratio even in starvation conditions. Cells with higher levels of TMBIM4 displayed increased levels of the two key transsulfuration enzymes. This suggests a role for de novo synthesis of cysteine in this process, and possibly of GSH, since its synthesis is highly dependent on the availability of this amino acid. Supplementation of culture media with N-acetylcysteine or GSH ethyl ester enhanced long-term cell survival in starvation conditions. This supports the importance of cysteine and GSH availability in TMBIM4-mediated protection from cell death and provides a potential mechanism for the observed resistance to oxidative.

Conclusion

Our findings demonstrate that the GA plays a role in cancer cell metabolic adaptation, allowing cancer cells to survive even in starvation conditions. These results also highlight the importance of cysteine availability for cancer cell survival and suggest TMBIM4 and the GA as regulators of cysteine and GSH synthesis.

Acknowledgments: FCT (UID/DTP/04567/2016, UIDB/04567/2020, UIDP/04567/2020, UI/BD/151424/2021), and COST (CA16112-42814, CA18133-48426).

EACR25-0877

Role of the collagen hydroxylase LEPRE-1 in breast cancer

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Introduction

Oncogenic enzymes upregulated in human cancers represent potential therapeutic targets. In this context, we investigated LEPRE-1, a prolyl hydroxylase whose deficiency causes Osteogenesis Imperfecta, a genetic disorder leading to bone malformations due to defective collagen hydroxylation. Our analyses reveal that LEPRE-1 is overexpressed across all breast cancer subtypes, particularly in triple-negative breast cancer (TNBC) cells as well as in MCF7 cells undergoing epithelial-to-mesenchymal transition (EMT), a key process in metastatic progression.

Material and method

LEPRE-1 expression was analyzed at both RNA and protein levels in cellular and murine models of breast cancer. We examined its expression through immunofluorescence and FACS analysis, especially in all epithelial subtypes of the murine mammary gland. Additionally, the correlation between LEPRE-1 and E-

Cadherin expression was assessed by western blot in different murine breast cancer cell lines to investigate its potential involvement in EMT. To assess the functional role of LEPRE-1 in metastatic progression, we took advantage of the highly metastatic 4T1 TNBC cell line. LEPRE-1 knockdown was performed using shRNA, followed by injection into a syngeneic mouse model to assess lung metastasis formation.

Result and discussion

Our findings show that LEPRE-1 as well as the LEPRE-1-associated protein CRTAP are enriched in basal epithelial cells of the murine mammary gland. Moreover, LEPRE-1 expression inversely correlates with E-Cadherin levels, suggesting its direct involvement in EMT. Finally, LEPRE-1 depletion in 4T1 cells significantly reduces their ability to form colonies in vitro and lung metastases in mice, highlighting its critical role in metastatic progression.

Conclusion

Our study identifies LEPRE-1 as a candidate enriched in basal cells and acting as a driver of breast cancer metastasis, potentially by promoting EMT. These findings pave the way for further investigations into molecular mechanisms through which LEPRE-1 promotes tumor progression. These studies may define LEPRE-1 as a therapeutic target to fight metastatic cancers.

EACR25-0885

Small Extracellular Vesicle-Driven Ferroptosis: A Novel Strategy to Target Therapy-Resistant Ovarian Cancer

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Introduction

Epithelial ovarian cancer (EOC) is the deadliest gynecological cancer, largely due to the difficulty of early diagnosis and the eventual development of resistance to treatment. Ovarian cancer cells (OVCA) exhibit altered iron metabolism, making them susceptible to ferroptosis – a form of non-apoptotic cell death triggered by high levels of free iron. Small extracellular vesicles (sEV) play a key role in tumor communication, potentially influencing ferroptotic signaling in the tumor microenvironment (TME). However, the ability of ferroptotic OVCA-derived EV to propagate ferroptosis in a paracrine manner remains unexplored.

Material and method

To investigate ferroptosis propagation via sEV, OVCA were treated with ferroptotic modulators, and the ferroptosis phenotype was confirmed by assessing key ferroptosis markers. sEV were isolated from ferroptotic OVCA, characterized, and used to treat naïve OVCA to evaluate their ability to induce ferroptosis. Furthermore, sEV from these newly ferroptotic OVCA were collected and applied to another round of naïve OVCA to determine whether the process could be propagated further.

Result and discussion

sEV derived from ferroptotic OVCA successfully triggered ferroptosis in non-ferroptotic OVCA. Additionally, the secondary ferroptotic OVCA released sEV that continued to propagate ferroptosis. Notably, these sEV exhibited increased Fe²⁺ levels, suggesting a key role in the transmission of ferroptotic signaling.

Conclusion

This study provides evidence that ferroptotic OVCA-derived sEV can mediate the paracrine propagation of ferroptosis, potentially offering a novel therapeutic strategy for overcoming treatment-resistant ovarian cancer. Future research will focus on elucidating the molecular mechanisms underlying this process to explore its potential in clinical applications.

EACR25-0896

Molecular and Epigenetic Signatures of Senescence: Investigating Clonal Expansion and Chromatin Remodeling in HUVECs

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Introduction

Cellular senescence is a stable state of proliferation arrest that plays a dual role in aging and tumor suppression. While it prevents uncontrolled cell proliferation, it also contributes to vascular dysfunction and disease progression. Endothelial cell senescence, in particular, remains poorly understood, and its molecular drivers require further investigation. This study aims to characterize the transcriptomic and epigenetic changes associated with senescence in human umbilical vein endothelial cells (HUVECs) over multiple passages and identify pre-existing subpopulations predisposed to senescence.

Material and method

HUVECs were continuously passaged to induce replicative senescence. Barcode lineage tracing was performed to track clonal expansion over time. Morphological assessment, senescence-associated β-galactosidase (SA-β-gal) staining, and proliferation assays were conducted to confirm senescent phenotypes. Transcriptomic profiling (NanoString analysis) was used to identify gene expression changes in pathways related to cell cycle regulation, chromatin remodeling, and inflammation. Immunofluorescence staining and mass spectrometry were performed to assess epigenetic modifications, including H3K79 and H4K20 methylation, associated with senescence progression.

Result and discussion

Prolonged passaging led to morphological changes, increased SA-β-gal staining, and reduced proliferation, confirming the induction of senescence. Gene expression analysis revealed a significant downregulation of HMGB1, a chromatin-associated protein involved in DNA repair and inflammatory signaling, suggesting its role in senescence progression. Furthermore, genes associated with cell cycle arrest, innate immune signaling, and chromatin remodeling exhibited

differential regulation, reinforcing the hypothesis that endothelial senescence follows a distinct molecular trajectory. The observed epigenetic modifications, particularly in H3K79 and H4K20 methylation, suggest chromatin restructuring as a hallmark of deepening senescence. While barcode lineage tracing suggests clonal expansion, further validation is required to determine whether senescence-prone subpopulations pre-exist or emerge over time.

Conclusion

Our findings provide insights into molecular and epigenetic changes in endothelial senescence, with potential implications for vascular aging and disease progression. Future studies will investigate parallels with breast cancer dormancy, aiming to uncover shared regulatory mechanisms that may influence tumor dormancy and therapeutic resistance.

EACR25-0936

Targeting CKAP2 to boost Chemotherapy Efficacy and alleviate immunosuppressive environment in non-small cell lung cancer

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Introduction

Lung adenocarcinoma (LUAD) is a leading cause of cancer-related mortality worldwide. Recent advances in multiomics and artificial intelligence have identified previously elusive molecular pathways of clinical significance. Cytoskeleton-Associated Protein 2 (CKAP2), a cytoplasmic, microtubule-associated protein regulating cell division and migration, has been linked to multiple cancers but remains poorly understood in LUAD. This study aims to elucidate the function, regulation, and therapeutic potential of CKAP2 in LUAD.

Material and method

Publicly available LUAD datasets were mined to identify genes associated with poor patient survival, and immunohistochemistry (IHC) was used to assess CKAP2 and LRRK2 expression in human lung cancer tissues. In vitro experiments employed CKAP2 siRNA or miR-497-3p mimics to evaluate cell viability (MTT), spheroid formation (high-content imaging), and invasiveness (Matrigel-coated transwells). Flow cytometry measured cytotoxicity and lipid peroxidation, while total RNA sequencing characterized transcriptomic alterations following CKAP2 depletion. A high-throughput virtual screening (HTVS) approach identified small-molecule CKAP2 inhibitors, validated via cellular thermal shift assays. In vivo tumorigenicity and metastasis were evaluated in subcutaneous and tail-vein LUAD models, with platinum-based chemotherapy. Finally, bio-electrical profiling and artificial intelligence (AI) modeling assessed the preclinical safety of these novel CKAP2 inhibitors.

Result and discussion

Through integrative bioinformatic analyses, CKAP2 emerged as a top biomarker of poor prognosis in LUAD. CKAP2 knockdown reduced LUAD cell viability and invasiveness, markedly increasing sensitivity to oxidative

stress and platinum-based agents. Transcriptomic profiling and mechanistic studies revealed that CKAP2 inhibition suppresses LRRK2/NRF2/HMOX1 signaling, thereby enhancing cytotoxicity. High-throughput virtual screening (HTVS) identified BFN2 as a novel CKAP2 inhibitor, which decreased tumor cell proliferation and invasion *in vitro*, while synergistically enhancing platinum-based chemotherapy in both subcutaneous and metastatic mouse models. Additionally, BFN2 reduced MDSC infiltration *in vivo*, suggesting an immunomodulatory effect. Finally, bio-electrical profiling and AI modeling confirmed a favorable safety and tolerability profile for BFN2.

Conclusion

Our findings underscore the pivotal role of CKAP2 in driving LUAD progression, chemoresistance, and immune evasion. By targeting CKAP2 with BFN2, we effectively inhibit tumor growth and metastasis, enhance the cytotoxic effects of conventional chemotherapy, and favorably modulate the tumor microenvironment through reduced MDSC infiltration. These results establish CKAP2 as a promising therapeutic target in LUAD and support BFN2 as a clinically safe, effective strategy for improving patient outcomes.

EACR25-0946

Regulation of Cancer Stemness by Hypoxia-Induced Metabolic Shifts and BNIP3-Driven Mitophagy in OECM-1 CSCs

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Introduction

Mitochondrial metabolism plays a critical role in cancer cell adaptation to hypoxia, influencing cancer stem cell (CSC) properties. Mitochondrial dynamics, including fission and fusion, regulate cellular energetics and may contribute to cancer progression. Mitophagy, a key mitochondrial quality control process, removes damaged mitochondria and protein aggregates via lysosomes, maintaining homeostasis and limiting oxidative stress. This study investigates how hypoxia-induced metabolic reprogramming and BNIP3-mediated mitophagy regulate CSC features in oral cancer OECM-1 cells.

Material and method

OECM-1 CSCs and non-CSC OECM-1 cells were exposed to hypoxia for 16 hours. Mitochondrial function was assessed using a Seahorse XF analyzer to measure oxygen consumption rate (OCR) and extracellular acidification rate (ECAR). BNIP3-driven mitophagy was examined via lentiviral-mediated siBNIP3 transfection, followed by analysis of BNIP3 expression, autophagy markers (LC3), mitochondrial respiration, and reactive oxygen species (ROS) levels.

Result and discussion

Hypoxia significantly increased basal and maximal OCR, as well as ATP production, in OECM-1 CSCs compared to non-CSC OECM-1 cells, indicating enhanced oxidative phosphorylation (OXPHOS) metabolism. ECAR was mildly elevated, suggesting a limited glycolytic contribution. Knockdown of BNIP3 in OECM-

1 CSCs reduced BNIP3 expression, suppressed autophagy, and impaired mitochondrial respiration, leading to increased mitochondrial ROS accumulation. These findings suggest that BNIP3-driven mitophagy supports mitochondrial metabolic adaptation in hypoxia-treated CSCs.

Conclusion

Hypoxia-induced mitochondrial metabolic reprogramming and BNIP3-mediated mitophagy are key regulators of CSC properties in OECM-1 cells. Targeting these pathways may offer potential therapeutic strategies for oral cancer by disrupting the metabolic adaptability of CSCs under hypoxic conditions.

EACR25-0947

Triple-negative breast cancer cell survival strategies under acute and chronic hypoxia

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Introduction

Triple-negative breast cancer (TNBC) is a particularly notable breast cancer subtype due to its aggressive, heterogeneous, and refractory nature, along with limited treatment options, posing a significant challenge to clinical management. One of the hallmarks of many cancers, including TNBC, is resistance to apoptosis, which can occur through the downregulation or inhibition of pro-apoptotic proteins or the upregulation of anti-apoptotic proteins. Hypoxia, a common feature of solid tumours, has been linked to increased invasiveness, malignant progression, and death evasion mechanisms that contribute to therapy resistance. The severity and duration of hypoxia define the response and, thus, the subsequent signalling mechanisms initiated by the cancer cells. Here, we explore the induction of apoptotic priming and apoptotic dependencies in TNBC cell lines under physoxia, acute and chronic hypoxia.

Material and method

Apoptotic priming is defined as the threshold at which a cell can commit to apoptosis. The baseline BH3 (bBH3) profiling assay, utilising BH3-only sensitizer peptides, determines whether a cell relies on one or more anti-apoptotic Bcl-2 family proteins for survival by targeting and inhibiting specific anti-apoptotic proteins. Apoptotic priming and anti-apoptotic protein dependencies in TNBC cell lines were assessed using image-based bBH3 profiling under physoxia (8% O₂), acute (2% O₂, 24h) and chronic (2% O₂, 72h) hypoxia.

Result and discussion

Preliminary findings indicate that each TNBC cell line exhibits unique dependencies on anti-apoptotic proteins, highlighting heterogeneity and underlining the diverse survival strategies that cells employ. Assessment of these dependencies could provide a foundation for developing more tailored strategies to target TNBC cells.

Conclusion

Baseline BH3 profiling enables the identification of apoptotic dependencies in TNBC cells that could be targeted through the application of BH3 mimetics as a combination therapy.

EACR25-0965**Metabolic rewiring in the therapy-induced secretome facilitate glioblastoma resistance to radiotherapy**J. Poh¹, H. Hoi¹, C. Koh¹¹Nanyang Technological University, School of Biological Sciences, Singapore, Singapore**Introduction**

The heterogeneous nature of glioblastoma (GBM) contributes to its poor prognosis and high recurrence rates. Adaptive radioresistance, driven by complex intratumoral communication, plays a critical role in treatment failure. Following radiotherapy, therapy-stressed cells secrete factors that shape the tumor microenvironment, thereby influencing therapeutic response. However, the molecular composition and functional consequences of these secretions remains poorly understood, particularly with regards to GBM radioresistance. In this study, we aimed to characterize the molecular composition of the therapy-induced secretome, providing insight into potential vulnerabilities that can be exploited for improved therapeutic intervention.

Material and method

Proteomic profiling was performed on conditioned media from irradiated GBM cells of distinct tumor subtypes using LC-MS/MS. Bioinformatics and proteomic enrichment analysis was conducted to identify the key enriched biological processes pertaining to the therapy-induced secretome. Functional assays then examined the impact of these secretions on tumor subtype response to radiotherapy.

Result and discussion

Proteomic analysis of the therapy-induced secretome revealed a metabolic shift in the mesenchymal GBM subtype. The apparent enrichment of metabolic enzymes and intermediates associated with glycolysis, pentose phosphate pathway, and nucleotide biosynthesis reflect metabolic rewiring post-radiotherapy, which are key adaptive responses that can play pivotal roles in shaping therapeutic response. Notably, some of the identified glycolytic enzymes have extracellular functions that can promote tumor proliferation and DNA repair, providing survival advantages post-radiotherapy. Furthermore, the upregulation of nucleotide biosynthesis processes is a crucial factor for DNA repair following radiotherapy-induced DNA damage. The pentose phosphate pathway also emerged as a critical metabolic node, balancing glycolytic flux and nucleotide production. These findings collectively suggest that metabolic rewiring within the therapy-induced secretome sustains both energy demands and DNA repair capacity, ultimately enhancing resistance to radiotherapy.

Conclusion

This study highlights the role of metabolic adaptations that reinforce radiotherapeutic resistance. The secretion of metabolic enzymes and intermediates not only sustains tumor cell survival under stress but also modulates the metabolic landscape to promote adaptive resistance. These findings emphasize the need for integrative treatment strategies targeting both metabolic and pro-survival pathways to overcome tumor resistance in GBM.

EACR25-0981**Inhibition of arginase-2 differentially affects cell proliferation in breast cancer cell lines representing different clinical subtypes in vitro**A. Röglind¹, P. Zoroofchi¹, F. Kleinsang¹, V. Osterhage¹, R. Böger¹, J. Hannemann¹¹Institute of Clinical Pharmacology and Toxicology, University Medical Center Hamburg-Eppendorf, Hamburg, Germany**Introduction**

Arginases (ARGs) are enzymes that convert L-arginine into L-ornithine. ARG1 forms part of the urea cycle, whilst the mitochondrial ARG2 is part of a pathway leading to synthesis of polyamines, which are involved in the regulation of cell proliferation. The relationship between ARGs and cell proliferation in the different subtypes of breast cancer has not yet been clarified. Therefore, we aimed to study the effect of ARG inhibition on cell proliferation in breast cancer cell lines representing different clinical subtypes.

Material and method

Five breast cancer cell lines (MCF-7, BT-474, SK-BR-3, MDA-MB-468, MDA-MB-231), representing hormone receptor-positive (HR+), HER2-positive and triple-negative breast cancer, were compared to MCF-12A cells representing normal breast epithelial cells. Pharmacological inhibition of ARG was performed with 1 mM 2-(S)-amino-6-boronohexanoic acid (ABH) for 72 h in medium containing a physiological L-arginine concentration (100 µM). For siRNA-mediated knockdown of ARG2 mRNA expression, cells were transiently transfected with a specific siRNA and harvested after 72 hours. To confirm success of inhibition and knockdown, respectively, qRT-PCR, Western Blotting, and a photometric enzyme activity assay were used. Proliferation was analysed by a) assessment of cell counts, b) mitotic index in H&E-stained cytopsins, and c) immunofluorescent staining of Ki-67 antigen.

Result and discussion

Baseline ARG2 expression and ARG activity greatly varied between cell lines; ARG1 was not expressed in any of the cell lines. ARG2 knockdown significantly reduced ARG2 mRNA and protein expression in all cell lines. However, in MCF-7 and BT-474 cells, ARG2 knockdown did not significantly reduce cell count, mitotic index, and KI-67 staining. By contrast, ARG2 knockdown caused reductions in mitotic index (-16% and -31%, respectively) and KI-67 staining (-72% and -33%, respectively) in both triple-negative cell lines, MDA-MB-468 and MDA-MB-231, which was consistent with MCF-12A cells. Pharmacological inhibition of ARG confirmed reduction in cell proliferation in triple-negative cell lines (mitotic index, MDA-MB-468: -47%; MDA-MB-231: -30%; both $p < 0.05$).

Conclusion

Our results show a differential pattern in the response of breast cancer cell lines to ARG2 knockdown. The HR+ cell lines MCF-7 and BT-474 showed unchanged or increased proliferation and the HER2-positive cell line SK-BR-3 showed variable results, whereas in the triple

negative cell lines proliferation was significantly and consistently reduced; this was comparable to MCF-12A cells. Thus, arginase inhibition may be an additional treatment option in triple-negative breast cancer, but it might be futile in HER2-positive and detrimental in HR+ breast cancer subtypes.

EACR25-0999

Macrophages stimulate glioblastoma motility and invasion through paracrine signaling leading to the regulation of Rho GTPases

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Introduction

Glioblastoma multiforme (GBM) is one of the most common and deadliest primary malignant cancers of the central nervous system. The main challenge in treating GBM remains their high ability to infiltrate healthy brain tissues. GBM growth and invasion can be modulated by communication between tumor cells and their microenvironment. Microglia and infiltrating macrophages constitute up to one third of GBM tumors. We thus investigated the mechanisms by which macrophages stimulate GBM cell invasion.

Material and method

U-87 MG and T98G astrocytoma cells were cultured with or without activated M1-type THP-1 macrophages or with activated THP-1 conditioned media (CM). Astrocytoma cell migration was measured through live random cell migration assays. Cells were also stained with Rhodamin Phalloidin to observe actin structures. We also examined the activation status of the actin regulators RhoA and Cdc42 via pull down assay and using Förster resonance energy transfer (FRET) biosensors to image the localization of their activation.

Result and discussion

We first examined the effects of activated M1-type THP-1 macrophages paracrine signaling on cell motility and adhesion of U-87 MG and T98G cancer cells. Time lapse analysis results showed that co-culturing cancer cells with activated THP-1 led to a significant increase in 2D cell motility (40%). We also observed a decrease in adhesion to collagen in astrocytoma cells treated with activated THP-1 conditioned media (CM), as compared to the control. Interestingly, the CM induced morphological changes in both cancer cell lines including the formation of membrane protrusions and membrane ruffles as well as an increase in cell-cell connecting tunnel-like structures. In addition, cells co-cultured with the activated THP-1 cells showed an increase in invasion as well as an increase in invadopodia structures that stained for TKS-4 and TKS-5 in immunostaining. Further analysis by pull down assay and using FRET biosensors showed an increase in the activation of Cdc42 and RhoA in the GBM cancer cells in response to treatment with CM.

Conclusion

Altogether the data indicate that M1-type activated macrophages stimulate GBM motility and invasion in a paracrine manner by upregulating key structures involved

in cell motility and invasion. Further work is still warranted to determine the molecular targets implicated in the macrophages-GBM paracrine signaling.

EACR25-1006

Colon cancer cells metabolic plasticity: oxidative metabolism helps cancer cells to survive under glucose-deprivation

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Introduction

Colon cancer cells exhibit metabolic plasticity, surviving glucose deprivation by shifting to oxidative metabolism, but the mechanisms underlying this adaptation remain poorly understood. This study aims to elucidate the metabolic adaptations facilitating the survival and proliferation of colon cancer cells under glucose-deprived conditions, with a particular focus on the transition to a glucose-deprivation resistant (GDR) state and the role of oxidative metabolism.

Material and method

To induce metabolic stress the colon cancer cells were cultured under glucose-deprived conditions. Metabolic adaptations were analyzed using Seahorse XF approach to measure glycolytic and oxidative phosphorylation (OXPHOS) activities. LC-MS based metabolomics was employed to profile tricarboxylic acid (TCA) cycle and one-carbon metabolism related metabolites.

Result and discussion

We found that glucose deprivation induced cell death and adaptive dormancy in selected colon cancer cells. Within this dormancy, the survived cells shifted their metabolism from glycolysis to OXPHOS. Lactate levels transiently increased during dormancy were significantly decreased when proliferation resumed in the GDR state. LC-MS metabolomics revealed significant activation of the TCA cycle, underscoring enhanced dependence on OXPHOS for ATP production. An increased ROS levels found in dormant cells, accompanied by massive DNA damage were eliminated in GDR cells through enhanced antioxidant defenses and upregulated DNA repair mechanisms, facilitating sustained proliferation with reduced apoptosis. Furthermore, the oxidative stress tolerance and glycolysis-independent proliferative capacity of GDR cells was confirmed by hydrogen peroxide (H₂O₂) and 2-Deoxy-D-Glucose (2-DG) treatment respectively. The acceleration of the one-carbon metabolism supporting redox homeostasis and associated with elevated levels of metabolites including methionine, folate, glutamine, pyridoxine, glycine, fumarate, cystine, and taurine was also observed.

Conclusion

This study reveals the remarkable metabolic flexibility of colon cancer cells in response to glucose deprivation. The transition to the GDR cells to OXPHOS along with the activation of one-carbon metabolism, supports energy production and redox balance. These adaptations enable the GDR cells to withstand oxidative stress through robust antioxidant systems and efficient DNA repair, highlighting potential vulnerabilities for therapeutic intervention. We believe that targeting these adaptive mechanisms may help overcome glucose deprivation resistance in colon cancer.

EACR25-1007**PD-L1 expression is essential for EGFR signaling- and fatty acid-enhanced metastasis in head and neck cancer***J. Chiou¹, W. Chang¹, B. Chen²*¹*Taipei Medical University, Graduate Institute of Medical Science, College of Medicine, Taipei, Taiwan*²*National Cheng Kung University, Department of Pharmacology, College of Medicine, Tainan, Taiwan***Introduction**

Recent research has illuminated the significant role of tumor-intrinsic programmed cell death ligand 1 (PD-L1) regulates tumor progression independently of the immune system. For immunotherapy and targeted therapy, PD-L1 expression is related to the tumor response and overall survival of head and neck squamous cell carcinoma (HNSCC) patients during anti-PD-1/PD-L1 immunotherapy. However, its effect on EGF-mediated metastasis and chemotherapy in HNSCC under lipid-enriched conditions remains unclear.

Material and method

Investigating the impact of ANGPTL4, PTX3, and PD-L1 on HNSCC progression, we analyzed the gene expression signatures in four phenotypic classes of HNSCC (atypical, basal, classical, and mesenchymal), as analyzed through the Gene Expression Profiling Interactive Analysis version 2 (GEPIA2) database. To clarify the molecular mechanism regulating PD-L1 expression in EGF-treated cells, inhibitors of MAPK/ERK and NF-κB were used, followed by examining mRNA, protein, and promoter activity of PD-L1. The metastatic regulators, such as epithelial-mesenchymal transition (EMT) markers, ANGPTL4, and PTX3, were also examined in cells. The in vitro trans-well migration and invasion assays and in vivo extravasation assay were applied to study the role of PD-L1 in HNSCC metastasis. The apoptotic and spheroid formation assays were used to further study the impact of gefitinib and PD-L1 on the cell viability in cisplatin-treated HNSCC cells.

Result and discussion

Our study showed a significant increase in PD-L1 expression in EGF-treated HNSCC cell lines. We determined that EGF-induced transcriptional activation of PD-L1 relies on ERK and NF-κB activation and ANGPTL4 expression. Importantly, depleting PD-L1 reduced EGF-induced expression of EMT markers, resulting in inhibition of migration, invasion, and anoikis resistance. Moreover, the enhancement of EGF-induced anoikis resistance and metastasis by oleic acid (OA) was diminished in PD-L1-knockdown cells. The combination of EGF and OA (EGF/OA)-induced cell metastasis and anoikis resistance were disrupted when treated with gefitinib. Although the EGF/OA attenuated cisplatin-induced apoptosis, only gefitinib, but not PD-L1 knockdown, restored sensitivity to cisplatin treatment. These findings suggest that intrinsic PD-L1 is essential for EGF- and lipid-induced cell metastasis but is not correlated to chemoresistance.

Conclusion

Targeting both EGFR and PD-L1 may provide a promising therapeutic strategy for managing metastatic

HNSCC with cisplatin resistance and dysregulated lipid metabolism.

EACR25-1020**OXPHOS targeting of MYCN-amplified neuroblastoma***S. Epp¹, D. Egan¹, K. Wynne², E. Poon³, J. Simpson⁴, M. Halasz¹, W. Kolch¹*¹*Systems Biology Ireland, Dublin, Ireland*²*Conway Institute, Dublin, Ireland*³*Institute of Cancer Research, Sutton, United Kingdom*⁴*Cell Screening Laboratory, Dublin, Ireland***Introduction**

Half of High-Risk Neuroblastomas (HR-NB) are driven by MYCN gene amplification (MNA). These HR-NBs require high dosage chemotherapy and often relapse. Moreover, current therapies can cause severe long-term side effects in young cancer patients. As MYCN, like many transcription factors, is considered undruggable, there is an urgent need for new therapies. Although precision therapy for individual patients remains limited, combination therapy presents an immediate solution at the level of patient population to reduce reliance on highly toxic chemotherapy. This study investigates a novel therapeutic approach targeting the metabolic vulnerabilities of MNA NB cells, which are found reliant on mitochondrial respiration for survival.

Material and method

MNA cell lines and 2D spheres derived from a MYCN-driven NB mouse model were employed as experimental systems. Phosphoproteomics and proteomics analyses were performed using LC-MS/MS, and confocal microscopy was used for imaging. Cell death, cell viability, neuronal differentiation, and ROS production were assessed using standard assays. Synergy models were applied to evaluate drug interactions.

Result and discussion

Preliminary data has identified Diphenyleneiodonium chloride (DPI) as a MYCN-selective anti-proliferative compound through a high-throughput screen on NB cell lines. DPI was found to induce MNA NB cell differentiation, and trigger apoptosis through cytochrome c release and caspase 9 activation. Additionally, DPI significantly reduced tumor size and the number of micrometastases in a transgenic zebrafish model of MYCN-driven NB, without apparent toxicity. We discovered that DPI synergizes with mitoquinone mesylate (MitoQ), a mitochondria-targeted antioxidant in 2D and 3D in vitro models of NB. Similarly to DPI, MitoQ affects MNA cells in a MYCN-dependent way, being more toxic when MYCN levels are high. Furthermore, low nanomolar concentrations of MitoQ significantly decrease MYCN protein expression and induce differentiation of MNA cells. The DPI and MitoQ combination further synergizes with vincristine, a chemotherapeutic agent used in NB treatment. Phosphoproteomics and proteomics analysis suggests that the drug combination induces MNA NB cell death by arresting the cell cycle and inhibiting oxidative phosphorylation (OXPHOS) in the mitochondria.

Conclusion

Thus, interfering with mitochondrial metabolism may serve as an effective strategy to enhance the activity of chemotherapeutic drugs while reducing the required concentrations in MNA-NB.

EACR25-1030

Targeting cell cycle vulnerabilities and signalling rewiring in p53 deficient cancers

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Introduction

TP53 is the most frequently mutated gene in human cancers, linked to genomic instability and poor patient outcomes. Targeting mutant p53 remains a major challenge. Our lab identified that TP53 mutation is essential for a defective G2/M checkpoint, which we call the Topo2A-dependent G2 checkpoint. This checkpoint functions independently of the DNA damage checkpoint and prevents premature mitotic entry in the presence of DNA catenanes. Without it, cells must engage a mitotic failsafe pathway to delay mitosis, allowing for resolution of DNA catenanes. This study explores how p53 dysregulation leads to Topo2A-dependent G2 checkpoint loss and aims to define the molecular requirements of the mitotic failsafe pathway to uncover novel therapeutic targets.

Material and method

We have assessed the competency of the Topo2A-dependent G2 checkpoint in a diverse panel of cancer cell lines, including HGSOC, NSCLC, TNBC, and colorectal carcinoma, alongside non-transformed RPE1 and FNE1. To examine the requirement for p53 pathway dysregulation in Topo2A-dependent G2 checkpoint loss, we used siRNA knockdown and inhibitors in cancer and non-cancerous cells. Additionally, a candidate-based drug screen identified potential synthetic lethal targets in cells with an active mitotic failsafe pathway.

Result and discussion

We have shown that a panel of TP53-deficient cancer cell lines have a defective Topo2A-dependent G2 checkpoint. To elucidate key regulators of this checkpoint, we used a panel of siRNAs targeting TP53, ATM, ATR and CHEK2 in the non-transformed RPE1 cell line to assess checkpoint competency after Topo2 inhibition with ICRF-193 to induce DNA catenation. Our screen revealed TP53 siRNA leads to loss of checkpoint and further studies are underway to determine the other molecular requirements. To explore the potential for therapeutic of targeting the mitotic failsafe pathway, we conducted a preliminary small molecule inhibitor screen focusing on proteins involved in either the G2 checkpoint or mitosis. Using this candidate-based approach, we observed synthetic lethality in the HGSOC cell lines, Kuramochi, upon combined inhibition of Topo2 (ICRF-193) and selected mitotic kinases. Importantly, ICRF-193 induced G2 arrest in RPE1 and FNE1 cells, suggesting a protective effect, as the combination of inhibitors had no deleterious effect on these non-cancerous cells.

Conclusion

A defective Topo2a-dependent G2 checkpoint creates a tumor-specific vulnerability, potentially a fundamental weakness of TP53-deficient cancers. This provides a therapeutic window where Topo2 inhibition protects normal cells while priming cancer cells for mitotic failsafe pathway activation which can then be targeted.

EACR25-1031

GATA4 drives metaplasia and cancer initiation from foregut keratinocytes in vivo

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Introduction

Barrett's Esophagus (BE) is a metaplasia in which the squamous epithelium of the esophagus is replaced by columnar epithelium due to chronic gastroesophageal reflux. Esophageal metaplasia is a major risk factor for adenocarcinoma (EAC) and adenosquamous carcinoma (ASC), both of which have poor prognoses. Although the cell of origin remains a topic of long-standing debate, it is tempting to speculate that more than one cellular origin may exist in the initiation of BE. Notably, the contribution of keratinocytes at the squamo-columnar junction (SCJ) to BE-like metaplasia through trans-differentiation was unambiguously demonstrated a few years ago. On the other hand, keratinocytes distant from the junction seem to be more resistant to cell fate rewiring. We revealed previously that reactivation of the hedgehog (HH) pathway in esophageal cells due to chronic acid reflux induces the dedifferentiation of esophageal cells into embryonic-like progenitors in vivo but is not sufficient to trigger the development of specialized metaplasia. Interestingly, foregut embryonic progenitors are bipotent and can give rise to either squamous or columnar epithelium depending on the expression of certain transcription factors such as GATA4. We therefore hypothesized that esophageal keratinocytes initially lack cell plasticity but that embryonic-like cells could be reprogrammed into a gastric lineage, thereby serving as a cellular reservoir for specialized metaplasia.

Material and method

We analyzed copy number variations in human EAC and developed a new transgenic mouse model mirroring EAC-associated gene amplification in esophageal epithelium. Through lineage tracing, histology, and single cell RNA sequencing, we characterized esophageal cells fate in vivo.

Result and discussion

During foregut development, GATA4 is crucial for gastric lineage specification. Intriguingly, while it is absent from normal esophagus, we found that it emerges as one of the most frequently amplified genes in EAC and is overexpressed in human BE. Thus, GATA4 could be a driver of cell reprogramming. Our results show that GATA4 overexpression in keratinocytes induces gastric-like metaplasia preferentially at SCJ. Strikingly, if HH pathway is activated first, GATA4 expression leads to the formation of specialized metaplasia and even ASC-like tumors over large regions of the foregut, suggesting a greater potential to initiate cancer. Single cell RNA

sequencing allowed to follow the transcriptomic trajectory between esophageal progenitors and metaplastic cells. It will be used to study metaplasia-to-cancer transition. Our study demonstrates that the HH pathway stimulates keratinocytes' plasticity and makes them competent to transdifferentiate into gastric-like metaplasia and initiate ASC-like tumors.

Conclusion

In conclusion, our study shows that esophageal cell plasticity can be modulated and that a fraction of BE and ASC may originate from squamous progenitors.

EACR25-1048

Targeting Fatty Acid Oxidation to Boost the Antitumor Effects of Nutrient Starvation in Solid Malignancies

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Introduction

In vitro starvation (STV) and in vivo fasting and fasting-mimicking diets (FMDs) have been shown to slow tumor progression and extend survival in tumor-bearing mice. However, they do not achieve complete tumor eradication when used alone. Data from clinical trials conducted by our group in breast cancer patients demonstrate that tumors exposed to FMD upregulate Fatty Acid Oxidation (FAO). Here, we investigated whether FAO is a resistance mechanism that is activated by cancer cells to acquire resistance to fasting/FMD.

Material and method

We investigated the effects of FAO in combination with STV conditions on the proliferation and survival of human or murine cell lines of Triple Negative Breast Cancer (TNBC) ($n = 16$), HR+HER2- breast cancer ($n = 3$), colorectal ($n = 1$), pancreatic ($n = 1$) and lung cancer ($n = 1$), as well as of non-transformed human epithelial cells ($n = 3$). STV consisted of 50% reduction of extracellular glucose and serum in cell growth media. To inhibit FAO, we used the Carnitine Palmitoyl transferase 1 inhibitors etomoxir or ST1326. To confirm in vitro results in vivo, we used mouse transplants of human MDA-MB-231/SUM159PT and murine 4T1 cells injected in the mammary fat pad of female NOD/SCID and BALB/c mice, respectively. Mice were treated with every-24-hour intermittent fasting (IF), alone or in combination with etomoxir; we assessed the impact of these treatment in primary tumor growth and animal survival.

Result and discussion

STV and FAO produced cooperative or synergistic antiproliferative effects in all investigated tumor cell models, but not in normal cells. In all mouse models, combining IF and etomoxir resulted in cooperative delay of primary tumor growth and in significant animal survival prolongation. Data from bulk RNA-seq analysis, performed in tumor masses collected at the end of fasting in NSG mice injected with MDA-MB-231 or SUM159PT cells, revealed that the combination of IF and etomoxir resulted in an enrichment of several signatures related to

immune processes, such as antigen processing and presentation, paralleled by a depletion of signatures related to DNA repair and several metabolic processes, such as oxidative phosphorylation and amino acid metabolism. We confirmed the mechanistic relevance of these biological processes in mediating the antitumor activity of the starvation-FAO combination through targeted in vitro experiments.

Conclusion

FAO is a potential resistance mechanism to nutrient starvation. Combining FAO with in vitro or in vivo starvation conditions results in cooperative antitumor effects that are mediated through the modulation of DNA repair and amino acid pathways. Our findings pave the way for conducting clinical trials to combine nutrient starvation approaches, such as FMD, with the modulation of FAO in combination with standard pharmacologic treatments.

EACR25-1050

PSRC1 promotes oncogenic dedifferentiation in TP53-mutated hepatocellular carcinoma

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Introduction

The tumor suppressor TP53 is critical in maintaining cellular self-renewal and differentiation in various human cancers and ranks as the most common driver mutation type in hepatocellular carcinoma (HCC). However, the mechanisms underlying the interplay between TP53 and stemness in HCC remain unclear. Herein, we identified proline and serine-rich coiled-coil 1 (PSRC1) as a critical player in promoting oncogenic stemness and dedifferentiation in TP53-mutated HCC.

Material and method

Human HCC cell lines, preclinical sporadic HCC tumor models, and tissues of patients with HCC were used to assess the role of PSRC1 in driving HCC stemness and dedifferentiation in a TP53 context.

Result and discussion

PSRC1 is significantly upregulated in TP53-mutated HCC and correlated with aggressive clinical features, including survival, tumor stage, metastasis risk, tumor size, and stemness signatures. Functionally, PSRC1 promotes tumor initiation, self-renewal, and metastasis as demonstrated in both in vitro and in vivo models. Mechanistically, wild-type TP53 is identified as an upstream repressor of PSRC1, while activation of the Notch signaling cascade was found as a downstream mediator of PSRC1's oncogenic activities.

Conclusion

Our findings highlight the critical role of PSRC1 in TP53-mutated HCC progression, particularly in promoting oncogenic stemness, dedifferentiation, and metastasis. PSRC1-induced tumor lineage plasticity may

represent an Achilles heel for TP53-mutated HCC. Inhibition of PSRC1 may represent a novel treatment opportunity in the clinic.

EACR25-1058

Activation of the p38/AP-1 Signaling Pathway by PFKFB4 Promotes ANXA2 Expression and Osteosarcoma Metastasis

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Introduction

Osteosarcoma, the most prevalent primary bone malignancy, is responsible for the majority of bone cancer-associated deaths due to its high metastatic potential. The bifunctional enzyme PFKFB4 (6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase-4) is induced by hypoxia and is strongly associated with glycolysis. PFKFB4 is highly expressed in various types of cancer and is essential for tumor survival. Over the past decades, extensive efforts have been made to elucidate the role of PFKFB4 in tumor progression. However, its effects on the metastasis of osteosarcoma cell lines remain unclear. Therefore, we investigated the role of PFKFB4 in the invasion and migration of human osteosarcoma cells, as well as the underlying molecular mechanisms.

Material and method

We conducted reverse transcription-polymerase chain reaction (RT-PCR), real-time PCR, Western blot, Boyden chamber assay, flow cytometry, luciferase reporter assay, and RNA sequencing to investigate human osteosarcoma cell lines.

Result and discussion

In this study, we compared the mRNA and protein expression levels of PFKFB4 in four osteosarcoma cell lines (HOS, MG63, Saos2, and U2OS). We established PFKFB4 siRNA systems and found that the invasion and migration abilities were dramatically decreased in Saos2/PFKFB4 siRNA and U2OS/PFKFB4 siRNA cells. Using Illumina RNA sequencing workflows, we also found that PFKFB4 knockdown reduced ANXA2 expression. After ANXA2 knockdown, the invasive and migratory abilities of Saos2 and U2OS cells were significantly reduced. In addition, PFKFB4 knockdown decreased ANXA2 promoter activity, c-Jun and c-Fos nuclear protein levels, and phospho-p38 signaling protein levels. Moreover, overexpression of p38 rescued the PFKFB4 knockdown-induced inhibition of ANXA2 expression and cell motility in U2OS cells. Furthermore, overexpression of PFKFB4 rescued the PFKFB4 knockdown-induced inhibition of phospho-p38, ANXA2 expression, and cell motility in U2OS cells.

Conclusion

According to the results, PFKFB4 induces ANXA2 expression via phosphorylation of the p38 signaling pathway, facilitating the nuclear translocation of c-Jun and c-Fos, which bind to the AP-1 site on the ANXA2 promoter and consequently promote osteosarcoma cell metastasis.

EACR25-1082

Interconnections among MET tyrosine kinase receptor, NMDAR, glutamine metabolism, and stress response in cancer progression

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Introduction

The MET oncogene encodes a tyrosine kinase receptor for the hepatocyte growth factor (HGF) with pleiotropic functions in cancer onset and progression. Fast tumor growth and survival under adverse conditions are stimulated by glutamate, generated through glutamine metabolism, a process known as “glutamine addiction”. Additionally, stress response mechanisms, like the integrated stress response (ISR) signaling and endoplasmic reticulum (ER) stress pathway, are crucial in cancer pathogenesis. Glutamine deprivation activates ISR mediators that supports cell survival and homeostasis, but severe or prolonged stress shifts them toward apoptosis regulation. While these processes are studied individually, their integration is less understood. In this study we hypothesize that MET can protect cancer cells from stress-dependent cell death by interacting with N-methyl-D-aspartate receptor for glutamate (NMDAR) and stimulating glutamine metabolism.

Material and method

Warburg effect and glutaminolysis were analysed by enzymatic assay in various breast and colon cancer cell lines. To evaluate the metabolic role of MET-NMDAR crosstalk a metabolomic analyses was conducted and explored using computational models. To evaluate the involvement of MET, glutamine metabolism, and NMDAR in cancer invasive program, specific inhibitors and molecular analyses were also exploited.

Result and discussion

MET activation in cancer cell lines facilitates the Warburg effect and glutaminolysis. Importantly, we found that NMDAR is involved in this MET-mediated metabolic rewiring. LC-MS/MS metabolomic analysis showed increased metabolic consumption in HGF-treated cancer cells, returning to basal levels after MET-specific inhibition. Interestingly, glutamate is among the most expressed metabolites after HGF treatment. Bio-informatic analysis of the metabolomic evaluations highlighted that HGF treatment enhances specific metabolic pathways: biosynthesis of amino acids and lipids, glutamate metabolism, TCA (tricarboxylic acid) cycle, and glutathione metabolism. Interestingly, glutamine deprivation or glutaminase inhibition induce: i) apoptotic cell death, ii) phosphorylation of eIF2α, marker of ISR stress response, and iii) counteraction of HGF-driven migratory and invasive cancer program. Accordingly, HGF treatment reduces apoptosis and ISR activation in both pro-death treatments.

Conclusion

In this study we demonstrate that MET, NMDAR, glutamine metabolism, and ISR intersections are involved in cancer progression. MET can regulate glutamine metabolism by interaction with NMDAR and protect cancer cells from stress-dependent cell death mechanisms. These results pave the way for future development of novel combinatory therapeutic approach for cancer patients.

EACR25-1083

Deciphering the role of MET/NMDAR complex in colorectal cancer pathogenesis

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Introduction

MET, the receptor of the natural ligand Hepatocyte Growth Factor (HGF), is a proto-oncogene well-known for its role in cancer cell invasion. However, the mechanisms underlying these HGF-induced pro-invasive features are still unclear. Recently, we demonstrated that MET physically interacts with the N-methyl-D-aspartate receptor (NMDAR), a glutamate-gated ion channel known for its role in synaptic transmission encoded by GRIN genes. Indeed, we observe that MET and NMDAR cooperate in the invasion program of Triple Negative Breast Cancer. In this study, we address the role of MET/NMDAR interaction in colorectal cancer (CRC).

Material and method

Different immunoassays such as proximity ligation assay and co-immunoprecipitation were exploited to evaluate physical association between MET and NMDAR receptors in different CRC *in vitro* models. To understand the biological role of this crosstalk in the HGF-driven invasive program, matrigel invasion and wound healing assays were performed in presence of two specific NMDAR (MK801 and Ifenprodil) and MET (JNJ-38877605) inhibitors. Moreover, we performed a RNA sequencing analysis on adenoma and CRC tissues in order to highlight differentially expressed genes in CRC in comparison with the adjacent tissue.

Result and discussion

Our results confirmed that MET and NMDAR receptors are physically associated in a subset of CRC models. Moreover, the inhibition of NMDAR impaired the HGF-driven invasion and migration in CRC models positive for MET/NMDAR complex, acting synergistically with MET receptor inhibition. In the context of HGF-driven invasion, those models lacking MET/NMDAR crosstalk showed no response upon NMDAR inhibition. Finally, RNA sequencing data of adenoma and CRC tissues highlighted, compared to the adjacent colonic tissues, a

significant upregulation of MET, GRIN2B, and GRIN2D expression, a finding corroborated by the analysis on TCGA database. Interestingly, clustering analysis associated Consensus Molecular Subtype (CMS) 1 and 2 or Adenomatous Polyposis Coli (APC) mutations to high expression of MET and GRIN genes.

Conclusion

Overall, these data emphasize that MET/NMDAR cross-talk is involved in the CRC invasive program, suggesting that a combinatorial therapy targeting this complex may be exploited as a new therapeutic strategy.

EACR25-1111

Characterizing the partial epithelial-mesenchymal transition and invasion in a colorectal cancer *in vitro* model

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Introduction

The high-plasticity states of tumor cells lead to drastic phenotypic changes, such as epithelial-mesenchymal transition (EMT), enabling high invasive and metastatic potential. Tumor cells may occupy a spectrum along an epithelial-mesenchymal axis, where the two ends are mixed by a hybrid partial EMT (pEMT) state. Colorectal cancer (CRC) is the second-third leading cause of cancer-related death. Gene expression-based CRC clustering identified a group (CMS4) with EMT, fibroblast (CAF) accumulation, invasion, and decreased survival, representing an aggressive subtype with high plasticity and no effective treatment. In this work, we aimed at characterizing pEMT by using the organoid technology and co-cultures. Patient-derived organoids (PDO) maintain the cellular heterogeneity of the original tissue, representing a popular tool for cancer research.

Material and method

PDOs were isolated from patients with CRC and cultured in the absence/presence of fibroblasts in different extracellular matrices (ECM), such as the laminin-rich Matrigel or collagen-I. Cells were characterized by imaging and statistical analysis, flow cytometry, whole-mount immunostaining and by RT-qPCR at the RNA level.

Result and discussion

IL1R1 was shown to be a marker of iCAFs in CRC, a subpopulation of fibroblasts with inflammatory phenotype. Whereas immune cell-derived cytokines, such as IL-17A, TNF-alpha, IL-22 or IFN-gamma had no effect on fibroblast polarization, co-culturing fibroblasts with CRC organoids resulted in the decrease in the percentage of IL1R1+ cells. Thus, tumor cells actively shift CAFs towards the myCAF phenotype that are a major source of collagens. Interestingly, co-culturing with fibroblasts resulted in an increase in EMT marker levels without the loss of epithelial identity, suggesting a pEMT phenotype, but with no invasion. pEMT with cellular migration was only induced when changing extracellular matrix to collagen-I. This resulted in a loss of organoid circularity and CRC organoids acquired an invasive phenotype. Furthermore, modifying the collagen-based ECM by

including other proteins had only a limited effect on the PDO phenotype.

Conclusion

Tumor cells with the most metastatic capacity reside in a pEMT state that can be characterized by the co-presence of mesenchymal and epithelial markers. Thus, this malignant cell population should be a primary target of therapies. Fibroblasts induce pEMT in CRC cells, however, this is coupled with an invasive phenotype only in a permissive ECM niche, such as the presence of collagen-I. These results highlight the importance of targeting ECM components in CRC.

Funding: OTKA137554 (National Research, Development and Innovation Office, Hungary), TKP2021-EGA-24 (Ministry of Innovation and Technology of Hungary). *Ethical permission:* TUKEB 2015, 51323-4/2015/EKU.

EACR25-1157

DNA methylation landscape defines metastatic routes in high grade serous carcinoma

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Introduction

High-grade serous ovarian carcinoma (HGSC) is the most lethal gynecological cancer, primarily due to late-stage diagnosis and extensive metastatic spread. While genetic drivers of HGSC have been extensively studied, the role of DNA methylation in metastasis and therapy response remains largely unexplored.

Material and method

We analyzed 400+ whole-genome bisulfite sequencing (WGBS) samples, along with matched RNA sequencing data from 125 HGSC patients enrolled in the prospective, longitudinal, multi-region DECIDER trial (NCT-04846933). A computational deconvolution approach was applied to separate tumor-specific methylation patterns from patient-specific, micro-environmental, and treatment-related effects, enabling precise epigenetic analysis.

Result and discussion

Our findings revealed three primary routes of metastatic dissemination in HGSC: solid tissue invasion, ascites-mediated spread, and lymphatic dissemination, each characterized by distinct DNA methylation patterns. Solid tissue invasion emerged as the predominant route, with differential methylation in estrogen signaling, interferon response, and androgen pathways. Ascites-mediated spread facilitated tumor dissemination to the pleural fluid, with epithelial-mesenchymal transition dysregulation playing a key role in ascites formation. Lymphatic spread, though less frequent, involved para-aortic lymph nodes and was associated with epigenetic changes in immune-related pathways. Analysis of treatment-induced epigenetic changes revealed site-specific responses to platinum-based treatment. The omentum showed the highest methylation changes after neoadjuvant chemotherapy (NACT), but only in well-

responding patients. In contrast, refractory patients exhibited no changes in promoter methylation, reflecting an absence of response in those with poor outcomes. Ascites displayed a unique pattern: while largely unchanged following NACT, they underwent substantial epigenetic shifts at relapse. This suggests a dynamic change of DNA methylation in ascites after the whole course of treatment, potentially driving disease progression and chemoresistance.

Conclusion

This study provides a comprehensive epigenetic characterization of metastatic spread in HGSC and tissue-specific response to platinum-based treatment. By defining distinct DNA methylation patterns across metastatic routes and therapy responses, our findings highlight epigenetic drivers of disease progression and potential therapeutic targets.

EACR25-1162

Plasticity of esophageal progenitors in fine-tuned by their microenvironment in vivo

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Introduction

Chronic acid reflux in the esophagus can trigger the conversion of squamous cells with columnar cells, a process known as metaplasia which increase the risk of developing esophageal adenocarcinoma. While keratinocytes demonstrate plasticity by their ability to undergo this transdifferentiation, mechanisms regulating progenitor cell fate remain poorly understood. We previously identified the transcription factor Sox9 as a regulator of esophageal cell plasticity downstream of the Hedgehog (HH) signaling pathway in vivo. We showed that gastroesophageal reflux induces the activation of the HH pathway in keratinocytes, and subsequently their dedifferentiation into an embryonic-like state, promoting a Sox9-dependent squamous-to-columnar conversion. Deciphering Sox9 regulation in esophageal progenitors could provide insight into the mechanisms initiating metaplasia, thus revealing new targets for cancer prevention and therapeutic.

Material and method

Esophageal organoids were derived from WT and K5:Smo transgenic mice with constitutive HH pathway activation, as well as human biopsies. ATAC-seq identified regulatory elements predicted to control Sox9 expression. Bulk and single-cell RNA seq were used to analyze transcriptional changes upon HH pathway activation. NSAIDs treatments were conducted to assess the effect of Sox9 inhibition on cell plasticity.

Result and discussion

Using organoids, we show that Sox9 is not directly regulated by HH pathway. In silico analysis of Sox9 promoter identified Smads, effectors of TGF-β/BMP signaling pathways, as potential transcription regulators. Transcriptomics data confirmed that HH signaling pathway is associated to an increase of TGF-β/BMP signaling in vivo. Activation of these pathways in vivo is the consequence of (1) local extracellular matrix

remodeling, which in turn may activate latent TGF- β , and (2) upregulation of BMP ligands in keratinocytes. Treating organoids with TGF- β and/or BMP demonstrate that TGF- β triggers Sox9 transcription and that BMP further increases Sox9 expression. Interestingly, we observed that NSAIDs inhibited HH-induced Sox9 expression and cell plasticity in vivo. Whereas NSAIDs slightly inhibit TGF- β /BMP signaling in vivo, these drugs prevented TGF- β /BMP-induced Sox9 expression by destabilizing protein expression.

Conclusion

Our findings highlight the role of the microenvironment in regulating esophageal cell plasticity through Sox9 modulation. In addition, that keratinocytes can locally remodel the extracellular matrix to promote their plasticity. Importantly, we report a new role for NSAIDs treatment, which modulates squamous-to-columnar conversion by destabilizing Sox9 expression in keratinocytes. Our results offer new perspectives for improving esophageal mucosal re-epithelialization, particularly after endoscopic resection of metaplasia or pre-cancerous lesions and offer potential strategies for cancer prevention.

EACR25-1167

Autophagic Cell Death in GBM Cells Following Arginine Deprivation is Mediated by Ferroptosis

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Introduction

Glioblastoma Multiforme (GBM) is characterized by high mortality and low survival rates secondary to poor prognosis and the lack of effective treatment options. We had previously demonstrated that GBM cells were auxotrophic for arginine, secondary to the insufficient or lack of expression of argininosuccinate synthetase 1 (ASS1), and sensitive to arginine deprivation induced by a recombinant human arginase [HuArgI (Co)-PEG5000]. We have shown that arginine deprivation is selectively cytotoxic to GBM cells with cell death being caspase-independent, and non-apoptotic. We have also shown, indirectly, that autophagy was activated without determining its impact on cell death. Here, we attempt to decipher the mechanism of cell death in GBM cells after arginine deprivation, by confirming the activation of autophagy, determining its impact on cell death and the potential mechanism of cell death.

Material and method

We tested two GBM cell lines for hallmarks of autophagy, the impact of autophagy on cell survival as well as the potential mechanism of cell death, including accumulation of cytosolic ROS, lipid peroxidation and ferroptosis, following arginine deprivation.

Result and discussion

Arginine deprivation led to marked and sustained activation of autophagy starting at 24 h and lasting up to 120 h post-treatment. Inhibition of autophagy, using chloroquine (CQ), reversed the cytotoxicity of arginine deprivation, indicating that cell death is mediated by

autophagy. Arginine deprivation also led to the accumulation of cytosolic ROS in both cell lines. Addition of the ROS scavenger NAC reversed cytotoxicity of arginine deprivation without affecting the activation of autophagy, indicating that ROS accumulation is downstream of autophagy and upstream of cell death. Next, we show that lipid peroxidation occurs following treatment, indicating that arginine deprivation induces ferroptotic cell death. To further confirm this and link it to the activation of autophagy, we investigated the expression of NCOA4 and FTH1. Results showed increased expression of NCOA4 and decreased expression of FTH1, indicating an increase in the shuttling of ferritin into autophagosomes and an increase in ferritin degradation leading to increased levels of free iron. We also show a decreased expression of the antioxidant protein GPX4 following arginine deprivation. The autophagy-dependent degradation of FTH1, following arginine deprivation, leads to the accumulation of free Fe²⁺, which generates ROS through the Fenton reaction. The loss of antioxidant proteins prevents the neutralization ROS leading to lipid peroxidation and death by ferroptosis.

Conclusion

Our results demonstrate that arginine deprivation in GBM cells leads to the autophagy-mediated activation of ferritinophagy resulting in ferroptotic cell death.

EACR25-1168

Revealing the tumor suppressive sequence within KL1 domain of the hormone Klotho

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Introduction

Klotho, a 1012 amino acid transmembrane protein, is a potent tumor suppressor in different cancer types. Klotho is composed of two internal repeats KL1 and KL2, and the tumor suppressor activity is primarily attributed to the KL1 domain. Despite its significant role in regulating various cancer-related pathways, the precise mechanism underlying its tumor suppressor activity remains unresolved. In this study, we aimed to identify the sequence responsible for the tumor suppressor function of Klotho and gain insights into its mechanism of action.

Material and method

We designed expression vectors of truncated KL1 that included C-terminal truncations: 1-340 (KL340), 1-320 (KL320), and N-terminal truncation: 88-340 (KL88-340). We assessed their anti-cancer activity or regulation of the Wnt- β /catenin pathway by colony formation assay and co-expression in cancer cells. We used several cancer cell lines MCF-7, MDA-MB-231, PANC1, MIA Paca-2 and HCT116. We then performed RNA seq analysis on MCF7 cells over-expressing either KL1 or one of the C-terminal truncated KL340, KL320 to decipher global transcriptome differences. Finally, we performed multiple sequence alignment and utilized the α -fold

predictor tool to further understand the structural properties of the truncated proteins.

Result and discussion

Our findings demonstrated that truncated KL340 effectively inhibited colony formation similar to KL1, while truncated KL320 lost this activity. Furthermore, this correlated with the inhibitory effect of KL1 and KL340 on the Wnt/β-catenin pathway whereas KL320 had no effect. Transcriptomic analysis of MCF-7 cells expressing the constructs revealed enriched pathways associated with tumor suppressor activity in KL1 and KL340. The results show significant enrichment of the necroptosis pathway, MAPK pathway, protein processing in the ER and the ribosome, suggesting that the tumor suppressor activity is driven by several processes leading to cell death. Interestingly, the α-fold predictor tool highlighted distinct differences in the α and β sheets of the TIM barrel fold of the truncated Klotho constructs, adding to our understanding of their structural variations.

Conclusion

This study identified the 340 N-terminal amino acids as the sequence that possesses Klotho's tumor suppressor activity and reveals a critical role the 320-340 sequence for this function. It also provides a foundation for the development of Klotho-based therapeutic approaches for cancer treatment.

EACR25-1174

Pathophysiological Mechanisms of Pancreatic Islet Dysfunction in Pancreatic Ductal Adenocarcinoma

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Introduction

Pancreatic Ductal Adenocarcinoma (PDAC) is the most common type of pancreatic cancer and is widely associated with Diabetes Mellitus (DM). Notably, up to 85% of patients with PDAC exhibit hyperglycaemia, which frequently manifests 2-3 years before the diagnosis of cancer. Interestingly, PDAC-associated DM may

resolve after tumour resection if sufficient pancreatic islets remain in the residual pancreas. Recent evidence suggests that PDAC not only increases the risk of DM but may also directly disrupt glucose homeostasis, leading to a new onset of DM, referred to as PDAC-associated DM. However, the exact pathogenic mechanisms driving PDAC-associated DM and its role in hyperglycaemia remain incompletely understood.

Material and method

To evaluate the impact of PDAC on pancreatic islets, we utilized a syngeneic mouse model based on the transplantation of organoids expressing the oncogenic KRAS-G12D mutation and p53 loss. This approach allowed us to assess tumor-induced alterations in islet architecture and function within an immunocompetent environment. To enhance the translational relevance of our findings, we further validated our results using a patient-derived xenograft (PDX) mouse model of PDAC.

Result and discussion

Our findings reveal a pronounced disruption of glucose homeostasis, driven at least in part by impaired pancreatic islet architecture and significant beta-cell dysfunction (insulin-secreting cells). These results were further validated using a patient-derived xenograft mouse model of PDAC. Additionally, we found that secreted factors from both murine organoids and human PDAC tumours reduced insulin content and secretion, potentially accounting for the hyperglycaemia observed in these patients.

Conclusion

Our findings indicate that PDAC induces pancreatic islet dysfunction, marked by β-cell impairment and disrupted blood glucose regulation, ultimately contributing to diabetes. Targeting these pathological mechanisms to restore glucose homeostasis could serve as a potential therapeutic strategy to counteract the impact of hyperglycaemia on PDAC progression.

EACR25-1210

Degradation of IDH1 via Chaperone-mediated autophagy inhibits cell cycle progression in glioblastoma

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Introduction

Chaperone-mediated autophagy (CMA) is a selective protein degradation pathway essential for maintaining cellular homeostasis. The dysregulation of CMA has been implicated in various diseases, including glioblastoma, a highly aggressive brain cancer.

Material and method

This study utilized a combination of established glioblastoma cell lines, patient-derived glioblastoma stem cells, and orthotopic mouse models to investigate CMA's role in glioblastoma.

Result and discussion

CMA deficiency enhances glioblastoma cell growth by impairing the degradation of isocitrate dehydrogenase 1 (IDH1), a critical metabolic enzyme and molecular marker in glioma classification. IDH1, which is

frequently upregulated in glioblastoma, contains a conserved CMA targeting motif and interacts with the CMA chaperone heat shock cognate 71 kDa protein (HSC70). Inhibition of lysosomal function results in IDH1 accumulation in both glioblastoma cells and patient-derived glioblastoma stem cells, indicating that CMA targets IDH1 for lysosomal degradation. Knockdown of the CMA receptor lysosome-associated membrane protein type 2A (LAMP2A) leads to elevated IDH1 expression, disruption of the RB1 cell cycle pathway, and increased glioblastoma cell growth. Suppression of IDH1 mitigates the tumor-promoting effects of CMA inhibition by restoring cell cycle regulation, whereas IDH1 upregulation fuels cell cycle progression, partly through its metabolic product alpha-ketoglutarate (alpha-KG), which stimulates Cyclin D1 expression. Notably, pharmacological activation of CMA suppresses cell growth and reduces IDH1 and Cyclin D1 expression.

Conclusion

These findings reveal a novel mechanism by which CMA modulates cell cycle progression by targeting IDH1 for degradation, thereby inhibiting glioblastoma progression.

EACR25-1214

Increased OGT activity promotes nuclear localization of RNA polymerase II to support oncogenic transcription

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Introduction

Prostate cancer is the second most common cancer in men. O-GlcNAc transferase (OGT), the enzyme responsible for all nucleo-cytoplasmic O-GlcNAcylation, is overexpressed in aggressive prostate cancer.

Previously we showed that combined inhibition of OGT and transcriptional cyclin-dependent kinases (tCDKs) is toxic for prostate cancer cells. tCDKs have a fundamental role in regulating transcription of all protein-encoding genes by phosphorylating RNA polymerase II (RNA Pol II) carboxy-terminal domain (CTD). RNA Pol II CTD is also O-GlcNAcylated and here we wanted to establish the functional significance of this modification.

Material and method

The functional significance of RNA Pol II O-GlcNAcylation has remained enigmatic due to the lack of reagents that selectively recognize the O-GlcNAcylated polymerase. Here we developed a novel approach to identify chromatin localization of O-GlcNAcylated RNA Pol II referred to as wChIP. By integrating the results of computational docking, lectin pulldown-experiments and peptide-mapping, we determined a site at which RNA Pol II CTD is O-GlcNAcylated. We used chemically synthesized CTD-peptides to identify proteins that bind to the O-GlcNAcylated RNA Pol II and probed the functional role of this interaction using cell fractionation and assays of cell fitness.

Result and discussion

Using our wChIP approach, we show that O-GlcNAcylated RNA Pol II localizes on promoters of highly expressed genes. These data propose that OGT can

integrate metabolic information to directly control transcription. We confirm serine 5 as a site that is O-GlcNAcylated on RNA Pol II CTD and identify nuclear import protein karyopherin subunit beta 1 (KPNB1) as a selective binding partner of CTD O-GlcNAcylated at this site. In the future, it is important to map the binding partners of the differentially O-GlcNAcylated CTD and probe if they differ across cell types. Functionally, we show that combined inhibition of OGT and KPNB1 reduces nuclear levels of RNA Pol II, and that the combination is more toxic to prostate cancer cells than to cells representing normal prostate.

Conclusion

Our results suggest that RNA Pol II O-GlcNAcylation promotes nuclear entry to maintain high levels of transcription.

EACR25-1231

The Potential Role of PAK4 in PDGF-BB-Mediated EMT and Metastatic Processes in Triple Negative Breast Cancer

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Introduction

Epithelial-Mesenchymal Transition (EMT) is a biological process enabling epithelial cells to acquire mesenchymal characteristics, including reduced cell-cell adhesion and enhanced motility, thereby facilitating processes such as cancer metastasis and tissue repair. Platelet-derived growth factor-BB (PDGF-BB) is a key regulator of cell proliferation, migration, and angiogenesis, playing a crucial role in wound healing, vascular remodeling, and pathological conditions. PAK4 (p21-activated kinase 4), a serine/threonine kinase, governs essential cellular functions, including cytoskeletal dynamics, proliferation, survival, migration, and invasion.

Material and method

This study aimed to investigate the potential role of PDGF-BB-mediated PAK4 function in EMT process and metastatic properties of MDA-MB-231 triple negative breast cancer (TNBC) cells. For this purpose, cells were stimulated with PDGF-BB and also treated with PAK4 inhibitor PF-3758309, separately and in combination. PAK4 activation and expression levels of EMT markers; E-cadherin, N-cadherin and vimentin were determined by Western blotting. Additionally, an *in vitro* wound healing assay was conducted to assess migration potential.

Result and discussion

According to obtained results, PDGF-BB stimulation did not result in a substantial alteration of the endogenous PAK4 levels, however it induced a 3.63-fold increase in p-PAK4 levels. As expected, PAK4 activation was suppressed in PF-3758309-treated cells despite PDGF-BB stimulation. PAK4 inhibition significantly upregulated E-cadherin (2.91-fold) while downregulating vimentin (2-fold), with no notable change in N-cadherin levels. Even though PDGF-BB stimulation, PAK4 suppression caused a 2.96-fold increase in E-cadherin, a 2.5-fold decrease in N-cadherin, and a 4-fold decrease in vimentin levels. Migration analysis showed that PDGF-BB stimulation promoted cell motility by 32% for 24

hours and 40% for 48 hours compared to control. PF-3758309 reversed this effect and suppressed the PDGF-BB-dependent cell motility. These findings suggest that PAK4 plays a key role in the PDGF-BB-mediated EMT and metastasis processes in TNBC cells.

Conclusion

Consequently, the PDGF-BB/PDGFR/PAK4 axis can be considered a significant therapeutic target in the management of cancer progression.

EACR25-1241

PSMG2 role in tumorigenesis and stemness mediated by protein accumulation, reticulum stress and autophagy

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Introduction

Studying genes involved in proteostasis is essential for understanding the mechanisms driving dedifferentiation in cancer, as this process enables tumor cells to acquire stem-like properties, promoting plasticity, resistance, and progression. In head and neck squamous cell carcinoma (HNSCC), a highly aggressive malignancy with poor prognosis, identifying molecular regulators of dedifferentiation is crucial for developing targeted therapies. PSMG2, a chaperone protein that forms a heterodimer with PSMG1, plays a key role in this context by facilitating the assembly of the 20S proteasome. Here, we characterized the role of PSMG2 in tumorigenesis and dedifferentiation in HNSCC cell lines.

Material and method

Protein-protein interaction (PPI) analysis using IntAct and BioGRID identified key interactors of PSMG2, followed by functional enrichment via Gene Set Enrichment Analysis (GSEA). PSMG2 was over-expressed and downregulated in two HNSCC cell lines. Tumorigenesis was analysed using various functional assays, including growth curves, clonogenic assays, tumorsphere assays, and in vivo models. Molecular mechanisms such as apoptosis and autophagy were tested by flow cytometry, Western blotting, and immunofluorescence. Additionally, a reprogramming assay was conducted to assess the impact of gene downregulation on the dedifferentiation process.

Result and discussion

High PSMG2 expression correlated with poor prognosis and reduced survival in HNSCC patients. To explore the molecular networks regulated by PSMG2, we analysed protein-protein interactions (PPI) identifying common interactors. GSEA revealed enrichment in proteostasis, unfolded protein response, and cancer-related signaling pathways. PSMG2 knockdown impaired cell proliferation both in vitro and in vivo and led to a significant reduction in stemness, dedifferentiation, and reprogramming properties. Mechanistically, PSMG2 reduction resulted in the accumulation of polyubiquitinated proteins, triggering endoplasmic reticulum (ER) stress and activating apoptosis and autophagy as compensatory mechanisms.

Notably, PSMG2 downregulation enhanced the response to proteasome inhibitors, suggesting a potential therapeutic vulnerability.

Conclusion

Our findings demonstrate that PSMG2 is a key regulator of proteasome assembly, ER stress, and cellular homeostasis in HNSCC, linking proteostasis with dedifferentiation and therapy resistance. Targeting PSMG2 or the proteasome machinery may represent a promising therapeutic strategy for overcoming tumor plasticity and improving treatment outcomes in HNSCC patients.

EACR25-1243

PP2A inhibitor proteins CIP2A and PME-1 as medulloblastoma oncoproteins and therapy targets

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Introduction

Inhibition of the tumor suppressor protein phosphatase PP2A, through the overexpression of its endogenous inhibitor proteins CIP2A, PME-1, and SET, has been implicated as a potential mechanism underlying kinase inhibitor resistance in various cancers. However, the role of these PP2A inhibitor proteins in the progression of pediatric brain tumor medulloblastoma has not been previously investigated.

Material and method

The expression of endogenous PP2A inhibitor proteins was preliminarily analyzed using publicly available transcriptome data. Functional studies were conducted in vitro and in vivo using medulloblastoma cell lines DAOY (SHH group) and D283-Med (Group 3/4). PME-1 and CIP2A were inhibited using CRISPR/Cas9 approaches [1].

Result and discussion

Transcriptome analysis of 763 primary medulloblastoma tumors revealed that high expression of CIP2A, PME-1, or SET correlates with poor prognosis. Additionally, CRISPR/Cas9 loss-of-function data from the DepMap portal identified PP2A and its regulatory proteins as among the top 10 preferentially essential genes in medulloblastoma, particularly in DAOY cells. In vivo studies further confirmed that knockout of CIP2A and PME-1 significantly reduced tumor growth and progression in mouse models. Notably, CIP2A knockout tumors exhibited a marked reduction in macrophage infiltration, suggesting a potential link between PP2A inhibitor proteins and the tumor immune microenvironment.

Conclusion

Our findings highlight the critical role of PP2A inhibitor proteins CIP2A and PME-1 as medulloblastoma oncoproteins and therapy targets. Further studies are needed to understand the mechanistic basis of the oncogenic activities of individual PP2A inhibitor proteins on tumor

recurrence and their potential as therapeutic targets to overcome resistance in medulloblastoma treatment.

[1] All animal studies were approved by the National Animal Experiment Board of Finland and conducted according to the Institutional Animal Care and Use Committees of the University of Turku.

EACR25-1248

AHSG exacerbates malignant progression in lung adenocarcinoma through ubiquitination mediated degradation of GSK3 β

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Introduction

AHSG, a glycoprotein is produced and secreted by tumor cells. It has a pleiotropic role in tumorigenesis. Lack of AHSG in mammary tumors prolongs mammary incidence and promotes the proliferation of bladder cancer cells. However, studies on AHSG regulation on tumor progression are minimal. Thus, we want to study its role in Lung Adenocarcinoma progression and heterogeneity.

Material and method

The GSE data set was retrieved from the GEO database. The results were validated on patient tissue samples at the gene and protein levels through qPCR and Immunohistochemistry (IHC). In-vitro studies were carried out to determine the AHSG expression in epithelial (BEAS 2B) and LUAD cell lines (A549 and H23). AHSG was silenced and the silencing efficiency was validated on gene and protein levels. The MTT assay ki67 staining and PI staining were performed to assess the effects of AHSG on LUAD cell proliferation. A wound scratch assay was done to assess the wound healing whereas transwell assays were carried out to check the migration and invasion capabilities in AHSG control and silenced LUAD cells. BioGRID analysis was done to check the downstream targets of AHSG. A co-immunoprecipitation was performed to check the interaction between AHSG and GSK3 β . A co-localization study was performed, and the correlation between the two proteins was calculated based on Pearson's coefficient. Western blotting was carried out to determine the influence of AHSG silencing on downstream protein targets of the GSK3 β /β catenin pathway.

Result and discussion

We identified that AHSG is upregulated in LUAD through bioinformatic analysis. This was further confirmed in LUAD patients at both gene and protein levels and in vitro. Through MTT assay, ki67 immunofluorescence staining, and cell cycle analysis it was observed that AHSG promotes LUAD cell proliferation. AHSG control LUAD cells had faster wound healing capacities as well as more migration and invasion capacities in scratch and transwell assays respectively. AHSG propels LUAD cells towards a higher Epithelial Mesenchymal transition (EMT) in a 3D unicellular LUAD model. Protein network analysis revealed that GSK3 β is a part of the AHSG interactome.

Mechanistically, AHSG interacts with glycogen synthase

kinase-3 beta (GSK3 β) and exacerbates the ubiquitination-facilitated degradation of GSK3 β by slowing down the dissociation of the ubiquitin ligase complex. Moreover, AHSG stimulates the Wnt/β-catenin signaling pathway through GSK3 β , thereby promoting the malignant transformation of LUAD. In conclusion, AHSG was a significant promoter of LUAD. The research we conducted identified AHSG as a promising theranostic target for LUAD.

Conclusion

The results of our research will shed light on the fundamental function of AHSG in controlling important cancer signaling networks and emphasize the biological backbone for the possible use of AHSG as a novel LUAD diagnostic indicator for LUAD as well as its therapy.

EACR25-1268

The effect of Ibuprofen on autophagic gene expression in Hepatocellular Cancer

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Introduction

Ibuprofen is an NSAID-derived drug and is generally used to treat pain, fever and inflammation. Its regular and long-term use has been shown to reduce the risk of cancers, with mostly positive results. Cancer is an abnormal proliferation of the body's cells that undergoes uncontrolled carcinogenesis with metastasis. Hepatocellular Cancer (HCC) is the most common type of primary malignant cancer of the liver. One of the hallmarks of cancer is a tumor promoted by inflammation. Autophagy is defined as the sequestration of bulk cytoplasm and organelles in vesicles, their delivery to their lysosomal system and subsequent degeneration. Autophagy is classified as type 2 cell death. When the studies in the literature were examined, no study was found on the relationship between ibuprofen and autophagic pathways in hepatocellular cancer cells. In this context, this study aims to determine the relationship between the effect of ibuprofen on autophagic pathways in hepatocellular cancer (Hep3B).

Material and method

For this purpose, Hep3B cells were cultured in 10% FCS containing high glucose DMEM and plated out in 6 well plates (5x10⁴ cells/well), then treated with previously determined ibuprofen IC₅₀ dose. As a control group, 1% of DMSO was applied as control. Also, cisplatin was used as positive control. After 24h, 48h and 72h, the cells were trypsinized and total RNA was isolated using cell pelet with RNA extraction kit. Total RNA was reverse transcribed into cDNAs using cDNA synthesis kit. cDNA was used as template for Real-Time Analysis. ATG3, ATG5, ATG7, LC3II and beclin gene expressions related to autophagic pathway were determined using specific primers at mRNA level. Human beta microglobulin gene was used as an internal control.

Result and discussion

As a result of the study, it was determined that there was a statistically significant increase in mRNA levels for

ATG-3, ATG-5, ATG-7 and LC-3II following 72 h of ibuprofen administration. Autophagy-related marker gene expressions were found to increase especially at 72 h compared to cisplatin-treated cell groups.

Conclusion

This study presents the first evidence in the literature regarding the association between ibuprofen and the autophagic cell death pathway in liver cancer cells. Understanding the underlying mechanisms of this effect at a fundamental level is crucial, especially for NSAID derivatives like ibuprofen.

EACR25-1294

Mechanisms regulating the formation of colorectal cancer brain metastasis

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Introduction

Colorectal cancer (CRC) remains a major cause of cancer-related mortality, with brain metastasis (BMs) posing a growing clinical challenge with limited treatment options. The brain microenvironment imposes metabolic constraints, including low oxygen levels and lipid scarcity, necessitating adaptive mechanisms for metastatic CRC cells. This study aimed to reveal molecular drivers of CRC BMs.

Material and method

KRAS CRC BMs (n = 9) and liver metastasis (LMs) (n = 6) FFPE samples were subjected to transcriptomic analysis, using RNAseq. FOXM1 and FASN expression were assessed by immunohistochemistry (IHC). CRC cell lines were used to validate transcriptomic results using qRT-PCR and western blot and reporter assays. Tumor microenvironment was simulated by culturing CRC cell lines in astrocyte-conditioned media (A-CM), hepatocyte-conditioned media (H-CM) or hypoxia (1% O₂). Analysis of CM was assessed via a growth factor array assay. Intracranial mouse model was used to assess gene expression within brain environment.

Result and discussion

Transcriptomic analysis showed that FOXM1 was significantly upregulated in CRC BMs compared to LMs and these results were confirmed by IHC. Furthermore, an increase in FOXM1 expression was observed following intracranial injection of CRC cells into mouse brain. CRC cells cultured in A-CM exhibited higher FOXM1 expression than in H-CM, suggesting that brain-derived soluble factors contribute to FOXM1 up-regulation, further supporting the finding that the brain microenvironment actively induces FOXM1 expression. Hypoxia, characteristic of brain conditions, induced FOXM1 expression as well as the transcriptional activity. A growth factor array identified HGF as one of the most abundant factors in A-CM. Indeed, HGF treatment upregulated FOXM1 in CRC cells, indicating a role for HGF in FOXM1-mediated tumor adaptation to the brain

microenvironment. Fatty acid synthesis was shown to play a role in cancer cell adaptation, and we observed FOXM1 correlated with FASN, highlighting a possible role for FOXM1 in metabolic reprogramming essential for CRC BM survival.

Conclusion

FOXM1 is a key regulator of CRC BMs, regulated by brain microenvironment. Furthermore, correlation between FOXM1 and FASN expression suggests it plays a role in the metabolic reprogramming required for CRC cells to survive in the brain microenvironment, making it a potential therapeutic target for CRC BM patients.

EACR25-1303

WNT alterations in CRC: Selfish Apc Vs Altruistic Rspo?

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Introduction

Aberrant WNT pathway activation is a hallmark of colorectal cancer (CRC). The principal mechanism of WNT hyperactivation is mutation of key WNT-genes, including loss-of-function mutations in APC and gain-of-function mutations in RSPO. These mutations cause Wnt hyperactivation in different manners and show differential distribution across CRC subtypes. Recently, Apc mutations in LGR5+ve cells have been shown to give rise to super-competitor cells, whereby mutant cells hold an endogenous fitness advantage provided by the intrinsic and constitutive WNT activation, coupled with suppression of neighbouring wild-type cells' stemness and promotion of their differentiation through secretion of NOTUM. This allows Apc-mutant cells to "fix" and outcompete wild-type cells. Here, we assessed the tumorigenic potential of Rspo3 alteration, compared to Apc mutations.

Material and method

Using mouse models of WNT disruption, we compared the impact of Rspo alterations and Apc mutations on the intestinal epithelium. We also evaluated the WNT mutation landscape in precancerous and cancerous CRC subtypes.

Result and discussion

Comparison of our mouse models showed that Rspo3-mutant lesions are phenotypically different from Apc-mutant lesions. Compared to the polyp formation with dysplasia extending through crypt-villus axis seen in the Apc-mutant models, Rspo3-mutant lesions were characterised by hyperplastic lesions marked by proliferating ectopic crypts. Furthermore, in contrast with the super-competitor nature of Apc-mutations, Rspo3 overexpression favoured Paneth cell differentiation and supported the formation of WNT-dependent ectopic stem cell niches. Given their role as source of WNT ligands, an increase in Paneth cells, coupled with the presence of elevated Rspo3, is believed to create a WNT-high environment that promotes stemness in all cells within the milieu. In accordance, cell competition studies showed that whilst Apc-mutant cells colonise the crypt overtime by outcompeting wild-type cells, Rspo3-mutant cells allow for the co-expansion of both Rspo3-mutant

and wildtype LGR5⁺ve clones. Interestingly, when comparing the distribution of these mutations in human CRC, colitis-associated lesions were found to preferentially select for RSPO-mutations, contrary to sporadic CRC precursor lesions, which have predominantly APC mutations. This may be because an RSPO alteration is more favourable in an environment requiring increased regenerative capacity.

Conclusion

Based on these observations, an Apc selfish versus Rspo altruistic model is proposed. In this, Apc mutations in LGR5⁺ve cells give rise to selfish super-competitors, which act by promoting their own stemness while suppressing that of their neighbouring cells. Conversely, Rspo3-mutant cells' expression of RSPO3 supports their own and their neighbouring cells' stemness by potentiating WNT signalling, through secreted RSPO3 and an expansion of Paneth cells.

EACR25-1308

POSTER IN THE SPOTLIGHT

Senescence Induction Sensitizes Hepatocellular Carcinoma Cells to Immunotherapy

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Introduction

Hepatocellular carcinoma (HCC) remains a major global challenge, requiring innovative therapeutic strategies to improve treatment outcomes. Inducing senescence in cancer cells offers a promising approach for tumor elimination, as senescent cells exhibit unique traits that can be targeted for therapy. However, effective strategies to exploit senescence in HCC remain limited. Our goal is to identify novel therapeutic targets, explore their mechanisms, and apply them to HCC treatment.

Material and method

Gene dependency, expression, and prognostic significance were analyzed using data from the DepMap and TCGA datasets. CRISPR screens were conducted to identify genes regulating cell proliferation and synthetic lethal candidates of Aurora B. Colony formation assays were used to assess cell viability. Senescence was confirmed through mRNA sequencing and β-galactosidase staining. Subcutaneous tumor model was established to evaluate the therapeutic efficacy of Aurora B inhibition and its combination with anti-PD-1 treatment. HLA expression, PD-L1 levels, and T cell activity were analyzed by FACS.

Result and discussion

Starting with 1,021 genes, we identified 26 HCC cell-dependent genes that are highly expressed in tumor tissues and associated with poor prognosis. To prioritize clinically translatable targets, we further refined this selection based on targetability and clinical relevance, highlighting Aurora B as a promising candidate. A genome-wide CRISPR screen confirmed that Aurora B knockout significantly inhibited HCC cell growth. Notably, Aurora B inhibition induced a strong senescence response. To explore potential vulnerabilities of Aurora B inhibition-induced senescent cells, we conducted a

secondary CRISPR screen, which identified TTK as a synthetic lethal candidate. However, validation experiments revealed that TTK inhibition further enhanced senescence rather than effectively eliminating senescent cells. Interestingly, Aurora B inhibition upregulated HLA and PD-L1 expression, suggesting a potential synergy with immunotherapy. We therefore combined an Aurora B inhibitor with immune checkpoint blockade anti-PD-1, which led to significant tumor growth suppression, accompanied by increased infiltration of CD8⁺ T cells and elevated IFNγ level. In vitro assays confirmed that Aurora B inhibitor-treated HCC cells stimulated CD8⁺ T cells to produce more IFNγ, and CD8⁺ T cells preferentially targeted the senescent cells.

Conclusion

This study demonstrates that immunotherapy is a potent approach to eliminate drug-induced senescent cells. Conversely, Aurora B inhibition may serve as an effective strategy to enhance the efficacy of HCC immunotherapy.

EACR25-1309

Age-associated mitochondrial dysfunction drives CAR-T cell failure

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Introduction

CAR-T cell therapy is one of the most promising cancer treatments developed over the last decades, and its application is expected to grow exponentially in the coming years. However, different known and unknown hurdles are limiting its application and efficacy. In this context, aging is the first factor associated to cancer, but how aging influences anti-tumor responses and CAR-T cell outcomes is largely unknown.

Material and method

To assess the effect of aging on CAR-T cell therapy we have generated Her2 or mCD19 directed CAR-T cells from young and old mice and have used a murine B16-Her2 or EL4-mCD19 model, respectively, to assess their functionality in vivo. Afterwards, to validate the results obtained using the murine models, we have used PBMCs derived from young and old healthy donors, as well as melanoma patients. Furthermore, to determine how the age of the patients and our target pathways influence the responsiveness to CAR-T cell therapy in the clinic we have analyzed publicly available transcriptomic data of responder and no responder patients.

Result and discussion

Here, we show that CAR-T cells generated from aged mice are unable to maintain an appropriate stem-like phenotype, a key feature of successful CAR-T responses. As a result, aged CAR-T cells fail to survive long-term in vivo and control tumor growth upon adoptive cell transfer. We unveil that in aged CAR-T cells cellular NAD levels are depleted, which drives major mitochondrial dysfunctions. Replenishing cellular NAD levels by supplementing NAD precursors and blocking age-

associated NAD consuming enzymes, such as CD38, restores mitochondrial fitness and functionality of CAR-T cells, ultimately leading to an improved long-term persistence *in vivo* and tumor control capacity.

Moreover, human data analysis reveals that both the age of the patient and NAD metabolism determine the responsiveness to CAR-T cell therapy. Finally, by targeting NAD pathways we were able to recover the mitochondrial function of CAR-T cells derived from older adults.

Conclusion

Altogether, our study demonstrates that aging is a limiting factor to successful CAR-T cell responses. Repairing metabolic and functional obstacles derived from age, such as NAD decline, is a promising strategy to improve current and future CAR-T cell therapies.

EACR25-1320

Comparative analysis of regorafenib's effects in human and canine osteosarcoma cell lines: an interdisciplinary and translational perspective on bone tumor therapy

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Introduction

Regorafenib (REG) is a multi-kinase inhibitor used to treat various cancers, including colorectal cancer, gastrointestinal stromal tumors, and hepatocellular carcinoma. Recently, REG's potential has extended beyond human oncology, gaining recognition as a promising therapeutic candidate in veterinary medicine. In this context, the effects of REG are being explored for their relevance in canine osteosarcoma (OSA), a highly aggressive bone tumor in dogs that shares many biological and molecular similarities with human OSA.

Material and method

Three cell line models were used: human primary osteosarcoma ARP-1, human cell line MG63, and canine cell line D17. REG's effects were determined using metabolic assays (MTS), cytometric techniques (Annexin V/PI staining, PI staining for cell distribution), while the bioenergetic shift was monitored using Agilent Seahorse XF Real-Time ATP Rate Assay. Changes in molecular markers, including c-MYC, ERK2, PI3K, and AKT, were analyzed using RT-qPCR, while proteins mTOR and PI3K were detected using Western Blot. Cell invasiveness was tested with a wound-healing assay.

Result and discussion

The lowest IC₅₀ for REG was noted in D17 cells. REG decreases ATP production by inhibiting oxidative phosphorylation (OXPHOS) in all cell lines. In APR-1 and MG63, it also significantly reduces glycolytic activity, suggesting a dual mechanism of metabolic disruption. At molecular levels, REG increased anti-apoptotic markers in human cells but induced pro-

apoptotic markers in canine cells. It also reduced invasiveness across all lines.

Conclusion

REG's dual metabolic disruption, species-specific apoptotic effects, and ability to reduce invasiveness highlight its potential as a targeted therapy for osteosarcoma in humans and dogs. These findings provide insights into its mechanisms of action and translational importance. By adopting an interdisciplinary approach that integrates molecular biology, veterinary and human oncology, and pharmacology, this study bridges the gap between species-specific differences and therapeutic applications.

EACR25-1324

Metastatic ovarian cancer drives fundamental tissue reprogramming in the human omentum

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Introduction

The omentum, a visceral adipose tissue with critical metabolic, immunological, and stem cell functions, is the preferred site for ovarian cancer metastasis. However, its role in maintaining homeostasis and responding to metastatic colonization remains incomplete. To address this, we aimed to generate a human omentum cell atlas and investigate mechanisms underlying cancer-induced organ transformation.

Material and method

Single-cell RNAseq was performed on a clinical cohort of 36 omentum tissue samples from patients with benign disease and (pre-) invasive, treatment-naïve high-grade serous ovarian cancer. Samples were collected from multiple anatomical regions within the omentum. Marker validation was conducted using an independent bulk RNA-seq cohort with matched proteomics, complemented by flow cytometry and spatially resolved confocal immunofluorescence.

Result and discussion

The analysis of >110,000 cells identified 12 cell types and over 50 transcriptionally defined states, with distinct enrichment in benign and metastatic samples. Cells adjacent to tumor nodules were classified as inflammatory, while cells in the tumor core aligned with myofibroblast cancer-associated fibroblasts (CAFs). Cancer-associated mesothelial cells and CAFs shared a gene expression program, including immunosuppressive markers, ECM remodelers, and angiogenic factors.

RUNX1 was distinct to trans-differentiating cells, a population enriched in metastases and originating from normal mesothelial and mesenchymal progenitor cells. Moreover, RUNX1 was absent in primary tumors, suggesting a key role in the differentiation of normal omental cells upon metastatic colonization.

Conclusion

Cancer cells orchestrate cell reprogramming through a repertoire of signaling factors affecting both proximal and distal omental tissue. Our cell atlas highlights the cellular and molecular determinants of omentum homeostasis, revealing extensive plasticity and cancer-driven cellular reprogramming.

EACR25-1351

Characteristics and Effects of Stemness Genes in Primary and Recurrent Ovarian Cancers and Ovarian Cancer Stem Cells

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Introduction

Ovarian cancer (OC) is one of the most lethal gynecological malignancies due to its asymptomatic progression and late-stage diagnosis. While primary OC responds to initial chemotherapy, recurrence is common and often associated with chemoresistance and poor prognosis mostly caused by cancer stem cells (CSC). Primary (POC) and recurrent (ROC) ovarian cancers exhibit distinct molecular profiles, with recurrent tumors often demonstrating enhanced stemness and resistance mechanisms. NANOG, SOX2, PROM1, ALDH1A1, ALDH1A2, and ALDH1A3 are key stem cell markers and potential therapeutic targets that have been implicated in OC progression, chemoresistance, and tumor-initiation.

Material and method

The genes of interest (GoI) were examined due to their roles in stem cell characteristics. The methodology includes data collection, processing, normalization and analysis. In data collection step, RNA-seq datasets including ROC and POC from TCGA (The Cancer Genome Atlas), and ovarian CSC (OCSC) from GEO (Gene Expression Omnibus) were collected. These datasets provide FPKM values (Fragments Per Kilobase of transcript per Million mapped reads) which is a widely used method for accurately quantifying gene expression, enabling comparative analysis across different conditions for the genes. Data were transferred into R (v4.4.1) for analysis. DESeq2 analysis was performed to identify differentially expressed genes between conditions, with a focus on log fold change transformation and p-value distribution to understand the statistical significance. The analysis examined the distribution of all genes also the GoI. For the further analysis of the GoI, min-max scaling and log2 transformation were applied. This normalization process rescaled the values to a range of 0 to 1 with preserving differences between datasets, allowing for the identification of those with significant changes in expression. Therefore, it was identified the significant expression patterns and potential biomarkers. Lastly,

gene ontology and network analysis were performed to identify the biological pathways of the GoI.

Result and discussion

Bioinformatics analysis of multiple datasets revealed that the expression levels of NANOG and SOX2 were inversely correlated between POC and ROC groups, with significantly higher levels observed in ROC.

Additionally, a positive correlation was found between the expression of NANOG, SOX2, and PROM1 in both OCSC and ROC, suggesting these genes may contribute to tumor aggressiveness and chemoresistance. ALDH1A1 and ALDH1A3 showed a positive correlation with ROC, similar to NANOG, SOX2, and PROM1, indicating a potential link between these genes and tumor progression.

Conclusion

Our findings suggest that the increased expression of NANOG, SOX2, ALDH1A1 and ALDH1A3 in ROC may play a critical role in tumor relapse and importance as potential biomarkers for tumor aggressiveness.

EACR25-1352

Mechanisms of AICAr-Induced Acute Myeloid Leukemia Differentiation: Role of Ribonucleotide Metabolism and Replication Stress

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Introduction

Acute myeloid leukemia (AML) is a hematological malignancy characterized by the proliferation of immature blasts arrested at specific differentiation stages, making differentiation-based therapies a promising alternative to traditional cytotoxic treatments. Our previous study demonstrated that 5-aminoimidazole-4-carboxamide ribonucleoside (AICAr) induces AML differentiation through pyrimidine synthesis inhibition, similar to the DHODH inhibitor brequinar. We also found that cytarabine (AraC), a standard chemotherapy agent, promotes differentiation via replication stress and Chk1 activation, a mechanism shared with pyrimidine synthesis inhibitors. However, the precise link between DNA damage, replication stress, and differentiation remains incompletely understood.

Material and method

Human AML cell lines U937, THP-1, and MOLM-13 were cultured in RPMI-1640 or αMEM medium supplemented with ribonucleosides (rNs) and deoxyribonucleosides (dNs). Metabolomic profiling was performed using liquid chromatography-mass spectrometry (LC-MS). Cell viability, cell cycle distribution, and differentiation were assessed using multiparametric flow cytometry, while Western blotting was used to evaluate ribonucleotide reductase subunit M2 (RRM2) and Wee1 signaling. Inhibition studies were conducted using COH29 and hydroxyurea (HU) as inhibitors of

ribonucleotide reductase (RNR), MK1775 as a Wee1 inhibitor, and siRNA targeting Wee1.

Result and discussion

Metabolomic analysis revealed that pyrimidine and purine nucleotides were among the most differentially regulated metabolites, decreasing in brequinar- and AICAr-treated samples while increasing in AraC-treated samples. When cells were cultured in αMEM supplemented with 10 mg/ml of both rNs and dNs, the effects of all tested agents on proliferation, differentiation, and cell cycle arrest were completely abolished. All agents increased RRM2 expression, suggesting a role for RNR in differentiation. Inhibition of Wee1 kinase with MK1775 blocked RRM2 upregulation and AML differentiation without preventing S-phase arrest. Similar effects on differentiation and the cell cycle were observed in cells treated with COH29, a recently described RNR inhibitor. However, HU, a well-known RNR inhibitor, failed to prevent differentiation. Experiments are underway to further investigate the role of RNR in cells with downregulated expression.

Conclusion

Our findings suggest that ribonucleotide metabolism regulates AML differentiation, with Wee1 and RNR activation occurring downstream of replication stress.

EACR25-1356

CFTR modulator drugs can reduce the invasive properties of colorectal cancer cells

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Introduction

Colorectal cancer (CRC) remains a leading cause of cancer-related mortality, driven by complex genetic, epigenetic, and microenvironmental factors. Recent findings implicate the cystic fibrosis transmembrane conductance regulator (CFTR) ion channel in CRC progression, as CFTR levels are notably reduced in sporadic CRCs, particularly in advanced and metastatic tumors, correlating with poorer patient outcomes.

Additionally, cystic fibrosis (CF) patients, who carry CFTR mutations, have a 6-fold increased risk of early-onset CRC. Given recent advances in small-molecule modulators that restore CFTR function in CF patients, this study explored the potential of repositioning these modulators to address CFTR downregulation in sporadic CRC.

Material and method

Using a panel of CRC cell lines, we investigated whether CFTR modulators can increase CFTR functional expression in cells with various genetic backgrounds and

whether such improvements could reduce their oncogenic properties.

Result and discussion

Our data show that treatment with the CFTR folding correctors VX-661 and VX-445 led to a significant, approximately three-fold increase in CFTR abundance in CRC cells expressing reduced but detectable levels of the channel. Additionally, these treatments significantly reduced the migratory and invasive behavior of Caco-2 and DLD-1 cells, particularly when combined with the CFTR potentiator VX-770.

Conclusion

Our findings suggest that CFTR modulators may hinder the oncogenic properties of CRC cells. Further *in vivo* studies are necessary to fully assess their potential benefits for repositioning as a CRC treatment.

EACR25-1362

CDK6 inhibition coupled with Vitamin D receptor activation synergistically promotes AML differentiation and cell death

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Introduction

Acute Myeloid Leukemia (AML), the most prevalent acute leukemia in adults, is a devastating cancer, with a 5-year survival rate of 35–40% in patients under 60 years and only 5–15% in older patients. AML is a heterogeneous disease caused by acquired genetic modifications in stem and progenitor cells. Leukemic cells fail to differentiate and invade the bone marrow, interfering with the renewal of mature blood cells. In this pathology, relapse and resistance to therapies are the primary causes of mortality. Many patients are not eligible for conventional intensive chemotherapy. The standard treatment for these unfit patients is a combination of venetoclax (a BCL2 inhibitor) in association with azacytidine (a DNMTs inhibitor). However, at least 60% of those patients will relapse. Therefore novel treatments remain critically needed to eradicate AML cells, including leukemic stem cells.

Material and method

AML cell lines and primary samples were treated with a combination of CDK6 inhibitors and VDR agonists. Differentiation and cell death were assessed via flow cytometry, caspase activity assays, and RNA sequencing. Clonogenic potential was evaluated using colony-forming unit assays. To better understand our transcriptomic results, chromatin accessibility was analyzed through ATAC-seq. Proliferation and cell death assays were conducted to assess venetoclax sensitivity in resistant models.

Result and discussion

AML cells, including resistant cells, are all sensitive to CDK6 inhibition. We demonstrate that the combination of CDK6 inhibition coupled with the activation of the VDR synergistically induces AML cell differentiation and decreases their clonogenic potential. Furthermore, this combination induces caspase-dependent cell death in AML cell lines and primary cells. Transcriptomic analyses confirm the activation of differentiation signatures, the decreased expression of genes associated with immaturity (HSPC signatures), and the deregulation of genes implicated in apoptosis signatures and other death and stress pathways following treatment. The combination induces extensive chromatin accessibility rearrangements. These accessibility changes correlate with transcriptomic alterations, highlighting the role of chromatin regulation in these changes. We also demonstrate that the combination increases venetoclax-induced cell death. We challenged several models of venetoclax-resistant cells, and our results indicate that some mechanisms of resistance to venetoclax could be completely bypassed by the triple therapy.

Conclusion

In conclusion, the combination of CDK6 inhibitors with VDR agonists exerts pleiotropic effects on leukemic cells including the restoration of cell differentiation, the decrease of immature potential and the promotion of cell death by apoptosis. Considering that all these molecules are FDA-approved drugs, our study advocates for the repositioning of CDK4/6 inhibitors and vitamin D in AML.

EACR25-1364

TGF- β induced EMT may promote tolerance of mitotic errors and genomic instability through inhibition of p53/p21 pathway

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Introduction

Transforming growth factor β (TGF- β) is a cytokine constitutively present in tumor microenvironment that has been associated with tumor progression, metastasis and drug resistance. TGF- β inhibits proliferation of normal epithelial cells, while inducing epithelial to mesenchymal transition (EMT) in epithelial cancer cells. We previously demonstrated that cells undergoing EMT induced by TGF- β are more prone to mitotic dysfunctions. Therefore, in this study we aimed to understand the mechanisms by which TGF- β induced EMT promotes tolerance of mitotic errors and genomic instability.

Material and method

MCF10A and RPE1 cells were treated or not with TGF-beta as well as different mitotic stressors and 5-fluorouracil. Number of chromosomes, DNA damage and P53/P21 expression were assessed through different methods.

Result and discussion

TGF-beta treatment promoted tolerance of mitotic errors and facilitated the appearance of cells with increased ploidy after challenging them with different mitotic stressors in both MC10A and RPE1 cell lines. In line with these results, DNA damage as determined by the number of γ H2AX foci was increased after TGF-beta treatment for 21 days. In contrast, p53 and p21 protein levels were downregulated as determined by western blot and timelapse imaging of RPE-1 cells expressing p53-NeonGreen and p21-mKate reporter genes.

Conclusion

TGF-beta may promote tolerance of mitotic errors and aneuploidy by downregulating p53 and p21 protein levels.

EACR25-1365

Oncogenic variants in GNAQ and GNA11 cause dysregulation of calcium signalling in uveal melanoma

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Introduction

Uveal melanoma (UM) is the most common eye cancer in adults, and it originates from melanocytes in the eye. Mutations in GNAQ and GNA11 genes are initiating events in 90% of UM. Up to 50% of patients develop lethal metastasis to the liver, where cancer cells can remain dormant (quiescent) for a long period before expanding. Identification of approaches to intercept and treat metastatic UM remains an unmet clinical need. GNAQ and GNA11 oncogenic variants also cause mosaic disorders characterised by vascular and pigmentary malformations, where we have previously shown their role in hyper-activating intracellular calcium pathway. Here we aimed to assess UM cells' dependence on mutant GNAQ/GNA11 and the role of these variants in shaping calcium signalling at different stages of tumour progression. As calcium pathways control several oncogenic properties, this analysis may unveil new specific targets.

Material and method

We developed allele-discriminating small interfering RNAs (siRNAs) to specifically silence mutant GNAQ or GNA11 in human UM cell lines. Constitutive calcium signalling activity and intracellular calcium accumulation were measured by IPone assay and fluorescence detection in calcium dye-loaded cells, respectively. Proliferation and apoptosis were measured by an automatic plate imager. We implemented a double genetic reporter strategy to simultaneously track the whole UM cell population and highlight cells undergoing quiescence. We developed a co-culture system of labelled UM cells

with human immortalised hepatocytes, and the fraction of UM cells undergoing dormancy was tracked over time. Calcium oscillations in individual cells loaded with calcium dye were imaged, and data processed via a tailored Fiji/Python pipeline.

Result and discussion

We show that variant GNAQ or GNA11 knock-down reduces both constitutive and ligand-induced calcium signalling in UM cells. This leads to decreased proliferation and triggers apoptosis. Comparison with wild type melanocytes also reveals that UM cells have altered calcium flux across the plasma membrane. We go on to show that co-culture of UM cells with hepatocytes significantly increases the fraction of quiescent UM cells over time compared to co-culture with control cells. Preliminary investigation of the role of hepatocytes in promoting dormancy suggests that calcium signalling may be involved in this process, as suggested by analysis of differential calcium signal activity in dormant and proliferative UM cells.

Conclusion

We demonstrated that GNAQ/GNA11 mutations aberrantly activate calcium signalling in both vascular mosaic diseases and UM. Recapitulating *in vitro* the interaction between UM cells and hepatocytes, we modelled metastatic dormancy and monitored alterations in calcium signalling. This study paves the way to identification of new therapeutic targets for interception of dormant disseminated cells and prevention of metastatic disease.

EACR25-1369

Patient-derived organoid models to investigate the role of tryptophan in colorectal cancer progression

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Introduction

Diet plays a crucial role in colorectal cancer (CRC) development and progression, influencing gut microbiome composition, inflammation, and metabolic pathways that contribute to tumorigenesis. Tryptophan is an essential amino acid and a regular supply via certain foods such as fish, meat, milk, eggs and nuts, is critical for the human body. It is mainly metabolized via the kynurenine pathway, generating metabolites with both pro- and anti-inflammatory properties. The kynurenine-

to-tryptophan (Kyn/Trp) ratio and several kynurenine metabolites have been associated with CRC progression and survival outcomes. Within the FOCUS Consortium, a large cohort of 2,102 prospectively followed stage I–III CRC patients, we have previously shown that tryptophan was significantly associated with improved all-cause mortality. This study therefore aims to elucidate the molecular role of tryptophan in CRC progression using patient-derived organoid models.

Material and method

Organoids were established from patients with stage I–III CRC who underwent primary surgery at the Heidelberg University Hospital, Germany. CRC organoids were treated with 100 µM tryptophan for up to five days. Microscopic imaging, Calcein-AM staining, and CellTiter-Glo 3D viability assays were performed to assess cell proliferation. Western blot analysis was conducted to evaluate the expression of PCNA (proliferating cell nuclear antigen), a marker for cell proliferation, and IDO1 (indoleamine 2,3-dioxygenase 1), the rate-limiting enzyme that catalyzes the initial step of the kynurenine pathway by converting tryptophan into kynurenine.

Result and discussion

The quantification of organoid colonies showed a slight, non-significant decrease in cell proliferation following tryptophan treatment. Additionally, the results of the Calcein-AM staining analysis also indicated a decrease in proliferation of organoids treated with tryptophan. CellTiter-Glo assays showed a significant decrease in proliferation at day 5 in organoids from stage III patients. Furthermore, a significant reduction in PCNA expression in organoids from stage I and II CRC patients after three days, and in organoids from stage III CRC patients after five days was observed. IDO1 levels were significantly elevated in advanced CRC stages compared to stage I, suggesting enhanced kynurenine pathway activation in later stages. Notably, IDO1 expression did not increase following tryptophan treatment.

Conclusion

Our findings suggest that tryptophan may have an anti-proliferative effect on CRC organoids. This aligns with previous studies linking higher tryptophan levels to improved prognosis in CRC patients. Future research will focus on analyzing the impact of kynurenine pathway metabolites on CRC proliferation and exploring potential therapeutic strategies to modulate this metabolic pathway for improved patient outcomes.

EACR25-1377

Modelling multiple FGFR1 mutations uncovers modulatory effects and novel tumorigenic mechanisms in glioneuronal tumors

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Introduction

Genetic alterations in fibroblast growth factor receptor (FGFR) genes have been associated with a broad spectrum of human diseases, from developmental disorders to cancer. In a previous report, we described a germline FGFR1 variant (R661P) in a family with Dysembryoplastic neuroepithelial tumors (DNETs), a subgroup of pediatric low-grade glioneuronal tumors, with secondary oncogenic somatic hits (N546K or K656E) in cis identified in the tumors of those individuals. The pattern of multiple FGFR1 mutations was confirmed in therapy-naïve sporadic cases, suggesting intrinsic mechanisms of selective pressure toward FGFR1 multiple mutational events arising in the context of a quiet genome. This occurrence of intragenic multiple mutations in low-grade, low mutation-burden and predominantly therapy-naïve tumors, represents a striking and unexplained phenomenon.

Material and method

Stable inducible (Flp-In T-REx®) HEK293 cell lines expressing FGFR1-BirA*-Flag fusion proteins have been generated. These cell lines have been used for Proximity-dependent Biotin Identification (BioID) coupled to Mass Spectrometry (MS)-based interactome profiling of FGFR1 Wild-type (WT) protein, single (germline R661P and oncogenic N546K, K656E) and double (N546K/R661P and K656E/R661P) mutants. To study the impact of FGFR1 mutations in a glioma background the human oligodendrogloma (HOG) cell line has been selected and engineered through CRISPR-Cas9 to obtain FGFR1 single- and double-mutant clones.

Result and discussion

The proximity interactome profiling revealed both unique and shared preys among FGFR1 mutants and with the WT protein. The germline R661P clustered differently, compared to the hotspot mutants, both single and double. Analysis of interactome data and western blot assays revealed novel oncogenic functionality for the oncogenic mutations N546K and K656E, linked to altered mechanisms of receptor turnover and increased levels of protein accumulation. Notably, we identified modulatory roles played by the R661P variant, hampering the oncogenic potential of N546K and K656E mutants by reducing affinity for downstream effector PLC γ and rescuing receptor degradation. Notably, the R661P mutation alone abolished the self-renewal capacity of oligodendrogloma cells, an effect that was rescued in double mutant clones. In R661P cells, differential

expression analysis revealed downregulation of genes involved in neurodevelopment and neuro-glial cell fate decisions, which was overcome in the double mutants, unveiling molecular defects associated with the variant R661P during brain development and highlighting potential novel mechanisms of early onset brain tumorigenesis driven by FGFR1 multiple mutations.

Conclusion

This study sheds light on the oncogenic effects associated with multiple FGFR1 alterations and their recurrence in low-mutation burden and therapy naïve brain tumors.

EACR25-1387

Mevalonate Pathway Suppresses Ferroptosis and Promotes Liver Cancer via CoQ10 Production and Selenocysteine-tRNA Modification

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Introduction

Hepatocellular carcinoma (HCC), particularly the steatotic subtype linked to metabolic dysfunction-associated steatotic liver disease (MAFLD), poses a significant therapeutic challenge due to its aggressive nature and rising prevalence. Ferroptosis, an iron-dependent cell death mechanism driven by lipid peroxidation, offers potential for HCC treatment, yet cancer cells exploit pathways like glutathione peroxidase 4 (GPX4)/glutathione and ferroptosis suppressor protein 1 (FSP1)/coenzyme Q10 (CoQ10) to evade ferroptosis. This study investigates novel targets to induce ferroptosis in both non-steatotic and steatotic HCC.

Material and method

Multi-omic screening identified the mevalonate pathway enzyme, mevalonate diphosphate decarboxylase (MVD), as a key target. Genetic ablation of TRSP (encoding selenocysteine-tRNA) or TRIT1 (tRNA i6A37 modification writer) and pharmacological inhibition of MVD using 6-Fluoromevalonate or Atorvastatin – a clinical inhibitor of the mevalonate pathway – were evaluated in HCC cell lines and mouse models, including steatotic HCC. Combination therapy with tyrosine kinase inhibitors (TKIs) and PD-1 antibody was tested.

Result and discussion

Isopentenyl pyrophosphate (IPP), a metabolite directly generated by MVD, was critical for CoQ10 synthesis and i6A37 selenocysteine-tRNA modification, stabilizing selenocysteine-tRNA to enable translation of selenoproteins, including GPX4. Disruption of TRSP or TRIT1 impaired selenoprotein production, inducing ferroptosis and suppressing tumor growth. Targeting MVD via 6-FMEV or Atorvastatin – which suppresses upstream mevalonate pathway activity – reduced IPP/CoQ10, destabilized tRNA modification, and triggered ferroptosis. *In vivo*, 6-FMEV monotherapy or combined with TKIs inhibited HCC progression, even in steatotic HCC models. Notably, Atorvastatin also demonstrated

anti-tumor efficacy, underscoring the therapeutic relevance of mevalonate pathway inhibition. This highlights a novel link between the mevalonate pathway, selenoproteins translation, and ferroptosis evasion. Dual targeting of CoQ10 and selenoproteins pathways via MVD or upstream inhibitors offers a synergistic strategy against HCC.

Conclusion

MVD inhibition disrupts IPP-dependent CoQ10 synthesis and selenocysteine-tRNA modification, sensitizing HCC to ferroptosis. The efficacy of 6-FMEV and Atorvastatin – both alone and combined with TKIs – underscores the potential of mevalonate pathway inhibition as a therapeutic strategy for non-steatotic and steatotic HCC, providing actionable insights for drug repurposing and combination therapies.

EACR25-1389

ROBO receptors act as tumor suppressors in cholangiocarcinoma

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Introduction

ROBO receptors were discovered as axon-guidance-cues during neuronal development, but were found to be deregulated in many cancers in a context-dependent manner. Genetic alterations resulting in deletion or truncation of ROBO receptors were identified in the invasive form of cholangiocarcinoma (CCA) but not the precursor lesions (Goeppert et.al, 2022). CCA is a rare but highly aggressive malignancy of the bile duct with inadequate therapy options and poor prognosis. The aim of this project was to functionally characterize ROBO receptors in CCA.

Material and method

ROBO1 and ROBO2 were introduced into CCA cell lines with a doxycycline-inducible expression system by lentiviral transduction. Functional assays were performed including viability, migration, and colony formation. RNA-seq analysis was performed to dissect downstream signaling pathways. The proximity-based protein labeling method BioID was used to identify interacting partners of ROBOs. Hydrodynamic-tail-vein injections (HDTV) in murine CCA models served to assess the function of ROBO1 and ROBO2 *in vivo*.

Result and discussion

ROBO1 and ROBO2 reduced cell viability, migration, and clonogenicity of CCA cells *in vitro*. RNA-seq revealed a decrease in cell cycle and DNA-replication whereas an increase in interferon response. BioID identified candidate interaction partners of which the majority is involved in MAPK signaling. *In-vivo* experiments with HDTV showed strong tumor suppressor features of ROBO1 and ROBO2. Mice with ROBO1/2 overexpression had no tumors or significantly less tumors compared to controls. Tumors expressing ROBO1/2 presented limited tumor stroma by reduced fibrosis and inflammation. In addition, a shift in the immune cell

phenotype was observed. ROBO receptors limit tumorigenicity in vitro and in vivo and restrict tumor stroma by means of reduced fibrosis and immune cell modulation. Further experiments will reveal the molecular mechanisms of ROBO downstream signaling. Therefore, our research on ROBO might be useful to provide better therapy options to patients with CCA.

Conclusion

ROBO receptors limit tumorigenicity in vitro and in vivo and restrict tumor stroma by means of reduced fibrosis and immune cell modulation. Further experiments will reveal the molecular mechanisms of ROBO downstream signaling. Therefore, our research on ROBO might be useful to provide better therapy options to patients with CCA.

EACR25-1391

Effects of Asparaginase-induced amino acids deprivation on Triple Negative Breast Cancer cell lines

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Introduction

Asparaginase (ASNase), a first-line drug for Acute Lymphoblastic Leukemia (ALL), exerts its anticancer activity hydrolyzing Asn and glutamine (Gln), two conditionally essential amino acids for ALL cells. Previously, we showed that ASNase affects cell cycle, growth, clonogenicity and proliferation in 786-O (renal) and A549 (lung) adenocarcinoma cell lines. Here, we expand our studies to triple negative breast cancer (TNBC), an aggressive tumor lacking molecular targets (ER, PR, HER2), with limited treatment options. We tested ASNase on two TNBC cell lines, BT549 and MDAMB231, to evaluate a possible cytostatic effect of Asn and Gln deprivation.

Material and method

To evaluate the effect of ASNase on proliferation, the doubling time (DT) and the inhibition of clonogenic efficiency (IC50) were determined as a function of increasing ASNase concentration after 72h or 10 d, respectively. Cell cycle analysis was done in the presence of increasing doses of recombinant ASNase. Newly synthesized DNA was marked by adding 5-ethynyl-2'-deoxyuridine (EdU), total DNA was marked using FxCycle (Invitrogen). The molecular effect of ASNase treatment was evaluated by western blot by analyzing canonical markers of drug sensitivity (ASN-synthetase, ASNS and GLN-synthetase, GS) and drug response (Akt, ERK and pS6) only on BT549, as no significant changes in cell cycle were observed in MDAMB231.

Result and discussion

In both cell lines, proliferation was significantly inhibited both in the short term (DT, CTR vs. 1 U/ml ASNase, BT549 18.3 ± 2.7 h vs. 54.8 ± 7.5 h, $p < 0.0001$, and MDAMB231 21.4 ± 3.4 h vs 37.5 ± 5.1 h, $p < 0.0001$), and in the long term (IC50, BT549 0.044 ± 0.003 U/ml, MDAMB231 0.30 ± 0.20 U/ml). The canonical response of the cell cycle to ASNase consists of a blockade in G1-

S transition, visible as an accumulation of cells in G1. In BT549, we observed an accumulation of cells negative for newly synthesized DNA (EdU-) in the S-phase, which suggests a reduced efficiency in DNA synthesis. The same effect was observed in our previous work on 786-O. No perturbation of MDAMB231 cell cycle was observed. Molecular analysis of the treatment effect in BT549 resulted in a significant reduction in S6 activation from 0.5 U/ml ($p = 0.0008$) upward which implies a reduced activation of the mTOR pathway, confirming the effect of ASNase on BT549 cell metabolism. Starting from the same dose of ASNase, ASNS and GS were reduced significantly as well ($p = 0.03$ and $p < 0.0001$, respectively), in accordance to the observed sensitivity of this cell line to the drug. However, no significant changes were seen in canonical upstream regulators of mTOR (Akt and ERK, $p > 0.05$), which suggests a possible alternative pathway of downregulation of mTOR mediated by ASNase in BT549.

Conclusion

We are currently performing single-cell gene expression analysis to better understand the mechanism of ASNase on BT549 cells and developing BT549 organoids to further investigate ASNase effects in a 3D cancer model.

EACR25-1411

Proteo-transcriptomic characterization of the CD14-CD34+ stem and progenitor cells in chronic myeloid leukemia

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Introduction

Deep characterization of the leukemic stem cells (LSC) in chronic myeloid leukemia (CML) may allow for the identification of novel therapeutic targets ultimately leading to a permanent cure of the disease. Compared to scRNA-seq, which provides the information of transcriptome at a single cell level, CITE-seq additionally pairs it with protein expression, which may enable the identification of cell phenotypes, subsequent FACS-based isolation as well as functional characterization. Detection of BCR-ABL1 transcripts, considered the only definitive marker to distinguish CML LSC from healthy hematopoietic stem cells (HSC), remains a significant challenge with current high-throughput 3' end capture-based single-cell sequencing methods.

Material and method

For the present study, we performed CITE-seq analysis of the expression of 597 genes and 51 proteins in >70,000 hematopoietic stem and progenitor cells (HSPCs) from 16 chronic phase CML patients and five healthy donors. Additionally, we combined it with an in-house optimized method allowing parallel BCR-ABL1 expression detection at the single-cell level.

Result and discussion

The results revealed pronounced expansion of mainly erythroid and myeloid progenitors within the CML CD34+ compartment, and high heterogeneity within the traditionally defined CD34+CD38-/low LSC compartment. A detailed analysis of the most immature CD34+ CD38-/low cells identified a subset of cells with a CD45RA-cKIT-CD26+ TKI resistant phenotype. The potential LSC showed expression of previously reported cell-surface markers CD25 and CD26, as well as some unreported gene and protein markers that allowed clear distinction of these cells from their healthy counterparts. Unlike HSC, the immature LSC showed upregulated expression of TIM3 at protein level and the von Willebrand factor gene (VWF) at the transcriptome level. Upregulation of VWF expression within the stem cell population may be linked to the aberrant myeloid-biased nature of the disease. High expression of TIM3 on the other hand could be beneficial for elimination of immature LSC in CML.

Conclusion

In conclusion, we performed detailed proteo-transcriptomic analysis of CML stem and progenitor cell compartment. The findings may have implications for the understanding of differences between leukemic and normal hematopoiesis as well as for the phenotypic definition of CML LSC.

EACR25-1427

Spastin Functions in Glioblastoma Multiforme Migration Through Focal Adhesion Kinase Signaling Pathway

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Introduction

Glioblastoma Multiforme (GBM) is a highly proliferative and invasive type of aggressive brain tumor. Since highly migratory GBM cells are known to be more resistant to therapy than proliferative ones, identifying the proteins involved in the invasion process is critical for improving existing therapies. Previously, we discovered that cis trans isomerase enzyme Pin1 interacts with microtubule-severing protein Spastin through phosphorylated Thr292Pro&Thr303Pro Pin1 recognition motifs located in its microtubule-binding domain (MBD), directing it to actin filaments upon this interaction.

Material and method

In order to investigate roles of Spastin phosphorylation in its MBD and molecular signaling pathways targeting Spastin to actin filaments and thereby triggering migration of T98G GBM cells, we analyzed the expression levels of proteins having roles in migration and invasion mechanisms upon Spastin depletion by high-throughput membrane arrays. To reveal signaling pathways connected to Focal Adhesion Kinase (FAK) activation, firstly, FAK expression and phosphorylation status were investigated via immunoblotting upon Spastin silencing or overexpression of phospho-mutant (M87_mutA) or phospho-mimetic (M87_mutD) Spastin mutants. We also examined the localization of FAK by immunocytochemistry analysis. Furthermore, immunoblotting was also used to investigate the level of

expression and/or release of proteins hypothesized to be involved in FAK-related migration or proliferation pathway owing to the absence or phosphorylation of Spastin.

Result and discussion

The results indicated that Spastin could trigger GBM cell migration and invasion only when it is phosphorylated in the MBD and its role in GBM migration is related to Focal Adhesion Kinase (FAK) activation via phosphorylation. Spastin silencing decreased the FAK phosphorylation, whereas M87_mutD overexpression triggered the autophosphorylation of FAK. Moreover, ICC results demonstrated that while inactivated FAK moved to the nucleus upon Spastin silencing, FAK was directed to the cell membrane to become fully activated only when M87_mutD was overexpressed. Western blot analysis revealed that phosphorylated Spastin triggered migration through SPARC-mediated FAK activation; whereas proliferation could be stimulated by reduced expressions of p53 and Snail upon Spastin silencing.

Conclusion

In conclusion, via reduction of p53 expression, highly migratory T98G GBM cells are converted into proliferative form which is known to be more sensitive to chemotherapeutic treatment and Spastin playing a key role in transition of GBM cells from migrative form to proliferative form could be a novel therapeutic target for GBM treatment.

EACR25-1430

Investigating the effects of culture medium derived from HEK293 cells expressing DDB2^APCNA- protein on microenvironment

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Introduction

DNA damage binding protein 2 (DDB2) is crucial in the early step of DNA damage recognition of the nucleotide excision repair (NER), which is triggered by UV-induced damage. DDB2 interacts with PCNA through a phylogenetically conserved sequence called PIP-box. This interaction is essential for proteosome-dependent degradation of DDB2 after recognizing the DNA damage, a prerequisite for further NER components recruitment. A mutation in the DDB2 PIP-box disrupts its ability to bind PCNA, resulting in defective DNA repair and an accumulation of DDB2 protein within cells. Additionally, when these cells are exposed to UV radiation, the efficiency of NER decreases, resulting in a more aggressive cellular phenotype.

Material and method

This work aims to study the influence of DDB2 in the inflammatory tumor microenvironment and monocyte differentiation. We used THP-1 cell line (monocytes), grown with a medium derived from the HEK293 control culture or HEK293 stably expressing DDB2 wild-type

protein or others unable to interact with PCNA (DDB2^APCNA-), both non-irradiated and collected at different times after irradiation with UV-C (10J/m²), to evaluate if the medium had any molecules inducing macrophage differentiation. The cell morphology and adhesion were assessed at various incubation times using gentian violet staining and then observed through an optical microscope. Immunofluorescence analysis was used to examine CD80 and CD68, which are markers for monocytes and macrophages. Western blot analysis was performed in all irradiated HEK293 cell lines to evaluate inflammatory protein levels.

Result and discussion

Observing the cells under the microscope highlighted that THP-1 cells grown with a medium derived from irradiated HEK293 DDB2^APCNA- adopt an irregular conformation with the presence of multiple extensions/filaments. Immunofluorescence analysis showed that in THP-1 cells treated with the medium from the DDB2^APCNA- cell line, both non-irradiated and at 3- and 7-days post-irradiation the signal for CD80 intensified in the cell membrane. An increase in the fluorescence signal specific for CD68 was also observed, in the intracellular compartment and on the plasma membrane. In both cases, the cells adopt progressively to a more irregular morphology. Preliminary results showed an increasing trend on IL-6, IL-8, and CD38 in irradiated HEK293 cells that express stably DDB2^APCNA- protein.

Conclusion

Taken together, our results suggest that the loss of DDB2-PCNA interaction may also influence the microenvironment.

EACR25-1431

DDB2-PCNA interaction is involved in maintaining genome stability in human UV-irradiated cells

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Introduction

The DDB2 protein plays a crucial role in the initial step of global genome Nucleotide Excision Repair (NER). Our previous studies have demonstrated that the interaction between DDB2 and PCNA is essential for efficient DNA UV-C lesion removal. Cells expressing a mutant form of DDB2 that cannot bind PCNA (DDB2^APCNA-) exhibit delays in the NER process and display tumor-like characteristics, including the ability to form colonies after UV-C exposure.

Material and method

We conducted clonogenic assays in soft agar using HEK293 cells, both non-irradiated and UV-C irradiated (10 J/m²). These included untransfected cells as well as those expressing either wild-type or mutant DDB2. No colony grew with non-transfected cells or cells expressing the mutated protein while two resistant clones (called Clone 1, and Clone 2) were isolated from cells expressing the mutant protein and subsequently expanded. To assess protein expression levels, flow cytometry, microscopy, and Western blotting (WB)

experiments were performed. Additionally, iCELLigence biosensor technology was used to evaluate cell adhesion, Boyden chamber and wound healing assays were employed to assess cell migration.

Result and discussion

The analysis of the clones is aimed to investigate their possible tumor-like phenotype in comparison with their parental cell line. To study the cellular proliferation, we analyzed the ability to form colonies and the results show that Clone 2 exhibited higher capability after cisplatin and caffeine treatment. The UVC-resistant clones demonstrated reduced adhesion in the first two hours after seeding and faster wound closure. Moreover, both the clones in comparison with their parental cell line displayed increased levels of CD117, CD44 and OCT4, markers associated with cancer progression and cellular plasticity. In the complexity of cancer, an important role is played by the immune system and in particular macrophages. Recent results suggested that the supernatant derived from Clone 1 culture had an effect on THP-1 adhesion, suggesting that factors secreted by these cells might promote monocyte differentiation. Finally, the capacity to form 3D structure was analyzed, and the clones formed spheroids on agar faster than their parental cell line. WB analysis revealed differences in Epithelial to Mesenchymal transition (EMT). SNAI1, Vimentin and ZEB1 expression is higher in parental cell line than in both the clones; instead, NF- κ B showed the highest value in Clone 1.

Conclusion

These findings confirmed that the DDB2⁺PCNA- cell lines exhibit tumor-like features and highlight interesting differences between the two clones. The heterogeneity observed among clones requires further investigation in future studies.

EACR25-1441

The BASP1 signaling protein interferes with the WNT signaling pathway

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Introduction

The MYC oncoprotein represents a transcription factor that regulates crucial cellular processes and is therefore highly regulated by several pathways. For instance, in colorectal cancer the WNT signaling pathway is perturbed resulting in aberrant MYC expression. We found that the brain acid-soluble protein 1 (BASP1), which is downregulated in a variety of MYC-dependent cancer cells, strongly interferes with the WNT signaling pathway not only leading to low MYC protein levels but also reduced MYC-induced cell transformation.

Material and method

Using the human colon cancer cell line SW480 featured by high MYC expression and a silenced BASP1 gene, we further investigated the putative tumor-suppressive property of BASP1. Three different cell types were established in which BASP1 is re-expressed. Whereas two cell lines are characterized by ectopic BASP1 expression, the third one was generated by CRISPR-mediated BASP1 gene activation. Relevant cell lines

were subjected to qPCR and Northern analysis, RNA sequencing, immunoblotting, reporter assays, agar colony assay, and liquid chromatography coupled to mass spectrometry (LC MS).

Result and discussion

Expression of BASP1 in SW480 leads to a decrease of MYC protein and mRNA levels, and a significantly reduced transformed phenotype. Proteome comparison of SW480 cells with those ectopically expressing BASP1 showed that 879 proteins are differentially expressed between the two cell lines, including proteins associated with beta-catenin function. Also, transcriptome analysis revealed a downregulation of the Traf2 and Nck-interacting kinase (TNK), a key protein in the activation of WNT signaling. A TCF reporter assay confirmed that the WNT signaling pathway is strongly impaired in the presence of BASP1 by showing significantly less transcription activity even upon ligand stimulation. By using a TNK inhibitor we could additionally show that MYC protein levels are heavily influenced by TNK functionality as they strongly decrease upon inhibition. We suggest that BASP1 influences MYC protein levels by interfering with the WNT signaling pathway.

Conclusion

Re-expression of BASP1 results in perturbed WNT signaling activity which might be the cause of the reduced MYC protein levels and the drastically reduced transformed phenotype that we observe in these cells.

EACR25-1479

The Golgi Apparatus Protein TMBIM4 Promotes Glioma Cell Invasion and Metabolic Adaptation

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Introduction

The ability of glioma cells to invade adjacent tissues is a key hallmark of tumour progression and a major determinant of patient outcome. Due to the high tumour heterogeneity and invasiveness of high-grade gliomas (Glioblastoma - GB), the current therapies remain largely ineffective, highlighting the need for novel therapeutic approaches. The Transmembrane BAX Inhibitor Motif Containing (TMBIM) protein family, consisting of six intracellular ion channels, is implicated in multiple cell processes associated with cancer hallmarks. The Golgi-localized TMBIM4 regulates intracellular Ca²⁺ fluxes, apoptosis, and cell motility. Here, we investigate the role

of TMBIM4 in glioma cell invasion and metabolic adaptation.

Material and method

Glioma transcriptomic datasets from TCGA, CGGA, Rembrandt, and Ivy_GAP were analysed to determine TMBIM1–6 mRNA levels and their association with tumour grade and patient survival. The cellular role of TMBIM4 was assessed in human glioblastoma U87 and U251 cells following TMBIM4 KD using siRNA. Cell invasion was measured in 2D and 3D cultures, and an orthotopic mouse model was used to analyse tumour growth/invasion. Lipid peroxidation, DNA damage, and Cystathione Beta-Synthase levels were evaluated by immunofluorescence. Quantitative mass spectrometry was used to assess protein expression.

Result and discussion

TMBIM1, 4, and 6 mRNA were significantly upregulated in gliomas, particularly in GB. Their expression correlated with tumour grade and was associated with reduced patient survival, supporting their potential prognostic value. Interestingly, TMBIM4 exhibited elevated expression in hyperplastic blood vessels and regions of microvascular proliferation, hinting at its involvement in tumour invasion or angiogenesis.

TMBIM4 KD significantly reduced GB cell invasion in both 2D and 3D models without affecting cell viability or proliferation and led to a marked reduction in tumour growth *in vivo*. Lower expression of TMBIM4 increased lipid peroxidation and DNA damage, indicating a role in oxidative stress resistance. Proteomic analysis further uncovered a reprogramming of amino acid and lipid synthesis-related proteins upon TMBIM4 KD. A decreased expression of CBS, a key enzyme in cysteine biosynthesis, was detected in the tumour cells *in vivo*. Supplementation of cell media with glutathione (GSH) rescued the 2D invasive phenotype of TMBIM4 KD cells, suggesting a role for GSH in TMBIM4-dependent tumour invasion.

Conclusion

Our findings suggest that TMBIM4 promotes glioma metabolic reprogramming, contributing to redox homeostasis and cell invasion. Targeting TMBIM4 could represent a novel therapeutic strategy to reduce glioma progression.

Acknowledgments: FCT (UIDB04567/2020, UIDP/04567/2020, UI/BD/151424/2021), SeedFunding (COFAC/ILIND/CBIOS/2/2024) and COST (CA15214-47282).

EACR25-1494

The RHO exchange factor VAV3 shows unexpected tumor suppressor roles in non-small cell lung cancer

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Introduction

RHO guanine nucleotide exchange factors (GEF), a group of enzymes associated with the catalytic activation of RHO GTPases, have been traditionally associated with protumorigenic functions in a variety of tumor contexts. However, we have found that the VAV3 GEF plays unexpected tumor suppressor roles in the case of non-

small cell lung cancer (NSCLC). In this abstract, we will provide information about this role and the potential signaling mechanism involved.

Material and method

We utilized two mouse NSCLC models: (i) urethane-induced NSCL using wild-type and Vav3^{−/−} mice; and (ii) KrasG12D/+ and KrasG12D+/Vav3^{−/−} mice. We also have used murine and human NSCLC cell lines in which endogenous VAV3 was knockdown by shRNA-mediated approaches. As control, we used similar models in which VAV2, another member of the RHO GEF family, was eliminated using analogous techniques. These models were characterized using signaling and cell biology analyses; rescue experiments with VAV3 mutants and downstream signaling elements; RNA-seq; and LC MS/MS-based proteomics.

Result and discussion

We found that animals lacking Vav3 exhibited increased tumor burden and aggressiveness. In line with this, the elimination of endogenous VAV3 in lung cancer cells lead to increased cell growth and cell cycle progression. This is specific for VAV3, because the elimination of VAV2 induces opposite effects in urethane- and K-RASG12D-driven lung tumorigenesis. Signaling and rescue analyses performed in control and VAV3 knockdown NSCLC cells reveal the implication of specific RHO and PKN kinase family members in this suppressor role. The distal elements that mediate the final suppression effect are now under characterization.

Conclusion

These results demonstrate that VAV3 acts as a tumor suppressor in NSCLC, possibly via the modulation of a RHO–PKN-dependent pathway that impacts on cell cycle progression.

EACR25-1497

Recreating a Metastatic Liver Model to Unveil the Role of Cancer Stem Cells in Pancreatic Ductal Adenocarcinoma Progression and Therapeutic Targeting

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Introduction

Pancreatic Cancer (PC) is a multifactorial disease with various endocrine and non-endocrine subtypes, the most common and aggressive being pancreatic ductal adenocarcinoma (PDAC), accounting for over 92% of cases. More than 50% of PDAC patients present with metastatic disease, primarily in the liver (>70%), making them inoperable. This late diagnosis results in a 5-year overall survival rate of only 2% for metastatic PC (mPC), with a median survival of one year. Available treatments are limited to chemotherapy and palliative care. Metastasis requires tumor cells with self-renewal, plasticity, dormancy, and differentiation capabilities – key traits of cancer stem cells (CSCs). Given these properties, CSC involvement in metastasis is likely. Here, we analyze CSCs' impact on metastatic PDAC (mPDAC) to the liver.

Material and method

We selected a batch of four PDAC cell lines (ASPC-1, MIAPaCa-2, BxPC-3 and PANC-1) with varying levels of aggressiveness and distinct genetic profiles to better simulate the diversity observed in human patients. For tracking purposes, all cell lines stably expressed a luciferase reporter. Following a well-established protocol, CSCs were generated from the 2D cell lines and subsequently characterized by western blot and flow cytometry. Different cell concentrations were then selected for intrasplenic injections, followed by splenectomy to properly develop liver metastasis. Initial analysis and characterization were performed using quantitative PCR, flow cytometry, and immuno-histochemistry.

Result and discussion

We examined CSC-specific markers in vitro, focusing on CD44 isoform v6, associated with PC metastasis, and observed differential protein expression across cell lines. Additional CSC markers, including CD24, ALDH1, and CD133, were assessed. In vivo, ASPC-1-derived CSCs exhibited the highest metastatic potential, surpassing PANC-1, MIAPaCa-2, and BxPC-3. PDAC liver metastases phenotyping revealed differences in CD44v6, CD133, CXCR4, and ALDH1 expression. These markers were crucial for characterizing CSC plasticity and correlating with disease progression. Our findings provide insight into potential CSC-targeted anti-metastatic molecules.

Conclusion

Our research underscores the complexity of PDAC liver metastasis and emphasizes the need for CSCs-targeted therapies to disrupt the metastatic process. By successfully recreating a metastatic liver model, we have provided a robust platform to study the plasticity of CSCs and their involvement in metastatic dissemination. By leveraging insights from cell line studies and molecular pathways, novel strategies can be developed to improve outcomes for patients with PDAC. In this sense, our model serves as a valuable tool for identifying potential therapeutic targets and advancing precision medicine strategies in metastatic PDAC.

EACR25-1500

Identification of TGM2 as a Key Mediator of Autophagy-Driven Chemoresistance in p53-R282W expressing Cancer Cells

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Introduction

TP53 is well-documented as a pivotal regulator of autophagy. Mutations in TP53, observed in approximately 50% of cancers, perturb its canonical transcriptional programs, thereby rewiring cellular homeostasis. This is particularly pertinent in High-Grade Serous Ovarian Cancer and Gastric Cancer where p53 acts as a driver mutation. Yet their precise role in regulating autophagy remains poorly investigated.

Material and method

A library of p53 hot-spot mutants curated from TCGA database was stably expressed in p53-null SKOV3 (ovarian) and KATO3 (gastric) cancer cells. LC3 turnover assay/p62 degradation, LC3/LAMP1 co-localization and transmission electron microscopy (TEM) were done to study autophagic flux. TGM2 expression was analyzed using RT-PCR. In-vitro migration and invasion were assessed through wound-healing and Boyden chamber assays. Bulk RNA transcriptomics was performed on the Illumina NovaSeq 2006 platform.

Result and discussion

Comprehensive screening of six TP53 hotspot mutants in ovarian cancer cells demonstrated that R282W, a highly resistant mutant, exhibited an active autophagic flux upon platinum treatment. Conversely, other cisplatin-resistant mutants displayed a blockade in autophagic flux suggesting the involvement of alternative resistance mechanisms. To delineate the molecular basis of this differential autophagic regulation, bulk RNA sequencing of R282W cells after cisplatin treatment was performed. Transcriptomic analyses revealed the dysregulation of 16 autophagy regulators in response to platinum. Among them, transglutaminase 2 (TGM2), a canonical p53 target gene involved in aiding autophagosome-lysosome fusion, was upregulated (2.5-fold) by cisplatin. Cisplatin treatment induced a 2-fold higher TGM2 expression in both ovarian and gastric cancer cells harboring R282W, whereas other mutants showed reduced expression of TGM2. Interestingly, LC3-LAMP1 co-localization studies also indicated that the autophagolysosome formation was highest in R282W post platinum exposure. This indicates a putative role of R282W in transcriptionally regulating TGM2 which might be responsible for a high autophagic flux. Additionally, blocking autophagy in R282W significantly attenuated its aggressiveness and re-sensitized the cells to platinum. This highlights autophagy to be a key pathway in acquiring chemoresistance.

Conclusion

Our findings identify R282W mutant of p53 to exclusively exhibit autophagy-mediated chemoresistance, potentially through TGM2 upregulation and enhanced autophagosome-lysosome fusion. The heightened

autophagic flux in R282W expressing cells contributes to their aggressiveness and platinum resistance. Therefore, targeting this pathway may offer a promising strategy to overcome chemoresistance in R282W harbouring cancers. Currently studies are ongoing to elucidate the role of p53R282W in TGM2 regulation associated with autophagy and chemoresistance.

EACR25-1502

FGF/FGFR System in Medulloblastoma: New Approaches for Tumor Treatment

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Introduction

Medulloblastoma (MB) is a highly aggressive childhood brain tumor, accounting for about 20% of pediatric brain cancers. It primarily affects children between 3 and 9 years, though it can also occur in adults. Symptoms often include cerebellar dysfunction, increased intracranial pressure, and hydrocephalus. MB is classified into four main groups based on genomic features: wingless/WNT (MB-WNT), sonic hedgehog/SHH (MB-SHH), group 3 (MB-Gr3), and group 4 (MB-Gr4). These groups differ in genetic profiles, clinical outcomes, and risk levels. Each group can be further subdivided into subtypes, influencing prognosis and treatment. Standard treatment involves surgery, radiotherapy, and chemotherapy, but recurrence rates and mortality remain high, with survival rates varying from 55% to 95% depending on the subtype. Among these groups, MB-Gr3 makes up about 30% of MB cases, often affects infants and young children, and has the worst prognosis with a 5-year survival rate below 30%, especially in cases with MYC gene amplification. Studies have demonstrated that the Fibroblast Growth Factor (FGF)/FGF receptor (FGFR) pathway plays a key role in cancer, impacting processes like cell proliferation and invasion, angiogenesis, and resistance to treatment. To date FGFR inhibitors (FGFRi) have been clinically approved for other cancers, while their potential in MB has yet to be fully investigated. This study seeks to assess the effectiveness of FGFR inhibition as a therapeutic approach for aggressive MB-Gr3, utilizing FDA-approved FGFRi and innovative FGF trap molecules developed in our laboratory.

Material and method

In this study we exploited MB-Gr3 cells (HD-MB03, D425Med and MED411) to assess the expression and activation of the FGF/FGFR system. The inhibition of FGF/FGFR was carried out using a clinical grade FGFR inhibitor (Erdafitinib) and a FGF trap small molecule (UPR1430). Cell proliferation, Western blot and flow cytometry were used to test the effect of the drugs. In vivo, subcutaneous tumor xenograft was performed to validate the therapeutic effect and immunohistochemistry for the analysis of tumor samples.

Result and discussion

Preliminary data confirm that FGFRs are highly expressed and activated in aggressive MB subtypes, correlating with worse prognosis. In vitro studies

demonstrate that both FGFRi (Erdafitinib) and a prototypic FGF trap (UPR1430) significantly reduce MB cell proliferation. Indeed, Western blot analyses confirmed that anti-FGF/FGFR treatments significantly reduced the activation of FGFR and levels of MYC. In vivo, FGFR inhibition resulted in reduced tumor growth and impaired intra-tumor angiogenesis in a subcutaneous model of MB-Gr3 (HD-MB03 cells).

Conclusion

In conclusion, our preliminary data suggest that the FGF/FGFR system plays a role and might represent a promising target for the treatment of MB-Gr3.

EACR25-1503

Loss of UFMylation supports prostate cancer metastasis and rewire cell metabolism towards hexosamine biosynthesis

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Introduction

Cancer metastasis requires the acquisition of genetic, transcriptional and translational features that enable invasion in distant organs. However, the role of non-canonical post-translational modifications and their impact in cancer metabolism and progression is less explored. UFMylation is the last identified ubiquitin-like post-translational modification and participates in essential biological functions.

Material and method

In order to identify key pathways in the metastatic process, we made use of publicly available Prostate Cancer databases using Cancertool and cBioPortal. Furthermore, we developed molecular tools to genetically modify the UFMylation pathway and perform several in vitro proliferation and invasion techniques, and in vivo metastatic assays. We also carry out metabolic tracing assays and glycoprotein identification experiments. Finally, to identify UFMylated proteins, we developed several stable cell lines expressing BioID and TurboID fusion proteins and analysed them via immunoblot, immunofluorescence, and biotin-affinity purification-based proteomics.

Result and discussion

Through a bioinformatics search we identified protein UFMylation as a process altered in prostate cancer metastasis, where the expression of UFL1 and UFM1 was consistently downregulated in metastatic lesions compared to primary tumors in multiple prostate cancer patient cohorts. Our in vitro and in vivo results showed that reducing UFMylation in prostate cancer cells favours cancer cell invasion and metastatic dissemination at the expense of proliferation. Mechanistically, we identified a novel UFMylated protein, namely the glycolytic enzyme phosphofructokinase (PFKAP). Consistent with a role for

UFMylation in the regulation of PFKAP activity, loss of UFMylation increases the flux of glucose from glycolysis towards the hexosamine biosynthesis pathway, the production of precursors for protein glycosylation, and the glycosylation of integrins that participate in the process of cancer cell dissemination.

Conclusion

Our results reveal PFKAP as a new UFMylation target and support a metabolic role for UFMylation in metastasis through the pivotal contribution of PFKAP regulation in this process.

EACR25-1506

Modulation of CITED2 Expression Unveils Different Biological Functions in Glioblastoma

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Introduction

Glioblastomas (GBM) are the most common and aggressive malignant brain tumors, characterized by high invasiveness, resistance to therapies, and poor prognosis. These tumors exhibit marked cellular heterogeneity, comprising a more differentiated bulk tumor population and a subpopulation of Glioblastoma Stem Cells (GSCs), which retain stem-like properties. Evidence suggests that GSCs play a crucial role in therapy resistance and tumor relapse, yet conventional treatments primarily target the bulk tumor cells, leaving this resilient subpopulation largely unaffected. Thus, a better understanding of the mechanisms governing stemness in GSCs is urgently needed to identify novel therapeutic targets. CITED2, a co-transcriptional modulator, is a key regulator of pluripotency in both embryonic and adult stem cells. Its role in cancer is complex, with both pro-tumorigenic and anti-tumorigenic functions reported across different malignancies. This study aims to investigate whether CITED2 influences GBM biology, with a particular focus on its impact on both GSCs and bulk tumor cells.

Material and method

To model both the bulk tumor population and Glioblastoma Stem Cells (GSCs), we cultured GBM cell lines under two distinct conditions: (i) differentiated bulk tumor cells, grown in 2D cultures with fetal bovine serum (FBS), which promotes cell differentiation, and (ii) GSC-enriched populations, cultured in 3D, low-adherence conditions using a neural stem cell-adapted medium,

which supports stemness properties. To assess the role of CITED2 in GBM biology, we modulated its expression through overexpression and knockdown approaches. The resulting cell populations were subjected to functional assays to evaluate self-renewal, proliferation, migration, viability, and tumorigenic potential.

Result and discussion

Functional assays revealed that CITED2 overexpression enhances cancer stem cell properties, including increased clonogenic potential and metabolic activity. Conversely, CITED2 knockdown significantly reduced the clonogenic capacity of GBM cells, suggesting a key role in tumor cell self-renewal. Additionally, sphere formation assays demonstrated reduced aggregation and smaller spheroid size in CITED2-overexpressing cells, which may indicate alterations in cell-cell adhesion or changes in the stem-like properties of these populations.

Conclusion

Altogether, our results suggest that CITED2 may contribute to the self-renewal and tumorigenic properties of GBM. Therefore, modulating CITED2 expression in this context may be an effective therapy approach that deserves further study.

This work was supported by Fundação para a Ciência e Tecnologia (FCT, ref. 2022.09209.PTDC).

EACR25-1512

Elucidating how nutrient dynamics in the tumor microenvironment modulates cancer metastasis

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Introduction

Metastasis is the leading cause of cancer-related mortality, yet the metabolic adaptations driving this process remain poorly understood. While primary tumor metabolism has been extensively studied, metastatic cells exhibit distinct metabolic demands. Nutrient availability in the tumor microenvironment contributes to metabolic heterogeneity, potentially promoting invasive cell states. Identifying the nutrients, metabolites, and metabolic pathways that enhance metastatic potential remains a major challenge. We hypothesize that nutrient fluctuations induce metabolic stress, triggering adaptive reprogramming that enhances metastatic potential via epigenetic memory. To investigate this, we developed a high-throughput, multiplexed screening platform to systematically assess metabolite influence on melanoma, the deadliest skin cancer.

Material and method

To determine whether nutrient perturbations drive metastatic states, we designed an innovative multiplexed strategy for evaluating metabolite contributions to metastasis. Our approach leverages lentiviral genetic barcoding, enabling the unique identification of cancer cell clones via PCR and NGS. We first performed untargeted metabolomics on tumor interstitial fluid (TIF) and plasma from melanoma-bearing mice, identifying

over 500 metabolites, with 25% showing significant differences. To functionally assess these metabolites, we utilize our platform, which enables parallel testing of 14 stable murine melanoma cell lines, each carrying a unique genetic barcode. The 14 barcoded cell lines are cultured under distinct nutrient conditions, pooled, and injected into the circulation of wild-type mice. Lung metastases are then analyzed via barcode DNA sequencing to quantify representation and assess metastatic impact. If significant changes are detected, single-cell RNA sequencing is performed to identify metabolic pathways driving metastasis.

Result and discussion

Using this platform, we have tested various metabolic conditions, including metabolites differentially present in tumor-derived TIF and others linked to distinct physiological or pathological contexts (e.g., essential amino acids, vitamins, microbiota-derived metabolites). Our results demonstrate that specific metabolites modulate the metastatic potential of melanoma cells. Furthermore, we are validating key findings and exploring the underlying molecular mechanisms through scRNA-seq, uncovering gene expression changes and pathways associated with the pro-metastatic phenotype.

Conclusion

We have developed a powerful multiplexed screening platform to systematically investigate how metabolic conditions shape melanoma metastasis. This scalable approach identifies nutrient-driven vulnerabilities in metastatic cells, providing new insights into tumor metabolism and potential therapeutic targets.

EACR25-1523

NGFR-Driven Lipid Metabolic Reprogramming in CR-CSCs: Implications for Chemoresistance in Colon Cancer

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Introduction

The nerve growth factor receptor (NGFR) plays a key role in neuronal plasticity and is implicated in tumor progression in colon cancer. Within the tumor bulk, colon cancer stem cells (CR-CSCs) are characterized by self-renewal capacity, tumor growth promotion, metastasis, and chemoresistance. Recent studies have highlighted that CR-CSCs reprogram their lipid metabolism to sustain stemness, enhance metastasis, and confer chemotherapy resistance. Notably, CR-CSCs accumulate lipid droplets (LDs) more than healthy cells. Under metabolic stress, LDs release free fatty acids (FFAs), which can be utilized for ATP generation via fatty acid oxidation (FAO). Dysregulated lipid metabolism is a critical factor in cancer development, and increased lipid uptake enhances the tumor-initiating capacity of CR-CSCs.

Material and method

CR-CSCs were transduced with lentiviral vectors to overexpress NGFR. Metabolism-associated gene expression profiles were analyzed via RT-PCR using a customized gene panel, including lipid metabolism genes, in NGFR-overexpressing cell lines. Intracellular lipid content was evaluated via Nile Red staining (1 µM). Additionally, NGFR-overexpressing cells were treated with chemotherapeutic agents – 5-fluorouracil, oxaliplatin, and irinotecan – at 1, 2, and 10 µM for 72 and 96 hours to evaluate whether NGFR is directly or indirectly involved in the acquisition of a chemoresistant phenotype.

Result and discussion

Analysis from public databases (GEPIA) was performed to identify genes mostly associated with NGFR expression in CRC, revealing several genes involved in CR-CSCs lipid metabolism, including GPIHBP1, CD36, PLIN1, and FABP4. Gene Set Enrichment Analysis (GSEA) highlighted significant enrichment in pathways related to adipogenesis, lipid localization, and transport. Transcriptomic analysis of NGFR-overexpressing cells showed upregulation of lipid metabolism genes such as FASN, FADS1, FADS2, PLIN1, and ACSL5, suggesting that NGFR regulates lipid metabolic pathways in CR-CSCs. Nile Red staining confirmed increased lipid droplet accumulation in NGFR-overexpressing cells, reinforcing NGFR's role in lipid metabolism. Given the putative association between high fatty acid content and resistance to chemotherapy-generated ROS, we hypothesized that NGFR plays a crucial role in the acquired chemoresistance to standard treatment. Our data confirmed this hypothesis, demonstrating that NGFR expression induces resistance to 5-FU, oxaliplatin, and irinotecan.

Conclusion

NGFR-overexpressing CR-CSCs boost lipid metabolism, with a consequent increase in intracellular lipid droplet accumulation, suggesting a critical role for NGFR in regulating lipid metabolism, which may contribute to chemoresistance in CRC. Accordingly, NGFR could represent a potential therapeutic target in overcoming chemoresistance in the future.

EACR25-1525

C/EBPα coordinates key metabolic pathways driving the resistance to targeted therapies in FLT3-mutated acute myeloid leukemia

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Introduction

Acute myeloid leukemia (AML) are heterogeneous malignancies characterized by the uncontrolled proliferation of immature myeloid cells in the bone marrow. FLT3 mutations, present in approximately 30% of patients, drive leukemic cell survival by enhancing

glucose metabolism, amino acid utilization, and redox homeostasis. These metabolic adaptations contribute to therapy resistance, leading to high relapse rates and poor prognosis. Therefore, targeting the specific metabolic vulnerabilities of this patient subgroup could offer new therapeutic opportunities.

Material and method

To investigate novel FLT3-specific non-genetic mechanisms of therapeutic resistance, we performed a combination of bulk and single-cell multiomics analyses with functional approaches, alongside *in vivo* experiments, using FLT3-mutant AML cell lines and primary specimens from AML FLT3-mutated patients.

Result and discussion

In this context, we identified the transcription factor C/EBP α as a key regulator of lipid metabolism and FLT3 inhibitors response in FLT3-mutated AML. We demonstrated that its inhibition perturbs lipid homeostasis by reducing phospholipid production and increasing PUFA accumulation, sensitizing leukemic cells to ferroptosis, a lipid peroxidation-driven cell death. Notably, we found that ferroptosis inducers potentiate the effects of FLT3 inhibitors through the C/EBP α -SCD pathway, uncovering a metabolic vulnerability that could be therapeutically exploited. Beyond resistance to FLT3 inhibitors, FLT3-mutated AML also exhibits poor response to venetoclax/azacitidine (VEN/AZA), the current frontline therapy for newly diagnosed patients ineligible for intensive chemotherapy. However, the mechanisms underlying the response to VEN/AZA remain poorly understood in this mutational context. Our current study reveals that mitochondrial transaminases play a critical role in VEN/AZA resistance in FLT3-mutated cells. Mechanistically, C/EBP α regulated mitochondrial function and amino acid metabolism through glutamate-pyruvate transaminase 2 (GPT2) expression. Since amino acid metabolism is crucial in VEN/AZA response, we further showed that genetic and chemical inhibition of mitochondrial transaminases GPT2 or glutamate-oxaloacetate transaminase 2 (GOT2) (and not cytosolic transaminase GOT1) triggered mitochondrial oxidative stress and markedly sensitized FLT3-mutant AML cells to VEN/AZA treatment *in vivo*.

Conclusion

Together, our findings establish a novel role of C/EBP α in regulating both lipid and amino acid metabolism, affecting therapeutic response in FLT3-mutant AML. By identifying lipid biosynthesis enzymes and mitochondrial transaminases as key metabolic drivers of FLT3 inhibitors or VEN/AZA resistance, respectively, our study highlights promising targets to enhance treatment efficacy in this high-risk patient subgroup.

EACR25-1530

Crosstalk between autophagy and apoptosis induced by unsymmetrical bisacridines in pancreatic cancer cells

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Introduction

Pancreatic cancer (PC) is an aggressive malignancy with a poor prognosis, highlighting the urgent need for novel therapeutic strategies. Our previous studies demonstrated that unsymmetrical bisacridines (UAs) exhibit high anti-cancer activity against PC cells by inducing apoptosis and decreasing c-Myc protein levels. Autophagy plays a complex, dual role in tumor progression, including PC, where increased autophagy correlates with poor prognosis. This study aimed to evaluate the impact of UAs on autophagy and its crosstalk with apoptosis in PC cells.

Material and method

We used four human pancreatic cancer cell lines (Panc-1, MIA PaCa-2, AsPC-1, BxPC-3), one normal pancreatic cell line (hTERT-HPNE), and three UAs (C-2028, C-2045, C-2053). Cell viability was assessed using the MTT assay, and apoptosis was detected with Annexin V-FITC and caspase 3/7 cytometric assays. Autophagy was evaluated by observing acidic vesicular organelles (AVOs) using acridine orange and Western blot analysis of autophagy-related proteins: LC3, p62, Beclin1, and Bcl-2.

Result and discussion

UAs exhibited lower activity against normal cells than PC cells, and IC₈₀ doses were several times higher in normal cells (C-2028: 0.04-0.10 μ M for PC vs 0.366 μ M for normal cells). These compounds preferentially induced apoptosis in PC cells (C-2028: 27.4-57.0% for PC vs 26.7% for normal cells). The strongest apoptosis induction was triggered by the derivative that also most severely caused autophagy (C-2028 for MIA PaCa-2 and AsPC-1; C-2045 for Panc-1; C-2053 for BxPC-3), confirmed by increased LC3-II levels, decreased p62, and change in the AVOs amount. In the AsPC-1 and BxPC-3 cells, a decrease in Beclin1 levels was observed with no significant changes in Bcl-2, while in Panc-1 cells, both Beclin1 and Bcl-2 levels decreased, suggesting Beclin1/Bcl-2-independent activation of autophagy in these three cell lines. In contrast, in MIA PaCa-2 cells, UAs caused an increase in Beclin1 and Bcl-2 levels, with the lowest Bcl-2 induction for C-2028. Only in MIA PaCa-2 cells was p62 accumulation observed with weak LC3-II induction after treatment with C-2045 and C-2053, suggesting autophagy inhibition. The use of chloroquine (CQ), an autophagy inhibitor, combined with UAs had little effect on cell survival, except in BxPC-3, where CQ enhanced C-2053's cytotoxic effect, evidenced by increased caspase 3/7 activity (C-2053 - 12.1%; C-2053 + CQ - 23.4%). This implies a protective role of autophagy in BxPC-3 cells.

Conclusion

UAs exhibit a preferential cytotoxic effect on PC cells, with significantly reduced activity in normal cells. These compounds induce autophagy in PC cells in a manner dependent on both the cell line and UA derivative. Strong autophagy induction correlates with increased apoptosis, suggesting autophagy's dual roles based on cellular stress. After exposure to UAs, the protective effect of Bcl-2 is insufficient, leading to autophagy disruption and apoptosis dominance.

EACR25-1541

Impact of prevalent KRAS mutations in PDAC resistance to FIRINOX

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Introduction

Pancreatic cancer (PC) is a disease with a poor prognosis due to its low survival. Despite the efforts made by medicine, current treatments to fight PC are not effective. Part of the problem is the late diagnosis of the disease. Once diagnosed, most patients receive chemo-therapy regimens as FOLFIRINOX, which is formed by the combination of leucovorin, 5-fluorouracil (5-FU), irinotecan and oxaliplatin. Regrettably, resistance mechanisms are recurrent causes of treatment failure. A potential strategy to overcome these resistance phenotypes is to dissect them in genetically-defined model systems that take into account the heterogenous genetic landscape of these tumors, to then develop better therapeutic strategies. KRAS is an oncogene, which is activated in more than 95% of patients with pancreatic ductal adenocarcinoma (PDAC), and can be found altered differently among patients. Recent studies have shown that common altered mechanisms of chemoresistance may be link to KRAS activation. The main objective of this project is to evaluate the mechanisms underlying acquired resistance to the combination of three cytostatic agents: 5-FU, SN-38 and Oxaliplatin (FIRINOX) in PDAC in the context of distinct KRAS mutations. To achieve this goal, we have set up two types of models: 1) in vitro by using mouse pancreatic organoids (KPOs) and 2) in vivo by performing orthotopic transplantation of KPOs in syngeneic mice.

Material and method

KPOs were previously generated by transfecting LSL-KrasMUT organoids (G12D, G12V and G12R) with a plasmid expressing Cre recombinase and a sgRNA targeting Tp53. We have chosen three prevalent mutations in PDAC as KRAS-G12D (40%), KRAS-G12V (32,5%) and KRAS-G12R (18%), to then, induced chemoresistance in the different KPOs by continuously treating them with increasing concentrations of FIRINOX

regimen. To assess the acquisition of chemoresistance, cell viability was measured. Then, chemoresistant (chr) and non-chemoresistant KPOs were transplanted orthotopically into the tail of the pancreas of syngeneic mice (C57Bl/6N). Harvested tumors have been profiled by immunohistochemistry and flow cytometry at final time point.

Result and discussion

We have already induced chemoresistance to FIRINOX in 3 KPO-KRASG12D, 3 KPO-KRASG12V and 3 KPO-KRASG12R lines. Preliminary data shows that in vitro chr-KPO lines tolerate a higher concentration of FIRINOX regimen. The initial characterization in vivo exhibits phenotypic changes among chr and non-chr-tumors expressing specific KRAS mutations.

Conclusion

This preliminary data is the seed to continue our research strategy, that seeks to unravel the connection between specific-KRAS mutations, tumor heterogeneity and treatment resistance. In the near future, we hope to identify new vulnerable targets to design new therapy approaches depending on the specific KRAS mutation in the context of chemoresistance.

EACR25-1545

Unbiased Phosphoproteomic and Metabolic Profiling in WT and KRAS Mutant Colorectal Cancer Cells Reveals Molecular Interplay Between Protein Regulation and Metabolic Rewiring

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Introduction

KRAS mutations cause significant metabolic and protein dysregulation in cancer, leading to alterations in cellular signaling pathways, promoting uncontrolled cell growth and proliferation. The metabolic reprogramming of altered KRAS function includes increased glucose, amino acid, and lipid metabolism. The changes in the cellular proteome stem from modifications in protein expression and phosphorylation. Understanding the interplay between these metabolic and protein-level alterations is crucial for developing targeted therapeutic strategies against KRAS-driven cancers. Accordingly, we performed an integrated metabolomic and phosphoproteomic analysis of HCT116 cells with and without a KRAS mutation.

Material and method

HCT-116 cells containing wildtype (WT, n = 5) and mutant KRAS (MUT, n = 5) were profiled to assess metabolite, protein, and phosphoprotein levels between WT and MUT cells. Metabolomic profiling was completed with LC/MS to capture polar and lipid metabolites. Data was processed through an in-house untargeted metabolomic analysis pipeline. For phosphopeptide analysis, enriched phosphopeptides and flow-through peptides were analyzed with DIA LC/MS/MS. The resulting data was processed with DIA-NN and combined into aggregated protein and phosphosite profiles.

Result and discussion

The multi-omic analysis profiled 2,025 metabolites, 6,654 proteins, and 10,034 phosphosites. When considering each data type individually, all three analyte types separated the WT and MUT cells through PCA analysis, underscoring the magnitude of dysregulation from KRAS mutation. When considering the metabolites, proteins, and phosphosites that reached statistical significance, 85 metabolites and 145 proteins were differentially abundant. Strikingly, 1,036 phosphosites were unique to either KRAS and WT samples, with ~800 of these being unique to KRAS samples. The results of a joint pathway analysis identified >70 pathways that reached significant enrichment ($p < 0.05$) and had support at both the protein, metabolite, and phosphosite level. The most enriched pathway was RAS signaling. Other enriched pathways include selenocysteine synthesis, sialic acid metabolism, ROS detoxification, and respiration.

Conclusion

This study showed extensive differential phosphorylation in KRAS mutant cells, suggesting a widespread rewiring of signaling networks that impacts diverse cellular functions. The multi-omic approach taken enabled the identification of previously known and novel pathways affected by KRAS signaling, offering potential new targets for therapeutic intervention in KRAS-driven cancers.

EACR25-1551

Evaluation of novel, high-affinity SSTR2/SSTR5 somatostatin analogs in pancreatic and pituitary neuroendocrine tumor models

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Introduction

Neuroendocrine tumors (NETs) are a heterogeneous group of neoplasias that originate from neuroendocrine cells in various organs, including the pancreas and pituitary gland. Pancreatic NETs (PanNETs) and pituitary adenomas/NETs represent clinically relevant subtypes, typically characterized by the expression of somatostatin receptors (SSTRs). These receptors represent key therapeutic targets for both somatostatin analogs (SSAs) and peptide receptor radionuclide therapy (PRRT). Currently approved SSAs exhibit high affinity for specific SSTR subtypes, with octreotide and lanreotide targeting SSTR2, while pasireotide binds to SSTR5, SSTR3 and SSTR2. Despite the availability of these

SSAs, these peptides may not be suitable for all patients due to variable SSTR expression, limited efficacy in aggressive or advanced tumors, and the potential development of resistance. As a result, developing novel compounds targeting NET-specific pathways is crucial to improving patient outcomes. In this study, we tested 5 newly synthesized SSAs having high affinity for SSTR2 and SSTR5 that are being developed as SSAs and PRRT.

Material and method

As experimental models, we selected the NT-3 and AtT-20 cell lines, derived from a human well-differentiated pancreatic NET (Pan-NET) and a murine pituitary adenoma cell line, respectively. NT-3 cells were chosen based on their low proliferation rate, sustained SSTR expression, and insulin-secreting capability, making them a representative in vitro model for Pan-NETs. Similarly, AtT-20 cells, which secrete ACTH, serve as a well-established model for pituitary adenomas/NETs. Since SSAs are expected to inhibit tumor growth and suppress hormone secretion, we assessed the short-term effects of the five peptides on these features using proliferation/viability assays (WST-1, cell count) and hormone secretion measurements (ELISA for insulin or ACTH) in 2D cultures.

Result and discussion

In the pancreatic NT-3 cell model, all tested peptides strongly inhibited insulin secretion at doses ranging from 5 to 100 nM, and significantly blunted proliferation compared to untreated controls. In the pituitary AtT-20 cell model, two out of five peptides had clear inhibitory effects on ACTH secretion, while the peptides also appeared to limit tumor cell growth.

Conclusion

These results indicate that the newly synthesized SSAs effectively inhibit both hormone secretion and cell proliferation in NET models. Further investigations in 3D cultures and in vivo models will be essential to confirm their therapeutic potential and optimize their clinical applicability.

EACR25-1555

CK2α and CHIP crosstalk: uncovering its role in tumorigenic processes

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Introduction

Casein Kinase II (CK2) is a ubiquitously expressed eukaryotic serine/threonine protein kinase known for its heightened activity in proliferating and cancerous tissues. The catalytic subunit, CK2α, phosphorylates a wide array of substrates, including both tumor suppressors and oncogenes, thereby playing a pivotal role in modulating signaling pathways that drive cell proliferation in cancer. The Carboxy Terminus of Hsc70 Interacting Protein (CHIP), a dual-function co-chaperone and E3 ubiquitin ligase, is crucial for maintaining protein homeostasis by targeting misfolded or damaged proteins for degradation.

Material and method

Assays for ubiquitination, overexpression, cycloheximide chase, and inhibition were used to measure changes in protein levels. Additionally, Liquid chromatography-mass spectrometry (LC-MS), immunocytochemistry

(ICC) and co-immunoprecipitation (Co-IP) were carried out. To assess the therapeutic importance of these results, additional studies included spheroid modelling, migration and invasion assays performed.

Result and discussion

Our findings explores the interaction between CK2 α and CHIP in cancer development. Preliminary analysis indicated an inverse relationship between the two proteins, suggesting CK2 α may regulate CHIP degradation and inhibit apoptosis. This degradation stabilizes the oncogenic kinase AKT that leads to possible phosphorylation by CK2 for its super-activation and promoting tumorigenesis. Functional assays supported these findings, demonstrating CK2 α 's role in controlling CHIP protein levels.

Conclusion

This study unveils a potential CK2 α -CHIP regulatory axis in cancer, offering new insights into the molecular mechanisms driving tumor progression. However, further research is essential to fully elucidate the complexities of this regulatory pathway and its broader implications in cancer biology. Understanding this mechanism could pave the way for novel therapeutic strategies targeting the CK2 α -CHIP axis in cancer treatment.

EACR25-1557

Investigating the role of PTX3 in the biology of glioblastoma

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Introduction

Glioblastoma Multiforme (GBM) is the most common and aggressive brain tumor in adults, classified as grade IV tumor by WHO. Common therapies include surgical removal, chemotherapy and radiotherapy; however, relapses are inevitable. In addition, it is hypothesized that the relapses are mainly due to a subpopulation of stem cells with self-renewal properties, called glioblastoma stem cells (GSC) localized in specialized niches. These cells are resistant to conventional treatments thanks to their ability to escape apoptosis and activate DNA repair mechanisms. Pentraxin 3 (PTX3) is a soluble pattern recognition receptor belonging to the humoral arm of the innate immunity that is also involved in several aspects of tumor growth, angiogenesis, metastasis and cancer immunoregulation. To date, a correlation between PTX3 and tumor aggressiveness in GBM has been described, but studies regarding its role in GSC are still missing, as well as the identification of the biological pathways involved. In order to investigate this issue, we used different in vitro and in vivo models, increasing the

complexity system and moving closer to the pathological scenario found in humans.

Material and method

We used human GSC BT302 cells, derived from glioblastoma specimens diagnosed according to WHO criteria, to obtain PTX3 silenced cells. PTX3 presence and production was assessed by Western blot, qPCR, ELISA and immunostainings. We analysed the cell culture features and we generated organoids as in vitro models, while we performed subcutaneous and orthotopic injection in immunodeficient mice to evaluate the effect in PTX3 knockdown in vivo. Proliferation, invasion and angiogenic assays were performed to analyse the effects of PTX3 silencing. In addition, RNA microarray analysis was conducted in order to identify the up- and down-regulated pathways, in response to PTX3 modulation. According to the output of the bioinformatic analysis, a further characterization through immunohistochemical stainings has been carried out.

Result and discussion

Preliminary observations revealed a wide expression of PTX3 in GSC, and specific silencing in a GSC line revealed a significant reduction of cell growth, invasiveness and angiogenic capacity in GSC after PTX3 knockdown. In vivo this resulted in decreased tumorigenic capacity. Furthermore, bioinformatic analysis revealed a modulation of apoptotic and hypoxia-related pathways, whose activation seems to be altered after PTX3 silencing. Accordingly, immunohistochemical characterization revealed an effective increase in hypoxia levels and a decrease of the proliferation rate in the PTX3 silenced conditions.

Conclusion

Our data suggest that PTX3 is expressed and may play a relevant role in GSC cells. In this context, PTX3 silencing may impair tumor features in vitro and in vivo. These data set the basis for further characterization of the pro-tumoral role of PTX3 in glioblastoma.

EACR25-1564

Trigger ferroptosis in breast cancer cells (BC) by targeting xCT and FATP1

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Introduction

Cancer cells can reprogram their cellular metabolism to proliferate. Cysteine and fatty acids metabolism are two pathways that work synergistically to support breast cancer (BC) progression and protect these cells against damages caused by lipid peroxidation. We aimed to induce BC cell death by inhibiting two transporters that play pivotal roles in cysteine and fatty acids uptake, xCT and FATP1, using the compounds selenium chrysin (SeChry) and 5k piperazine, through ferroptosis, a cell death caused by excessive lipid peroxidation.

Material and method

The human TNBC cell lines MDA-MB-231 and HCC1806, and Luminal A and Luminal B cell lines MCF7 and BT474 were used and exposed to SeChry (EC50 concentration: 24 μ M for HCC1806; 7 μ M for MDA-MB-231; 23 μ M for BT474, and 19 μ M for MCF7), and 5k (12.5 μ M) in separate or in combination,

in regular media and stearic acid (C18) enriched media. Cell death, reactive oxygen species (ROS), and lipid peroxide levels were assessed using Annexin-V and PI, and the DCF-DA (2 μ M) and Bodipy 581/591 C11 (2 μ M) probes by flow cytometry, respectively. xCT and FATP1 expression was observed by immunofluorescence and lipid droplet (LD) levels were examined by fluorescence microscopy, using Nile red (1 μ L /mL) staining. Xenograft orthotopic mice models of MDA-MB-231 cells were developed and treated with SeChry encapsulated in a nanoparticle (SeChry@PUREG4-FA2) and SeChry@PUREG4-FA2 + 5k. Primary tumors were collected, measured, frozen, and embedded in optimal cutting temperature (OCT) compound. Tumor sections were stained with Nile red and Bodipy 581/591 C11 probe and analyzed by fluorescence microscopy.

Result and discussion

The EC50 concentration of SeChry showed that the MDA-MB-231 cell line was the most sensitive to SeChry, which tended to decrease FATP1 and LD levels in all cell lines. Exposure to 5k resulted in increased cell death and lipid peroxide levels in BC cells cultured in C18-enriched media. It was confirmed that ferroptosis was occurring, as evidenced by the decrease in ROS levels caused by SeChry, along with an increase in lipid peroxides, indicating that ROS are consumed during lipid peroxidation. Exposure to a C18-enriched medium significantly enhanced ferroptosis in TNBC cell lines, suggesting they have a greater susceptibility to ferroptosis. Treatment with SeChry@PUREG4-FA2 reduced the volume of BC xenograft tumors with decreased LD accumulation and increased lipid peroxide levels, which was not seen in treatment with SeChry@PUREG4-FA2 and 5k. This disclosed a protective role that 5k has over SeChry, because it reduces fatty acid uptake.

Conclusion

Upon increased levels of cell death, decrease in mice tumor size, and accumulation of lipid peroxides both in vitro and vivo, we confirmed the potential of SeChry@PUREG4-FA2 as a promising therapeutic strategy. However, 5k decreased the anti-cancer efficacy of SeChry by lowering the amount of fatty acids to undergo peroxidation, thus inhibiting ferroptosis.

EACR25-1571

Attaching to and Crossing Endothelial Barriers

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Introduction

Leukocyte extravasation is a key component of the innate immune response, while circulating tumor cell extravasation is a critical step in metastasis formation. Despite their importance, the mechanisms underlying leukocyte and tumor cell extravasation remain incompletely understood.

Material and method

Here, we developed an imaging pipeline that integrates fast, label-free live-cell imaging with deep learning-based

image analysis to quantitatively track and compare the initial steps of extravasation – cell landing and arrest on an endothelial monolayer – under physiological flow conditions.

Result and discussion

We find that pancreatic ductal adenocarcinoma (PDAC) cells exhibit variable adhesion strength and flow sensitivity. Remarkably, some PDAC cells demonstrate comparable endothelial engagement as leukocytes, preferentially arresting at endothelial junctions, potentially due to increased stiffness at these sites, which leads to exposure to the underlying basal extracellular matrix. PDAC cells also tend to cluster in regions with high, heterogeneous expression of the endothelial CD44 receptor. Simulations suggest that clustering results from the combination of CD44-mediated attachment and localized flow disturbances that facilitate subsequent cell attachment. Targeting CD44 using siRNA or function-blocking antibodies, or degrading its ligand hyaluronic acid (HA), almost completely abolishes PDAC cell attachment.

Conclusion

Overall, we demonstrate that cancer and immune cells share both common and unique features in endothelial adhesion under flow, and identify CD44 and HA as key mediators of PDAC cell arrest.

EACR25-1573

Apoptotic and cytotoxic effect of hispolon in human colorectal adenocarcinoma cell line (HT-29)

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Introduction

Cancer remains a major global health challenge, characterized by uncontrolled cell growth and metastasis. Colorectal cancer (CRC) is one of the most common malignancies worldwide, and the need for effective treatments is imperative. This study aims to evaluate the therapeutic potential of hispolon in HT-29 human colorectal adenocarcinoma cells, focusing on its effects on cell viability, oxidative stress markers, and apoptosis.

Material and method

HT-29 cell line were obtained from National Centre for Cell Science (NCCS), Pune, India. For hispolon treatment, 8% purity of hispolon was obtained from Santa Cruz Biotechnology(sc-221726), Dallas, Texas, USA. HT-29 cells were treated with varying concentrations of hispolon. Cell viability was assessed using the MTT assay. Lipid peroxidation was measured by determining malondialdehyde (MDA) formation. The activities of antioxidant enzymes, including superoxide dismutase (SOD), catalase, and glutathione peroxidase (GPx), were evaluated. The levels of reduced glutathione (GSH) were also quantified. ROS generation was assessed using a fluorescent probe. Apoptosis was

analyzed using acridine orange/ethidium bromide (AO/EtBr) staining.

Result and discussion

Hispolon treatment resulted in a dose-dependent (10–100 μM) manner. The treated cell line showed significant cytotoxic activity against HT-29 cell line with an IC₅₀ value of 48 μM decrease in cell viability, as demonstrated by the MTT assay. A significant increase in lipid peroxidation and MDA formation was observed, indicating oxidative stress induction. Hispolon treatment also led to alterations in antioxidant enzyme activities, with increased SOD and catalase activities and decreased GPx activity. The levels of reduced glutathione (GSH) were significantly reduced in hispolon treated cells. Furthermore, hispolon treatment induced a substantial increase in ROS generation. AO/EtBr staining revealed a marked increase in apoptotic cell population following hispolon treatment.

Conclusion

The findings suggest that hispolon exerts potent anti-cancer effects on HT-29 cells by inducing oxidative stress, modulating antioxidant enzyme activities, and promoting apoptosis. The dose-dependent cytotoxicity, increased lipid peroxidation, ROS generation, and alterations in antioxidant defences highlight the potential of hispolon as a therapeutic agent for colorectal cancer. Further studies are warranted to elucidate the underlying molecular mechanisms and explore the clinical applications of hispolon in cancer treatment.

EACR25-1586

Cell-cell adhesion disruption promotes stress granules formation: A survival strategy in Inflammatory Breast Cancer

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Introduction

Cell-cell adhesion is a biomarker of circulating tumor emboli, a hallmark of aggressive Inflammatory Breast Cancer (IBC).

Material and method

This work aims to explore if cell-cell adhesion disruption has an impact on the survival of circulating IBC emboli. Using 3D spheroids of IBC models, E- and/or P-cadherin were silenced in SUM149PT and MDA-IBC-3 cells.

Result and discussion

Initial observation showed that E- and P-cadherin compensate each other to maintain adhesion in SUM149PT, while E-cadherin plays a dominant role in cell-cell adhesion in MDA-IBC-3. Disruption of cell-cell adhesion in SUM149PT cells reduced anoikis resistance ($p = 0.0040$) and slightly decreased calcein/PI intensity ($p = 0.0002$), but no significant changes in caspase 3/7 activation or ROS production were observed. In MDA-IBC-3 cells, anoikis resistance was unaffected, but the viability of cells increased with E-cadherin KD ($p = 0.011$) and double KD ($p = 0.045$). Thus, cell death induction was not as pronounced as initially expected. Proteomics was then conducted to uncover the mechanisms that enable IBC cells to resist cell death upon cell-cell adhesion disruption. Interestingly, in the double KD, the results indicated an upregulation of “negative regulation of apoptotic processes” and “regulation of stress granule assembly”. G3BP2, a key scaffolding protein for stress granule assembly, was highly expressed at protein, mRNA, and ultrastructural levels when E/P-cadherin were silenced in SUM149PT cells. This was accompanied by increased expression of stress granule-associated genes (e.g., G3BP1, YTHDF1-3, FMR1, ATXN2L) and pluripotency/EMT-related genes (e.g., OCT4, KLF4, ZEB1, CDH2, SLUG, SOX2, TWIST). Additionally, recent evidence indicates that treating SUM149PT IBC double knockdown (KD) spheroids with an integrated stress response inhibitor (ISRB) significantly reduces cell survival. However, no significant changes were observed in single knockdowns of either cadherin.

Conclusion

In conclusion, the upregulation of stress granule assembly may enable IBC cells to survive to cell-cell adhesion disruption. Then targeting cell-cell adhesion and stress granule assembly holds promise as a potential therapeutic strategy for this aggressive disease.

EACR25-1588

Dual Cyclin-CDK Inhibition by Plant-Derived Hexadecanoic Acid Triggers G1/S Arrest

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Introduction

Prostate cancer is the second most common cancer among men, with the Caribbean experiencing the highest mortality rates globally. Conventional treatments rely on chemotherapy, often leading to resistance and adverse effects. This has spurred interest in alternative therapies with targeted mechanisms. Hexadecanoic acid, a naturally occurring saturated fatty acid, has demonstrated anticancer properties, including apoptosis induction and cell cycle modulation. This study examines the effects of hexadecanoic acid-enriched extracts from *Dioscorea cayenensis* and *Dioscorea alata* on prostate cancer cells.

Material and method

Hexadecanoic acid-enriched extracts from *D. cayenensis* and *D. alata* yams were tested on DU145 prostate cancer

and RWPE-1 normal prostate cells. Cytotoxicity was assessed via MTS assay (IC₅₀ determination). Morphological changes were observed microscopically. Flow cytometry evaluated G1/S arrest, and Western blotting analyzed cyclin D and CDK4 expression.

Result and discussion

Treatment of DU145 cells with hexadecanoic acid-enriched extracts resulted in significant antiproliferative effects, with an IC₅₀ of 18 µg/mL, while RWPE-1 cells exhibited minimal cytotoxicity (IC₅₀ >200 µg/mL). Morphological changes were observed in DU145 cells following treatment, suggesting cellular stress or apoptosis. Flow cytometry analysis confirmed that treatment induced G1/S cell cycle arrest, indicating disruption of cell cycle progression. Western blot analysis revealed a marked reduction in cyclin D and CDK4 expression, further supporting the role of hexadecanoic acid in regulating cell cycle progression. These findings suggest that hexadecanoic acid exerts its anti-cancer effects through dual inhibition of cyclins and CDKs, leading to G1/S arrest in prostate cancer cells.

Conclusion

Hexadecanoic acid-enriched extracts from *D. cayenensis* and *D. alata* demonstrate selective cytotoxicity against DU145 prostate cancer cells while sparing normal prostate cells. The observed G1/S cell cycle arrest and downregulation of cyclin D and CDK4 indicate a targeted mechanism of action. Given its selective anticancer properties, hexadecanoic acid may serve as a promising natural therapeutic agent for prostate cancer. Further molecular and *in vivo* studies are necessary to validate its clinical potential.

EACR25-1590

The impact of the microsporidia *Encephalitozoon intestinalis* on colorectal carcinogenesis

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Introduction

Colorectal cancer is a multifactorial disease influenced by genetic, environmental and microbial factors. Among the latest, the intestinal microsporidia *Encephalitozoon intestinalis* which is known to disrupt the cell cycle and inhibit host cell apoptosis could be a potential pro-carcinogenic actor. Recent epidemiological studies, notably by our team (Nourrisson et al., 2024), suggest a link between microsporidia and CRC. Indeed, sero-prevalence of the *Encephalitozoon* genus is significantly higher in CRC patients. Aim of this study is to precise impact of infection on epithelial cells.

Material and method

In vitro cellular infection models using Vero E6 and HT-29 cells were used to study the effects of *E. intestinalis* infection for 24H et 48H, with infection efficiencies approaching 80%. Their impact on apoptosis, cell cycle, and proliferation was evaluated by flow cytometry (using

TUNEL assays and cell cycle analysis), by immunofluorescence (targeting p53 and Ki67), by RT-qPCR and Western Blot analyses to assess the expression of genes such as PCNA, Cyclins (E, A, and D), Retinoblastoma, and p21. A cell culture inserts system (which prevents the passage of spores) was also used to study the effect of the secretome from *E. intestinalis*-infected cells (above) on non-infected cells (below).

Result and discussion

We observed that *E. intestinalis* induced cell cycle arrests in the S and G2 phases, as well as increased protein accumulation of cyclins E (24H) and A (48H), and a decrease in the protein proliferation marker PCNA (48H). There was also a decrease in gene expression of p53 and bax and protein expression of p21. These results suggest that microsporidia block the host cell in metabolically and energetically favorable phases for reproduction, while inhibiting the induction of cell apoptosis. Recent experiments with the cell culture insert system have shown cell cycle arrest in S and G2 phases in uninfected cells below infected cells, suggesting that the secretome of infected cells can induce cell cycle arrest in neighboring uninfected cells.

Conclusion

These results indicate that *E. intestinalis* disrupts host cell cycle regulation by inducing cycle arrest, decreased proliferation and inhibition of apoptosis. In addition, these infected cells appear to secrete molecules that induce these same phenotypes in neighboring cells. This could enable potentially damaged adjacent cells to survive. Together, these alterations may initiate a pro-tumor environment favoring genomic instability and cell survival, potentially contributing to the development and progression of colorectal cancer.

EACR25-1596

Therapeutic approaches to primary NRAS-driven leptomeningeal melanoma

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Introduction

Primary NRAS-driven leptomeningeal melanoma is a rare childhood cancer most often seen in the context of mosaic disorder congenital melanocytic naevus syndrome. It is universally and rapidly fatal, although treatment with MEKi trametinib confers temporary symptomatic benefit. We sought to explore other therapeutic options, small interfering RNA (siRNA) to oncogenic NRAS, which has recently been shown to induce apoptosis in dermal melanocytic naevus cells¹,

and a high throughput FDA approved small molecule screen.

Material and method

Antisense siRNAs to siNRASQ61K (NM_002524.5 (NRAS):c.181C>A,p.(Q61K)) and siNRASQ61R (NM_002524.5(NRAS:c.182A>G,p.(Q61R))) were designed to be variant allele-specific, and transfected into the following cell lines: NRAS-variant primary leptomeningeal cells from three patients with leptomeningeal dysplasia, the precursor to leptomeningeal melanoma; NRAS-variant primary leptomeningeal cells from one patient with leptomeningeal melanoma; two control wild-type immortalised melanocyte cell lines hTERT-dermal melanocytes and HERMES-1. In parallel, an in vitro drug screen of 2529 FDA-approved drugs was performed in primary leptomeningeal cells from one patient with leptomeningeal dysplasia and the two wild-type melanocyte cell lines, with validation of potential hits in all patient and control lines. IncuCyte® repeated phase-contrast imaging was used to measure confluence and a DNA-binding fluorescent caspase 3/7 dye to measure apoptosis across both experiments.

Result and discussion

We report here that oncogenic driver knockdown with siNRASQ61K or siNRASQ61R triggers apoptosis in all leptomeningeal patient cells, dysplastic or malignant. Furthermore, addition of current clinical standard of care trametinib to siNRAS further elevates levels of apoptosis over siNRAS alone, despite a complete lack of apoptosis with trametinib alone. Mechanistic exploration demonstrates siNRAS treatment leads to downregulation of antiapoptotic genes BIRC5 and ARL6IP1, a gene protective against ER stress recently identified as downstream of NRAS. Additionally, we report here that the top four drug hits from the FDA screen epirubicin, tucidinostat, dasatinib and valemestat suppress proliferation in patient cells significantly more than in controls, with epirubicin having the greatest effect. None of these small molecules, however, has any effect on apoptosis. Lastly, we explore the genome-wide expressional profiles of dysplastic and malignant cells pre- and post-treatment with epirubicin, trametinib and siNRAS.

Conclusion

Precision targeting of oncogenic NRAS driver alleles using siRNA is more promising than repurposing of small molecule inhibitors in currently untreatable leptomeningeal dysplasia and melanoma, potentially in combination with trametinib.

EACR25-1598

Investigating the Therapeutic Potential of Antidiabetic Drugs, Metformin and Canagliflozin, in Papillary Thyroid Carcinoma

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Introduction

Thyroid cancer (TC) is the most common endocrine neoplasia. The incidence of TC has been increasing, mainly due to the increased incidence of papillary thyroid carcinoma (PTC). Generally, PTC patients have a good prognosis, however, 10 to 15% of cases can evolve to a more aggressive state, leading to recurrence and metastasis. The cause of this change in PTC's biological behaviour is unknown, making it essential to predict cases at risk of progression. Metformin and canagliflozin are antidiabetic drugs, and recent studies suggest that these drugs influence various cancer cell lines, affecting proliferation, invasion, migration, growth, morphology, metabolism, cell cycle, and autophagy. However, the available data are still controversial and limited. Our aim is to evaluate the effects of metformin and canagliflozin on PTC cells, assessing their impact on key cellular and molecular processes. This may help determine their potential as therapeutic agents for PTC patients who don't respond to conventional treatments.

Material and method

Two PTC cell lines, TPC-1 and BCPAP - with different genetic alterations (RET/PTC rearrangement and BRAFV600E, respectively) - and one non-neoplastic thyroid cell line, Nthy Ori 3-1, were chosen. We optimized drug concentrations to evaluate their effects on cellular and molecular parameters, including proliferation, viability, apoptosis, cell cycle dynamics, metabolic activity, mitochondria, and cell morphology and migration, using techniques such as Prestoblue and EdU assays, fluorescence bioimaging, Seahorse XFe24 Analyzer, flow cytometry, electron microscopy and wound healing assay.

Result and discussion

Our results show that the viability of the cancer cell lines decreased with statistical significance from the concentration of 20 mM metformin and 20 µM canagliflozin, with the strongest effect at 48h post-treatment. This effect wasn't observed in Nthy Ori 3-1. Based on these results, 20 mM metformin and 20 µM canagliflozin were selected for further studies. When treated, TPC-1 and BCPAP cell proliferation were significantly reduced ($p < 0.01$) with both drugs, while there was no effect in Nthy Ori 3-1. Regarding migration, canagliflozin delayed wound closure in cancer cells, while metformin inhibited wound closure only in BCPAP. In contrast, migration in Nthy Ori 3-1 wasn't affected by either drug. Treatment also altered mitochondrial morphology and increased mitochondrial number in BCPAP and TPC-1. Notably, lipid droplets emerged in treated tumour cell lines with a statistically significant increase. We are currently assessing the impact of these drugs on cell cycle and in metabolism.

Conclusion

The observed effects on viability, proliferation, migration, mitochondrial dynamics, and lipid droplet formation suggest a potential therapeutic benefit of metformin and canagliflozin for PTC patients. Further insights into their impact on cell cycle and metabolism would complement these findings.

EACR25-1599**Nucleotide imbalance sensed by p53 leads to an upregulation of pyrimidine salvage transporter hCNT1**

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Introduction

Increased biosynthesis of nucleotides (NTPs) and deoxy-nucleotides (dNTPs) is a hallmark of cancer, driven by the need to support DNA replication and repair, transcription, ribosome biogenesis and post-translational protein glycosylation. Nucleotide biosynthesis encompasses a complex network of enzymes and transporters that can be divided into de novo synthesis pathways and salvage reactions from nucleosides and nucleobases. Wild type p53 plays an important role in nucleotide metabolism regulation inhibiting pentose phosphate pathway and purine de novo synthesis. However, when activated upon DNA damage, p53 can activate dNTP synthesis through RRM2B to favor DNA repair. Moreover, gain of function activities of mutant p53 contribute to nucleotide metabolism reprogramming to support proliferation and invasion. This study aims to define a p53 regulation over some essential elements of nucleoside salvage, such as nucleoside transporters (NTs), which are also responsible for the internalization of nucleoside analog drugs. Our work focuses on Concentrative Nucleoside Transporter 1 (hCNT1, encoded by SLC28A1), a unidirectional pyrimidine transporter whose expression has been reported to be decreased in a wide variety of tumors.

Material and method

NTs expression was determined after wtp53 restitution by RT-PCR in different models. Possible binding sites for p53 in SLC28A1 promoter were found by bio-informatics approaches. Promoter-luciferase reporter assays and ChIP-RT-PCR were performed to validate them. Induction of hCNT1 expression has been studied in p53 WT and KO HCT-116 cells. In order to induce DNA damage or an imbalance on nucleotide pools, cells were subjected to different treatments, including Etoposide or Brequinar. The role of mutant p53 has been also studied.

Result and discussion

Our data indicates that wtp53 overexpression specifically induces hCNT1 expression in a non-tumoral background. Putative binding sites for wtp53 in hCNT1 promoter have been identified and validated. Blockage of pyrimidine de novo synthesis with Brequinar or induction of DNA damage with Etoposide lead to an upregulation of hCNT1 expression largely dependent on p53 activation,

suggesting that p53 could play a role in connecting de novo and salvage pathways to maintain nucleotide pools. Interestingly, this regulation induced by wtp53 is not replicated by restoring different mutant p53 expression in a p53 KO model, which could contribute to hCNT1 expression reduction observed in tumors and the consequent decrease in some nucleoside analogs incorporation.

Conclusion

wtp53 regulates nucleoside salvage pathway transporter hCNT1 expression. Besides, in cell contexts where wtp53 is induced by DNA damage or imbalance of nucleotide pools transcription of hCNT1 is potentiated. However, mtp53 fails to induce hCNT1 expression.

EACR25-1608**Deciphering the Role of Gasdermin B and D in Endometrial Cancer: A Multidisciplinary Approach**

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Introduction

Pyroptosis is an inflammatory cell death process in which Gasdermin family protein plays a central role as final effectors. Gasdermins (GSDM, six members in human GSDMA-F), consist of a pyroptotic N-terminal domain (NTD) with the pyroptotic role, and a C-terminal domain (CTD), with auto-inhibitory capacity, both linked by a hinge region. In response to specific stimuli, the NTD is released, oligomerizes at the plasma membrane and forms pores that ultimately lead to cell death by pyroptosis. Interestingly, while activating GSDM lytic function in tumors could be a promising therapeutic anticancer approach, the overexpression of GSDMB or GSDMD has also been associated with pro-tumor activities. Thus, GSDMB promotes anti-HER2 therapy resistance and poor prognosis in HER2+ breast and gastric tumors, whereas high GSDMD associates with chemotherapy resistance and lower survival in lung cancer and osteosarcoma. However, the functional roles of GSDMB and GSDMD in endometrial cancer (EC) remain unknown. EC is the most common malignant gynecological tumor in developed countries and the eighth in incidence in women. While surgery is the primary treatment, it is often insufficient for advanced or recurrent cases with poor prognosis and there are few effective targeted therapies. Consequently, to identify novel predictive and prognostic biomarkers and to develop new therapeutic opportunities for aggressive

EC tumors, we focus our multidisciplinary study on the implication of GSDMB and D in these tumors.

Material and method

We first evaluated GSDMB and GSDMD expression by immunohistochemistry in a cohort containing different subtypes of ECs. We also analyzed the pyroptotic capacity of GSDMB and GSDMD in different EC cell lines, by western blotting and live-cell confocal microscopy.

Result and discussion

In the EC cohort, GSDMB and GSDMD expression associated with distinct clinicopathological variables, such as prognosis and histological subtype. Furthermore, we observed a differential expression of GSDMB and GSDMD within different EC cell lines. We generated diverse models expressing the cytotoxic NTDs of GSDMB or GSDMD and observed increased cell death capacity. Moreover, we also proved that distinct GSDMB isoforms exhibit differential pyroptotic capacity, when EC are cocultured with NK cells.

Conclusion

Our study advances in the characterization of pyroptosis in EC, discovering key differences between the expression of GSDMB or GSDMD in EC tumor samples and cell lines, as well as in the ability of these Gasdermins to conduct cell death under different cellular contexts.

EACR25-1615

Evaluation of Anti-Cancer Effects of Omeprazole in Prostate Cancer Cells

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Introduction

Prostate cancer is the second most common cancer in men worldwide. Proton pump inhibitors (PPIs), widely used for gastritis, are being repurposed as anti-cancer agents. Omeprazole, an FDA-approved PPI, has shown anti-cancer potential in various cancers. WZB117 is a selective GLUT1 inhibitor. This study aims to investigate the effects of Omeprazole on prostate cancer cells and its potential synergy with WZB117.

Material and method

The prostate cancer cell lines were treated with Omeprazole to evaluate its effects on viability, colony formation, migration, and lipid droplet accumulation. The crystal violet staining assessed cell viability, while fluorescence microscopy visualized lipid droplets. The molecular docking simulations analyzed Omeprazole's binding affinity. To assess synergy with WZB117, the combinatorial treatments were applied, followed by viability assays. 3D spheroid formation assays were performed, and the glucose consumption and pH changes were measured to evaluate metabolic shifts.

Result and discussion

IC50 values for PC-3, LNCaP, and CCD1072-SK cells were determined as 175.3, 77.4 and 481.4 μM respectively. Omeprazole reduced PC-3 colony formation and inhibited lipid droplet accumulation in LNCaP cells. The docking analysis confirmed high binding affinity to

target proteins. Omeprazole and WZB117 showed synergistic effects, reducing spheroid size in 3D cultures. While the glucose consumption and acidity of medium were increased in omeprazole treated cells, the combined treatment with WZB 117 and omeprazole lowered the acidity and glucose consumption in LNCaP cells. Our findings suggest that Omeprazole exerts anti-cancer effects in prostate cancer by inhibiting colony formation, migration, and lipid metabolism. Molecular docking analysis confirmed its strong binding affinity to target proteins. The synergistic effect observed with WZB117 indicates that dual targeting of lipid and glucose metabolism may enhance therapeutic efficacy. The metabolic shift, evidenced by increased acidity in treated cancer cells, suggests significant alterations in cellular metabolism. Notably, the differential response in normal cells highlights potential selectivity.

Conclusion

Omeprazole, especially in combination with WZB117, shows promise as an anti-cancer agent in prostate cancer, warranting further investigation for preclinical applications.

EACR25-1635

Therapy-induced senescence shapes tumor biology and treatment outcome in hepatoblastoma

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Introduction

Hepatoblastoma is the most common malignant liver tumor in children with poor survival rates in high-risk cases. Due to the very few recurrent genetic aberrations targeted therapies remain unavailable. Therefore, dynamic changes in hepatoblastoma cell states upon cytotoxic therapies, such as senescence, need to be characterized to unveil new targets for synthetic lethal or senolytic treatment approaches. Therefore, we investigate changes in hepatoblastoma biology upon therapy-induced senescence (TIS) with the goal to identify senescence-specific vulnerabilities that can be exploited to improve the therapy with “one-two punch” treatment approaches.

Material and method

Hepatoblastoma cell lines (HepG2, Hep293TT, Huh6), patient-derived xenografts (PDX), Myc-/Ctnnb1-driven mouse models generated by hydrodynamic tail vein infection (HTVI) in C57Bl/6 mice with defined senescence-specific genetic defects (Suv39h1- or CDKN2A-/-) were treated with doxorubicin or cisplatin. TIS was assessed in cell lines in vitro and tissue sections in situ by H3K9me3 and Ki67 immunofluorescence (IF) as well as SA-β-gal staining. Similarly hepatoblastoma patient samples from different molecular risk groups were analyzed to correlate senescence status to treatment outcome. Changes in tumor cell biology and micro-

environment were analysed by RNA sequencing, flow cytometry and spatial multiplex imaging (MACSima, Miltenyi). A high-throughput pharmaceutical screen with the European Biology Chemical pilot library (ECBL) combined with cytological profiling by Cell Painting was performed to identify senescence-specific vulnerabilities.

Result and discussion

Senescence was induced upon treatment with Doxorubicin in hepatoblastoma and PDX-derived cell lines, and Myc- and Ctnnb1-driven hepatoblastomas upon exposure to Doxorubicin and Cisplatin *in vivo* as evidenced by increased H3K9me3 levels, reduced Ki67 staining and higher SA- β -gal activity. Hepatoblastoma patient samples showed signs of senescence by RNAseq and immunofluorescence analysis. Importantly, low molecular risk groups (MRS1, MRS2) displayed higher levels of senescence than with improved overall survival by Kaplan Meier analysis. Senescent cells displayed higher immunogenicity, including the upregulation of MHC class I proteins and CD80. MACSima analysis demonstrated increased infiltration of macrophages and T cells into senescent hepatoblastomas. In the ECBL screen cell painting analyses of untreated vs. senescent HepG2 cells identified five highly selective senolytic compounds targeting mitochondrial and endoplasmic reticulum function.

Conclusion

The results highlight senescence as a key treatment response in hepatoblastoma that strongly impacts on tumor biology and overall survival. Given the absence of targeted therapies, senolytic “one-two punch” approaches represent a promising strategy to improve outcomes for high-risk hepatoblastoma patients.

EACR25-1640

Characterization of Patient-derived xenograft (PDX)- derived organoids (PDXOs) to Find New Treatment Strategies for LKB1-Mutated NSCLC

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Introduction

Lung cancer is the leading cause of cancer-related death, with 2.2 million new cases and 1.8 million deaths in 2020. Non-small cell lung cancer (NSCLC) accounts for 85% of cases, with lung adenocarcinoma (LUAD) as the most common subtype. Despite improved NSCLC survival due to personalized therapies, LKB1-inactivating mutations, found in 30% of cases, reduce response to immune checkpoint inhibitors (ICIs) and exclude targeted therapy options, conferring poor prognosis and an unmet clinical need. Our research group explores ERK inhibitors as alternative treatments using patient-derived xenografts (PDXs) and PDX-derived organoids (PDXOs). Thanks to the collaboration with Fondazione

Ca' Granda in Milan, we access fresh tumor samples, as PDXOs better model tumor complexity than 2D cultures, which fail to fully replicate NSCLC heterogeneity and biology.

Material and method

PDXOs were generated by mechanically disaggregating fresh tumor biopsies, followed by enzymatic digestion with collagenase to obtain single-cell suspensions, which were cultured in ultra-low attachment (ULA) plates. STR analysis confirmed genetic matching between PDXOs and original tumors. Organoid growth curves were assessed by seeding disaggregated PDXOs in 96-well ULA plates at xx cells/ml, monitoring viability using CellTiter-Glo assay every 24 hours. Human and murine cell composition was evaluated by real-time PCR using actin gene analysis. Flow cytometry and immunofluorescence analyses of fixed PDXOs identified lung, epithelial, and mesenchymal markers. Whole exome sequencing (WES) determined specific alterations. Pharmacological treatments on suspended PDXOs commenced 48 hours post-seeding, followed by seven-day incubation and viability assessment via CellTiter-Glo.

Result and discussion

In the past year, we successfully generated five new PDXOs and re-established four previously obtained. Two newly derived long-term (>10 passages) LKB1-mutant PDXOs were stabilized, adding to three pre-existing ones. Histological and flow cytometry analyses confirmed the expression of lung cancer, epithelial, and mesenchymal markers, while molecular profiling demonstrated preservation of alterations in both patient samples and PDXs. PDXOs displayed heterogeneous growth rates, partially mirroring their PDX counterparts. Preliminary pharmacological characterization validated ERK inhibitor efficacy in LKB1-null cells. WES data will guide combination strategies to enhance treatment responses based on specific alterations identified.

Conclusion

PDXOs represent reliable preclinical models for studying NSCLC biology and vulnerabilities, particularly in LKB1-mutant tumors, for which therapeutic options remain limited. Our findings support the use of ERK inhibitors in combination with other targeted therapies, opening new perspectives for treating this tumor subpopulation.

EACR25-1645

Highlighting the Small GTPase RhoA as a New Therapeutic Target in ALK+ ALCL

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Introduction

Rho GTPases are critical players in the biology of T cell lymphoma. Recurrent genetic lesions of RhoA and dysregulation have been reported in different T cell lymphoma, including Angioimmunoblastic T-cell lymphoma (AITL) and Peripheral T-cell lymphoma, not otherwise specified (PTCL-NOS). In ALK+ anaplastic large cell lymphoma (ALCL) oncogenic ALK regulates the Rho family GTPases CDC42 and RAC. We have demonstrated that the cytoskeleton regulators WASP and WIP are frequently downregulated resulting in hyper-activation of the RhoGTPase CDC42 and the MAPK pathway. Therefore, hyperactivated CDC42 and MAPK pathway represent a therapeutic vulnerability in ALK+ ALCL. We have previously demonstrated that RhoA activity is repressed by ALK, but its specific role in ALK-driven lymphomagenesis is not yet elucidated.

Material and method

To explore the contribution of RhoA in NPM-ALK lymphomagenesis, we generated the NPM-ALK/CD4Cre/RhoAfl/fl Tg mouse models from which we derived primary murine cell lines to dissect cell-autonomous alterations in vitro. We also abrogated RhoA expression by the CRISPR/Cas technology in human ALK+ lymphoma cell lines TS, JB6 and COST. We assessed cellular rewiring in the absence of RhoA by measuring cell proliferation and metabolism, analyzing actin organization through immunofluorescence and signaling by Western blot. We also performed RNA Sequencing analysis to evaluate changes of the transcriptome profile in RhoA knock-out cells. We tested in vitro the combination of ALK tyrosine kinase inhibitors (TKIs) and the ROCK inhibitor Y-27632.

Result and discussion

In vivo RhoA deletion impaired lymphoma development resulting in significant extension of survival of NPM-ALK/RhoAfl/fl mice. In vitro, in both murine and human cell lines, RhoA deletion affected lymphoma cell proliferation and migration, and caused changes in cell morphology and actin organization. RhoA knock-out ALK+ lymphoma cells underwent transcriptional rewiring to repress genes for mitotic spindle organization, cell cycle checkpoint regulation and reprogram metabolic processes for energy production. Moreover, the ratio of glucose/lactate production and oxygen consumption were significantly decreased in RhoA knock-out cells. In addition, RhoA knock-out ALK+ lymphoma cells showed increased sensitivity to ALK TKI crizotinib and inhibition of the RhoA downstream effector ROCK in combination with ALK-TKI, further potentiated the effects of crizotinib.

Conclusion

Overall, our findings suggest that RhoA plays a critical role in ALK-driven lymphomagenesis, with its deletion impairing lymphoma progression and increasing sensitivity to ALK-targeted therapies. In addition, RhoA deletion alters cell proliferation, migration, and metabolism, suggesting its potential as a therapeutic target in ALK+ lymphomas.

EACR25-1661

Deciphering the Role of CMA in Platinum Resistance: The Impact of LAMP2A in OCCC

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Introduction

Ovarian clear cell carcinoma (OCCC) is often diagnosed at early stages; however, its poor responsiveness to chemotherapy remains a significant clinical challenge. The limited efficacy of platinum-based chemotherapeutic agents frequently leads to disease recurrence and poor patient outcomes. Identifying alternative therapeutic targets is therefore critical. Chaperone-mediated autophagy (CMA), a selective autophagy pathway, regulates protein turnover in cancer cells, influencing their survival and progression. CMA has been reported to play dual roles in cancer, acting as both a tumor-promoting and tumor-suppressive mechanism depending on the context. As lysosomal-associated membrane protein type 2A (LAMP2A) is the key mediator of CMA, elucidating its role in drug resistance mechanisms in OCCC may provide valuable insights for novel treatment strategies.

Material and method

The JHOC-5 OCCC cell line was used to investigate the role of LAMP2A in platinum-based drug responses. LAMP2A knockout (KO) cells were generated using CRISPR/Cas9 genome editing. Whole-genome sequencing confirmed the absence of off-target effects and the successful knockout of the LAMP2A-specific sequence. Drug response was assessed in parental, plasmid control, and KO cells using the Sulforhodamine B (SRB) assay following cisplatin and carboplatin treatment. Further characterization of LAMP2A depletion effects included analysis of apoptosis-associated proteins, drug resistance mechanisms, and cell cycle distribution. Additionally, cell migration was assessed via the wound healing assay, while the colony formation assay was used to evaluate proliferative capacity.

Result and discussion

Loss of LAMP2A expression conferred increased resistance to cisplatin and carboplatin in JHOC-5 cells. In the wound healing assay, LAMP2A KO cells exhibited more aggressive behavior and enhanced migration. Similarly, in the colony formation assay, LAMP2A KO cells displayed an increased colony-forming ability compared to control cells. Further molecular analyses, including apoptosis-associated protein expression and cell cycle distribution, provided additional insights into how LAMP2A modulates drug response pathways.

Conclusion

These findings suggest that LAMP2A plays a modulatory role in chemotherapy resistance in OCCC. A deeper understanding of CMA's involvement in platinum-based drug response mechanisms may contribute to the development of more effective therapeutic strategies, particularly for drug-resistant ovarian cancer subtypes.

EACR25-1662

Ferrodoxin 2 is critical for tumor suppression and lipid homeostasis but dispensable for embryonic development

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Introduction

Ferrodoxin 1 and 2 (FDX1/2) constitute an evolutionarily conserved FDX family of iron-sulfur cluster (ISC) containing proteins. FDX1/2 are cognate substrates of ferredoxin reductase (FDXR) and serve as conduits for electron transfer from NADPH to a set of proteins involved in biogenesis of steroids, hemes, ISC and lipoylated proteins. Recently, we showed that Fdx1 is essential for embryonic development and lipid homeostasis. However, the biological function of FDX2 remains to be characterized.

Material and method

Genetic mouse model generation; Cell culture, siRNA transfection, RNA isolation and RT-PCR analysis, Plasmid construction and cell line generation, Western blot analysis, Nile Red staining, Measurement of cellular cholesterol and triglycerides, Histological analysis, Untargeted lipidomic analysis using LC-MS/MS

Result and discussion

To explore the physiological role of FDX2, we generated Fdx2-deficient mice. Interestingly, we found that unlike Fdx1-null embryos, which were dead at embryonic day 10.5 to 13.5, Fdx2-null mice were viable. We also found that both Fdx2-null and Fdx2-heterozygous mice had a short lifespan and were susceptible to spontaneous tumors and steatohepatitis. Moreover, we found that FDX2-deficiency increased whereas overexpression of FDX2 decreased cytoplasmic accumulation of lipid droplets. Consistently, we found that FDX2 deficiency led to accumulation of cholesterol and triglycerides. Mechanistically, we found that FDX2 deficiency suppressed expression of cholesterol transporter ABCA1 and activated master lipid transcription regulators SREBP1/2, thus leading to altered lipid metabolism. Untargeted lipidomic analysis showed that FDX2 deficiency led to altered biosynthesis of various lipid classes, including cardiolipins, cholesterol, ceramides, triglycerides, and fatty acids.

Conclusion

In summary, our findings underscore an indispensable role of FDX2 in tumor suppression and lipid homeostasis at both cellular and organismal levels without being a prerequisite for embryonic development.

EACR25-1668

Modeling gastric intestinal metaplasia in organoids: role of TP53 loss-of-function

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Introduction

The gastric bacterium *Helicobacter pylori* chronically colonizes approximately half of the world's population

and triggers a cascade of inflammatory responses that can ultimately lead to gastric intestinal metaplasia (GIM) and gastric cancer (GC). While the early stages of this pathological cascade are reversible with *H. pylori* eradication via antibiotic treatment, GIM often represents a point of no return, placing patients at high risk for cancer. GIM is defined as the replacement of the gastric mucosa with an epithelium of intestinal identity, characterized by the appearance of goblet cells in the stomach. While the architectural features of this lesion are well characterized, the underlying molecular mechanisms remain largely unclear.

Material and method

Using CRISPR/Cas9 technology, we generated TP53 loss-of-function mutations in gastric organoids and functionally characterized them through RNA-seq, microscopy and infection experiments. Moreover, we validated our findings in TP53-mutant mice and patient-derived samples.

Result and discussion

TP53 mutant gastric organoids showed clear signs of reprogramming toward intestinal cells, including the activation of key intestinal markers such as the mucin MUC2 and CDX2, a hallmark of GIM. Interestingly, TP53 mutation in gastric organoids increased the production of antimicrobial peptides, including DMBT1. This antimicrobial protein, which was only expressed in metaplastic cells, showed potent activity against *H. pylori*.

Conclusion

Our results suggest that TP53 loss-of-function mutations drive GIM in the stomach and may contribute to its irreversible progression to gastric cancer. Furthermore, the identification of DMBT1 as a mutant p53-dependent antimicrobial factor provides new insights into the mechanisms underlying *H. pylori* clearance in the precancerous stage of GIM.

EACR25-1675

Modulation of SUMOylation machinery complex proteins induces cell death in human colorectal cancer cells

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Introduction

Colorectal cancer is the second most frequently diagnosed cancer in women and the third most common cancer in men. There are multiple therapies available for the treatment of colon cancer, but the problem of drug resistance and metastasis is a major concern.

Understanding drug resistance pathways such as post-translational modification will provide insights into disease progression including new therapeutic opportunities. SUMOylation is a multi-step enzymatic post-translational modification cascade that facilitates the attachment of SUMO proteins to target substrates. This process involves an E2-conjugating enzyme (Ubc9, encoded by the UBE2I gene) and is regulated by deSUMOylating enzymes such as sentrin-specific

protease 1 (SENP1). This deSUMOylation step has been implicated in various tumours, including processes such as cell proliferation, survival, and DNA repair, making it a potential target for cancer therapies. The SUMOylation pathways in naked mole-rats (NMRs) are not well understood. NMRs have unique traits such as their longevity, resistance to cancer, and hypoxic adaptations and gaining insight into how SUMOylation is regulated in these pathways may provide insights. This study aimed to investigate the regulation of SENP1 and Ubc9 machinery between NMR intestinal tissues compared to human colorectal cancer cells, to find novel targets for cancer therapy.

Material and method

DLD-1 and HCT116 cell lines were used as colorectal cancer cell models. We used techniques such as cell culture, qPCR, Western blotting, colony formation, and invasion assays including genetic manipulations such as sh-knockdown and CRISPR knock-out cells.

Result and discussion

Here we report low expression levels of SENP1 and Ubc9 gene and protein in NMR tissues compared to DLD-1 colorectal cancer cells. SUMOylation machinery expression levels from NMR are recapitulated in DLD-1 and HCT116 colorectal cancer cells either by knockdown or knockout of the SENP1 and UBE21 to study functional effects. The knockdown and knockout of SENP1 or UBE21 reduces the colony formation, and invasions. The genetic manipulation also induces apoptotic mechanisms looking at Bcl-2 and BID expression in colorectal cancers compared to controls.

Conclusion

Our data revealed significant differences in the SUMOylation machinery between NMRs and colorectal cancer cells. This work provided substantial proof for further study to understand SUMOylation pathways to provide new strategies to target colorectal cancer.

EACR25-1695

α -Mangostin as a potential adjuvant in HER2+ breast cancer treatment

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Introduction

Breast cancer (BC) is the most prevalent neoplasia among women worldwide. It is classified into: estrogen receptor-positive (ER+), human epidermal growth factor receptor 2-positive (HER2+), and triple-negative (TN) subtypes. Despite subtype-specific treatments, adverse effects often limit their clinical efficacy. Our previous in vitro studies demonstrated that α -mangostin (AM), a phytonutrient from mangosteen, enhances the anti-proliferative effects of 5-fluorouracil and tamoxifen in TN and ER+ BC cell models, enabling dose-reduction

without losing efficacy. This study evaluates the in vitro effects of AM in combination with the HER2 inhibitor lapatinib (LAP) on cell growth, LAP dose-reduction, and pharmacological interactions in HER2+ BC cells.

Material and method

All reagents were of analytical grade. Cell culture treatment conditions and pharmacological interactions: MBCDF (HER2+) BC cells were previously generated from an explant obtained during a radical mastectomy of a patient diagnosed with stage IV ductal infiltrating carcinoma. The cells were treated in sextuplicate with LAP [0–6 μ M], AM [0–10 μ M], or a combination of LAP [0–4 μ M] with AM [0, 2, 4, and 6 μ M] for six days. Cell proliferation was assessed by the sulforhodamine B (SRB) assay. The inhibitory concentration at 50% (IC50) was determined using nonlinear logistic regression. Combination index (CI), efficacy index (EI), and dose reduction index (DRI) were calculated to evaluate the compounds' interaction; anticancer efficacy; and drug reduction potential.

Result and discussion

The LAP-AM combination significantly inhibited MBCDF cell proliferation in a dose-dependent manner. Notably, AM enhanced LAP's antiproliferative effects, reducing its IC50 from 0.94 μ M (LAP alone) to 0.84 μ M, 0.60 μ M, and 0.34 μ M when combined with AM at [2 μ M, 4 μ M, and 6 μ M], respectively. Higher AM concentrations in the co-treatment induced a synergistic effect. DRI analysis indicated that LAP concentration could be reduced without compromising efficacy. The anticancer efficacy, measured by EI, was significantly enhanced in a dose-dependent manner. The addition of AM at 6 μ M to the LAP curve showed the maximum efficacy. These findings suggest that AM could be an adjuvant in HER2+ BC treatment, potentially reducing LAP dosage and its associated toxic effects. These results warrant future *in vivo* studies.

Conclusion

AM enhances the antiproliferative effect of LAP in HER2+ BC cells, enabling dose reduction without efficacy loss. Moreover, improved EI supports AM's potential as an adjuvant agent for LAP therapy.

This study was partially funded by the Department of Reproductive Biology, INCMSZ (BRE-2606). D.A.-M. received a research assistant fellowship from SECIHTI (Mexico) under the supervision of L.D. No conflicts of interest were declared.

EACR25-1712

Epigallocatechin-gallate (EGCG) augments the anti-leukaemic effect of L-asparaginase in childhood B-cell acute lymphoblastic leukaemia cells

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Introduction

The dependency of childhood acute lymphoblastic leukaemia (CALL) cells on exogenous asparagine has made L-Asparaginase (ASNase) one of the key therapeutics in this haematological malignancy. Besides the relatively frequent immune-mediated inactivation of the enzyme, a number of molecular mechanisms of

resistance to ASNase have been described. The complex interplay between the activation of amino acid response, autophagy, MEK/ERK, mTORC1, Wnt signaling and other pathways, implicated in the response to asparagine (and glutamine) deamination and subsequent metabolic adaptation and survival, has raised interest not only in cALL, but in a number of other malignancies. Therefore, in order to tackle ASNase-resistant refractory or relapsed cALL, new therapeutic approaches addressing the bioenergetic plasticity of lymphoblasts need to be investigated. The green tea polyphenol flavonoid EGCG is a well-established inhibitor of glutamate dehydrogenase 1 and 2 (GLUD1/2), but also of IDH1 and other cell metabolism related targets making it an attractive pleiotropic therapeutic. In this study we aimed to characterize the response to ASNase and to EGCG and to investigate the potential of EGCG to concomitantly block potential rescue metabolic mechanisms in cALL cells and thus augment the anti-leukaemic effect of ASNase.

Material and method

REH, Sup-B15, and SEM B-cell cALL cell lines were examined after treatment with L-asparaginase and the epigallocatechin gallate (EGCG). Metabolomics analysis of cells, MTT assays, glutamine/glutamate luminescent assay, Annexin V/PI apoptosis test were applied, as well as assessment of mitochondrial function and ATP production by Seahorse XFp Analyzer (Agilent).

Result and discussion

We found that L-Asparaginase alters a number of amino acid biosynthesis pathways as well as purine and pyrimidine synthesis. These metabolic changes are accompanied by a significant reduction of mitochondrial function and ATP production. Furthermore, there is a distinct cellular response to the minimal clinically recommended concentrations of 0.1 IU/ml of ASNase, and a relatively wide range of IC₅₀ values for the three cell lines for EGCG – from ~8, 18 and 35 uM. Upon following the progression of cell proliferation and cell death on days 1, 3 and 7 after treatment, the three lines exhibited diverse dynamics spanning from apoptosis as early as on day 1 to delayed additive effects of the combination that were observed only beyond 72h.

Conclusion

We observed distinct metabolomic alteration patterns and different dynamics in the response to L-Asparaginase in all three cell lines. However, targeting cALL metabolism with EGCG holds potential to enhance the cytostatic and cytotoxic effects of this key chemotherapeutic.

ACKNOWLEDGEMENTS: National University Complex for Biomedical and Applied Research with participation in BBMRI-ERIC, NUCBPI-BBMRI.BG contract no. DOI-371/15.12.2023

EACR25-1722

Age-related effects in cancer therapy are driven by local and systemic changes in immune response

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Introduction

There is a strong bidirectional relationship between ageing and cancer. Ageing of the immune system, immunosenescence, is characterised by immune dysfunction. This compromises immune-surveillance and is associated with an increased development of malignant tumours. Therapies that can directly activate anti-cancer immunity, such as radio-and immunotherapy, may also be affected by age-related changes in the immune system which may limit their efficacy. Therefore, it is important that cancer therapies are understood through the lens of ageing, by using aged mouse models in preclinical cancer research.

Material and method

Old mice (18-month-old ~65 years in humans) and their younger counterparts (8–10 weeks old mice ~20-year-old humans) on C57/Bl6 background were subcutaneously injected with 4434 melanoma cell line and treated with dual radio-immunotherapy (3x8Gy and aPD-L1, respectively) or left untreated. Tumour growth was monitored to compare age-related efficacy of therapy combination. A separate cohort was culled immediately prior to treatment (when tumours were ~100mm³) and tumours, draining and contralateral lymph nodes (LN), spleen and blood profiled using multi-parameter flow cytometry. Photoconvertible KikGR (Kikume Green Red) mice were used to monitor patterns of migration from draining LN into tumour and other immune organs, and how those change with age.

Result and discussion

The efficacy of combination therapy was significantly reduced in aged versus young mice. High-dimensional analysis of early tumours revealed a dominance of exhausted T cell clusters in old, whilst in the tumour microenvironment (TME) of young mice, clusters with higher IFNg and Granzyme B production, and a higher percentage of “stem-like” TCF1+ cells were more prevalent. This suggests that the more functionally suppressed TME of aged mice is leading to poorer therapy response. Similar age-related differences were seen in other immune-related organs from aged mice, except for draining lymph nodes where, despite increased PD-1+ phenotype, robust immune response, assessed through IFNg production, was observed. Migration experiments using KikGR mice demonstrated that T cells were less migratory in older mice, with a higher percentage of photoconverted cells staying trapped in the LN over time compared to younger counterparts. Thus, it appears that effector IFNg-producing T cells do not readily migrate to tumours in old mice.

Conclusion

Older mice show a significant decrease in the efficacy of anti-cancer therapy that is associated with localised age-related changes within immune-relevant tissues, including the TME and secondary lymphoid organs, as well as systemic changes in migration of immune effector cells. These data suggest that age-related changes in the anti-cancer immune response are important to consider when assessing the efficacy of cancer therapy.

EACR25-1724

Functional Characterization of UBX Domain Protein 7 (UBXN7) in

Glioblastoma: Implications for Tumor Progression

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Introduction

Glioblastoma (GB) is the most common primary brain tumor, with limited therapeutic options and a median overall survival of approximately 15 months. The GLIOCAT group conducted a genome-wide expression analysis of 151 GB patients treated homogeneously with the Stupp protocol, identifying UBX Domain Protein 7 (UBXN7) as an unfavourable prognostic biomarker (Clin Cancer Res, 2021). UBXN7 is involved in proteasomal degradation and ubiquitin-mediated signaling, and previous studies suggest that its overexpression may reduce HIF1α degradation, potentially contributing to GB progression. The aim of this work is to validate the role of UBXN7 in GB.

Material and method

We generated a UBXN7 knockout (KO) LN229 cell line using the CRISPR/Cas9 system, the absence of expression of UBXN7 was by western blot. To assess the impact of UBXN7 depletion, we performed proliferation and migration assays, comparing the UBXN7-KO-LN229 model to the non-targeting control (NT-LN229). Proliferation was monitored using the Incucyte® live-cell imaging system, while migration was evaluated using both wound-healing and transwell Boyden chamber assays. Additionally, cell viability was assessed using the MTT assay following a 5-day treatment with temozolomide (TMZ). HIF1α expression was analyzed at different time points under hypoxic conditions (O₂: 0.01%) during 24 hours. RNAseq was performed to compare the transcriptome patterns between the KO cell line and its negative control (NC). Data was analyzed using GraphPad Prism 8.

Result and discussion

The LN229-UBXN7-KO cells exhibited reduced proliferation under normoxic conditions ($p = 0.039$) but showed no significant differences under hypoxia compared to NC-LN229. A significant decrease in migration was observed in UBXN7-KO cells, as demonstrated by the transwell (t-test $p = 0.023$) and wound-healing assays (2way ANOVA $p = 0.002$). In a 24-hour hypoxia experiment, HIF1α expression was lower in LN229-UBXN7-KO cells compared to the NT condition (2way ANOVA $p = 0.04$). No significant differences in cell viability were detected after 5 days of temozolomide (TMZ) treatment in normoxia conditions. The expression of several genes was found to be altered between KO and NC cells.

Conclusion

KO of UBXN7 in LN229 cells resulted in reduced proliferation and migration, as well as decreased HIF1α expression under hypoxia. These findings suggest that UBXN7 could be a potential therapeutic target in GB patients by modulating the HIF1α pathway.

EACR25-1730

NRF2 activation by myeloid cell-derived oxidative stress induces SNAI-driven EMT and metastasis in breast cancer

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Introduction

Metastasis is facilitated by epigenetically regulated transcription programs that enhance epithelial-to-mesenchymal transition (EMT). In this process, epithelial cancer cells gain mesenchymal features with migratory and invasive capacity. Breast cancer tumors are commonly infiltrated by myeloid cells that have been shown to stimulate EMT and metastatic outgrowth by mechanisms that include production of pro-inflammatory cytokines. Myeloid cells are also major producers of reactive oxygen species (ROS) via the NOX2 enzyme, but the potential impact of NOX2-derived ROS for breast cancer metastasis remains poorly understood.

Material and method

Co-culture experiments were conducted to investigate the role of ROS from wild type (WT) and Nox2-deficient (Nox2-KO) myeloid cells in triggering EMT in human and murine breast cancer cell lines. The EMT status of breast cancer cells was defined by expression of EMT transcription factors and migratory capacity. Moreover, we administered the NOX2-inducing peptide WKYVMVm intratumorally to orthotopically implanted 4T1 or EO771 breast cancer tumors in WT and Nox2-Ko mice. The effects of exogenous hydrogen peroxide (H₂O₂), the ROS scavenger catalase, and a NOX2 inhibitor were also evaluated in vitro and in vivo.

Result and discussion

Our study demonstrated that both exogenously added H₂O₂ and NOX2-derived ROS released from neighboring myeloid cells induced an EMT response in adjacent breast cancer cells, resulting in enhanced migratory and metastatic potential. ROS upregulated the EMT transcription factors SNAI1 and SNAI2 via the redox-sensitive transcription factor NRF2. Inhibition of NRF2 significantly reduced H₂O₂-induced SNAI1 and SNAI2 gene expression. In vivo, intratumoral administration of H₂O₂ and the NOX2 activator WKYVMVm increased EMT marker expression and metastasis, effects reversed by NOX2 inhibition. In accordance, myeloid cells from Nox2-deficient mice were less prone to induce EMT in breast cancer cells in vitro and in vivo. Analysis of publicly available human breast cancer datasets showed correlations between NOX2 and EMT-related gene expression within the tumor microenvironment. Additionally, high expression of NOX2 subunit genes and SNAI1 associated with reduced distant metastasis-free survival.

Conclusion

Collectively, these findings suggest that myeloid cell-derived ROS initiate SNAI-driven EMT in breast cancer cells. Targeting myeloid cell-derived ROS could be a potential strategy to limit metastasis in breast cancer.

EACR25-1739

Vemurafenib impairs mitochondrial bioenergetics and modulates stress-related transcript levels in the RAB-1 cell line: a multidisciplinary perspective in translational medicine for histiocytic lesions

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Introduction

Histiocytic disorders are marked by the uncontrolled proliferation of histiocytes, frequently driven by oncogenic mutations contributing to disease progression. Among these, the BRAFV600E mutation has emerged as a critical molecular target, leading to the clinical adoption of vemurafenib (VEM), a selective BRAF inhibitor, as part of targeted therapeutic approaches. Although VEM has proven effective in limiting tumor growth, its broader influence on cellular bioenergetics and regulating molecular stress responses remains inadequately characterized. The study aimed to evaluate the cytotoxic effects of vemurafenib (VEM) on the mitochondrial bioenergetics of the RAB-1 cell line, a primary cell line derived from histiocytic lesions (DSM ACC3377; patent: P.447749). Additionally, the investigation assessed VEM's impact on the expression of critical molecular biomarkers linked to mitochondrial metabolism.

Material and method

Vemurafenib cytotoxicity was assessed using an MTS assay to establish IC₅₀ and IC₂₅ doses. Mitochondrial bioenergetics included measuring basal respiration, maximum respiratory capacity, and ATP production rates in real time through oxygen consumption rate (OCR) and extracellular acidification rate (ECAR) measurements. The measurements were performed using a Seahorse XF HS Mini Analyzer (Agilent). The transcript levels of mitochondrial biomarkers were examined with RT-qPCR to explore the molecular changes induced by VEM.

Result and discussion

The study revealed that VEM decreased mitochondrial respiration in the RAB-1 cell line in a dose-dependent

manner, with reductions in both basal respiration and maximum respiratory capacity. Furthermore, VEM reduced ATP production, as reflected by lower OCR and ECAR values, though this effect was not dose-dependent. Molecular analysis showed increased mRNA levels of fission-1 (FIS-1) and myeloid cell leukemia-1 (MCL-1), along with elevated levels of lncRNA TUG-1 following VEM treatment.

Conclusion

Vemurafenib impairs the mitochondrial bioenergetics of the RAB-1 cell line primarily by reducing respiration and ATP production. These changes are associated with altered expression of mitochondrial biomarkers, suggesting a disruption of cellular energy metabolism. Exploring the molecular mechanisms by which VEM influences mitochondrial dynamics and stress responses may offer insights into the drug's efficacy and potential side effects. By integrating a multidisciplinary approach, the study highlights the importance of combining molecular, cellular, and clinical insights to optimize therapeutic strategies for histiocytic lesions.

EACR25-1743

Aurora-A-mediated Maf1 phosphorylation plays a novel role by regulating mitochondrial function in hepatocellular carcinoma

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Introduction

Transfer RNA (tRNA) biosynthesis is essential for processes like protein synthesis, cell cycle progression, mitochondrial function, and cell proliferation. Dysregulated tRNA biosynthesis contributes to diseases such as hepatocellular carcinoma (HCC). Maf1 is a key inhibitor of RNA polymerase III (Pol III), regulating tRNA biosynthesis by blocking the interaction between Pol III and transcription factors TFIIB and TFIIC. Maf1 also suppresses tumor growth and the AKT/mTOR pathway by enhancing PTEN expression. mTOR phosphorylates Maf1 at Ser-75, leading to its export to the cytosol and subsequent ubiquitination, promoting protein synthesis and cell cycle progression in HCC. Aurora-A, a serine/threonine kinase, is crucial for cell cycle regulation, and its overexpression is linked to cancer progression. However, the relationship between Maf1 and Aurora-A remains unclear.

Material and method

HCC cells were transfected with pGFP-Aurora-A and pHA-Maf1 to investigate their correlation. Alisertib was added to inhibit Aurora-A activity. RT-qPCR was performed to determine the RNA expression of Aurora-A, Maf1, pre-tRNALeu and pre-tRNAiMet. Western blot was performed to analyze expression levels of Maf1 and Aurora-A. Translational inhibitor cycloheximide was added to observe the protein stability of Maf1. The

protein interaction between Aurora-A and Maf1 was determined by co-immunoprecipitation and proximity ligation assay. The subcellular localization of Maf1 was observed by cell fraction assay and immunofluorescence. The cell proliferation was analyzed by CCK-8. The glycolytic activity was analyzed by Extracellular Acidification Rate (ECAR) assay.

Result and discussion

Aurora-A interacts with the Maf1 C domain to promote its cytosolic localization and enhance its protein stability in a kinase-dependent manner. Mutation of one potential Aurora-A phosphorylation site on Maf1 results in the nucleolus localization and decreased the protein stability. In the cytosol, Maf1 may regulate mitochondrial function by reducing OXPHOS levels. Notably, overexpression of the Maf1 mutant (Maf1-MT) failed to suppress tRNA biosynthesis and restore mitochondrial function. Additionally, HCC cells with Maf1 overexpression were more sensitive to Aurora-A inhibitor.

Conclusion

Aurora-A promotes the protein stability and cytosolic localization of Maf1 via interacting with its C domain in a kinase-dependent manner in HCC cells. Aurora-A phosphorylation site mutant of Maf1 can promote nucleolar localization and lost the RNA polymerase III suppressor function and regulation of mitochondria. In Maf1 overexpressed HCC cells, Aurora-A inhibitor has a higher efficacy in inhibiting cell growth. In HCC patients with high Aurora-A and Maf1 expression, treatment with Aurora-A inhibitors can be a promising strategy in cancer therapy.

EACR25-1748

Serum-related Metabolic stress and Breast Cancer: Differential Responses in Metastatic versus Non-Metastatic Cells

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Introduction

Starvation in breast cancer (BCa) cells is a complex adaptive phenomenon that promotes tumor survival and progression through diverse molecular mechanisms and signaling pathways. Understanding these mechanisms is critical for developing targeted therapeutic strategies. This study investigates the impact of serum-related metabolic stress on BCa cells in a dose- and time-dependent manner, focusing on morphology, metabolism, metastatic potential, stemness, gene expression, and drug sensitivity.

Material and method

BCa cells were subjected to short-term (72 h) and long-term (≥ 30 days) serum restriction and high-serum conditions. Morphological changes were monitored via inverted microscopy. Glycogen and lipid accumulation were analyzed using Periodic Acid-Schiff (PAS) and Oil Red O staining. Fourier-transform infrared (FTIR) spectroscopy was employed to assess biochemical alterations. Metastatic potential was evaluated through

wound-healing and invasion assays. Cancer stem cell (CSC) properties were assessed by flow cytometric analysis of CD44+/CD24- markers, colony formation, and spheroid assays. Drug response was determined using the XTT assay. RNA sequencing was performed to identify gene expression changes.

Result and discussion

Serum related metabolic stress triggers a reaction in cancer cells, leading to noticeable transformations in cell composition. Specifically, we found that metastatic BCa cells display distinct modifications in morphology and behavior such as migration and spheroid formation capacity when deprived of nutrients. Based on the IR data that supported by RNA sequencing results, significant alterations are observed in the spectral regions of proteins, lipids, nucleic acids and glycogen in BCa cells, indicating structural/compositional alterations. Although serum-related stress caused an increase in the expression of CSCs markers, it also increased drug sensitivity. PAS and Oil Red staining images reveal increments in the glycogen and lipid droplet contents in metastatic BCa compared to non-metastatic counterpart under starvation, suggesting a high metabolic activity.

Conclusion

Our integrative approach combining spectroscopic, transcriptomic, and functional assays provides critical insights into the metabolic plasticity of BCa cells under nutrient deprivation. The differential adaptive responses observed between metastatic and non-metastatic cells underscore the role of metabolic reprogramming in tumor progression. These findings offer a foundation for therapeutic strategies targeting metabolic vulnerabilities in aggressive breast cancer subtypes.

EACR25-1761

Increased intracellular hydrogen sulfide production reduces chemically-induced hepatocarcinoma progression and extends lifespan in mice

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Introduction

Liver cancer is the 6th most diagnosed and 3rd leading cause of cancer-related death. Hydrogen sulfide (H2S), which diffuses through cell membranes to induce intracellular responses, is generated by enzymatic and non-enzymatic production and modulates protein activity through cysteine persulfidation. This study examined the effects of increased H2S production on liver cancer carcinogenesis and progression.

Material and method

For a short-term study, male C57/Bl6J mice were treated with the sulfur donor compound α (150 mg /kg of food) for a week. Then, mice were injected with diethyl-nitrosamine (DEN) (100 mg/kg) to induce carcinogenic events. 48 hours after, mice were injected with 5-bromo-deoxyuridine (BrDU; 100 mg/kg). 2 h after BrDU injection mice were euthanized and liver tissue harvested. Liver sections were stained with γ H2ax, p53 and BrDU antibodies to determine DNA damage, stress responses

and compensatory proliferation, respectively. mRNA levels of inflammatory mediators and detoxifying enzymes were measured by PCR. For a long-term study, male mice were injected with DEN (5 mg/kg) at day 16 of life. The same day mice were treated with compound α , in a treatment sustained for the remaining of their lives. A High Fat Diet treatment was initiated on day 21. Body weight and caloric intake were monitored weekly. Two cohorts ($n \leq 12$) were sacrificed to assess tumor progression, while the remaining mice ($n \leq 45$) were monitored for health and lifespan. Physical health was assessed by rotarod, wire hang, grip strength and indirect metabolic calorimetry tests ($n \leq 25$). Finally, H₂S production was measured using the lead sulfide method, and persulfidated proteins were detected by DAz-2: Cy-5 Click labeling.

Result and discussion

In the short-term study, compound α -treated mice showed lower amount of DNA breaks (γ H2ax), higher p53 expression and unchanged compensatory proliferation (BrDU). mRNA levels of inflammatory regulators Ppary and Tnf α were lower, while IL-10 (anti-inflammatory marker) was higher. Finally, other interleukins and detoxification enzymes were unaffected. In the long-term study, compound α increased mean life expectancy (12.96 %) and reduced body weight gain without affecting energy intake. At first sacrifice, treated mice showed reductions in number and weight of tumors. Tumor weight remained reduced in the advanced stage of the disease. Furthermore, compound α increased grip strength, rotarod performance and wire suspension, but did not affect treadmill performance. Lastly, tumor enzymatic H₂S production was lower than in the healthy liver.

Conclusion

Compound α improved health and survival in mice with chemically induced liver cancer and reduced body weight gain. Compound α protected against damage induced by DEN and reduced tumor progression, reflected in fewer and smaller tumors in the early stages of the disease, maintaining a lower weight of tumors in advanced stages.

EACR25-1767

The role of CEMIP and CTCF in castration-resistant prostate cancer: A novel insight from 3D spheroid model

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Introduction

Prostate cancer (PCa) affects 12.5% of men globally, making it one of the leading causes of mortality and morbidity. Cell migration inducing hyaluronidase 1 (CEMIP) facilitates hyaluronan catabolism and has a role in cell metastasis, invasion, and motility. Dysregulated CEMIP expression results in tumor development and prognosis. In this study, we aim to investigate the epigenetic change and possible role of cell migration inducing hyaluronidase 1 (CEMIP) that drives tumor metastatic phenotypes in castration-resistant prostate cancer.

Material and method

We analyse public database from ChIP-Atlas and UCSC Genome Brower to investigate epigenetic shift of CEMIP among prostate cancer cell lines. To further investigate, CEMIP were knock-downed (si-RNA) and overexpressed (OE) with lentivirus in androgen-insensitive (DU145, PC-3) and androgen-sensitive (LNCaP) human prostate cancer cell lines. Total RNA was extracted from the cells using the Trizol reagents before performing RT-qPCR with different cDNA concentration (25 ng, 50 ng and 100 ng) to confirm a successful knockdown and overexpression. We then performed MTT assay, 3D spheroid model (aggregwell method), transwell migration, invasion assay and cell cycle analysis furtherly confirmed that the knock-down and overexpression of CEMIP were more significant in castration-resistant prostate cancer cell lines with statistical analysis from GraphPadPrism software.

Result and discussion

The results from ChIP-Atlas and UCSC Genome Brower revealed that there is significant epigenetic shift of CEMIP at promoter region with 11-zinc finger protein or CCCTC-binding factor (CTCF) binding to it. The results from RT-qPCR confirmed the successful knock-down and overexpression. The knock-down displayed cytotoxic effects against cancer cells ($p < 0.05$) while the overexpression of CEMIP increased cancer cells proliferation ($p < 0.05$). Moreover, cancer cells form less aggregation (3D spheroid) after si-CEMIP but oe-CEMIP showed that cancer cells form better 3D structure. The MTT assay, migration, invasion, cell cycle analysis furtherly confirmed that the knock-down and over-expressed effect were more significant in castration-resistant prostate cancer cell lines ($p < 0.05$). Thus, we confirmed that CEMIP drives tumor metastasis and 3D spheroid formation in castration-resistant prostate cancer cell lines. Our investigations provide compelling and novel evidence that CEMIP has role as oncogene in androgen-resistant prostate cancer.

Conclusion

Our research provides fresh perspectives into the epigenetic change of CEMIP in prostate cancer with novel 3D model for validation. CEMIP can be a key oncogene in androgen-resistant prostate cancer. Further in vitro and in vivo validation of these results are required to examine whether the promising results are clinically translational. Overall, our outcomes reveal that CEMIP may have potential as a future therapeutic target in prostate cancer.

EACR25-1771

The mTORC2-AKT-GSK3-FBXW7 Axis Regulates ELP1 Stability and U34 tRNA Modifications in Lung Cancer

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Introduction

Lung cancer remains a leading cause of cancer-related mortality, with therapeutic resistance driven by tumor heterogeneity and adaptive survival mechanisms. tRNA modifications at the U34 position have emerged as critical regulators of mRNA translation, influencing tumor progression and drug resistance. Our previous work identified mTORC2-PI3K signaling as a key modulator of U34 tRNA modifications, specifically regulating ELP1, the structural subunit of the Elongator complex, in melanoma. While therapy-resistant non-small cell lung cancer (NSCLC) exhibits mTORC2 overexpression, its impact on U34 tRNA modifications remains unexplored.

Material and method

To investigate U34-enzyme regulation in lung cancer, we utilized NSCLC cell lines (A549, H460, H23) and assessed ELP1 stability under different genetic and pharmacological conditions. Immunoprecipitation assays were performed to evaluate direct binding and ubiquitination of ELP1 by FBXW7. The effect of GSK3 inhibition on ELP1 ubiquitination was assessed to determine its role in ELP1 turnover. To identify global translational changes, proteomic analysis was conducted to examine codon-dependent mRNA translation patterns in lung cancer cells upon FBXW7 depletion. Gene Set Enrichment Analysis (GSEA) was applied to the FBXW7-ELP1 proteome to identify key biological pathways regulated by this axis. Functional assays, including wound healing and transwell migration assays, were performed to evaluate the role of ELP1 in tumor cell migration.

Result and discussion

We demonstrate that ELP1 stability is regulated by the mTORC2-AKT-GSK3-FBXW7 axis. Immunoprecipitation assays confirmed direct binding and ubiquitination of ELP1 by FBXW7, with this ubiquitination abolished upon GSK3 inhibition, validating the role of mTORC2-AKT-GSK3-FBXW7 signaling in ELP1 turnover. Proteomic analysis revealed that FBXW7 loss enhances the translation of U34-sensitive codon-enriched genes, establishing a mechanistic link between mTORC2-FBXW7 signaling and codon-biased translation in lung cancer. GSEA analysis of the FBXW7-ELP1 proteome identified migration as a key pathway regulated by this axis. Functionally, ELP1 depletion blocked the increased migration of lung cancer cells induced by FBXW7 loss, demonstrating its critical role in FBXW7-mediated tumor cell motility.

Conclusion

This study identifies mTORC2-AKT-GSK3-FBXW7 as a key regulatory axis of ELP1 stability, linking U34 tRNA modifications to oncogenic translation and tumor cell migration in lung cancer. Our findings reveal a mechanistic connection between FBXW7 loss, ELP1 regulation, and lung cancer progression, suggesting that targeting this axis may present novel therapeutic opportunities for drug-resistant NSCLC.

EACR25-1783

PRKC ζ benefits melanoma brain metastases

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Introduction

While current standard therapies have shown effectiveness against metastasized melanoma, achieving lasting responses in melanoma brain metastases remains challenging. This is largely due to brain-specific resistance mechanisms, highlighting the need to investigate underlying differences between intracranial and extracranial metastases. Although the mutational profile is mainly unaltered, epigenetic regulation is diverging. Through methylome and transcriptome analyses of 16 matched intra- and extracranial metastases, 38 protein candidates with distinct promoter methylation and corresponding expression were identified. Eight promising candidates were validated by immunohistochemistry and had a significantly higher protein expression in intracranial metastases (PMID: 36716920). In this study, we wanted to investigate the role of two likely cancer-promoting candidates, named protein kinase C zeta (PRKC ζ) and growth factor receptor bound protein 10 (GRB10), which are upregulated in intracranial metastases.

Material and method

Various melanoma cell lines were screened on their PRKC ζ /GRB10 expression level and PRKC ζ /GRB10 protein level, siRNA-mediated knockdown was applied to selected lines. Functional validation was performed in a cell viability crystal violet assay, a propidium iodide-based cell cycle analysis, a 2D wound closure scratch assay and pathway analyses.

Result and discussion

We found a wide range of PRKC ζ and GRB10 gene expression in various established as well as in-house patient-derived melanoma cell lines, independent from the metastasis site. Cell lines with either high or moderate PRKC ζ or GRB10 expression were chosen for further functional investigation. Under GRB10-knockdown, only BRAF-mutated melanoma lines showed increased phospho-AKT levels, a component of the cancer-promoting PI3K/AKT signaling pathway, and increased viability, concomitantly. A decreased viability and G1 cell cycle arrest was seen in PRKC ζ -knockdown cells independent from mutation status. No clear link to PI3K/AKT and MAPK signaling could be drawn, though. A network-based pathway analysis with RNA data

predicted a connection between PRKC ζ and cell adhesion processes. Therefore, a 2D wound closure scratch assay was developed and revealed a decreased wound-closure capacity (migratory potential) in PRKC ζ -knockdown cells, accordingly.

Conclusion

Different from initial findings, GRB10 acted as a tumor suppressor in our cell culture-based setting. Contrastive, PRKC ζ promoted cell viability, cell cycle progression and migration in melanoma cells. Melanoma brain metastases could therefore benefit from high PRKC ζ levels in comparison to lower levels in extracranial metastases.

EACR25-1804

Loss of LKB1 in non-small cell lung carcinoma favours EMT-independent metastasis

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Introduction

Lung cancer is the most diagnosed type of cancer and the leading cause of cancer-related deaths. Non-small cell lung carcinoma (NSCLC) is the most common type of lung cancer accounting for 85% of lung cancer cases. Multiple oncogenic drivers underlie NSCLC but hyperactivating mutations in Kras (K) are frequently found in patients in combination with loss-of-function mutations in p53 (P) or/and LKB1 (L). Occurrence of co-mutations increases NSCLC aggressiveness as indicated by the higher metastatic potential of KP tumors upon LKB1 loss (KPL tumors) as compared to KP tumors bearing wild-type LKB1. Despite progress in therapeutic strategies, the 5-year survival rate of advanced NSCLC patients is less than 20%. This poor prognosis is in part due to therapy resistance, tumor relapse and metastasis. Indeed, most NSCLC patients reach terminal states with metastatic outgrowths. In this regard, the epithelial-to-mesenchymal transition (EMT) occurring in tumor cells is widely accepted as a key cellular program for acquisition of metastatic potential. As metastasis represents a threat for NSCLC patients' prognosis, we aim to unravel the molecular mechanisms of NSCLC metastasis beyond EMT to target lung cancer spread in the future.

Material and method

To study the metastatic potential of LKB1 loss in Kras- and p53 mutated NSCLC tumors, we generated NSCLC mouse models which develop either double-mutated KP or triple mutated KPL lung tumors in CRISPR/Cas9 mice upon intubation with mutation-containing viruses.

Result and discussion

Whereas KPL tumors significantly shorten mouse survival to a greater extent as compared to KP tumors, KPL tumor cells isolated from transgenic mice display a more epithelial-like phenotype. Indeed, KPL tumor cells are characterized by a cobblestone-shaped morphology, upregulation and downregulation of canonical epithelial

and mesenchymal markers, respectively as well as less focal adhesion contacts and reduced cell motility in vitro. Accordingly, RNA sequencing shows a significant enrichment of EMT signatures in KP tumor cells. In addition, as compared to NSCLC patients' tumors that were ordered according to their EMT score calculated based on transcriptomic data, KP and KPL tumor cells were positioned closer to the most mesenchymal and most epithelial NSCLC tumors, respectively.

Interestingly, when growing in the lungs of the transgenic mice, only KPL tumor cells form lymphnode metastasis. However, KP cells home the lungs faster than KPL cells when engrafted into mice via systemic circulation.

Conclusion

This study points towards the existence of molecular pathways beyond the canonical EMT program that govern NSCLC metastasis upon LKB1 loss within the native tumoral niche. Our research might reinforce the concept of hybrid and reversible EMT phenotypes and in parallel, shed light on a novel model of metastasis, where the metastatic potential of LKB1-mutated NSCLC is rather dictated by EMT-independent cellular processes.

EACR25-1818

Kv1.3 potassium channel-linked signaling networks based on STAT3 function are crucial for melanoma tumorigenesis

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Introduction

Ion channels are emerging oncological targets since their aberrant expression and function have been linked to altered proliferation, apoptosis, and migration of cancer cells both in vitro and in vivo. Importantly, most ion channels can be pharmacologically targeted by specific inhibitors to block ion fluxes. However, the view is emerging that these proteins may also affect cancer cell behavior by taking part in signaling complexes and actively modulating cancer-relevant intracellular signaling events. The Kv1.3 voltage-gated potassium channel represents a promising case; however, how it is involved in cancer progression is not yet clear. We hypothesize that Kv1.3, showing enhanced expression in various cancer cells, modulates cancer-relevant cell signaling through its direct interaction with STAT3, leading to STAT3 activation and to p53 stabilization. We furthermore hypothesize that interruption of these connections leads to profound changes in cancer-related signaling events.

Material and method

Given that Kv1.3 is functional both in the plasma membrane and in mitochondria (mitoKv1.3), we aim to understand i) whether inhibition of the former or latter population is sufficient to decrease STAT3 activation and increase p53 stabilization; ii) whether inhibition of the channel activity or lack of Kv1.3 protein is required to exert these effects. To this end, we exploited membrane-impermeant toxin inhibitors Margatoxin (Mgtx) and Stichodactyla toxin (Shk) or the mitochondria-targeted Kv1.3 inhibitor PAPTP. To exploit differences in biological processes between WT and KO cells, an

RNAseq has been performed, while, effects on the global protein translation have been confirmed by flow-cytometry experiments based on a click chemistry-based assay.

Result and discussion

Our results show that deletion of Kv1.3 resulted in stabilization and activation of p53, while decreasing stability and activation of STAT3. At the same time, pharmacological inhibition of the mitochondrial Kv1.3 channel also induces p53 stabilization and STAT3 inactivation. Moreover, the interaction between STAT3 and Kv1.3 is strongly suggested by proximity ligation assay on HEK239 cells overexpressing Kv1.3 channels. The RNAseq performed on Kv1.3-KO and WT melanoma cells analyses indicated a reduced expression of genes related to protein translation. Western blot, performed on B16F10 WT and Kv1.3 KO, suggests that in KO cells or WT cells treated with PAPTP, expression of three ribosomal proteins and a mitochondrial ribosomal protein is down-regulated. Down-regulation of ribosome proteins correlates with the reduction of the global protein synthesis, in the same conditions.

Conclusion

This represents a novel tumor-promoting Kv1.3-interactome landscape and mechanism of protein translation inhibition, that is driven by the down-regulation of potassium channel expression/activity and is currently being elucidated.

EACR25-1821

TGF- β Resistance as a Collateral Mechanism to Senescence Escape and Tumor Aggressiveness in Hepatocellular Carcinoma

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Introduction

Transforming growth factor- β (TGF- β), a potent senescence inducer, paradoxically promotes aggressive cancer cell traits in hepatocellular carcinoma (HCC), such as epithelial-to-mesenchymal transition (EMT), reshaping the tumor microenvironment, and enhancing invasion and metastasis. The underlying mechanisms of the transition from a TGF- β -sensitive state to a TGF- β -resistant state remain understudied.

Material and method

In this context, we developed TGF- β -sensitive and TGF- β -resistant subclones in epithelial HCC cell lines, Huh7 and Hep3B. We assessed senescence markers, cell cycle dynamics, and TGF- β signaling using various techniques, including SA- β -Gal staining, BrdU incorporation, flow cytometry, Western blotting, and reporter assays. We established the correlation between TGF- β resistance and aggressive characteristics through EMT gene expression

analyses, 2D and 3D assays, and invasion and metastasis. Transcriptomic analysis was performed to identify gene expression changes. Gene function was validated using RNAi and CRISPR/Cas9-mediated gene knockout, followed by functional assays.

Result and discussion

The transition from a sensitive state to a resistant state induced escape from senescence in epithelial HCC cells through defective Smad3 activation and nuclear trafficking, leading to increased proliferation and mesenchymal characteristics. Transcriptomic analyses revealed upregulation of EMT and angiogenesis pathways in resistant cells. MARK1 and GRM8 were identified as critical regulators of TGF- β resistance by disrupting Smad signaling dynamics. Depletion of these genes restored TGF- β sensitivity and Smad nuclear localization. These findings highlight the dynamic nature of senescence and suggest that HCC cells can exploit senescence plasticity to counteract TGF- β -mediated tumor suppression and progress towards more aggressive phenotypes.

Conclusion

Loss of TGF- β sensitivity in epithelial HCC cells is associated with aggressive cancer hallmarks. Our findings help further explain the differential responses of epithelial and mesenchymal HCC cell lines to TGF- β treatment. Furthermore, our findings suggest potential avenues for restoring the TGF- β /senescence axis in advanced HCCs exhibiting elevated TGF- β , representing a promising area of investigation, particularly concerning anti-tumor mechanisms and senescence escape.

EACR25-1845

C6ORF15 is a driver of lymph node metastasis through upregulation of EMT in colorectal cancer

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Introduction

Lymph node involvement in colon cancer affects clinical treatment and portends a worse overall survival. Molecular features associated with lymph node metastasis in colon cancer remain poorly described. In this study, we sought to identify and validate molecular targets of lymph node metastasis.

Material and method

Analysis of TCGA data was performed. Colon cancers were divided into two groups. The "expanding" phenotype represented cancers which were >5cm without lymph node metastasis, while the "metastatic" group denoted cancers which were <=5 cm with lymph node metastasis. Transcriptomic data from these two groups were compared, and analysed using GSEA. SW480 and HCT116 cell lines were used to validate findings by generating isogenic C6orf15 knockout clones. RNAseq was performed to analyse transcriptomic differences. Further validation of the effect of C6orf15 was performed using western blot, as well as proliferation, invasion and migration assays.

Result and discussion

378 colon cancers were analysed on TCGA. The median size of colon cancer in this group was 5.375cm, such that

5cm was used as the cutoff for the "expanding" and "metastatic" phenotype groups. There was no statistically significant difference in nodal positivity between this size cutoff and lymph node positivity, suggesting that these were independent variables (Fishers exact test, $p = 0.6381$). Kaplan-Meier comparison of "expanding" and "metastatic" phenotypes confirmed worse overall survival in the latter group ($p = 0.0145$). Comparing transcriptomic differences between these two groups demonstrated that one of the top differentially expressed gene was C6orf15 ($\text{LogFC} = 2.37$, $p = 2.06E-07$). GSEA using the Hallmark gene sets revealed epithelial-mesenchymal transition (NES 1.81, FDR q-value = 0) as the most enriched Hallmark in the "metastatic" group. C6orf15 knockout clones in SW480 and HCT116 cell lines were generated and validated using western blot, and Sanger sequencing. RNAseq of HCT116 wildtype and C6orf15 mutant clones demonstrated that EMT was down-regulated in the C6orf15 mutant population. This was validated using both HCT116 and SW480 cell lines on western blot, which revealed decreased presence of SNAI1, SLUG and CDH2. Functional assays of proliferation, invasion and migration also revealed decreased activity in C6orf15 knockouts. Finally, C6ORF15 expression was analysed on immunohisto-chemistry staining of patient derived tissue which fulfilled our criteria of the "expanding" and "metastatic" phenotypes. This revealed stronger expression of C6ORF15 in the "metastatic" group compared to the "expanding" group.

Conclusion

Using TCGA data, we identified C6orf15 mediated EMT as a potential pathway of lymph node metastasis in colon cancer. This raises the possibility of C6orf15 directed interventions as novel therapeutic targets to reduce the incidence of lymph node metastasis, therefore improving colon cancer overall survival.

EACR25-1846

Investigation of novel heterocyclic fused naphthalimides as DNA-intercalating agents with antitumor potential

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Introduction

The 1,8-naphthalimides are a class of heterocycles distinguished by a π -deficient planar aromatic system, exhibiting remarkable antiproliferative effects largely due to their ability to intercalate into DNA. The structures we introduce are conceptually novel and have no known analogues in the scientific literature. These molecules were designed to enhance the therapeutic potential of the well-established cytotoxic agent mitomycin C by combining its scaffold with the DNA intercalating tricyclic planar chromophore dibenzofuran into a single entity. The aim of this study is to evaluate the biological activity of these newly developed compounds.

Material and method

A549 and NCI-H1299 human non-small cell lung carcinoma cell lines, along with MRC-5 normal lung fibroblasts, were utilized for analysis. A series of experiments were performed to examine the cytotoxic and antiproliferative properties of over twenty novel derivatives. Additionally, various assays were conducted on selected candidates to explore their impact on the cell cycle and the mechanisms underlying cell death.

Result and discussion

The results revealed that the benzofuran-1,8-naphthalimides exhibited greater cytotoxic potency compared to mitomycin C and other intercalating agents. Notably, incorporating an additional nitro group into the benzofuran core led to an increase in cellular toxicity. Moreover, a distinction was observed among three positional isomers containing this modification. Cell cycle analyses indicated a different pattern of response to treatment in the two cancerous cell lines. These naphthalimide-based compounds were shown to induce apoptosis, inhibit DNA replication, and cause accumulation of damage in the genetic material.

Conclusion

The present study demonstrates that benzofuran-1,8-naphthalimides exhibit potent cytotoxic activity by inducing apoptosis and disrupting DNA replication.

These findings support their potential as promising anticancer agents. Acknowledgments: This work was supported by grant K11-06-H61/1 from 13.12.22 of the Bulgarian National Science Fund.

EACR25-1847

Tackling oxidative phosphorylation of glioblastoma-initiating cells by specifically impairing 1 transmembrane Chloride Intracellular Channel 1

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Introduction

One of the major challenges in cancer treatment is tackling the metabolic reprogramming of tumor cells, which requires higher energy and biomolecules than healthy cells. Glioblastoma (GB) cells, as well as the other cancer types, adapt their metabolism by switching between glycolysis and oxidative phosphorylation (OXPHOS). These processes are dependent on transmembrane proteins' response to the extracellular environment. Our research identified the transmembrane form of Chloride Intracellular Channel 1 (tmCLIC1) as a marker of malignancy and a potential therapeutic target. Thus, tmCLIC1 is upregulated in several solid tumors promoting cancer growth and progression, while it is mainly absent in healthy cells. Here, we found that the antidiabetic drug metformin, an OXPHOS inhibitor in cancer cells, reduces tmCLIC1 activity.

Material and method

All the experiments were performed in GB cells with several CLIC1 genetic background obtained with CRISPR-Cas9-Technology. We performed NMR and patch clamp recording to demonstrate tmCLIC1-metformin interaction. Moreover, we analyzed metabolism modulation given by metformin through OCR and EACR measurement. In addition, PP2A-GSK3 β -MCL-1 axis was analyzed through Western Blot. Our in

vitro results were further confirmed in vivo with Zebrafish embryo and in murine.

Result and discussion

Metformin impairs the tmCLIC1 function by a direct and specific binding coordinated by arginine 29 in the tmCLIC1 sequence. Mutation of this residue nullifies metformin inhibitory effects on cell proliferation, mitochondrial respiration, and tumor progression in *in vitro* and *in vivo* models. Additionally, in conditions of hypoglycemia, metformin induces cancer cell apoptosis by inhibiting the Cancerous Inhibitor of Protein Phosphatase 2A (CIP2A) and activating PP2A subunit B56 δ . This, dephosphorylates Glycogen Synthase Kinase 3 Beta (GSK3 β), leading to degradation of the pro-survival 53 protein MCL-1 and subsequent cell death. Based on previous data, it is possible to sustain a reasonable metformin mechanism of action in cancer cells. We suppose that tmCLIC1's role in reactive oxygen species (ROS) balance is crucial for GB cells metabolic re-programming, and its impairment sensitizes resistant tumor cells. Metformin is an extremely low-priced drug that can be repurposed as an adjuvant in cancer treatment with minor side adverse effects.

Conclusion

We demonstrate that tmCLIC1 is essential for this metformin-mediated antineoplastic effect, particularly through regulating the PP2A-GSK3 β -MCL-1 axis under hypoglycemic conditions. Given tmCLIC1's role in GB progression, our findings pave the way to optimize the molecular interactions between tmCLIC1 and metformin or related molecules, enhancing their therapeutic efficacy.

EACR25-1848

Structural Regulation of Gasdermin B Cytotoxic Activity: Insights into its Potential as a Therapeutic Target in Cancer

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Introduction

Gasdermin B (GSDMB) is a pore-forming protein involved in inflammatory cancer cell death and immune responses. Its activation requires the proteolytic release of the N-terminal domain (NTD) by granzyme A (GZMA), a protease secreted by cytotoxic lymphocytes. However, in solid tumours where GSDMB is over-expressed but immune infiltration is low, this mechanism may be inefficient, making alternative activation strategies therapeutically relevant. GSDMB over-expression correlates with poor prognosis in cancers like HER2+ breast cancer, though its precise role remains controversial. This may be linked to the existence of four isoforms (GSDMB1-4) which differ in sequence within the linker region between NTD and C-terminal domain (CTD), influencing its pyroptotic potential upon GZMA

cleavage. Before cleavage, the pore-forming NTD remains inhibited by intramolecular interactions with the CTD. Recent structural studies have predicted key residues within this inhibitory interface, but their functional relevance is unclear. Since full-length GSDMB has not been crystallized in its auto-inhibited state, direct structural validation is also limited. Given the isoform-dependent cytotoxicity, understanding the structural determinants of isoform-specific activation is crucial for therapeutic purposes. Here, we assess whether disrupting these inhibitory interactions is sufficient to activate GSDMB isoforms.

Material and method

In this study, we mutated various CTD residues predicted to mediate NTD inhibition, based on preceding structural analyses and comparisons across the Gasdermin family. The functional impact of mutations was assessed via propidium iodide staining and lactate dehydrogenase (LDH) release in cancer and control cell lines. Western blot and confocal microscopy were used to analyse expression and subcellular localization respectively.

Result and discussion

Tandem mutations disrupting hydrophobic and hydrogen-bond interactions in the full-length (FL) GSDMB3 isoform failed to induce cell-death, suggesting a stronger autoinhibition compared to other gasdermins. Moreover, structural elements may reinforce auto-inhibition in the GSDMB3 FL variant compared to other isoforms, with the linker region contributing to these stabilizing structural forces. Ongoing studies will determine whether equivalent mutations activate other isoforms, which would support the existence of isoform-specific regulatory mechanisms.

Conclusion

Our results demonstrate that disrupting predicted NTD-CTD interactions is not sufficient to activate GSDMB3 cytotoxic capacity, highlighting additional structural forces that reinforce its auto-inhibited state. Understanding isoform-specific regulation could guide the development of therapeutic strategies to induce GSDMB-mediated cancer cell death in cold tumours, potentially boosting anti-tumour immunity.

EACR25-1853

Unraveling CIP2A's impact on DNA damage response in basal-like triple-negative breast cancer

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Introduction

Basal-like triple-negative breast cancer (BL-TNBC) is an aggressive malignancy characterized by a high mutational burden, frequently driven by BRCA mutations or defects in homologous recombination (HR) pathways. CIP2A, a known inhibitor of protein phosphatase 2A (PP2A), is a driver protein for BL-TNBC initiation and progression. Emerging phosphoproteomic data suggest that CIP2A impairs PP2A-mediated dephosphorylation of

several DNA damage response (DDR) proteins. However, the functional consequences of these phosphorylation events in BL-TNBC remain unclear. This study investigates the role of CIP2A in DDR regulation and its impact on BL-TNBC cell survival and therapeutic response.

Material and method

A functional dependency analysis using DepMap data was conducted to assess CIP2A's association with key DDR proteins. Site-specific CRISPR/Cas9 mutagenesis screening was employed to examine the impact of CIP2A-regulated phosphosites in BL-TNBC cells. Additionally, CRISPR/Cas9 mutagenesis under ATR inhibitor treatment was performed to evaluate phosphosite-specific contributions to therapy response. Functional assays, including cytotoxicity and colony formation assays, were carried out in TNBC cells expressing a PP2A-binding defective CIP2A mutant in combination with DDR-targeting agents to further elucidate the relationship between CIP2A and DDR.

Result and discussion

CIP2A was found to exhibit functional co-dependency with multiple DDR proteins. Phosphoproteomic analysis confirmed CIP2A-mediated inhibition of PP2A-dependent dephosphorylation within the TopBP1-associated DDR complex. CRISPR/Cas9 mutagenesis revealed differential cell fitness effects upon phosphosite-specific modifications. Furthermore, TNBC cells expressing the PP2A-binding defective CIP2A mutant displayed altered sensitivity to DDR inhibitors, underscoring the functional significance of CIP2A-mediated regulation in therapy response.

Conclusion

Our findings demonstrate that CIP2A plays a crucial role in DDR phosphoregulation, influencing BL-TNBC cell survival and therapeutic vulnerability. By leveraging site-specific CRISPR/Cas9 mutagenesis, we aim to identify additional DDR targets modulated by CIP2A. These insights may pave the way for novel therapeutic strategies targeting CIP2A-dependent DDR vulnerabilities in BL-TNBC.

EACR25-1865

MyomiR-133b suppresses colorectal cancer cell proliferation and modulates expression of multiple gene targets

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Introduction

Colorectal cancer (CRC) is one of the most common types of cancer worldwide. Epidemiological and experimental evidence suggests that regular physical activity reduces CRC risk and progression partly due to exercise-induced circulatory factors, exerkines. Here, we investigated the effect of muscle-derived miR-133b on CRC cell proliferation and gene expression and studied

the effect of exercise-conditioned human plasma, a potential source of exercise-regulated miR-133b, on CRC cell proliferation. Muscle-derived miRNAs, myomiRs, can modulate cancer-related molecular pathways, such as myomiR-133b, that has been shown to regulate cell growth, differentiation, and apoptosis in different cancer types. However, its direct role in CRC remains unclear.

Material and method

HCT116 cells (a colorectal cancer cell line) were transfected with miR-133b mimic (30 nM), inhibitor (50 nM), or their negative controls. Cell proliferation was determined by BrdU and MTT assays at 0h, 24h, 48h, and 72h after transfection. HDAC4, LIF, SIRT1, SRF, TIMP1, TP53 and VEGF expression was assessed by qPCR 48h after transfection. Human plasma was obtained before, after and 60 min after indoor cycling (70% VO_{2max}, sedentary individuals with obesity; age $31,6 \pm 1,3$ years; BMI $35,4 \pm 4,4$ kg/m²), and its effect on HCT116 cell proliferation was assessed by BrdU. Exercise-related changes of miR-133b in human plasma were measured by qPCR.

Result and discussion

Transfection of HCT116 cells with miR-133b mimic decreased the proliferation rate of cancer cells at the 72-h time point, as measured by both MTT assay and BrdU incorporation ($n = 3$; $p < 0.01$). This was accompanied by a concomitant decrease in lactate release into the media ($p < 0.01$), suggesting a reduced cell number. Gene expression analysis ($n = 3$) revealed downregulation of SIRT1 and TP53 ($p < 0.01$), alongside with upregulation of HDAC4, LIF and SRF ($p < 0.001$), and no change in TIMP1 and VEGF. Despite no effect of miR-133b inhibitor on HCT116 cell proliferation, possibly due to low miR-133b levels in cancer cells, we observed upregulation of TP53 ($p < 0.01$), SRF ($p < 0.05$) and VEGF expression ($p < 0.05$). Exercise-conditioned plasma collected 60 min after acute exercise significantly reduced HCT116 proliferation ($n = 14$; $p < 0.01$). This reduction was not associated with increased miR-133b in exercise-conditioned plasma ($n = 4$) suggesting other inhibitory exercise mediators in plasma.

Conclusion

This study provides evidence that myomiR-133b suppresses CRC proliferation, which may be mediated through the regulation of transcription factors (SRF), deacetylases (Sirtuin1, HDAC4) and genes related to cancer cell proliferation and apoptosis (P53, LIF). The mechanisms of tumour-suppressing effect of exercise-conditioned human plasma will require further research.

Grant support: VEGA-2/0144/23, Sedláčková-Rabanová scholarship, APVV 19-0411, APVV 23-0604

EACR25-1866

Genetic ablation of NOX2 boosts anti-tumor immunity yet accelerates metastasis in the PyMT-MMTV breast cancer model

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Introduction

Myeloid cells are strong determinants of metastatic spread and outgrowth. Myeloid cells express the enzyme NOX2 that generates reactive oxygen species (ROS). We and others have demonstrated that NOX2-derived ROS contribute to an immunosuppressive microenvironment by suppressing the function of cytotoxic lymphocytes, polarizing macrophages towards a tumor-promoting M2 phenotype, and increasing regulatory T cell (Treg) populations. Additionally, our previous study suggests that NOX2-derived ROS stimulates EMT in breast cancer cells. To further investigate the effects of myeloid-derived ROS on the tumor microenvironment, we genetically ablated Nox2 in the PyMT-MMTV breast cancer model, allowing systematic evaluation of tumor progression and the immune landscape.

Material and method

Nox2^{-/-} mice were crossed with PyMT-MMTV mice. Female Nox2^{-/-} PyMT-MMTV mice were compared with age-matched littermate wild-type (WT) PyMT-MMTV controls. Mice were sacrificed, tumor weight was measured, lung metastasis was quantified via H&E staining, and splenic weights were recorded as an indicator of systemic inflammation. Tumor and spleen single-cell suspensions were assessed by flow cytometry for T cell activation, regulatory T cells, macrophage polarization, and neutrophil populations. Expression of EMT markers was evaluated by flow cytometry and Western blot, while intratumoral cytokine levels were quantified via qPCR.

Result and discussion

Nox2^{-/-} PyMT-MMTV mice showed accelerated tumor growth and enhanced metastasis. This concurred with a pronounced systemic expansion of granulocytic myeloid cells and splenomegaly. The Nox2^{-/-} animals showed signs of improved anti-tumor immunity with enhanced CD8+ T cell activity, reduced levels of Tregs, and reduced frequency of immunosuppressive M2 macrophages in the tumor and systemically, but this was not sufficient to prevent tumor progression. The paradoxical role of NOX2 in the PyMT-MMTV model may stem from the dysregulated inflammatory response, evident by the significant myeloid cell expansion. The tumors of the Nox2^{-/-} mice contained increased IL-6 levels and showed elevated EMT markers. In vitro experiments supported that macrophages differentiated in the presence of pharmacological inhibition of NOX2 produce enhanced levels of IL-6. Collectively, these inflammatory factors may override the benefit of a heightened CD8+ T cell response.

Conclusion

This study demonstrates that a favorable immune landscape does not guarantee improved outcomes in cancer and suggests that inflammatory cytokines, such as IL-6, may drive disease progression. Our findings challenge the current paradigm in cancer biology that mesenchymal tumors inherently possess more immunosuppressive microenvironments. These insights underscore the importance of comprehensive therapeutic strategies that address both adaptive immunity and myeloid cell regulatory networks for optimal clinical efficacy.

EACR25-1878

Targeting glycation stress in chemoresistant colon cancer: Effects on cancer stem cells

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Introduction

Our team recently demonstrated that KRAS-mutated colorectal tumors exhibit methylglyoxal (MG) glycation stress. This stress is linked to the reprogramming of glucose metabolism, a crucial event observed in many cancers during tumor progression and therapy resistance. A growing body of evidence suggests that cancer stem cells (CSCs) are primarily responsible for cancer aggressiveness, drug resistance, and tumor relapse. Based on these results, we decided to prioritize the study of MG stress in colorectal cancer CSCs.

Material and method

We compared cells grown under 2D conditions to 3D spheroids to assess their enrichment in CSCs. High levels of stem cell markers such as ALDH1A1, OCT-4, and CD133 were successfully detected in 3D cultures and correlated with elevated levels of CD44 and cyclin D1, indicating activation of the Wnt pathway. RNA sequencing analysis performed on colorectal cancer cells under endogenous MG stress revealed enhanced expression of several Wnt activators, including CTNNB1 (coding for β-catenin), WNT6, WNT7B, WNT9A, LEF1, and TCF4.

Result and discussion

Altogether these data suggest that MG stress could, at least in part, contribute to Wnt activation in colorectal cancer cells. Consistently, preliminary results point to a dose-dependent accumulation of MG protein adducts paralleled by increased TCF1 expression in colorectal cancer cells. CSCs characterized by high ALDH activity showed a significant increase in intracellular MG and MG protein adducts, suggesting a correlation between MG and this stemness marker.

Conclusion

The recent demonstration of chemotherapy-induced promotion of stemness in colorectal cancer indicate that therapy resistant preclinical models under study may recapitulate the sequence of CSCs enrichment associated with glycolysis, followed by MG stress and terminated by Wnt activation. We propose that neutralizing MG in CSCs could significantly enhance the efficacy of therapy in colorectal cancer. Understanding the energy metabolic modalities of CSCs and their ability to adapt in response to therapy promises numerous applications that can help improve cancer patient care.

EACR25-1889

Identifying the role of PLEKHS1 in cancer

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Introduction

The PLEKHS1 gene is mutated in multiple cancers, with approximately 40% of bladder cancer cases and 9% of thyroid cases carrying this mutation. However, very little is known about the consequences of this or the function of the protein for which PLEKHS1 codes. PLEKHS1 tumour DNA can be detected in urine of those with bladder cancer, and therefore interest has been paid to the possibility of its use as a detection biomarker with very little physiological understanding of PLEKHS1. As we have shown previously, PLEKHS1 is present in cell lines from multiple cancer types as well as 'normal' cell lines. Now the aim has moved to identifying the possible function of PLEKHS1, and to further our understanding of the protein.

Material and method

Immunofluorescence and immunoprecipitation experiments were carried out on bladder, prostate, and breast cancer cell lines.

Result and discussion

It was observed that PLEKHS1 is present in the cytoplasm but is largely localised to the nucleoli and the Golgi apparatus, which was further confirmed when analysing the immunoprecipitation results showing proteins that interact with PLEKHS1 are largely nucleolar and Golgi apparatus based proteins.

Conclusion

These results confirm the localisation of the PLEKHS1 protein and are vitally important in starting to unravel the function of PLEKHS1.

EACR25-1897

Miro1 depletion modulates intracellular ATP distribution and adhesion dynamics, suppressing cell migration and metastasis in B16 melanoma cells

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Introduction

Mitochondrial localization is crucial for meeting the energetic demands required for cell migration. The leading edge of migrating cells requires an adequate supply of ATP, which is facilitated by the redistribution of mitochondria toward this area. In the context of cancer, cell migration is a critical step in the invasion

process, being the initial step of metastasis. Miro1, a Rho GTPase located in the outer mitochondrial membrane, is a key regulator of mitochondrial trafficking. However, its role in cancer cell migration and the mechanisms underlying its function are poorly understood. The present study aims to evaluate the role of Miro1 in regulating cell invasion and the formation of metastases.

Material and method

Miro1-deficient B16 melanoma cells were generated by CRISPR-Cas12a. Oxygen consumption rates and extracellular acidification rate were measured using Seahorse XFe96 extracellular flux analyzer, and the ATP/ADP ratio was assessed by ratiometric imaging of Perceval HR. Cell migration and invasion were evaluated by scratch assay (Incucyte), spheroid outgrowth, invadopodial activity (gelatin degradation assay), and vinculin and F-actin staining. For in vivo studies, C57BL/6J mice were injected intravenously with B16 and B16 Miro1-deficient cells, with subsequent examination of the lungs for the presence of metastatic tumors through H&E staining.

Result and discussion

Miro1-deficient B16 cells showed a decrease of approximately 20% in ATP production rate and reduced ATP/ADP ratio in the leading edge of migrating cells, driven by impaired glycolysis rather than mitochondrial respiration. Consistently, depletion of Miro1 also led to a decrease in the wound healing area (50%), as well as the invasion index (60%), and the outward migration rate of cells from spheroids (45%). Additionally, Miro1-deficient cells showed reduced focal adhesion size, lower ability to degrade gelatin (60%), and alterations in the actin cytoskeleton. These findings support the idea that mitochondrial positioning, facilitated by Miro1, is crucial for efficient energy distribution during migration and invasion. Notably, in vivo data showed that mice injected with Miro1-deficient cells developed fewer metastatic foci in the lungs when compared to control cells, highlighting Miro1's essential function in metastatic progression.

Conclusion

Our results demonstrate that Miro1 plays a crucial role in regulating the migratory and metastatic potential of B16 cancer cells by controlling the positioning of mitochondria, ATP distribution, and coordinating the actin cytoskeleton. These findings suggest that targeting Miro1 could be a promising therapeutic strategy.

Funding: Funded by the Czech Science Foundation (GA21-04607X), CZ.1.05/1.100/02.0109, RVO: 86652036, and by Portuguese national funds via Fundação para a Ciência e Tecnologia (2020.04765.BD).

EACR25-1902

Identifying novel regulators of the BH3-only protein PUMA using whole genome CRISPR/Cas9 KO screens to enhance the efficacy of cancer treatment

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Introduction

The intrinsic apoptotic pathway, regulated by the BCL-2 protein family, is a key mechanism in regulating cell

death. BH3-only proteins, such as PUMA, BIM, BID, and NOXA, are crucial for initiating apoptosis, particularly in response to anti-cancer agents. PUMA (Bbc3) is a critical BH3-only protein that triggers apoptosis via both Trp53-dependent and Trp53-independent pathways. The expression of PUMA is directly regulated by Trp53, a tumor suppressor commonly mutated in cancers. This study aims to identify novel regulators of Puma expression in both Trp53-dependent and -independent ways in blood cancers, with the potential for developing new therapeutic targets.

Material and method

We engineered a Puma-tdTomato reporter mouse model by replacing the Puma coding region (Bbc3) with the tdTomato gene, maintaining the endogenous regulatory regions to track Puma expression in real-time. We generated pre-clinical AML models by introducing retroviruses expressing AML-driving oncogenes into hematopoietic stem and progenitor cells (HSPCs) from the Puma-tdTomato reporter mice. These modified HSPCs were injected into irradiated recipient mice, resulting in AML-like tumors. We also crossed the Puma-tdTomato mice with the Eu-Myc transgenic mouse model to generate lymphoma cell lines. To identify Puma regulators, we performed CRISPR/Cas9 whole-genome knockout (KO) screens in Puma-tdTomato-expressing AML cell lines. After isolating the top and bottom 3% of tdTomato-expressing cells, we conducted Next Generation Sequencing (NGS) to identify enriched single-guide RNAs (sgRNAs). Validation was performed in both Trp53-expressing and Trp53-deficient AML cell lines.

Result and discussion

The Puma-tdTomato model allowed real-time tracking of Puma expression in response to apoptotic stimuli in AML and lymphoma cells. Treatment with anti-cancer drugs led to upregulation of Puma, indicated by increased tdTomato fluorescence. In the CRISPR/Cas9 KO screen, several novel genes regulating Puma expression were identified. Validation in Trp53-deficient cell lines confirmed that some of these genes regulate Puma expression independently of Trp53.

Conclusion

Our study demonstrates that the Puma-tdTomato reporter model is an effective tool for identifying regulators of Puma expression in blood cancer models. The CRISPR/Cas9 screen revealed novel genes that could serve as potential therapeutic targets for enhancing Puma expression, especially in Trp53-deficient cancers. These findings suggest new strategies to promote cancer cell death and provide a foundation for future therapeutic development.

EACR25-1909

Flow-Based Cell Deformation Reveals EMT-Driven Mechanical Phenotype Changes in Metastatic Prostate Cancer

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Introduction

Mechanical alterations in cancer cells are critical markers of metastasis, reflecting profound molecular and metabolic reprogramming. In this study, we explore the interplay between cytoskeletal reorganization, metabolic shifts, and cellular stiffness in prostate cancer.

Material and method

To assess cellular mechanics, adherent cells were exposed to controlled fluid shear in microchannels, and their deformation was analyzed using a Kelvin–Voigt viscoelastic model. Complementary finite element simulations were performed to capture the complex mechanical response, incorporating not only the actin cortex but also atypical contributors such as intermediate filaments, microtubules, and organelles. This integrative approach enabled robust correlation of molecular alterations – such as EMT marker redistribution and metabolic reprogramming – with observed changes in cell stiffness.

Result and discussion

By employing zinc treatment as a modulatory intervention, we induced phenotypic changes in metastatic PC-3 cells that mirror a partial epithelial-mesenchymal transition (EMT) reversal. Specifically, zinc exposure led to reduced vimentin expression, enhanced E-cadherin levels, and a distinct perinuclear redistribution of caveolin-1. These molecular alterations were paralleled by increased ATP production, enhanced spare respiratory capacity, and modified mitochondrial distribution, collectively indicating a shift toward a more energetically efficient state. Importantly, the zinc-induced molecular and metabolic modifications were directly associated with an increase in cell stiffness. Complementary computational simulations suggest that this stiffening results not solely from actin cortex reorganization but also from contributions by intermediate filaments and organelle dynamics.

Conclusion

These findings highlight a critical link between the regulation of EMT markers, metabolic reprogramming, and the biomechanical properties of cancer cells. Overall, our results provide valuable insights into the molecular mechanisms underpinning metastasis and suggest that targeting the interconnection between cellular metabolism and mechanics could inform novel diagnostic and therapeutic strategies in cancer management.

EACR25-1933

The Role of Brown Adipose Tissue in Pancreatic Cancer Progression

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Introduction

Pancreatic ductal adenocarcinoma (PDAC) is the most common type of pancreatic cancer and is strongly associated with cancer-associated cachexia (CAC), a systemic metabolic disorder that worsens prognosis. In the prediagnostic phase of PDAC, profound metabolic alterations and soft tissue changes occur, which may serve as early biomarkers. While white adipose tissue (WAT) has been widely recognised as a key player in tumour progression by supplying growth factors, inflammatory cytokines, and metabolites, recent evidence suggests that brown adipose tissue (BAT), a thermogenic fat depot, may also modulate tumour metabolism. BAT is a critical regulator of systemic energy homeostasis and glucose metabolism, both of which are central to PDAC progression. We hypothesise that metabolic dysfunction driven by alterations in BAT activity contributes to tumour progression in PDAC.

Material and method

To investigate the role of BAT in PDAC, we established a mouse model of pancreatic cancer in which BAT was surgically removed (BATectomy). Tumour growth, metastasis, and CAC parameters were assessed and compared between BAT-intact (Sham) and BAT-depleted (BATectomy) mice. To characterise the metabolic alterations associated with BAT loss, we performed comprehensive metabolic profiling, including metabolomic and lipidomic analyses, as well as glucose homeostasis assessments. Additionally, histological and molecular characterisation of tumour and adipose tissues was conducted.

Result and discussion

Our findings indicate that BAT plays a crucial role in PDAC progression. Mice subjected to BATectomy exhibited significantly increased tumour burden and metastatic spread compared to their Sham counterparts. Furthermore, BAT depleted mice displayed impaired glucose and lipid metabolism, alongside severe lipid

dystrophy, highlighting the systemic metabolic consequences of BAT loss in the context of cancer.

Conclusion

These results underscore the critical impact of BAT on tumour progression and metabolic dysfunction in PDAC. Understanding the interplay between thermogenic fat and cancer metabolism could pave the way for novel therapeutic strategies aimed at restoring metabolic homeostasis and improving patient outcomes.

EACR25-1938

Peroxidasin promotes epithelial-mesenchymal transition and invasion in metastatic melanoma

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Introduction

Peroxidasin, an extracellular peroxidase enzyme that generates the strong oxidant hypobromous acid, is upregulated in many types of cancer including invasive metastatic melanoma, breast, brain, ovarian and prostate cancer. Its expression is associated with increased cell invasion and cancer progression. However, it is not known how peroxidasin contributes to cancer malignancy.

Material and method

We have analysed the transcriptomes of 70 metastatic melanoma cell lines with variable levels of peroxidasin expression from the New Zealand melanoma cell line panel. To determine transcriptional patterns associated with high and low peroxidasin expression, cell lines with high peroxidasin expression ($n = 22$) were compared against cells with low peroxidasin expression ($n = 22$). Pathway analysis (ReactomePA, R) and gene set enrichment analysis (FGSEA) using genes of the epithelial-mesenchymal transition (EMT) pathway and genes linked to different melanoma phenotypes (invasive/undifferentiated, neural crest-like, proliferative/melanocytic) were performed. To assess the functional role of peroxidasin in metastatic melanoma, we performed a knockout of peroxidasin in a highly invasive, high peroxidasin expressing cell line (NZM40) using CRISPR-Cas9 technology. We investigated the effect of knockout and inhibition of activity with the inhibitor phloroglucinol on cancer cell invasion using functional invasion assays and gene transcription (RNAseq).

Result and discussion

Transcriptomic analysis of metastatic melanoma cell lines highlighted the strong association between high peroxidasin expression and the expression of EMT genes as well as hallmark genes of the undifferentiated invasive melanoma phenotype, inferring a role of peroxidasin in melanoma cell invasion. Pathway analysis on down-regulated genes in the low peroxidasin expressing cell lines identified biological processes pertaining to

extracellular matrix remodelling including ECM organisation, collagen and elastic fibre formation and extracellular matrix degradation. Both knockout of peroxidasin and inhibition of its activity with phloroglucinol led to reduced cancer cell invasion in invasion assays. Transcriptomic analysis showed decreased EMT and invasive gene expression in both the peroxidasin knockout and the wild type treated with the inhibitor phloroglucinol. Phloroglucinol led to peroxidasin-specific differential gene expression, however, fewer genes were differentially expressed and smaller effect sizes were observed compared to the knockout.

Conclusion

Transcriptomic analysis of the NZM cell line panel confirmed peroxidasin as strongly associated with an invasive phenotype. Knockout and inhibition of peroxidasin identified a functional role in melanoma invasion by reducing EMT and invasive gene expression. These findings highlight peroxidasin as a promising novel target to reduce cancer cell invasion.

EACR25-1948

mTORC2 but not mTORC1 governs cellular metabolic landscape in Calreticulin (CALR) mutated

myeloproliferative neoplasm (MPN)

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Introduction

Myeloproliferative neoplasm (MPN) is classified as the disorder of hematopoietic progenitor cells with various mutations being implicated in its pathogenesis, including JAK2, MPL, and CALR driver mutations. We focused on CALR, an ER chaperone involved in protein folding and calcium homeostasis. The CALR mutations are primarily observed in Exon 9, resulting in partial (CALR ins5) or complete (CALR del52) deletion of negatively charged amino acids present in the CALR WT tail. While both the CALR mutations activate TpoR mediated JAK/STAT signalling, they differ significantly in their phenotype and prognosis, hinting at differential regulation of downstream signalling cascades. Consistently, co-targeting PI3K-mTOR and JAK-STAT signalling through BEZ235 and Ruxolitinib, increased the efficacy of these drugs in mice models and in MPN progenitor cells. Therefore, we aimed to dissect the interplay between CALR mutations implicated in MPN and mTOR signalling to pave the way for potential future targeted therapeutics.

Material and method

We utilized CALR overexpression systems (WT, Del52, Ins5) in HEK cells to investigate the regulation of mTOR (mTORC1 and mTORC2) signalling cascade through immunoblotting. As these mutations target the CALR tail, we created CALR WT truncation mutants to understand the extent of activation of mTOR signalling. Furthermore, we used inhibitors to assess any distinction between CALR mutations in the inhibition of mTOR cascade. We have compared the glucose uptake and cellular energetics through ATP levels in the cell between CALR WT and mutations. We also performed mass

spectrometry studies, siRNA-based knockdowns, and overexpressed mTOR complex-specific protein overexpression to establish the molecular axis behind the observed phenotype.

Result and discussion

We have established differential activation of mTORC2 but not mTORC1 by CALR mutants, with mTORC2 being significantly downregulated in CALR Del52. This further resulted in a shift in the lysosomal count, distribution, mTOR colocalization, and transcriptional activity of the TFE3 gene, a key component for lysosomal biogenesis and autophagy induction. Our mass spectrometry data reveals the potential interactors that play a role in the lysosomal distribution in the cells.

Conclusion

We have studied the effect of CALR mutants on different pathways and found that CALR ins5 mutant triggers high mTORC2 (S2481) activation compared to CALR WT or CALR del52. This selective activation of mTORC2 by CALR mutant and its downstream implications on lysosomal distribution and biogenesis is an interesting finding. This study highlighted the difference between CALR mutants del52 and Ins5 in differential modulation of mTOR pathway and its downstream impact on lysosomes.

EACR25-1950

Modification of β-Catenin by MCPIP1 endonuclease Activity regulates the metastatic potential of ccRCC

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Introduction

Renal cell carcinoma (RCC) is one of the most common urological neoplasms in the world, with ccRCC being the most common subtype, accounting for approximately 75% of RCC cases. Among the many pathways implied in ccRCC, the Wnt/β-catenin pathway has emerged as a key contributor. To overcome these challenges, acquiring a comprehensive understanding of the underlying biology of ccRCC is crucial. Among the endonucleases, the ZC3H12a gene encoding Monocyte Chemoattractant Protein-1 Induced Protein, has a suppressive effect in renal cell carcinoma. Our recent study revealed that MCPIP1 may act as a tumor suppressor in ccRCC that prevents EMT by stabilizing Wnt inhibitors and decreasing the levels of active β-catenin and EMT inducers.

Material and method

To investigate the role of MCPIP1 in ccRCC development and progression, we used *in vivo*, *in vitro* models and the samples from patients. We conducted a comprehensive analysis, including the assessment of

crucial genes and proteins of the Wnt/β-Catenin pathway, using Western blot, microarray analysis, quantitative PCR, immunofluorescence, and immunohistochemistry staining.

Result and discussion

In this study we demonstrate that the endonuclease activity of MCPIP1 may mediate tumor progression by controlling the transcriptional and post-transcriptional modification of β-catenin. We found that MCPIP1 regulates the level of genes binding to the CTNNB1 gene promoter, which encodes β-catenin. Moreover, MCPIP1 influences not only on the level of β-catenin, but also on its post-transcriptional modifications, including phosphorylations of Y654 and S675 which regulate its activity. Furthermore, we observed a dramatic decrease in the level of inactive, degradation-promoted, phosphorylated β-catenin (Ser45) in advanced stages of ccRCC compared to early clinical stages. In contrast, the level of active, phosphorylated β-catenin significantly increases with the progression of the disease. Microarray analysis indicated that the expression of genes binding to the CTNNB1 gene promoter, encoding β-catenin, may affect the progression of ccRCC.

Conclusion

Our studies suggest that MCPIP1 plays a significant role in the progression of ccRCC by regulating the post-transcriptional modifications of β-catenin. The level and location of phosphorylated β-catenin may be crucial at every stage of ccRCC development.

EACR25-1962

The pro-invasive MAP4 kinases promote tissue invasion and selectively modulate interactions with the neuralTME in medulloblastoma

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Introduction

Medulloblastoma (MB) is the most common malignant pediatric brain tumor, sub-grouped into WNT, SHH, Grp3, and Grp4 MB with different biological and clinical characteristics. Despite advancements in molecular diagnostics, therapeutic intervention for patients with MB remains limited. The neural contributions of the tumor microenvironment (TME) of MB are incompletely characterized. Specifically, the interferences between tumor and neural cells, as well as their functional consequences, remain largely unknown and present a significant barrier to advancing targeted and effective interventions for pediatric MB.

Material and method

We combine classical cell biology, 3D-invasion studies, and quantitative imaging with an advanced organotypic cerebellum slice culture model of SHH and Grp3 MB,

to uncover molecular insights into tissue invasion and the interactions between MB tumor cells and the neural tumor microenvironment (nTME) in the cerebellum. By implementing tissue perfusion and functional fluorescence imaging, we enabled the longitudinal analysis of tumor cell interaction with the nTME, cerebellar tissue invasion, and the visualization of dynamic processes in the tumor-nTME interaction.

Result and discussion

We found that the pro-invasive Ser/Thr kinase MAP4K4 cooperates with striatin 3 (STRN3), a central component of the Striatin-interacting phosphatase and kinase (STRIPAK) complex, to drive tissue invasion downstream of growth factor signaling. Mechanistically, STRN3 links MAP4K4 to the protein phosphatase 2A, which suppresses the growth-inhibitory functions of MAP4K4. Simultaneously, STRN3 enables growth factor-induced PKCθ activation and direct phosphorylation of VASP at S157 by MAP4K4, both essential for efficient cell invasion. High-resolution tumor imaging in the cerebellar tissue environment revealed the tight interaction between invading tumor cells and cerebellar astrocytes, accompanied by increased astrocytic calcium signaling. Notably, the pharmacological interference with MAP4K function increased these interactions, leading to the formation of extended junctional complexes and altered astrocyte reactivity. Phosphoproteomic and functional studies suggest that MAP4Ks drive MB-nTME interaction by promoting the targeted phosphorylation and re-localization of cell-cell junctional proteins. Collectively, our findings uncover a direct, functional interplay between MB tumor cells and astrocytes and identified MAP4 kinases as pivotal regulators of this process.

Conclusion

The interaction between MB tumor cells and the neural microenvironment in the cerebellum, along with the resulting TME remodeling, is intrinsically regulated by MAP4 kinases, opening new avenues for innovative therapeutic strategies.

EACR25-1967

Cell-free DNA (cfDNA) regulates metabolic remodeling influencing cancer cell proliferation, quiescence, and chemoresistance

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Introduction

Cell-free DNA (cfDNA) holds significant potential as a valuable tool in cancer diagnosis, monitoring, and patient management. While its clinical relevance in oncology has gained recognition, cfDNA biological relevance in tumor

malignancy remains unknown. Tumor-derived systemic signals are crucial for coordinating metabolic and signaling pathways that support cancer survival and dissemination. In a biological point of view, cfDNA emerges as a potential master regulator of metabolic adaptation and cell functioning control, allowing disease progression. Our aim is to unravel the role of cfDNA in cancer cells metabolic regulation, contributing to cancer establishment and progression.

Material and method

CfDNA was isolated from the conditioned media of breast cancer (MDA-MB-231) and ovarian cancer (ES-2) cell lines cultured at different glucose availability conditions. Four cfDNA variants were obtained based on glucose availability and cell culture duration: Early-cfDNA-Gluc (6 h with glucose), Early-cfDNA-NoGluc (6 h without glucose), Late-cfDNA-Gluc (48 h with glucose), and Late-cfDNA-NoGluc (48 h without glucose). The impact of these cfDNA variants on cell proliferation (proliferation curve), migration (wound-healing assay), metabolism (¹H-NMR spectroscopy), and cisplatin resistance (flow cytometry), was evaluated in new batches of MDA-MB-231 and ES-2 cultures. Additionally, we studied the protein levels of DNA-sensitive Toll-like receptor 9 (TLR9) by immunofluorescence and its colocalization with lysosome associated membrane protein 1 (LAMP-1).

Result and discussion

In MDA-MB-231 cell line, cfDNA influenced the metabolic adaptation by regulating glucose and glutamine consumption. In ES-2 cells, cfDNA prompted metabolic profiles similar to the initial cell cultures (cell-of-origin of cfDNA). Both cell models revealed that certain cfDNA variants favor cisplatin resistance, with specific cfDNA variants offering protection depending on the cell line and culture conditions. Our findings showed that TLR9 levels decreased in both cell lines over time, with inversed trends: ES-2 cells exhibited increased TLR9-LAMP-1 colocalization, while MDA-MB-231 cells displayed decreased TLR9-LAMP-1 colocalization. These results suggest the involvement of TLR9 in cfDNA-mediated remodeling, with cell-specific dynamics. Additionally, in both models, extending cfDNA exposure from 48 hours to 4 weeks induced quiescence in a subset of cancer cells, emphasizing the long-term impact of cfDNA on tumor behavior.

Conclusion

These findings highlight cfDNA as a key player in the tumor microenvironment, facilitating communication between cancer cells, supporting their survival and adaptation under stress, modulating the metabolic reprogramming, cell proliferation, and quiescence thereby contributing to different steps of disease progression and therapy resistance.

EACR25-1982

Novel liposomal nanoformulations containing cannabidiol, celecoxib and 2,5-dimethylcelecoxib induce cell cycle arrest and apoptosis in glioblastoma cells

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Introduction

Glioblastoma (GBM) is the most common primary brain tumor with patient survival below 18 months following diagnosis. The rigorous treatment regimen including surgical resection, radiotherapy, and chemotherapy with temozolamide, remains inefficient. Cannabidiol (CBD), celecoxib and 2,5-dimethylcelecoxib (DMC) exert promising anticancer properties, while liposome-based drug delivery systems offer a new way to reach tumor cells. Thus, the aim of this study was to verify the anti-glioma properties of CBD, celecoxib and DMC-loaded nanocarriers, and analyze their impact on cell cycle and apoptosis in U-138 MG glioblastoma cells.

Material and method

Liposomal nanoformulations loaded with CBD, celecoxib, DMC, and their combinations, as well as empty carriers were prepared via thin-film hydration method, followed by extrusion. The effect on U-138 MG cells viability following 48 h incubation with the analyzed nanoformulations (in a concentration range of 1–50 µM) was verified using the MTT assay, while their impact on cell cycle distribution and apoptosis was determined using flow cytometry with Muse Cell Cycle kit, and Muse Annexin V & Dead Cell Kit, respectively.

Result and discussion

Our results revealed that among the single agent-loaded nanoformulations, the one with celecoxib was the most cytotoxic, followed by CBD, and DMC. Combinatory treatment also induced dose-dependent reduction in cell viability. Empty carriers did not have negative impact on the viability of U-138 MG cells. All the analyzed nanoformulations in 5 µM and 10 µM concentration halted the cell cycle and induced apoptotic cell death of glioblastoma cells. The most pronounced effects were observed after the treatment with CBD.

Conclusion

The pro-apoptotic properties of novel liposomal formulations containing CBD, celecoxib and DMC have been highlighted in our research. The observed alterations in cell cycle phases distribution and apoptosis induction require further investigation to clarify the role of these nanoformulations in modulation of cellular signaling. Continued research, including *in vivo* models, will be essential to thoroughly evaluate their full potential as anticancer agents.

Funding: This work was funded by grant no. 2021/43/O/NZ5/02346 from the National Science Centre, Poland.

EACR25-1990

Understanding the role of desmoglein 2 in preserving the epithelial phenotype of breast cancer cells

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Introduction

Desmoglein 2 (DSG2) is a surface protein that plays a physiological role in desmosomal cell-cell adhesion and in maintaining epithelial tissue integrity. In cancer, the role of DSG2 remains controversial, as it has been reported to exhibit both pro-tumorigenic and tumor-suppressive effects. Given that the connection between DSG2 and the metastatic cascade in cancer is under-explored, we focused on its association with epithelial-mesenchymal transition (EMT) traits in breast cancer cells.

Material and method

CRISPR-Cas9 knockout (KO) cells were validated using western blotting, immunofluorescence, and sequencing of gRNA targets to confirm the loss of DSG2 in the T-47D cell line. DSG2 KO cells were tested for their attachment to ECM matrix components utilizing the ECM Select® Array Kit Ultra-36 (Advanced Biomatrix). Multicolor spectral flow cytometry was used to detect the epithelial-mesenchymal transition (EMT) surface profile of the DSG2 KO cells, assessing markers such as EpCAM, Trop2, CD9, CD29, CD49c, GD2, and ITGB5.

Result and discussion

To evaluate the impact of DSG2 loss in vitro, we established a T-47D DSG2 CRISPR knockout model and successfully confirmed the deletion of DSG2. We investigated the capacity of DSG2 knockout cells to adhere to various extracellular matrix (ECM) proteins and noted a general reduction in cell adhesion to most individual ECM components. The measurement of an in-house developed and validated EMT surface panel indicated the upregulation of multiple mesenchymal markers in DSG2 knockout cells and the downregulation of epithelial markers.

Conclusion

Our study demonstrates that in the T-47D breast cancer cell model, the loss of DSG2 results in a reduced capacity to adhere to various ECM components. These alterations are also evident in the overall mesenchymal-like cell surface profile of DSG2 KO cells. We conclude that DSG2 in the tested model is associated with EMT and may play a role in suppressing the metastatic process.

Acknowledgment: This work was supported by the Czech Science Foundation, grant no. 24-11793S.

EACR25-1996

Impact of androgen receptor expression on the efficacy of anti-androgen therapies in triple negative breast cancer

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Introduction

Triple negative breast cancer (TNBC) is the most aggressive type of breast cancer, characterized by the lack of expression of estrogen and progesterone

receptors, and HER-2 overexpression. However, most of TNBC cases express the androgen receptor (AR) and its variants, such as ARV7. Also, ARV7 has been associated with resistance to endocrine therapies. Besides, the hormonal environment seems to play a determining role in tumor progression. Therefore, this study aims to analyze the impact of AR antagonists on cell proliferation, cell migration, and hormonal environment in TNBC cell lines, evaluating the effectiveness of inhibiting the AR expression and its variants, and the potential of those compounds as a targeted therapy for TNBC.

Material and method

To this purpose, the TNBC cell lines, IPC-366 and SUM149, were maintained in culture flasks in their corresponding supplemented media under controlled conditions (5% CO₂ and 37°C). Cells were treated with different AR antagonists: bicalutamide (that bind to AR inhibiting its function), and ailanthone (that block AR at transcriptional level), at dose of 1 μM of each compound. Cell viability and migration assays were performed in order to evaluate the effect of the compounds in TNBC cell lines. To determine the AR, phosphorylated AR (p-AR), and ARV7 expression, western blot techniques were performed. Besides, dihydrotestosterone (DHT) and 17β-estradiol (E2) levels were measured to evaluate the DHT/E2 ratio as a good indicator of endocrine therapy effectiveness.

Result and discussion

Results revealed that IPC-366 had higher expression of AR, p-AR, and ARV7 than SUM149, which may explain the different responses to treatment observed in both cell lines. Although bicalutamide and ailanthone produced significant reductions in cell viability and cell migration in both cell lines, only ailanthone reduced cell viability in SUM149. The DHT/E2 ratio increase under both treatments with IPC-366, while in SUM149 decrease with bicalutamide. Thus, a higher DHT/E2 ratio seems to be associated with increased effectiveness of endocrine therapies. Finally, while in IPC-366, AR and ARV7 expression decrease with all treatments, p-AR expression increases. In SUM149, all treatments decrease AR, p-AR, and AR-V7 expression. Specially, ailanthone blocks ARV7 expression in both cell lines. Consequently, a reduction in AR expression and its variants has been observed to decrease cell growth and migration, being anti-androgen therapies a viable treatment option for TNBC.

Conclusion

These results revealed that targeting AR and its variants may serve as an effective therapeutic approach for TNBC, especially with the use of ailanthone. In addition, the DHT/E2 ratio could be a valuable indicator for predicting the efficacy of endocrine therapies.

EACR25-2006

Exploring the oncogenic role of the deubiquitinase USP7 in pancreatic cancer using PROTAC technology

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Introduction

Ubiquitin serves as a versatile post-translational modifier, regulating both degrading and non-degrading cellular processes through the activity of E3 ligases. Counter-balancing these processes, deubiquitinases (DUBs) play a critical role in maintaining protein homeostasis by modulating ubiquitin-encoded signals. Targeting DUB activity with small molecules offers a promising approach to modulate previously "undruggable" proteins implicated in cancer biology. However, chemical tools that interrogate DUBs with high specificity in cancer and that can be used to distinguish catalytic from non-catalytic functions have remained scarce.

Material and method

We synthesized a USP7-targeting degrader library and identified potent USP7 PROTACs in our panel of cell lines. Compound specificity was subsequently characterized in a suite of assays, including mass spectrometry analysis. To distinguish between USP7-catalytic and scaffolding effects we performed Seahorse analysis, multiplexed live cell migration and tested metastatic seeding of PDAC cells w/o USP7 targeting compounds *in vivo*.

Result and discussion

Here, we employ an integrated chemical biology strategy to study the deubiquitinase USP7 in pancreatic cancer using complementary approaches of pharmacological inhibition and targeted degradation. In a multiplexed live cell migration screen with a curated library of DUB inhibitors, treatment with USP7 inhibitors led to an invasive cell state switch in pancreatic cancer. Furthermore, cells reprogrammed their metabolism as treatment with the USP7-inhibitor FT671 led to a disruption of mitochondrial respiration and the switch to a glycolytic program. Notably, we observed significant phenotypic differences among commonly used USP7 inhibitors, which led to the development of a USP7-targeting chemical toolbox, comprising a novel USP7 inhibitor and a potent and selective USP7 proteolysis-targeting chimera (PROTAC). Strikingly, cells exhibited distinct cellular phenotypes when USP7 was depleted through targeted protein degradation or pharmacological inhibition.

Conclusion

Collectively, our work provides a curated toolbox of highly specific and well characterized small molecules to distinguish on-target catalytic-phenotypes which will aid to understand the role of DUBs in malignant diseases. More broadly, our data highlights the value of elevated specificity through PROTAC-mediated target degradation.

EACR25-2009 Mitochondrial damage triggers therapy-induced senescence

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Introduction

Glioblastoma (GBM) is a fatal brain tumor with a critical need for better therapies. It is known that the PI3K, MAPK, and CDK4/6 signaling pathways are hyper-activated in these tumors; however, previous studies have used very high concentration of inhibitors to assess their importance, with mixed results. Here we developed PMCI, a combination approach that targets all three pathways simultaneously, at clinically-relevant doses.

Material and method

To characterize the mechanism of PMCI-induced senescence *in vitro*, we use a combination of techniques: cell proliferation assays, quantitative Western blot, SA-β-galactosidase assays, single cell colony outgrowth assays, live-cell imaging, immunofluorescence and electron microscopy, RNAseq, proteomics, flow cytometry, chemo/cytokine arrays, qPCR, p0 cells and Seahorse assays. *In vivo*, we employed an orthotopic glioblastoma mouse model for efficacy experiments, monitored pharmacokinetics using LC-MS/MS, and assesses pharmacodynamics and toxicity using blood parameter analysis and immunohistochemistry.

Result and discussion

PMCI effectively suppresses GBM cell proliferation *in vitro* and *in vivo*, and outperforms monotherapies and dual combinations. PMCI acts by inducing cellular senescence, which is mediated solely by the mitochondria, and, unlike other forms of senescence, is independent of nuclear damage. This phenotype is caused accumulation of dysfunction mitochondria, triggering a reactive oxygen species (ROS)/cGAS-STING senescence-associated secretory phenotype (SASP) signaling cascade that acts in a paracrine manner to establish and maintain senescence.

Conclusion

Our results demonstrate that mitochondrial damage can be sufficient to drive senescence, and that this can be leveraged to target GBM cells.

EACR25-2018

SUMOylation of S6K2 May Confer Its Stabilization Contributing To The Survival of Breast Cancer Cells

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Introduction

S6K2 (S6 Kinase 2) protein is an PI3K/Akt/mTOR signaling pathway effector protein, which augments the evasion of breast cancer cell from apoptosis by activating

Akt via a positive feedback mechanism. Besides, S6K2 has many other substrates which contribute to the survival of breast cancer cells. Therefore, elucidation of the mechanisms which regulate S6K2 signaling has an important role in breast cancer therapy. Many post-translational mechanisms which play roles in S6K2 regulation still remains to be unraveled. SUMO (small ubiquitin like modifier) is a 11kDa protein which binds to target proteins via their Lys residues generally found in ψ KxE consensus motif (ψ - hydrophobic amino acid, x - any amino acid). There are 4 different SUMO proteins named as SUMO 1-4. SUMOylation of proteins, a post-translational modification, regulate proteins by altering their longevity, subcellular localization, protein interactions and functions. SUMOylation uses a mechanism similar to ubiquitination. In general, SUMOylation prevents ubiquitination and therefore prolongs longevity of proteins. We aim to identify whether S6K2 is SUMOylated and how this SUMOylation affects the longevity and pro-survival function of S6K2 protein.

Material and method

Our bioinformatics analysis revealed that human S6K2 has a ψ KxE consensus motif at residue 196 (189-GIIYRDLKPENIMLS-203). Therefore, we speculate that S6K2 is susceptible to SUMOylation. We will transfet MCF7 cells with FLAG-S6K2 and Myc-SUMO 1/2/3/4 and perform immunoprecipitation (with anti-FLAG antibody) followed by Western blot to observe whether S6K2 is SUMOylated by any of these SUMO proteins. FLAG-S6K2 K196R mutant plasmid will also be transfected and then immunoprecipitation and Western blot will be conducted to determine K196 is the actual SUMOylation site. Moreover, with the same experimental setting (FLAG-S6K2 WT or S6K2 K196R and Myc-SUMO transfection), MCF-7 cells will be treated with cycloheximide with increasing timepoints to investigate whether SUMOylation affects the half-life of S6K2 protein. Also, MCF-7 apoptosis upon TNF treatment will be tested using flow cytometry in the same experimental setting to investigate if S6K2 SUMOylation supports breast cancer cell survival. Finally, the addition of HA-Ub to the same experimental setting along with MG132 treatment will reveal whether S6K2 ubiquitination is affected by SUMOylation.

Result and discussion

We expect to find S6K2 K196 is the S6K2 SUMOylation site and this modulation will prolong S6K2 longevity by preventing its ubiquitination. Also, we expect an increase in MCF-7 cell survival upon TNF treatment when SUMO is overexpressed. On the other hand, the K196R mutant S6K2 overexpression is expected to decrease in S6K2 half-life and MCF-7 cell survival upon TNF treatment.

Conclusion

Based on our findings, S6K2 pro-survival function will be modulated. Therefore, one of the barriers of chemoresistance in breast cancer will be overcome.

EACR25-2025

Endothelial pyrimidine synthesis restricts tumor growth

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Introduction

Cancer cells rely on nucleotide synthesis for proliferation, and antimetabolites targeting nucleotide metabolism remain a cornerstone of cancer therapy. Despite their clinical success, these agents often face high rates of resistance, potentially due to interactions with stromal cells within the tumor microenvironment.

Material and method

To investigate the impact of altered nucleotide metabolism in the tumor microenvironment, we suppressed de novo pyrimidine synthesis in mice by generating a whole-body inducible knockout of dihydroorotate dehydrogenase (DHODH), the essential enzyme in this pathway.

Result and discussion

Unexpectedly, systemic DHODH deficiency led to accelerated growth of orthotopic lung tumors. Single-cell transcriptomics of tumor-bearing lungs revealed that DHODH loss significantly affected stromal cells, including immune cells and, notably, endothelial cells. To dissect the specific role of endothelial metabolism, we generated a mouse model with inducible, endothelium-specific DHODH deletion. Strikingly, selective loss of de novo pyrimidine synthesis in endothelial cells recapitulated the whole-body knockout phenotype, creating a more permissive environment for tumor growth. Transcriptomic analysis of the endothelial-specific model identified alterations in the immune composition of the tumor microenvironment, particularly in monocytes, which was further validated by spectral flow cytometry. Ongoing studies aim to elucidate the precise mechanisms by which endothelial pyrimidine deficiency promotes tumor progression.

Conclusion

Our findings reveal an unexpected pro-tumorigenic effect of systemic pyrimidine synthesis inhibition, driven by changes in the endothelial compartment. These results highlight a previously unrecognized role of endothelial pyrimidine synthesis in shaping the tumor microenvironment and raise concerns about the unintended consequences of targeting this pathway in cancer therapy.

EACR25-2031

Targeting Tumor Metabolism to Prevent the Development of Resistance to Cisplatin in Ovarian Cancer

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Introduction

Ovarian cancer is the deadliest gynecological malignancy in Western countries, with a 5-year survival rate of approximately 40%. Although 85% of patients initially respond to cisplatin (DDP)-based therapy, around 70% relapses with a resistant disease. Among the various mechanisms of resistance, metabolic plasticity is emerging as a key factor and potential therapeutic target. Metformin (MET), a widely used antidiabetic drug,

modulates metabolism and exhibits promising anticancer effects. Our previous studies demonstrated that the addition of MET to DDP can delay the onset of DDP resistance in ovarian cancer patient-derived xenografts (PDXs). This project aims to explore how the combination of DDP and MET can delay resistance, with the purpose of preventing its emergence.

Material and method

Murine ovarian cancer ID8 F3 cell lines sensitive to DDP (S) were used to develop DDP-resistant cells (R) by gradually increasing DDP concentrations. Parallel to this, cells were treated with a combination of DDP and MET (SDM). Resistance was assessed using cytotoxicity assays. Metabolic profiling was performed using a Seahorse® extracellular flux analyzer in both S ID8 cell lines and PDXs, as well as their R sublines derived from *in vitro* and *in vivo* DDP treatments and co-treatment with DDP and MET (SDM, partially resistant to platinum). Protein and gene expression assays were conducted to investigate the mechanisms underlying the delayed development of resistance.

Result and discussion

Resistant ID8 cells showed a five-fold increase in IC₅₀ (50% inhibitory concentration) compared to parental S ID8 F3 cells, while SDM cells exhibited intermediate sensitivity, with only a two-fold increase in IC₅₀. Metabolic profiling revealed that R cells relied more on oxidative phosphorylation, while S and SDM cells depended more on glycolysis. Similar results were observed in ex-vivo cultures derived from S, R and SDM PDX models. Western Blot analyses indicated that the preventing effect of the addition of MET is AMPK-dependent and likely linked to autophagy. An increase in the pAMPK/totAMPK ratio, leading to the inhibition of mTOR, was observed in co-treated cells compared to resistant cells. In the same manner, the co-treated SDM cells exhibited a higher expression of autophagy markers (LC3-I and LC3-II) compared to the R, similar to the parental S cells.

Conclusion

These findings suggest that combining cisplatin and metformin effectively delays the development of cisplatin resistance by modulating tumor metabolic plasticity. Ongoing and future research aims to clarify the underlying mechanisms and explore more specific approaches to prevent resistance development.

EACR25-2041

Utilizing BiOID to Identify Interaction

Partners of Haspin Kinase

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Introduction

Haspin is an atypical Ser/Thr kinase that is critical during mitosis. It has been specifically shown to regulate chromosome alignment and ensure the timely segregation of sister chromatids. Although its well-characterized functions are limited to mitosis, Haspin is present throughout the entire cell cycle. This observation raises the possibility that Haspin may have additional roles in other cell cycle phases. Understanding these potential roles requires investigating the Haspin interactome, which encompasses the network of protein-protein interactions involving Haspin.

Material and method

To analyze the interactome of Haspin, we employed the BioID method. This proximity-dependent biotinylation technique labels potential interaction partners based on their spatial proximity to the protein of interest. BioID was executed in HeLa cells, followed by mass spectrometry analysis to identify biotin-tagged proteins. Subsequent bioinformatics analyses were conducted to determine the putative interaction partners and evaluate their association with various cell cycle stages.

Result and discussion

We identified 127 proteins as potential interaction partners of Haspin kinase through mass spectrometry. Some proteins, such as Pds5b, were previously recognized as Haspin interaction partners. However, most identified proteins had no known association with Haspin, suggesting novel functional links. Furthermore, a subset of these proteins exhibited variations in their interaction patterns across different cell cycle stages, indicating that Haspin's interactome may be dynamically regulated throughout the cell cycle. These findings imply that Haspin might have broader cellular roles beyond mitosis, possibly influencing other cell cycle phases. In the next step, we refined our approach by employing the Fucci2 reporter system, which allowed cell sorting based on specific cell cycle phases before proteomic analysis. This strategy facilitated the elucidation of the dynamic changes in Haspin's interactome and its broader biological significance.

Conclusion

Our study offers insights into the interactome of Haspin kinase and its potential role in cell cycle regulation beyond mitosis. The discovery of new interaction partners emphasizes untapped facets of Haspin's function.

Acknowledgment: This work was supported by Czech Science Foundation grant nr. 23-06472S, and the project National Institute for Cancer Research (Programme EXCELES, ID Project No. LX22NPO5102) - Funded by the European Union - Next Generation EU. We acknowledge the CEITEC Proteomics Core Facility of CIIBS, Instruct-CZ Centre, supported by MEYS CR (LM2018127).

EACR25-2046

An oncogenic ubiquitin E3 ligase promotes lung cancer metastasis by regulating the HIPK2-Slug axis

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Introduction

Lung cancer remains the leading cause of cancer-related deaths worldwide, with metastasis being a major factor in poor prognosis. Epithelial-to-mesenchymal transition (EMT) plays a crucial role in metastasis, with Slug acting as a master regulator by promoting cell migration and invasion. Our group previously demonstrated that Slug levels are tightly regulated by the p53-MDM2 and GSK3 β -CHIP ubiquitin-proteasomal pathways. Deficiencies in these pathways lead to non-degradable Slug accumulation, E-cadherin repression, and increased metastasis. However, it remains unclear how metastasis suppressors or promoters influence Slug degradation under normal and disease conditions.

Material and method

A yeast two-hybrid screen identified novel Slug-interacting partners. The migratory and invasive abilities of CL-series lung cancer cells were assessed using transwell assays. Western blot and immunohisto-chemistry were used to analyze protein levels in cell lines and patient tissues. The metastatic activity of CL1-5 lung cancer cells was evaluated in an orthotopic lung cancer model via intrathoracic implantation in nude mice. In vitro kinase assays and mass spectrometry (MS) identified Slug phosphorylation sites via its key binding partner, HIPK2. Using Global Protein Stability (GPS) profiling with a CRISPR/Cas9 ubiquitin degradation sgRNA library and IP-MS analyses, we identified an oncogenic ubiquitin E3 ligase regulating the HIPK2-Slug axis.

Result and discussion

We found that HIPK2 interacts with and destabilizes Slug by phosphorylating it at Serine (S)104, promoting degradation via ubiquitin-proteasomal pathways. HIPK2 may also prime GSK3 β -dependent Slug phosphorylation at S100, S96, and S92, facilitating CHIP-dependent degradation. Overexpression of HIPK2 in highly invasive CL1-5 cells reduced Slug levels, increased E-cadherin expression, and suppressed metastasis, while these effects were absent in kinase-dead HIPK2 mutants. Although HIPK2 protein levels negatively correlate with invasiveness in CL-series cell lines, its mRNA levels do not match protein expression, and no significant differences in HIPK2 mRNA levels were observed across lung cancer stages. These findings suggest that HIPK2 protein stability control plays a critical role in metastasis, potentially regulated by an oncogenic ubiquitin E3 ligase. Using a CRISPR/Cas9 GPS screen and IP-MS analysis, we identified Zinc Finger Protein X (ZFPX) as a key regulator of HIPK2 stability. Inhibiting ZFPX increased HIPK2 stability, leading to Slug destabilization and impaired EMT-driven metastasis.

Conclusion

Our findings provide insights into the molecular mechanisms controlling HIPK2 stability and propose targeting ZFPX as a novel therapeutic approach to inhibit Slug-mediated lung cancer metastasis.

EACR25-2059

Characterization of Cancer Stem Cells in Osteosarcoma Progression

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Introduction

Osteosarcoma (OS) is a malignant bone tumor that affects adolescents and young adults. Its high heterogeneity and low prevalence hinder research and the development of targeted therapies. Despite treatment advances, survival rates remain poor, especially for metastatic OS. Cancer stem cells (CSCs) contribute to OS progression and poor outcomes due to their ability to evade therapies, drive metastasis, and promote tumor recurrence. This study aimed to characterize CSCs by examining key hallmarks, including immortality, metastatic potential, chemoresistance, and by using scRNA-seq transcriptomic profiling to uncover changes in cellular subpopulations and molecular pathways governing these processes.

Material and method

The study was performed on osteosarcoma samples from a single patient, collected at three stages of disease: diagnosis, post-chemotherapy resection, and recurrence. Immortality was characterized by measuring hTERT expression by qRT-PCR, and the relative telomere length by qPCR. Changes in metastatic potential were monitored through a spheroid formation efficiency (SFE) test. Cell migration was assessed using a wound healing assay, where the migration rate was observed after 24 hours. Chemoresistance was evaluated in 2D and 3D cultures treated with cisplatin, doxorubicin, and methotrexate. Transcriptomic changes were analyzed using scRNA-seq, and the data were processed with Cell Ranger. Downstream analysis was performed using Seurat to identify CSC subpopulations and related gene expression.

Result and discussion

Using scRNA-seq analysis, this study identified a cluster with aggressive properties, suggesting it may represent the CSC population. This cluster was significantly increased in the recurrent sample, showing expression of CSC markers, metastasis-associated genes, epithelial-mesenchymal transition-related genes, and transcription factors promoting CSC properties. CSC isolated from these samples did not show hTERT expression, suggesting they achieved immortality via alternative telomere extension, with transcriptomic analysis confirming the presence of enzymes associated with the ALT mechanism and their expression levels increasing as the disease progressed. As the disease progressed, telomere length shortened, while tumor initiation

capacity and invasiveness increased, as evidenced by higher SFE and enhanced migratory ability. These cells demonstrated selective responsiveness to chemotherapy, showing sensitivity only to doxorubicin.

Conclusion

This study reveals the key role of CSCs in OS progression. Using experimental data and scRNA-seq analysis, we identified mechanisms driving tumor evolution, chemoresistance, and recurrence. These findings emphasize how CSCs adapt to therapeutic pressure, enabling the tumor to acquire immortality, enhanced migration, and selective chemoresistance, ultimately resulting in a more aggressive phenotype.

EACR25-2065

The long non-coding RNA H19 promotes cell proliferation, migration, and invasion in breast cancer through the H19/miR-X/BST2 axis

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Introduction

H19 is the first identified lncRNA and is expressed from the H19/IGF2 locus, located on chromosome 11 (p15.5) and spanning 140 kbp. Previously, our team has shown that H19 overexpression in breast cancer cells promotes key tumorigenic processes, including proliferation, migration, and cell invasion. More recently, transcriptomic analyses conducted on cell lines stably overexpressing H19 revealed a direct correlation with increased expression of BST2, a type II transmembrane protein initially described for its antiviral role. However, a growing number of studies highlight its involvement in tumor progression, particularly in gastric cancer, where BST2 contributes to metastasis formation.

Material and method

Four cell lines were used : MDA-MB-231, MCF-7, T47D, and SUM159-PT. To overexpress or inhibit H19 and BST2, stable transfection and siRNA transfection were performed. Relative mRNA level were measured by RT-qPCR and protein expression by western blot or flow cytometry. To perform tumorigenesis assay, SCID mice were used. Finally, to explore the binding of the miR-X on H19 and BST2 RNA, we used reporter systems allowing luciferase expression combined with miRNA mimics and anti-miRNAs.

Result and discussion

We demonstrated that in our cell lines, H19 overexpression or inhibition is associated with an increase or decrease, respectively, in BST2 expression, both at the transcriptional level and protein level. Remarkably, we also observed that BST2 modulation directly influences H19 expression: BST2 overexpression leads to an increase in H19 expression, while its inhibition reduces it. This finding highlights a cross-regulation between these two molecular players. Furthermore, our in vitro and in vivo experiments revealed that BST2

overexpression reproduces the oncogenic effects of H19, resulting in enhanced proliferation, migration, invasion, and tumor growth. Finally, our analyses revealed that the cross-regulation between H19 and BST2 relies on post-transcriptional regulation involving a microRNA capable of simultaneously controlling the expression of both molecular players. To explore this hypothesis, we used reporter systems combined with miRNA mimics and anti-miRNAs, allowing direct evaluation of this microRNA's impact on H19 and BST2. Our results show that microRNA X specifically binds to nucleotide sequences of H19 and BST2, leading to a coordinated regulation of their expression. This post-transcriptional regulatory mechanism suggests that H19 and BST2 share a common control network, where the activity of microRNA X finely modulates their expression levels.

Conclusion

These findings highlight a novel molecular regulatory axis involved in breast cancer progression and open new perspectives for the development of therapeutic strategies targeting this microRNA-dependent interaction. Finally, these results have led us to the preparation of a scientific article currently under submission.

EACR25-2077

Investigating the role of miR-500 in metastasis-related processes in LLC1 cells

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Introduction

Metastasis is the leading cause of cancer-related deaths. Early detection of tumor progression before metastasis formation could improve treatment outcomes, requiring biomarkers specific to different metastatic stages. Epigenetic regulation, including microRNA (miRNA)-mediated control, plays a crucial role in metastasis by enabling cancer cells to adapt to new environments. By regulating gene expression post-transcriptionally, miRNAs are involved in epithelial-mesenchymal transition, migration, invasion, and organ colonization. Their expression profiles correlate with tumor development, progression, and therapy response, highlighting their potential as biomarkers and therapeutic targets. Previous studies in our laboratory identified 77 miRNAs, including miR-500, that are potentially related to non-small cell lung cancer metastasis. This study aims to further investigate the role of miR-500 in metastasis-related processes.

Material and method

The CRISPR/Cas9 system was used to knockout (KO) miR-500 in LLC1 cells. Transcriptional analysis of KO cells was performed using RNA sequencing, and differential gene expression was analyzed with DESeq2 software. GO and KEGG enrichment analyses were carried out using clusterProfiler. In vitro functional analysis of LLC1 miR-500 KO cells was performed in

monolayer culture, including cell proliferation assay with AlamarBlue, cell cycle analysis by flow cytometry, and cell migration assay via the wound healing method. 3D spheroid formation was assessed using a scaffold-free, self-assembly method.

Result and discussion

Genome editing using CRISPR/Cas9 successfully generated LLC1 cell lines with biallelic deletions of the miR-500 sequence, leading to significantly reduced expression of miR-500. RNA sequencing of LLC1 miR-500 KO cells revealed 1163 differentially expressed genes (DEGs), of which 391 were upregulated and 772 downregulated. GO enrichment analysis identified that these genes are associated with development, morphogenesis, cell migration regulation, cell-substrate adhesion, and actin cytoskeleton organization. KEGG analysis showed DEGs were enriched in signaling pathways, including extracellular matrix-receptor interactions, Rap1, Wnt, TNF, MAPK, and focal adhesion. Functional assays demonstrated that miR-500 does not affect proliferation or the cell cycle, whereas the wound healing assay revealed that it is involved in migration. Additionally, miR-500 was found to be important for LLC1 cells in forming 3D spheroids.

Conclusion

Our study demonstrates that miR-500 is involved in metastasis-related processes in LLC1 cells. However, further research is required to elucidate the molecular mechanisms underlying its role in lung cancer metastasis.

EACR25-2079

IDH1 mutation induces differential DNA repair and metabolic profiles in glioma

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Introduction

Gliomas are heterogeneous primary brain tumors arising from glial cells, with aggressive nature and poor prognosis. Mutations in isocitrate dehydrogenase (IDH) genes, found in 80% of gliomas, lead to neomorphic enzyme activity, resulting in 2-hydroxyglutarate (2-HG) accumulation. 2-HG has been implicated in several oncogenic effects, and studying its impact on glioma metabolism and genomic instability is crucial. This study characterizes the DNA damage response (DDR) and metabolic profile in IDH1-mutant glioma cells.

Material and method

Experiments were conducted in U-87 MG glioma cells (wildtype IDH1) and IDH1-mutant U-87 (isogenic, R132H). Chromosomal damage (cytokinesis-block micronucleus assay) and double-strand breaks (DSB; fast halo assay) were evaluated in basal conditions and after exposure to four DNA-damaging/repair-interfering agents, to establish repair kinetics (0, 1, 24, 48 h). For metabolic studies, glucose and mitochondrial dependence, glycolytic and fatty acid/amino acid oxidation capacity were assessed by SCENITH™ assay. To confirm the identified metabolic profiles, metabolic activity was measured (resazurin assay) after incubation with a glycolysis inhibitor (2-deoxy-D-glucose; 2-DG), oxidative phosphorylation inhibitor (oligomycin), and culture in glutamine-depletion (glutamine-free media). Glucose uptake was measured with 18F-FDG. Metabolism-related genes were evaluated by qPCR. Mitochondrial membrane potential (JC-1 probe), superoxide anion levels (DHE probe), and intracellular peroxides (DCF probe) were analyzed by flow cytometry.

Result and discussion

IDH1-mutant cells presented higher baseline chromosomal damage (2.2-fold, $p < 0.0001$) and DSB levels (1.3-fold, $p=0.0053$) than U-87 MG. Sensitivity to the DNA damaging/repair-interfering agents was also higher in IDH1-mutant cells, as shown by increased damage levels immediately after exposure (0 h), higher maximum damage peaks, and impaired repair kinetics ($p < 0.05$). IDH1 mutation increased mitochondrial dependence (2.6-fold, $p = 0.0353$), fatty acid and amino acid oxidation capacity (1.6-fold, $p = 0.0064$), and 18F-FDG uptake ($p < 0.05$). Cells' metabolic activity was reduced by 2-DG and oligomycin in a cell line-dependent manner, thus confirming the identified dependences. The mutation also rendered cells more sensitive to glutamine depletion ($p < 0.05$), which was linked to increased GLS1, GLUD1, and GLUD2 expression ($p < 0.05$). MYC levels also increased (1.5-fold, $p < 0.05$). Mitochondrial membrane potential was reduced (1.4-fold, $p = 0.0027$), and super-oxide anion and intracellular peroxides elevated (1.2-fold, $p = 0.0464$ and 1.6-fold, $p = 0.0024$), possibly indicating mitochondrial dysfunction.

Conclusion

IDH1 mutations induce DNA repair deficiencies and distinct metabolic profiles. Such alterations can render IDH1-mutant gliomas more susceptible to DNA repair inhibition and metabolism-interfering strategies.

EACR25-2085**Hypoxia-Driven Fatty Acid Metabolism Regulates Angiogenesis in Gastrointestinal Cancers**

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Introduction

Gastrointestinal cancers represent a heterogeneous group of diseases including gastric, pancreatic and colorectal cancer and hepatocellular carcinoma. In this complex scenario of different etiology and clinical management, oxygen limitation is one common condition that fosters cancer malignancy through a metabolic rewire, stimulating glycolysis and inhibiting mitochondrial activity. In particular, hypoxia is involved in fatty acids (FA) and lipid de novo synthesis and import, which in turn play an important role in cancer cell migration, invasion and angiogenesis regulation. Indeed, the hypoxic microenvironment is usually generated by scarce or aberrant vascularization. In this scenario, FA are reported to be capable on one hand of stimulating the expression of FA-binding proteins which are positive regulators of angiogenesis and on the other, drugs blocking FA oxidation decrease endothelial cell proliferation.

Material and method

Nine different cancer cell lines (gastric, pancreas and colon cancer) were subjected to hypoxia (0.5% O₂) for 24 or 72 hours as acute or chronic stimuli in a gas-tight manipulator and their mitochondrial mass and membrane potential assayed through MitoTracker and TMRM via flow cytometry, respectively. Their metabolic profile and the capacity to β-oxidate FA were evaluated via the Seahorse platform, while the hypoxic gradient was monitored continuously thanks to the Hypoxia Green dye. FA were measured in cells and conditioned media (CM) via Nile Red staining. The vessel formation capacity was assessed through 2D and 3D angiogenic models.

Result and discussion

A chronic hypoxic stimulus was capable, with respect to an acute one or normoxic oxygen tension, to induce an increased FA storage, as intracellular neutral lipids. The secretion of FA produced by cancer cells in lipid-depleted media was measured and human endothelial cells were subjected to the collected CM in standard conditions. We observed that the CM could regulate vessel formation in an inversely proportional manner to the amount of FA in the media. We validated this negative tendency by using drugs blocking the FA oxidation (Etomoxir), the FA synthase (Denifanstat) or the FA uptake (Sulfosuccinimidyl oleate) and by administering increasing doses of palmitic acid.

Conclusion

Our findings suggest that chronic hypoxia induces a metabolic shift in gastrointestinal cancer cells, leading to increased FA storage and secretion, which in turn modulates endothelial cell behavior and angiogenesis.

The inverse correlation between secreted FA levels and vessel formation highlights a potential mechanism for aberrant tumor vascularization. Interestingly, gastric cancer cells appear to follow a distinct metabolic pattern, warranting further investigation. Overall, these results provide new insights into the interplay between hypoxia, FA metabolism, and angiogenesis, offering potential therapeutic targets for modulating tumor vascularization.

EACR25-2093**The Oncofetal Protein ROR1 Drives Cancer Stemness and Metastasis in Hepatocellular Carcinoma through Actin Cytoskeleton Remodelling**

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Introduction

Hepatocellular carcinoma (HCC) is one of the most common and aggressive types of liver cancer worldwide, with poor prognosis, and high relapse and metastasis rates, highlighting the need for novel therapeutics. Phenotypic plasticity is recognised as one of the hallmarks of cancer and has been proposed to play a key role in driving tumor relapse and metastasis. Oncofetal reprogramming, the process by which cancer cells hijack the stem cell signaling pathway to reactivate fetal transcriptional programs, has been linked as a potential mechanism for cancer cells to acquire phenotypic plasticity. Although some oncofetal proteins, such as AFP, have been widely accepted as markers of less differentiated tumors, their poorly defined functional roles and presence in the regenerative liver make them less than ideal targets for HCC treatment. Therefore, we aim to identify a targetable oncofetal antigen in HCC and assess its role and mechanism in HCC tumorigenesis.

Material and method

Transcriptomics data from the TCGA-LIHC, human hepatocyte and fetal liver, and the Cell Surface Protein Atlas datasets were used to identify oncofetal antigens in HCC. In vitro and in vivo assays were performed using human HCC cell lines and xenograft mouse models to assess the functional role of the oncofetal candidate.

Transcriptome sequencing and affinity purification mass spectrometry (AP-MS) were used to determine the downstream pathway.

Result and discussion

We identified the receptor tyrosine kinase-like orphan receptor 1 (ROR1) as a potential oncofetal antigen in HCC. Although ROR1 is well characterized for regulating cell polarity and differentiation during fetal development, research of ROR1 in HCC is limited. Clinically, high expression of ROR1 was correlated with advanced tumor stage and worse prognosis. Functionally, ROR1 increased the ability to migrate, invade and initiate tumors in both in vitro and in vivo models.

Transcriptome profiling revealed the enrichment of the actin cytoskeleton in HCC cells with ROR1 over-expression. In vitro assays confirmed that ROR1 enhances actin polymerization. Treatment with actin polymerization inhibitors rescued the enhanced actin polymerization and reversed the pro-tumorigenic effects of ROR1. Since ROR1 functions as a pseudokinase and requires a binding partner for downstream signaling, we performed AP-MS coupled with interactome analysis and identified CD147 as the downstream effector of ROR1 for remodelling the actin cytoskeleton. Mechanistically, actin remodelling by ROR1 drove epithelial-mesenchymal transition (EMT), potentially leading to enhanced HCC tumorigenesis.

Conclusion

Our research demonstrates that the oncofetal protein ROR1 promotes HCC stemness and metastasis by facilitating actin remodelling and activating EMT. Current efforts are directed towards evaluating the therapeutic benefits of inhibiting ROR1 in HCC.

EACR25-2118

Influence of imbalanced progesterone receptor isoforms on breast cancer metabolism-related protein expression

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Introduction

Most diagnosed breast cancers express progesterone receptor (PR) isoforms A (PRA) and B (PRB).

Imbalanced levels of PR isoforms (PRA>PRB: PRA-H; PRB>PRA: PRB-H) are associated with differential responses to mifepristone treatment. Few studies investigate the biological characteristics of PRA-H and PRB-H tumors and the impact of PR isoforms on breast cancer metabolism remains under-explored.

Material and method

We performed transcriptomic and proteomic studies on frozen breast cancer specimens from the "Magdalena V de Martinez" Hospital, Argentina. The study was IRB-approved. Primary tumors were categorized as PRA-H or PRB-H by western blot. Eleven PRA-H and eight PRB-H samples were Poly(A) RNA sequenced. Nuclear (Nuc)

and cytosolic (Cyt) protein fractions from nine PRA-H and nine PRB-H samples were studied by LC-MS/MS.

Result and discussion

The transcriptomic study unveiled 129 deregulated genes, while the proteomic assay displayed 289 differentially deregulated proteins in Cyt and 301 in Nuc extracts ($\log_{2}FC > 1$, $p\text{val} < 0.05$). In PRA-H tumors, Gene set enrichment analysis (GESEA) of protein fractions showed an up-modulation of biological processes related to pyruvate metabolism and citric acid cycle ($p\text{val} = 0.0014$), fatty acid oxidation ($p\text{val} = 0.001$), and the metabolism of amino acids and derivatives ($p\text{val} = 0.006$). An up-modulation of arginine, proline, alanine, aspartate, and glutamate metabolism (KEGG, $p\text{val} < 0.05$) was also observed. Accordingly, proteins involved in BCAA metabolism, cystine uptake, and glutathione production ($p < 0.1$), all related with redox homeostasis and linked to cell invasiveness, were overrepresented. In PRB-H tumors, GESEA indicated an up-modulation of Glycolysis ($p\text{val} = 0.0415$) and Peroxisomal lipid metabolism ($p\text{val} = 0.01$) suggesting that these metabolic pathways provide their main energy source.

Transcriptomic data supported proteomic results

Conclusion

In summary, the proteomic and transcriptomic studies highlight metabolic differences related to imbalanced PR isoform expression in breast cancer and provide potential metabolic vulnerabilities that might be therapeutically exploited.

EACR25-2128

PIK3CA modulates oncogenic and stemness properties in Cervical Cancer

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Introduction

Human Papillomaviruses (HR-HPVs) infection are known to be the major cause of cervical cancer along with other factors. Cancer stem cells (CSCs) are also known to be involved in tumorigenesis and drug resistance, which is one of the challenges in treatment. It is therefore important to identify novel genetic alteration(s) which can serve as target(s) for effective treatment of cervical cancer. Hence, we performed a meta-analysis of exome sequencing data of cervical cancer patients from public databases and found that PIK3CA is having highest 37% gene alteration. PIK3CA, the catalytic subunit of PI3K, plays crucial role in cell growth and metabolism. We also found that PIK3CA was highly expressed in cervical CSCs. However, the molecular mechanism of PIK3CA in cancer stemness is still unclear and hence, we aimed to unravel the role of PIK3CA in modulating stemness properties in cervical cancer using CRISPR/Cas9 approach.

Material and method

CRISPR-Cas9 constructs targeting PIK3CA were designed at two different exon 2 and 9 (harbouring E542K and E545K hotspot regions) and transfected into SiHa cervical cancer cells. PIK3CA knock out (KO)

clones were screened by western blotting and sanger sequencing. PIK3CA KO clones were characterized using various phenotypic experiments. Cervical cancer stem-like cells were isolated and enriched from SiHa cells using flow cytometry. The self-renewal capacity of these CSCs was evaluated by spheroid formation assays. Expression levels of oncogenic genes and stemness markers were checked via qPCR.

Result and discussion

PIK3CA Knockout cells were generated using CRISPR-Cas9 gene editing. Total 46 PIK3CA KO clones forming single colonies were screened through western blotting and sanger sequencing, where 2 clones were identified as knockout clones. PIK3CA KO showed loss of AKT activation, impaired proliferation and colony formation. A remarkable reduction in cellular migration was observed in PIK3CA KO cells along with reduction in EMT markers such as ZEB1 and TWIST. Further, PIK3CA KO cells underwent G2/M phase arrest leading to cellular apoptosis due to microtubular defects. The stemness markers (OCT4, ALDH1) were downregulated in PIK3CA KO cells suggesting a reversal of stemness phenotype. Observing high expression of PIK3CA and c-Myc oncogenes in SiHa derived CSCs suggested a potential PIK3CA/c-MYC axis. Notably, PIK3CA KO cells lost c-Myc expression, which is reported to regulate stemness via PDK1/PLK1 axis, which is under investigation. These results provide new insights into CSC regulation in cervical cancer and underscore the potential of targeting the PIK3CA-c-Myc axis to enhance treatment efficacy.

Conclusion

PIK3CA regulates CSCs most likely via c-Myc, and targeting this axis may offer a promising therapeutic strategy to combat CSCs and enhance the effectiveness of current treatments.

EACR25-2141

Unraveling the PKM2 Nexus: Metabolic Reprogramming, Nuclear Regulator, and p38 MAPK-Signaling Driven Drug Resistance in Colorectal Cancer

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Introduction

Colorectal cancer (CRC) remains a leading cause of cancer-related mortality, with therapy resistance driven by metabolic reprogramming, stress signaling, and cancer stem cells (CSCs). Pyruvate kinase M2 (PKM2) plays a dual role in CRC by regulating glycolysis and translocating to the nucleus, where it interacts with transcription factors like HIF-1α to promote tumor survival and drug resistance. PKM2 has been linked to the modulating of p38 MAPK, facilitating adaptation to oxidative stress and chemotherapy-induced DNA damage. High glucose levels in the tumor microenvironment further enhance PKM2 expression and nuclear translocation, reinforcing CRC cell survival under therapeutic stress. Additionally, the p38 mitogen-activated protein kinase (p38 MAPK) pathway is activated in response to metabolic stress and chemo-

therapy, further supporting CRC cell adaptation and resistance mechanisms. Understanding the interplay between glucose-driven PKM2 regulation, nuclear PKM2 functions, p38 MAPK signaling is essential for overcoming CRC chemoresistance. This study explores how glucose sensing influences PKM2 expression and function and investigates PKM2 and p38 MAPK inhibition as potential therapeutic strategies.

Material and method

Using CRC cell lines and patient-derived tumor samples we investigate the expression and functions of PKM2, and MAPK pathway signaling. Flow cytometry were used to evaluate CSC populations. RNA interference (RNAi) and small-molecule inhibitors were employed to modulate PKM2 and p38 MAPK activity. Chemoresistance assays were conducted applying 5-fluorouracil (5-FU) and oxaliplatin. Phosphoprotein microarray, western blotting and qRT-PCR were performed to assess changes in gene and protein expression and cell signaling.

Result and discussion

Our findings reveal that glucose availability significantly upregulates PKM2 expression and promotes its nuclear translocation, as observed in CRC cells exposed to high-glucose media. In contrast, glucose deprivation reduced nuclear PKM2 levels, suggesting a strong link between glucose sensing and PKM2 function. PKM2 overexpression correlated with chemoresistance, as drug-resistant CRC cells exhibited increased PKM2 expression and nuclear localization. Additionally, down regulation of PKM2 enhanced p38 MAPK pathway activation, further supporting chemoresistance.

Conclusion

Our results feature glucose-driven PKM2 regulation as a key mechanism in CRC drug resistance and CSC survival.

EACR25-2157

Assessing the impact of endolysosomal Two-pore channel 2 (TPC2) on malignant melanoma aggressiveness

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Introduction

Two-pore channel 2 (TPC2) is a Na⁺/Ca²⁺ ion permeable channel located on the membrane of intracellular acidic organelles, including endo-lysosomes and melanosomes. It plays a crucial role in tumour progression across various cancers, including melanoma, one of the most aggressive and treatment-resistant malignancies, often driven by mutations in the serine/threonine kinase BRAF. This project aims to explore the involvement of TPC2 in melanoma progression,

comparing its role in primary and metastatic disease, to determine whether it could serve as a potential therapeutic target for tailored melanoma treatments.

Material and method

Two pairs of human melanoma cell lines (IGR and WM) were analyzed, each pair derived from the same patient at different stages of tumor progression and genetically characterized by BRAF mutation. The primary melanoma cell lines were IGR39 and WM115, while the metastatic melanoma cell lines were IGR37 and WM266-4. TPC2 expression levels were compared between the primary (IGR39 and WM115) and metastatic (IGR37 and WM266-4) cell lines. The expression of EMT markers, including N-cadherin, E-cadherin, and Vimentin, was assessed to examine the EMT transition. Additionally, migratory capability was measured using a scratch assay, and the ability of the cells to adhere to type 1 collagen was evaluated. To investigate the role of TPC2 in cellular behaviour, the TPC2 inhibitor SG-094 was applied to these cell lines.

Result and discussion

We found that all these human melanoma cell lines exhibit heterogeneous expression of EMT markers and display different behaviours in terms of migratory capability and adhesion to type 1 collagen. Notably, cells derived from the primary tumour site (IGR39 and WM115) have lower TPC2 expression than metastatic cells (IGR37 and WM266-4). Our results demonstrated that, pharmacological inhibition of this channel using the TPC2 inhibitor SG-094 significantly reduces the migratory capability of IGR and WM cell lines, as well as the adhesiveness of IGR lines to type 1 collagen, highlighting the role of TPC2-dependent intracellular Ca²⁺ signalling. Notably, SG-094 treatment also led to a decline in the proliferative potential of these cells, suggesting an additional impact on tumour progression. Additionally, our studies reveal that the effect of SG-094 may involve the microphthalmia-associated transcription factor (MITF), a master regulator of melanocytes implicated in metastasis tumour aggressiveness and survival.

Conclusion

The data demonstrate that TPC2 activity plays a crucial role in the aggressive traits of melanoma. The complex regulation of TPC2 in melanoma progression warrants further investigation. These findings suggest potential therapeutic strategies that combine TPC2 inhibition with existing treatments to try to overcome therapy resistance in melanoma patients.

EACR25-2169

The SuSe Model: A Dual-Marker Approach to Investigate the Impact of Senolytic Therapy in Cancer

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Introduction

Cellular senescence is a terminal cell cycle arrest induced by a variety of stresses, including oncogenes or certain

anti-tumor therapies. The effect of cellular senescence on tumor progression remains a contentious issue. While some reports show that cellular senescence is a tumor defense barrier, others show that senescent cells may contribute to tumor progression. Several mouse models have been developed to study cellular senescence, all of them based exclusively on the expression of p16. However, these models can also target non-dividing cells, including terminally differentiated ones, that overexpress p16. To address this limitation, we considered that senescent cells also undergo functional changes, such as the secretion of inflammatory factors like interleukin-6 (IL-6), and developed the SuSe (Suicidal Senescence) model. This novel transgenic system uses both p16 and IL-6 as dual markers of senescence, providing a more accurate approach to studying senescence *in vivo*.

Material and method

We developed a double transgenic mouse model, SuSe, expressing IL6pro-FKBP/Cas8-GFP and p16pro-FRB/Cas8-Cherry, which allows tracking and elimination of senescent cells upon AP21967 treatment. SuSe mice were crossed with the MMTV-PyMT model to study oncogene induced senescence. Mice were treated at early and late tumor progression stages, and tumor growth and metastasis were monitored. Additionally, we established two syngeneic models based on the SuSe system to study therapy induced senescence. Mice were treated with chemotherapy to induce senescence, followed by AP21967 administration. Tumor growth and metastasis were assessed. Single Cell RNAseq and IHC were used for analysis.

Result and discussion

Our findings show that early senolysis accelerates tumor growth and metastasis, whereas late senolysis has the opposite effect, suggesting that senescent cells initially restrain tumor growth but later promote progression and metastasis. Results from the syngeneic models further reinforce the idea that persisting senescence is detrimental. When senolysis was combined with chemotherapy, tumor regression and reduced metastasis were observed, emphasizing the therapeutic potential of targeting senescent cells at later stages. Additionally, we found that the interplay between senescent cells and the immune system is crucial in understanding their dual role. In the case of early senolysis, the accelerated tumor progression is mediated by an accumulation of immunosuppressive macrophages, which prevent infiltration of CD4+ and CD8+ T cells. In contrast, the beneficial outcome of late senolysis is associated with increased lymphocyte infiltration, promoting an effective antitumor response.

Conclusion

Using our transgenic model, which tracks and eliminates senescent cells, we highlight the importance of timing in senolytic therapies, influencing immune modulation and, therefore, tumor progression.

EACR25-2217

Dual-targeted therapy: Novel interventions to inhibit metastasis and chemoresistance in cervical cancer

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Introduction

Cervical cancer remains a critical global health issue, largely driven by high-risk human papillomavirus (HPV) infections. Despite treatment advances, chemoresistance and metastasis limit therapeutic effectiveness, resulting in poor outcomes. This study investigates the role of cluster of differentiation 44 (CD44) in these processes and evaluates the potential of resveratrol as an alternative treatment option to toxic chemotherapy drugs. By utilising ex vivo and in vitro models, we aim to uncover the molecular mechanisms behind treatment resistance and metastatic behaviour in cervical cancer.

Material and method

Ex vivo model - Ethical approval was obtained from Stellenbosch University (HREC - N22/04/037). Cervical tumour biopsies were cultured and treated with doxorubicin, carboplatin, gemcitabine, and paclitaxel. Cell viability was assessed via MTT assay, and western blotting analysed CD44 and EMT marker expression. A scratch assay measured wound closure rates, while immunofluorescence detected circulating tumour cells (CTCs) in patient blood. In vitro 2D model - An MTT assay evaluated cell viability in CaSki, SiHa, and HeLa cervical cancer cell lines. Western blotting assessed CD44 and EMT marker expression levels. A scratch assay measured wound closure, and clonogenic survival assays visualised survival post-treatment. In vitro 3D spheroid model - Immunocytochemistry examined CD44 expression both before and after carboplatin and resveratrol treatment. Scanning electron microscopy analysed spheroid topography.

Result and discussion

Ex vivo model: Patients exhibited significant chemo-resistance to multiple drugs, accompanied by elevated EMT markers and CD44 expression. Immunofluorescence confirmed CTC presence, indicating enhanced metastatic potential.

In vitro 2D model: Treatment significantly reduced cell viability across all lines. Carboplatin and resveratrol showed effective IC₅₀ values. Western blotting revealed enhanced CD44 and EMT marker expression levels.

In vitro 3D spheroid model: Immunocytochemistry indicated elevated CD44 expression, suggesting treatment resistance.

Conclusion

Evidence of treatment resistance and metastasis in advanced-stage cervical cancer patients requires the need to further investigate the molecular underpinnings of causality through targeting CD44 metastatic mechanisms and exploring the beneficial use of an alternative treatment option, such as resveratrol.

EACR25-2219

Novel molecular insights of gastric cancer dissemination and prognosis

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Introduction

Gastric Cancer (GC) is a major health problem, with 968,350 new cases and 659,853 deaths per year worldwide. GC exhibits a high tendency for recurrence and early-stage metastasis development. Consequently, the prognosis for GC patients is poor, with an average 5-year survival rate of 36%, dropping to only 10% for patients presenting distant metastases. However, the molecular determinants governing GC progression and metastasis are currently unknown.

Material and method

We took advantage of publicly available GC datasets from TCGA for the identification of matched genes among the 100 genes most associated with overall survival (OS) and disease-free survival (DFS). We confirmed the association of the identified genes with poor prognosis in the ACRG GC cohort and in over 2000 GC cases obtained from the integration of several cohorts using our own analysis pipeline. The Kaplan-Meier method and multivariate Cox regression analyses were used to determine prognostic significance, and linear modelling and correlation analyses were used to assess associations with clinicopathological parameters and biological hallmarks. Moreover, we performed in vitro and in vivo functional studies and transcriptomics (RNA sequencing) in GC cells to assess the role and mechanism of the most promising candidate gene among the initially identified genes.

Result and discussion

ANKRD6, ITIH3, SORCS3, NPY1R, and CCDC178 were the matched genes among the 100 genes most associated with overall survival (OS) and disease-free survival (DFS) in the TCGA GC cohort. High expression of these genes, individually and as a signature, was associated with reduced survival, recurrence, and node invasion in different GC cohorts. Moreover, the expression of ANKRD6 and ITIH3 was significantly higher in metastasis, and their levels correlated with markers of Epithelial to Mesenchymal Transition (EMT) and stemness in GC samples. In line with this, RNAseq analysis identified EMT among the pathways significantly altered in ANKRD6-silenced GC cells.

Accordingly, ANKRD6 silencing in GC metastatic cells showed abrogation of tumorigenic and metastatic traits in vitro and in vivo.

Conclusion

Our investigation uncovered a novel gene signature implicated in GC malignancy and prognosis and identified, for the first time, the pro-metastatic activity of ANKRD6 in the disease.

EACR25-2221

Epigenetic and Metabolic Consequences of JMJD5 Loss in Hepatocellular Carcinoma

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Introduction

Liver cancer is the third leading cause of cancer-related deaths worldwide, with hepatocellular carcinoma (HCC) accounting for 85% of primary liver cancers. HCC progression involves profound metabolic and epigenetic alterations, including transcription factor dysregulation, increased lipogenesis, and reactive oxygen species generation. JmjC domain-containing protein 5 (JMJD5), a 2-oxoglutarate-dependent dioxygenase predominantly expressed in hepatocytes, is significantly downregulated in HCC patients, suggesting a potential role as a tumour suppressor.

Material and method

Here, we employ a multi-omics approach to investigate the consequences of JMJD5 silencing in liver cancer cells. Proteomic analysis of JMJD5 knockout HepG2 cells revealed dysregulation of proteins involved in liver metabolism, DNA damage repair, histone methylation, and transcriptional regulation. Metabolomics using heavy carbon labelling demonstrated increased TCA cycle activity, nucleotide biosynthesis, and fatty acid production upon JMJD5 loss. Lipidomic profiling further revealed elevated phospholipid synthesis and diglyceride accumulation in JMJD5-deficient cells. These changes were indicative of increased cell-proliferation, which was confirmed by our proliferation, cell-survival and clonogenic assays.

Result and discussion

Additionally, we identified an enzymatic activity-dependent interaction between JMJD5 and histone methylation regulators NSD2 and DOT1L. Consequently, JMJD5 silencing led to increased dimethylation of H3K36 and H3K79, accompanied by DNMT3A upregulation. Notably, we observed a striking anti-correlation between JMJD5 and DNMT3A expression in both cell lines and patient data, suggesting that JMJD5 loss drives DNMT3A-mediated DNA hypermethylation, potentially contributing to transcriptional regulation of the metabolic signature.

Conclusion

Overall, our findings indicate that JMJD5 silencing disrupts proteome homeostasis, leading to the loss of a ‘liver-like’ phenotype, increased proliferation, and potential cellular dedifferentiation. Moreover, JMJD5 deficiency phenocopies the metabolic remodelling observed in HCC patients, reinforcing its role as a tumour suppressor. We further demonstrate that this metabolic shift is a downstream consequence of JMJD5-dependent epigenetic reprogramming.

EACR25-2227

Nuclear receptor NR5A2 negatively regulates HIF-1alpha signaling to inhibit tumor-specific metabolic adaptations in glioblastoma cells: potential novel therapeutic implications via NR5A2 targeting

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Introduction

Glioblastoma multiforme (GBM) is a highly lethal brain tumor characterized by altered metabolism. To sustain rapid proliferation rates, GBM cells favor glycolysis over oxidative phosphorylation (Warburg effect), driven by hypoxia-inducible factor-1α (HIF-1α). High HIF-1α expression correlates with more aggressive tumor phenotypes and poor prognosis, making it a key therapeutic target. To this end, by using a series of transcriptomic analyses in human GBM cells, we identified the orphan nuclear receptor NR5A2 as an inhibitor of HIF-1α signalling and associated GBM metabolic reprogramming. Specifically, RNA sequencing reveals NR5A2’s role in modulating hypoxia response, glycolysis, and fatty acid metabolism. Here we further explore NR5A2 function in GBM metabolic regulation and its potential as a therapeutic target.

Material and method

Human GBM cells were treated with DLPC to activate NR5A2 and with cobalt chloride to mimic hypoxia. Gene expression was analyzed using RNA-seq (transcriptomic changes), real time RT-qPCR, and Western blot. Cotransfection of HIF-1α and NR5A2 plasmids in GBM cells assessed their functional interaction. An orthotopic xenograft mouse model was used to evaluate DLPC effect on tumor growth, monitored via molecular and histological markers.

Result and discussion

RNA sequencing approaches showed that NR5A2 activation by its direct agonist (DLPC) in human GBM cells altered key metabolic pathways, including hypoxia response, glycolysis, and fatty acid metabolism. Over-expression of NR5A2 reduced glucose uptake and repressed HIF-1α downstream targets. NR5A2 over-expression in GBM cells decreased HIF-1α protein levels under both normoxic and hypoxic conditions, independently of prolyl hydroxylase pathway. Pharmacological activation with DLPC mimicked these effects, reducing tumor growth and aggressiveness in an orthotopic mouse xenograft model.

Conclusion

These results render NR5A2 as a key regulator of glioblastoma metabolism via the suppression of HIF-1α. Pharmacological targeting of NR5A2 reverses the HIF-1α-mediated metabolic adaptations in GBM cells, reducing glucose uptake, glycolysis, and tumor cell proliferation ex vivo as well as tumor growth in vivo. Targeting NR5A2 offers a promising strategy to suppress the GBM specific metabolic rewiring and tumor progression, warranting further preclinical and clinical investigation.

EACR25-2233

In vitro transformation of primary uveal melanocyte into uveal melanoma

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Introduction

Uveal Melanoma (UM) is a rare and aggressive eye tumor arising from melanocytes of the uveal tract. The tumorigenesis process is thought to rely on solely 2 genetic events: the acquisition of a Gαq pathway activating mutation as first event, and deleterious or change-of-function mutation in either BAP1, SF3B1 or EIF1AX gene as the second so called BSE event. Although these BSE events are well-established UM drivers, their molecular and cellular impacts triggering tumorigenesis remain largely unknown. Here, we aim to recapitulate the stepwise UM transformation in vitro to identify and decipher the molecular and cellular oncogenic mechanisms triggered by the Gαq constitutive activation and BSE events.

Material and method

Choroid and tumor samples were collected from UM patients undergoing enucleations at Institut Curie. Melanocytes and UM cells were purified and seeded in culture to establish cell lines. The GNAQQ209L activating mutation and BAP1 inactivating mutations were introduced in normal melanocytes using lentiviral transduction and CRISPR/Cas9 system, respectively. Both Gαq constitutive activation and BAP1 knock-out were validated using Western Blot, immunofluorescence and PCR.

Result and discussion

We established 27 uveal melanocyte and 23 uveal melanoma cell lines. Moreover, we successfully introduced both the GNAQQ209L activating mutation and BAP1 inactivation by CRISPR/Cas9 in one uveal melanocyte cell line. BAP1 knock-out was validated by TIDE (Tracking of Indels by Decomposition) analysis at the DNA level and Western-Blot and immunofluorescence at the protein level. Wild-type, GNAQQ-209L and GNAQQ209L + BAP1 knock-out uveal melanocytes are being characterized and will be compared to tumor cells from the same patient

Conclusion

As a proof of concept, we successfully introduced UM specific mutations in primary uveal melanocytes derived from UM patients. The development of an in vitro model will allow us to decipher the tumorigenic mechanisms involved in UM and to address key questions such as genetic predispositions, and BSE mutations contribution in UM. Furthermore, such models will constitute a unique tool for in vitro drug testing.

EACR25-2238

Luminal breast cancer cell migration is reduced by depletion of BCL6 transcriptional repressor

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Introduction

Breast cancer (BC) incidence has risen over the past two decades, now being the most prevalent cancer worldwide and the second leading cause of cancer-related deaths. Despite advancements in BC treatment, challenges like acquired resistance, recurrence, and metastasis persist. BCL6, a transcriptional repressor, acts as an oncogene in BC, being overexpressed in about half of primary tumors of all subtypes and associated with disease progression and poor patient prognosis. This underscores the need to better understand BCL6 role in BC development.

Material and method

This study used RNA interference to explore the impact of BCL6 depletion on the oncogenic progression of MCF-7 cells, a low-tumorigenic estrogen receptor-positive cell line.

Result and discussion

While BCL6 is known to regulate mammary cell proliferation and differentiation, its depletion did not affect MCF-7 cell proliferation or viability but significantly reduced their individual and collective migratory properties. RNA microarray analysis identified a set of genes upregulated following BCL6 depletion, including S100A7, previously reported to inhibit MCF-7 cell migration and invasion by reducing MMP9 secretion. However, our findings showed that S100A7 downregulation alone did not affect MCF-7 migration. Moreover, simultaneous depletion of BCL6 and S100A7 failed to restore MCF-7 cell migratory behavior.

Conclusion

Our results suggest that increased expression of BCL6 is linked to increased cell migration but independent on S100A7 upregulation. Further studies are required to clarify the role of BCL6 in BC, including disease progression.

EACR25-2251

Exploiting senolytic drugs to strike brain tumors metabolic vulnerabilities

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Introduction

Brain tumors remain one of the most challenging issues in pediatric oncology, since they represent the primary cause of cancer-related death in childhood. Despite extensive efforts to discover new treatments, the standard-of-care has not radically changed over the past decades, including highly toxic and non-specific chemotherapy, and resulting in treatment failure and high risk of recurrence. Most of drug screening campaigns described so far were not able to translate identified drugs into clinic, due to unrealistic culturing conditions using monolayer cell cultures with overestimated nutrients and oxygen tension. On the contrary, it has been widely described that the in vivo tumor microenvironment is characterized by low oxygen tension, glucose and growth factors deprivation, lactate accumulation and consequent acidosis. The identification of novel therapeutic

vulnerabilities for pediatric brain tumors thus necessitates the use of more accurate and realistic preclinical models.

Material and method

Patient-Derived Brain Tumor (PD-BT) cell cultures have been cultured in two opposite conditions: standard PD-BTs (st-PD-BTs) were cultured as monolayers, enriched in nutrients and growth factors, while exhausted PD-BT (ex-PD-BTs) were chronically adapted to hypoxic, acidic, exhausted medium conditions and grown as neuro-spheroids. St-PD-BTs and ex-PD-BTs were characterized from both transcriptomic and metabolomic point of view and then tested against a library of more than 3500 compounds using a High-Throughput Screening (HTS) approach.

Result and discussion

Here we present a simplified in vitro 3D model able to recapitulate a more representative tool for brain tumors drug screening. Multiomic characterization revealed that ex-PD-BT exhibited a more aggressive phenotype, sustained by purine metabolism rewiring, and altered DNA damage repair along with senescent features. Exposing both st-PD-BTs and ex-PD-BTs to High-Throughput Screening, we demonstrated that many identified hits in standard setting resulted unsuccessful in hostile tumor-like conditions. Conversely, we identified the class of senolytics as highly selective drugs for ex-PD-BTs, nonetheless strongly synergizing with conventional chemotherapy in st-PD-BTs, suggesting a possible role as adjuvant therapy in first-line chemotherapy.

Conclusion

Collectively, the proposed in vitro model, closely resembling the in vivo brain tumor microenvironment, allowed the identification of underestimated druggable vulnerabilities, suggesting senolytics as new therapeutic option for pediatric brain tumors.

EACR25-2267

Therapeutic overactivation of oncogenic signaling to improve outcomes in Ewing sarcoma

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Introduction

Ewing sarcoma (EwS) is a pediatric cancer of the soft tissue and bone with a 5-year survival rate of 15% for metastatic disease. The standard of care for EwS is multimodal therapy, which carries a risk for long-term disabilities, including lower fertility and severe cardiac and pulmonary complications. Therefore, there is an unmet clinical need for therapies to increase overall survival without long-term disabilities. The cytostatic and cytotoxic consequences of Fibroblast growth factor 2 (FGF2) in EwS have been previously described, and we have shown that sustained overactivation of the MAPK pathway underlies this toxicity. The toxicity of MAPK

overactivation by FGF2 in EwS conceptually aligns with our published proof-of-concept paper demonstrating the efficacy of “paradoxical activation”, now known as Therapeutic OVERactivation of oncogenic signaling (TOVER), in colorectal cancer. We hypothesize that FGF2-induced overactivation of MAPK signaling is a vulnerability of EwS and that combination of overactivation and stress-targeted inhibition is a viable therapeutic strategy.

Material and method

We previously designed a stress-focused drug library of 164 compounds targeting stress response pathways associated with malignant phenotypes (DNA damage, metabolic, mitotic, oxidative, and proteotoxic stresses and senolytics). These drugs were screened in 4 EwS cell lines (SKES-1, A673, MHES-1/TR/shEF, TC-71/TR/shEF) at 15 concentrations in the presence and absence of a sub-lethal dose of FGF2 to identify the compounds to which there was increased sensitivity with FGF2 treatment. We also utilized EwS cell lines with a doxycycline-inducible shRNA construct targeting the oncogenic driver, EWS-FLI fusion protein, to determine whether toxicity of potential combinations can be attenuated by depletion of EWS-FLI.

Result and discussion

Consistent with the literature, we show that FGF2 toxicity in EwS is driven by overactivation of MAPK signaling, which is attenuated by MEK inhibition and EWS-FLI knockdown. Moreover, in a stress-focused drug screen, we identified compounds exploiting proteotoxic and oxidative stress-related vulnerabilities of EwS models under MAPK overactivation. These findings were validated in a panel of EwS cell lines with varying intrinsic sensitivities to FGF2 in both short- and long-term viability assays. Ongoing experiments aim to dissect the mechanistic network underlying the toxicity of FGFR/MAPK agonism in the presence and absence of inhibition to stress-response in EwS. Furthermore, we are evaluating alternative activators of MAPK in combination with the aforementioned stress targets.

Conclusion

These data show that EwS is a promising tumor type for TOVER as a treatment strategy as evaluated in a panel of EwS cell lines. With FGF2 as our activator, we identify multiple potential combinations with targets of stress-response pathways, forming a basis for further preclinical investigation.

EACR25-2287

The Androgen Receptor Mediates Immune Evasion and Progression of Melanoma

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Introduction

Melanoma is the most aggressive skin cancer due to its high metastatic potential. Despite the beneficial effects of targeted therapies, drug-resistance frequently arises, leading to melanoma progression and metastatic disease. Recent evidence links the androgen receptor (AR) expression with poor prognosis and metastatic potential of melanoma, and AR blockade even enhances the response to BRAF/MEK-targeted therapy in preclinical melanoma models. However, the molecular mechanisms by which AR promotes melanoma progression appears still debated. The present data aim to fill this gap and dissect the role of AR in melanoma invasion and immune escape.

Material and method

A multi-approach strategy was employed. AR expression was assessed by Western blot, while the impact of androgens was evaluated by migration, invasion and spheroid growth assays. Co-immunoprecipitation approach was used to analyze AR-ADAM10 interaction and expression of the NKG2D ligand, MICA/B, was evaluated by western blot and immunofluorescence. Soluble MICA/B (sMICA/B) levels from cell culture supernatants and patient sera were quantified by ELISA. The impact of AR in immune evasion was assessed by measuring cytotoxicity in co-cultures of melanoma cells with primary human NK cells. AR blockade was achieved using bicalutamide, while GI254023X was used to inhibit the ADAM10 activity. Cytotoxicity assays and live-dead staining were lastly done in spheroid co-cultures with NK cells to assess the involvement of AR and ADAM10 in melanoma immune escape.

Result and discussion

Our results show that ligand-bound AR enhances the melanoma invasion, the growth of melanoma-derived spheroids as well as the immune-escape of melanoma cells from NK cell attack. Biochemical approaches indicated that androgens promote the assembly of AR/ADAM10/β1-integrin complex, which then activates ADAM10 and triggers MICA shedding. The latter event lowers MICA/B surface expression and increases sMICA levels, thus impairing NK cell function through NKG2D downregulation. Pharmacological blockade of AR or ADAM10 counteracts these effects and rescues the immune recognition of melanoma cells. Taken together, these findings support the role of AR in diagnostic guidance and therapeutic approach of melanoma.

Conclusion

The present study underscores the therapeutic relevance of AR blockade in melanoma outcome. The finding that high serum levels of sMICA/B correlate with a poor prognosis in melanoma patient's further highlights the clinical relevance of AR-mediated immune escape. Combining a more tailored AR blockade with immunotherapy would enhance immune-response and improve melanoma patient's survival. Future investigations are envisaged to optimize this strategy and validate our findings in clinical setting.

EACR25-2289

Inflammatory secretome from triple negative breast cancer cells drives EMT and enhances migration in non-cancerous breast cells

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Introduction

Triple-negative breast cancer (TNBC) is an aggressive subtype of breast cancer that presents significant treatment and management challenges due to its poor response to standard therapies. Research into the tumor secretome, the set of proteins secreted by cancer cells, is providing key insights into the molecular mechanisms underlying tumor progression. These include the epithelial-mesenchymal transition (EMT), a process associated with invasion and metastasis. In this context, the aim of this study was to investigate the role of secretome-derived proteins from TNBC MDA-MB-231 cells in response to an inflammatory condition, and their impact on the inflammatory response and invasion of non-cancerous human breast epithelial cells (MCF10A).

Material and method

The TNBC cell line MDA-MB-231 was exposed to an inflammatory treatment combination of estradiol (E2, 10 nM), TNFα (10 ng/ml) and IL-6 (50 ng/ml), termed ETI. Conditioned medium from these treated cells was collected and evaluated in MCF10A cells, focusing on key inflammatory signaling pathways identified by previous proteomic analysis of the secretome. Gene expression levels of EMT (CDH1, CDH2, MMP9) and inflammation-related genes (CXCL8, IL6, IL6R) were analyzed by qPCR. Activation of STAT3, a key regulator of inflammation and cancer progression, was assessed by Western blot. In addition, the effect of the conditioned medium on MCF10A cell migration was evaluated using the wound healing assay.

Result and discussion

Gene expression analysis revealed a significant upregulation of EMT markers (CDH2, MMP9) and pro-inflammatory genes (CXCL8, IL6, IL6R) in MCF10A cells treated with the conditioned medium of ETI-exposed MDA-MB-231 cells. Interestingly, STAT3 was highly activated in these cells, further supporting the involvement of inflammatory pathways. In addition, MCF10A cells treated with the conditioned medium showed increased migratory capacity in the wound healing assay, suggesting that the secretome of ETI-treated breast cancer cells promotes an EMT-like phenotype and enhances cell migration.

Conclusion

These findings highlight the key role of the inflammatory secretome in promoting EMT and cell migration in non-cancerous breast epithelial cells. Therefore, focusing on understanding the involvement of cellular signaling processes may be critical for identifying novel prognostic biomarkers and potential therapeutic targets for TNBC, which may lead to improved treatment strategies to mitigate the aggressive behavior of this disease.

EACR25-2297

Synergistic cell cycle blockade with the combination of CDK4/6 PROTACs and a CDK2 inhibitor as a potential anticancer strategy

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Introduction:

Combination therapies represent a highly effective strategy to cancer treatment. This approach offers the possibility of inhibiting different molecular targets, increasing efficacy, and minimizing the risk of treatment resistance. Proteolysis-targeting chimeras (PROTACs) are chimeric compounds that bind to target proteins and lead to their selective proteasomal degradation. Monotherapies based on PROTACs are currently in clinical trials with promising results, however, their effectiveness in combination therapies has not yet been described. Our study focused on PROTACs targeting cyclin-dependent kinases 4 and 6 (CDK4/6), which are based on FDA-approved CDK4/6 inhibitors for breast cancer treatment.

Material and method

We incubated various mouse and human cancer cell lines (CT26.WT, B16-F10.WT, MC38-hPD-L1, and RKO.WT) with increasing concentrations of CDK4/6 PROTAC, cyclin-dependent kinase 2 (CDK2) inhibitor, and their combination. We determined the protein levels (such as phosphorylated retinoblastoma and CDK4/6) by western blot method, while BrdU-based cell cycle analysis was conducted using flow cytometry. Further, we evaluated the effects of these therapeutic strategies on the proliferation of human peripheral blood mononuclear cells (PBMCs). For this purpose, we isolated PBMCs from whole blood, activated them for proliferation, stained with dilution dye and incubated with increasing concentrations of inhibitors. The proliferation inhibition was likewise observed by flow cytometry. The results

were analyzed in GraphPad Prism 8.0 software, and statistical significance was calculated using t test.

Result and discussion

We investigated the effect of combining CDK4/6 PROTACs with a cell cycle inhibitor targeting CDK2 on cancer cells. Our results demonstrated that this combination synergistically promotes cell cycle arrest in the G1/G0 phase in both mouse and human cancer cell lines. Furthermore, western blot hybridization revealed a dose-dependent reduction of the level of CDK4/6 proteins after treatment of cells with CDK4/6 PROTAC, as well as changes in the level of phosphorylated retinoblastoma protein (Rb) for cells treated with individual compounds and their combination. Additionally, treatment with either CDK4/6 PROTAC or CKD2 inhibitor alone showed partial inhibition of the proliferation of PBMCs, while their combination led to an almost complete arrest of those cells proliferation.

Conclusion

Our findings highlight the potential of CDK4/6 PROTACs and the CDK2 inhibitor in combination therapies to enhance therapeutic strategies in oncology while excluding the application of this dual cell cycle blockade in synergy with immunotherapy.

EACR25-2301

Induction of ferroptosis and senescence in epithelial ovarian cancer cell line: Implications of targeted therapeutic strategies

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Introduction

Senescence is a critical cellular process in both aging and cancer, marked by irreversible growth arrest, DNA damage, and the secretion of pro-inflammatory cytokines, collectively known as the senescence-associated secretory phenotype (SASP). Senescent cells play tumor-suppressive roles but can also alter the tumor microenvironment, promoting cellular proliferation. Ferroptosis, a form of iron-dependent cell death driven by lipid peroxidation, has emerged as a promising therapeutic strategy against chemotherapy-resistant cancer cells. This study investigates the effects of senescence and ferroptosis inducers on two distinct epithelial ovarian cancer (EOC) cell lines, SKOV-3 and OVCAR-3, using qPCR to assess the expression of various biomarkers.

Material and method

SKOV-3 and OVCAR-3 EOC cell lines were treated with ferroptosis inducers (RSL3, Erastin, FSP1i, Brequinar) and senescence inducers (Etoposide, Olaparib, Carboplatin, Cisplatin). Cells were treated with varying

drug concentrations (0.25–2 µM) and harvested for RNA extraction at Day 7. Gene expression of biomarkers associated with ferroptosis (NCOA4, CP) and senescence (p16 (CDKN2A), p21 (CDKN1A), IL6, IL8) was assessed by quantitative PCR. Cell viability was measured by crystal violet staining.

Result and discussion

In response to RSL3 (1 µM) and FSP1i (2 µM), both SKOV-3 and OVCAR-3 cells showed ferroptosis induction. After treatment with RSL3, SKOV-3 showed an increase in CDKN1A and a decrease in CDKN2A, while no significant modulation was observed in other genes. In contrast, in OVCAR-3, there was an increase in CDKN1A and IL8, along with a decrease in NCOA4 and CP. Notably, these ferroptosis-related biomarkers were modulated independently of the cell line, indicating a common response to RSL3 treatment across both SKOV-3 and OVCAR-3. For SKOV-3, treatment with Etoposide (1 µM) led to a reduction in CDKN1A, CDKN2A, and IL8, with a further decrease in IL8 at 2 µM, suggesting that IL8 downregulation could serve as a genetic marker of senescence in SKOV-3. In OVCAR-3, Etoposide (1 µM) increased CDKN1A, CDKN2A, IL8, and NCOA4, and at 2 µM, CDKN1A and CDKN2A were further elevated. This suggests that p16 and p21 could be markers of senescence induction in OVCAR-3 cells.

Conclusion

This study highlights the potential of targeting both senescence and ferroptosis in epithelial ovarian cancer. Ferroptosis-related biomarkers, such as NCOA4 and CP, were modulated independently of cell line, indicating a common response to ferroptosis induction. Senescence was more cell line-dependent, with CDKN1A and CDKN2A serving as markers of senescence induction in OVCAR-3. These findings provide valuable insights into the molecular pathways governing ferroptosis and senescence in ovarian cancer, suggesting that combining therapies that target both processes could be a promising strategy to overcome chemotherapy resistance and improve treatment outcomes in EOC.

EACR25-2310

Screening for senolytics by live monitoring the cell fate of senescent cancer cells

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Introduction

Cellular senescence is a highly regulated cell-cycle arrest, important for embryonic development and aging, that can be induced prematurely for instance by DNA damage. Therapy-induced senescence (TIS) is a promising anti-cancer strategy, especially when combined with senolytic compounds which clear senescent cells by targeting anti-apoptotic factors expressed by these cells. We developed a dual senescence and apoptosis reporter system to live monitor the cell fate of senescent cells, allowing to screen for novel senolytic agents.

Material and method

To monitor senescence, we made a reporter construct based on transgenic lamin A and B1 expression, and to monitor apoptosis we used a FRET reporter. The reporter constructs were validated in cancer cells by inducing senescence with irradiation and apoptosis with staurosporine. To identify potential senolytic compounds, cells were first irradiated to induce senescence followed by treatment with a panel of drug candidates. To follow cell fate, time-lapse imaging was performed by confocal or high-content imaging. Senescent and non-senescent cells were identified based on the mean intensities of transgenic lamins. The loss of FRET signal in the nuclei and the translocation of mNeogreen into the cytoplasm were used to identify apoptotic cells.

Result and discussion

Our dual reporter system allowed imaging of four different fluorescent proteins in cells without cross-talk. The FRET efficiency of the uncleaved apoptosis reporter was around 40%. Senescent cancer cells could be distinguished from non-senescent cells by their increased transgenic lamin intensities. The apoptosis cleavage site was effectively and specifically cleaved after induction of apoptosis, resulting in decreased FRET signal and translocation of mNeogreen from the nucleus to the cytoplasm. The signal of the senescence reporter was not influenced by apoptosis and vice versa. The efficiency of senolytics could be measured by monitoring senescent cells that went into apoptosis. Our dual reporter system is both compatible with confocal imaging and the Opera Phenix high content screening system.

Conclusion

We show that our dual reporter system can be successfully applied for live monitoring of senescence and apoptosis cell fates and for live screening of the efficacy of senolytics in cancer treatment.

EACR25-2333

Investigating the relationship between IGF signalling, androgens and amyloid precursor protein (APP) processing in prostate cancer

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Introduction

Both prostate cancer and Alzheimer's disease (AD) are major causes of death globally and despite having similar risk factors, epidemiological studies have shown an intriguing inverse relationship between the two. A key hallmark of AD is amyloid β (A β), a peptide produced by the cleavage of amyloid precursor protein (APP) by β -secretase (BACE1). APP may also be cleaved by α -secretases, including ADAM-10 and ADAM-17; this cleavage pathway is protective in an AD context as it precludes A β production, but has been shown to promote cell proliferation and migration in some cancers, including prostate. Effects of androgens and insulin-like growth factor-I (IGF-I) on APP processing have previously been investigated in AD, but in prostate cancer are currently unknown.

Material and method

In prostate epithelial cells, APP expression was silenced using siRNA in the presence or absence of IGF-I or dihydrotestosterone (DHT). The impact of inhibiting the IGF1R and androgen receptor (AR) on APP and its processing was investigated using AG1024, a receptor tyrosine kinase inhibitor, and enzalutamide, an anti-androgen, respectively. Effects on cell viability were assessed using a Muse Cell Analyser and the impact on components of the APP processing pathways, the IGFIR and AR were assessed by western blot.

Result and discussion

Silencing APP expression did not induce cell death in normal prostate epithelial cells, but cell viability was significantly reduced in both androgen sensitive LNCaP and androgen insensitive PC3 cancer cells. APP silencing reduced the abundance of AR in LNCaP cells and the IGFIR in PC3 cells, consistent with the inability of exogenously added IGF-I or androgen to rescue cells from apoptosis induced by APP silencing. In PC3 cells, IGF-I caused an increase in abundance of APP, α - and β -secretases and soluble APP- α and APP- β cleavage products. AG1024 resulted in increased cell death and reduced abundance of APP, α -secretases and soluble APP- α and APP- β . Similarly, in LNCaP cells, DHT caused a significant increase in levels of APP, α -secretases and soluble APP α , whilst enzalutamide resulted in reduced APP, α -secretases and soluble APP α .

Conclusion

Androgens are the key driver of development and progression of androgen sensitive prostate cancer, whilst IGF-I signalling has been shown to contribute to development and progression, metastasis, castration resistance and more aggressive disease. Previous studies in the brain have shown that both IGF-I and androgen regulate APP and promote survival, and our results indicate similar regulation of APP and associated growth in prostate cancer cells. Prostate cancer still has significant unmet therapeutic limitations and a better understanding of the relationship between APP and both AR and IGF signalling in prostate cancer biology may provide new approaches to optimising current treatments.

EACR25-2336

LZTR1 acts as a potent tumor suppressor gene in liver cancer by safeguarding aberrant MAPK activity via posttranslational control of RAS GTPases

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Introduction

Hepatocellular carcinoma (HCC) ranks among the cancers with the highest rate of mortality, yet treating this carcinoma remains challenging due to late diagnosis and poor patient stratification, thereby preventing the utilization of targeted therapeutical approaches. Using human genome sequencing data, we identified frequent deleterious alterations of Leucine-zipper-like

transcriptional regulator 1 (LZTR1), which plays a crucial role in regulation of RAS-like GTPases (e.g. RIT1) and downstream pathways, such as the mitogen activated protein kinase (MAPK) pathway. Elucidating the oncogenic driver potential of LZTR1 loss of function could subsequently warrant the usage of MAPK inhibitors, which are currently utilized in other cancers with aberrant MAPK activity.

Material and method

In vivo approach: Hydrodynamic tail-vein injection (HTVI) allows us to achieve liver-specific knockouts/overexpressions by inducing *in vivo* transfection of hepatocytes. This system allows stable expression of transgenes via Sleeping-Beauty transposons as well as editing genomes of cells via delivery of CRISPR/Cas9 constructs. Additional perturbations are introduced utilizing CRISPR/Cas9 or Cas12a(Cpf1) system, as well as knockdowns by short hairpin RNAs delivered via lentiviral vectors. **In vitro approach and read-outs:** Cell viability and/or proliferation is the primary cytometric read-out, measured in settings most appropriate for investigation: Colony formation assay (CFA) for low-seed, long-term cultivation experiments; multicolor competition assay (MCA) for long-term co-cultivation measured by flow-cytometry (FwC) and CellTiter-Blue®(CTB) assay for short cultivation experiments determining cell viability. Immunoblotting of phosphorylated ERK was set as the primary read-out of MAPK activation. Western blotting (WB) and quantitative polymerase chain reaction (qPCR) were used to estimate levels of protein or gene expression, respectively.

Result and discussion

We reveal that loss of function of LZTR1 promotes tumorigenesis *in vivo* as well as cell growth *in vitro*, an effect accompanied by elevated RIT1 expression and subsequent MAPK pathway activation. Moreover, truncated forms of LZTR1 lacking domains crucial for its interaction with RAS molecules phenocopied the effect of LZTR1 loss, further suggesting that this interaction is crucial for tumor suppression. Finally, expression of mutant RIT1 proteins rendering RIT1 non-degradable by LZTR1 in murine livers resulted in liver tumorigenesis comparable to LZTR1 loss.

Conclusion

Our findings suggest that LZTR1 safeguards MAPK signaling by controlling RAS GTPases in the liver and could therefore potentially be utilized to stratify HCC patients for usage of small molecule inhibitors targeting MAPKs, which are currently only employed in other carcinomas.

EACR25-2345

Targeting endometrial cancer stem cells with an ALDH-mediated photodynamic therapy

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Introduction

Endometrial cancer (EC) remains a clinical challenge, where cancer stem cells (CSC) have been associated with chemoresistance and recurrence. In particular, a relevant expression of aldehyde dehydrogenase (ALDH) on EC has been involved in disease progression and is associated with stemness pathways, proposing its double potential as a biomarker and a molecular target. To investigate more effective and minimally invasive treatments for EC, our research focuses on a novel approach using CSC-targeted photodynamic therapy (PDT) based on modified 4,5,6,7-tetrahydropyrazolo[1,5-a]pyridine-fused chlorins (PX).

Material and method

A refined sphere-forming protocol was performed to obtain endometrial CSC from ECC-1, RL95-2 and HEC-1-A cell lines. To evaluate the effectiveness of a CSC-targeted PDT strategy in EC, the colocalisation of a novel class of tetrahydropyrazolo[1,5-a]pyridine-fused chlorins functionalised with aldehyde moieties (A-PX) with ALDH was verified using confocal microscopy. Then, endometrial CSC were incubated with a range of A-PX concentrations (2.5 – 10 µM) for 2 hours, followed by a light irradiation (7.5mW/cm², 10J). To assess the expression of CSC markers, the effect of CSC-targeted

PDT on CD44 and CD24 was examined through flow cytometry. Viability and cell death were determined through luminescence and confocal microscopy.

Result and discussion

After evidence that ALDH interacts with A-PX in vitro and converts aldehydes into carboxylic acids and A-PX can internalise and accumulate in endometrial CSC, preliminary confocal studies pointed out that A-PX localise in CSC ALDH-enriched zones, facilitating the expected action mediated by ALDH. Moreover, initial findings revealed a high percentage of CD44+/CD24+ cells, confirming CSC-like characteristics. CSC-targeted PDT seems not to modulate the stemness features of the ECC-1 CSC-enriched populations. Nevertheless, A-PX-based PDT notably contributed to eliminating endometrial CSC, resulting in a significant reduction in viability and the induction of cell death.

Conclusion

In conclusion, these results emphasise the potential of the A-PX-mediated strategy to target ALDH in vitro and within CSC, as well as the effectiveness of A-PX-based PDT for eradicating EC cells. This research represents the first steps towards developing a conservative and minimally invasive therapeutic option for challenging cases of EC.

FCT supports CIBB (10.54499/UIDB/04539/2020; 10.54499/UIDP/04539/2020; 10.54499/LA/P/0058/2020) CQC (10.54499/UIDB/00313/2020; 10.54499/UIDP/00313/2020; TEMA (10.54499/UIDB/00481/2020), UIDP/00481/2020 (10.54499/UIDP/00481/2020); Projects CarboNCT (10.54499/2022.03596.PTDC) and Chem4LungCare (10.54499/PTDC/QUI-QOR/0103/2021); PhD Scholarship from FCT and European Social Fund to BS (10.54499/2020.07672.BD), BC (10.54499/2022.12013.BD) and RT (SFRH/BD/116794/2016).

EACR25-2351

MBNL1 as regulator of doxorubicin resistance in triple negative breast cancer

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Introduction

Triple-negative breast cancer (TNBC) accounts for ~15% of breast cancer cases. Due to the lack of targeted therapies, chemotherapy remains the main strategy. However, drug resistance limits its efficacy. This study explores the role of MBNL1 in doxorubicin resistance in TNBC.

Material and method

To model chemo-resistance, we used MDA-MB-231 cells with acquired resistance to doxorubicin (MDA-MB-231R) and its parental counterpart MDA-MB-231. Cytotoxicity was assessed by WST-1, flow cytometry, and western blot (c-CASP3, c-PARP). Drug accumulation was tested by flow cytometry and fluorescence microscopy. In vitro gain/loss of function studies were performed via transient transfection of plasmids or siRNAs, respectively. For in vivo experiments, MDA-MB-231 cells with stable MBNL1 KD/OE were orthotopically injected into BALB/C nude mice ($n = 8/\text{group}$) and treated with vehicle or doxorubicin (1.6 mg/kg, twice weekly, 4 weeks). An in-house cohort of 29 TNBC primary tumors treated with anthracycline + taxane neoadjuvant regimens were analyzed for MBNL1 and BIN1 gene expression by qRT-PCR. Results were validated in silico (GSE164458, $N = 407$). Ethical approvals were obtained. Statistical analyses were conducted by GraphPad Prism.

Result and discussion

MBNL1 expression in treatment-naïve tumors positively correlates with response to chemotherapy in our cohort ($p < 0.01$) and in public patients' data. MBNL1 was down-regulated in MDA-MB-231R. In vitro MBNL1 KD increased doxorubicin resistance ($\text{IC}_{50} (\mu\text{M})$: Scr = 0.07, siMBNL1-A = 0.20, siMBNL1-B = 0.27; $p < 0.001$) while OE restored sensitivity ($\text{IC}_{50} (\mu\text{M})$: pBABE = 0.45, MBNL1 = 0.22; $p = 0.001$). This result was confirmed in mouse models. MBNL1 KD tumors treated with doxorubicin showed a final volume of 184.6 and 532.8 mm³ for shMBNL1-A and B, respectively, compared to 37.7 mm³ in doxorubicin-treated control tumors ($p < 0.01$). MBNL1 OE tumors showed enhanced response to doxorubicin as shown by a final volume of 57.82 mm³ compared to 170.5 mm³ in treated control tumors ($p < 0.01$). Moreover, 50% of MBNL1 OE tumors achieved complete regression. Mechanistically, MBNL1 KD upregulates ABCC1 and ABCG2, reducing intracellular doxorubicin accumulation (~20%, $p < 0.01$), whereas MBNL1 OE increases drug retention (~20%, $p < 0.01$). Besides, MBNL1 directly inhibits BIN1, leading to increased doxorubicin resistance through downregulation of c-MYC and PARP1, ($\text{IC}_{50} (\mu\text{M})$: Scr = 0.15, siBIN1-A = 0.35, siBIN1-B = 0.31; $p < 0.001$). BIN1 OE restored doxorubicin sensitivity ($\text{IC}_{50} (\mu\text{M})$: pBABE = 0.58, MBNL1 = 0.35; $p < 0.001$). Furthermore, BIN1 expression was also validated as a predictive biomarker for doxorubicin response in the above-mentioned patient cohorts.

Conclusion

Loss of MBNL1 drives doxorubicin resistance in TNBC through regulation of ABC transporters and Bin1/Myc/ PARP1 axis. In vivo studies and clinical data analysis highlight its potential as a predictive biomarker of chemotherapy response and therapeutic target.

EACR25-2359

Translational control of BRAF and NRAS oncogene-driven MAPK signaling in malignant melanoma

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Introduction

Oncogenic BRAF and NRAS mutations, which activate the RAS/RAF/MEK/ERK mitogen-activated protein kinase (MAPK) pathway, are key drivers of malignant melanoma. However, due to the strong activating potential of the mutations, excessive extracellular signal-regulated kinase (ERK) MAPK signaling must be tightly regulated by negative feedback mechanisms to allow for optimal tumor growth. The upstream regulators that orchestrate ERK signaling negative feedback control and the extent of the MAPK pathway's spare signaling capacity remain largely unknown. The eukaryotic translation initiation factor eIF4F is a promising therapeutic target in various cancers. In melanoma, eIF4F has been implicated in the development of resistance to BRAF and MEK kinase inhibitors. However, its role in controlling ERK activity in melanoma remains to be fully elucidated.

Material and method

To investigate the potential role of eIF4F in modulating ERK activity in melanoma, we employed western blotting and luciferase reporter assays in human melanoma cell lines harboring BRAF and NRAS mutations. Additionally, we used eIF4F inhibitors and RNAi-mediated knockdown of eIF4F subunits to dissect its functional role.

Result and discussion

Our data reveal that eIF4F-mediated translation plays a crucial role in restraining ERK MAPK pathway activity in melanoma via the dual-specificity phosphatase DUSP6. This phosphatase, characterized by its short half-life in melanoma cells, requires continuous production in an eIF4F-dependent manner to prevent excessive ERK activation driven by oncogenic RAF/RAS mutations. Moreover, we demonstrate that small-molecule compounds disrupting the eIF4F-DUSP6 axis can induce potent hyperactivation of the ERK pathway and over-expression of its downstream targets FOS and EGR1. Our results further suggest that, under steady-state conditions, only a small fraction of the total ERK signaling capacity is utilized in BRAF- and NRAS-mutant melanoma cells, as eIF4F-dependent feedback actively keeps the majority of ERK molecules in an inactive state.

Conclusion

This study uncovers a critical interplay between the eIF4F translation initiation complex, which contributes to melanoma resistance, and the ERK signaling pathway, the primary therapeutic target in melanomas bearing BRAF and NRAS mutations. Targeting eIF4F-dependent regulation of ERK activity may offer new therapeutic opportunities to overcome resistance to MAPK pathway inhibitors.

Acknowledgments: This work was supported by the Czech Science Foundation (GA22-30397S, GA25-17766S), the European Union – Next Generation EU – the project National Institute for Cancer Research (Programme EXCELES, Project No. LX22NPO5102), and Masaryk University (MUNI/A/1760/2024).

EACR25-2366**Metabolic Rewiring in Chemoresistant Pancreatic Cancer Redox Metabolism and Lipid Alterations**

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Introduction

Combination therapies, such as chemo- and radiotherapy, are the first choice in the treatment of pancreatic ductal adenocarcinoma. Induction of chemoresistance can induce different metabolic alterations acting at several levels from membrane composition alteration to detoxification efficacy of drugs and reactive oxygen species (ROS), adversely impacting on the efficacy of therapeutic options based on ROS production such as radiotherapy. Understanding common and cell-specific mechanisms linked to chemoresistance acquisition could enable the development of improved therapeutic strategies based on co-administration of adjuvant agents.

Material and method

Two pancreatic cancer cell lines (PANC-1 and MIA PaCa-2) were made resistant to a generic drug mixture (Folfirinox©) by gradually increasing drug concentration during cell culture. Mitochondrial and cytosolic ROS levels were assessed using MitoSOXTM and dichlorodihydrofluorescein diacetate (DCFH-DA). Glutathione reductase (GSR), glutathione S-transferase (GST), and catalase activities were assessed spectrophotometrically. Cell viability was measured by protein content (BCA) and resazurin assay. Membrane order alterations were visualized by confocal microscopy using Nile Red dye, and activation of cell damage and stemness markers (p38, p53) by immunofluorescence. Changes in lipid profile were determined by lipidomic analysis.

Result and discussion

Both cell lines show increased resistance to the drug, with no sign of cell damage and morphology alteration. GSR activity increases only in MIA PaCa-2 cells, whereas catalase activity decreases in both cell lines. GST activity significantly increases in both cell lines. Cytosolic and mitochondrial ROS decrease in PANC-1 and increase in MIA PaCa-2 cells. Ratio between resazurin- and BCA-derived viability curves evidences a significant increase in reductase activity of resistant MIA PaCa-2 cells. Membrane order analysis evidences alteration correlated with chemoresistance acquisition, with increased fluidity in PANC-1 resistant cells.

Lipidomic analysis evidences alteration in both cell lines that encompasses both unsaturation degree and average length of lipid chains. These findings suggest that increased resistance of both cell lines is related to a rewiring of redox homeostasis, although exploiting not superimposable strategies.

Conclusion

Induction of chemoresistance in different cell lines highlights a variegated scenario with common traits and cell-specific features that affect both redox homeostasis and lipid composition. These findings underscore the

heterogeneity of chemoresistance mechanisms and provide new insights into potential vulnerabilities that could be targeted with adjuvant strategies to overcome resistance and improve treatment outcomes.

EACR25-2387**Role of MRPL15 in mitochondrial dynamics, cell cycle, and EMT regulation in different breast cancer subtypes.****Implications for tumor progression**

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Introduction

Mitochondrial ribosomal proteins (MRPs) are crucial for mitochondrial function and have been linked to cancer progression. MRPL15, a component of the large mitochondrial ribosomal subunit, has been associated with tumor growth and metastasis, but its role in breast cancer remains unclear. This study aims to evaluate the effects of MRPL15 silencing on migration, mitochondrial dynamics, and epithelial-to-mesenchymal transition (EMT) markers in different molecular subtypes of breast cancer cell lines.

Material and method

MCF7 (luminal A), BT-474 (luminal B), and MDA-MB-231 (triple-negative) breast cancer cell lines, were transfected with small interfering RNAs targeting MRPL15. Cell migration was evaluated through wound-healing assays. Cell cycle was evaluated by flow cytometry. Quantitative PCR (qPCR) was performed to analyze the expression of mitochondrial markers (COXI and COXIV), mitochondrial dynamics (MFN1, MFN2, OPA1, OMA1, DRP1, FIS1) and EMT-related genes (VIM, CDH1, CDH2). Moreover, an *in silico* proliferative analysis was performed using the cBioPortal tool.

Result and discussion

MRPL15 silencing led to a slight reduction in mitochondrial markers, and induced cell cycle modifications characterized by an increase in subG0/G1 and G0/G1 phases and a decrease in G2/M phase, suggesting a shift towards lower proliferation, except in MDA-MB-231 cells. These findings were consistent with *in silico* analysis of a cohort of ER+PR+HER2- breast cancer patients, which revealed more proliferative tumors associated with high expression of both MRPL15 and CDH2. Additionally, MRPL15 silencing significantly reduced migration in all breast cancer cell lines, with the strongest effect observed in MDA-MB-231 cells. EMT marker analysis revealed a notable decrease in vimentin (VIM) and N-Cadherin (CDH2) expression, suggesting a switch to a more epithelial phenotype. Moreover, qPCR analysis of mitochondrial dynamics related genes showed

an increase in mitochondrial fusion in MDA-MB-231 cells and a decrease in MCF7 cells, highlighting a subtype-dependent response to MRPL15 silencing in breast cancer cells. These findings indicate that mitochondrial dynamics may be linked to both cell cycle modifications and EMT regulation in breast cancer cells.

Conclusion

Our findings suggest that MRPL15 plays a role in cell cycle, mitochondrial dynamics, and EMT in breast cancer cells lines, with its inhibition leading to reduced tumor aggressiveness, modulating proliferative features or driving a shift towards an epithelial-like phenotype. Targeting MRPL15 could represent a novel therapeutic approach to limit breast cancer progression.

EACR25-2401

ABCC1 is responsible for invasive phenotype development in colon cancer

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Introduction

Colorectal cancer is one of the leading causes of morbidity and mortality from cancer in the world. Most deaths due to colon cancer are associated with metastatic disease. It is believed that one of the key processes involved in metastasis progression is an epithelial-mesenchymal transition (EMT). Recently, a novel possible role of EMT in cancer drug resistance has been suggested. One of the best-described mechanisms of cancer drug resistance involves reducing drug accumulation mediated by the ATP-binding cassette (ABC) transporter family proteins.

Material and method

We employed colon cancer cells isolated from different stages of tumour development and a panel of cells expressing multidrug resistance proteins. The elongation ratio, cell migration, invasion assay, and MMP-7 secretion were applied to analyse the cell behaviour. Western blot assay examined important epithelial and mesenchymal markers characteristic of EMT at the protein level. The role of ABCC1 in modulating the EMT process was confirmed by its up- and down-regulation, activity inhibition, secretion of leukotriene, and targeting leukotriene-dependent action.

Result and discussion

In this study, we investigated the role of the ABCC1 protein in developing an invasiveness phenotype by EMT-mediated changes in colon cancer cells. We demonstrated that chemotherapeutics inducing the multidrug resistance phenomenon by ABCC1 over-expression lead to the activation of the EMT process and the enhancement of cells' invasion abilities. We also observed that stable transfection with ABCC1 induced EMT and increased cell invasiveness. Furthermore, silencing ABCC1 expression by siRNA resulted in the opposite effect, i.e., EMT reversal and cell invasion capacity inhibition. A similar effect on the inhibition of invasive capacity was observed after the use of ABCC1 inhibitors. We revealed that ABCC1-dependent invasion abilities were due to leukotriene efflux, one of the primary ABCC1 substrates.

Conclusion

Our report provides new insights into the relationship between drug resistance and the development of invasion phenotypes, which may be important in finding optimal strategies for cancer treatment.

EACR25-2416

Additive Effect of Equol on Chemotherapeutics Using 3D Bioprinted Colorectal Cancer Cells

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Introduction

Colorectal cancer (CRC) is the second leading cause of cancer-related deaths worldwide. Because of CRC's increasing incidence, chemotherapy resistance and lack of drug effectiveness, the hypoxic tumor environment demands further research. The tissue microenvironment is oxygen-poor and leads to processes promoting progression of CRC. Therefore, new therapeutic approaches are needed. 3D bioprinted models provide reliable platforms for drug testing including natural products like Equol, which is associated with high anticancer activity. 3D bioprinted tumors are morphologically and biochemically like primary carcinomas and demonstrate more pronounced resistance to standard chemotherapeutics. There are no published reports on the additive effect of Equol on chemo-therapeutics within 3D bioprinted CRC models.

Material and method

This study investigates the efficacy of therapeutic regimens consisting of the standard chemotherapeutic (5-FU), autophagy inhibitor (Chloroquine) and Equol, alone, and in combination, on 2D models and 3D bioprinted HCT-116 CRC cells under normoxic and hypoxic conditions. The HCT-116 CRC cell line was grown as 2D and 3D cultures; each of them in conditions of normoxia and hypoxia. All cells were treated for 72 hours with 5-FU, Chloroquine, Equol and combinations of 5-FU + Equol and Chloroquine + Equol and compared to untreated control cells. The cells were 3D bioprinted and the spheroids were cultured for 10 days. The MTT assay was used to determine IC₅₀ values. Additionally, fluorescence assessment of cell viability was performed.

Result and discussion

HCT-116 cells were treated with 5FU - 5.5 μM, Chloroquine - 40 μM and Equol - 19.28 μg/mL. The cells were also treated with a combination of 5FU + Equol and Equol + Chloroquine under normoxic and hypoxic conditions. A higher inhibitory effect of Equol and 5-FU in hypoxia was detected for both 2D and 3D cultures compared to normoxia. When a combination of 5-FU + Equol and Chloroquine + Equol was applied on hypoxic 3D cultures, the additive effect of Equol on cell proliferation was more pronounced when used in combination. The results of the MTT test were confirmed by fluorescence analysis of cell viability.

Conclusion

In conclusion, our data demonstrate the application of Equol in combination with 5-FU and Chloroquine result in higher cytotoxicity on 3D CRC cell models and gives grounds for subsequent tailoring of anticancer treatment regimens. Therefore, Equol has the potential to be employed as an alternative supplement in the adjuvant therapy of CRC.

Acknowledgments: to the Ministry of Education and Science of the Republic of Bulgaria." Project Д01-165/28.07.2022.

EACR25-2421

Role of the NEDD4 family of E3 ubiquitin ligases in regulating the size of connexin43-based gap junctions in cervical cancer cells

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Introduction

Intercellular communication via gap junctions has important roles in maintaining tissue homeostasis and is frequently lost during cancer development. Such loss may contribute to increased cancer cell growth and affect their response to radio- and chemotherapy. Connexin43 (Cx43) is the most ubiquitously expressed gap junction channel protein in human tissues. Cx43 has a relatively high turnover rate, and by modulating the rate at which Cx43 undergoes endocytosis and degradation, cells can rapidly adjust the level of functional gap junctions in response to intracellular or extracellular cues. Many oncogenes and tumor promoters, such as the protein kinase C activator TPA, induce loss of gap junctions by increasing the Cx43 endocytosis and degradation rate. The NEDD4 family of E3 ubiquitin ligases comprises nine members, several of which have been shown to display oncogenic or tumor suppressor functions. Here, we have investigated the role of the NEDD4 family in controlling the size of Cx43-based gap junctions in cervical cancer cells under basal conditions and in response to TPA treatment.

Material and method

Experiments were performed on HeLa cells or HeLa cells stably transfected with Cx43 as well as c-33a cells, which express Cx43 endogenously. Cells were transfected with plasmids or siRNA for overexpression or knockdown studies, respectively, using cationic lipid-mediated transfection. The subcellular localization of Cx43 was determined by confocal immunofluorescence microscopy. The size of Cx43-based gap junctions in confocal microscopy images was quantified by using NIS-elements AR software. Co-immunoprecipitation was

used to study Cx43 ubiquitination and for protein-protein interaction analyses. Gap junction intercellular communication was measured using the quantitative scrape loading-dye transfer assay. Protein levels were analyzed using SDS-PAGE and western blotting.

Result and discussion

Simultaneous depletion of three members of the NEDD4 family - NEDD4, ITCH, and SMURF2 - by siRNA resulted in significantly reduced Cx43 ubiquitination and Cx43 degradation rate compared with their single depletion. The combined knockdown of these E3 ubiquitin ligases also caused an additive increase in the cellular level of Cx43 and in gap junction size. In addition, the triple knockdown strongly counteracted the TPA-induced degradation of Cx43.

Conclusion

Collectively, these data suggest that NEDD4, ITCH, and SMURF2 act together to mediate the basal and TPA-induced degradation of Cx43 in cervical cancer cells. To our knowledge, this study represents the first evidence that Cx43 ubiquitination and degradation and, consequently, the size of gap junctions, is controlled by the concurrent participation of multiple E3 ubiquitin ligases.

EACR25-2434

Naringenin's Role in Mitophagy and Apoptosis: A Study in Cancerous and Healthy Cells

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Introduction

Mitochondria regulate cellular homeostasis, energy production, and apoptosis through fusion, fission, and mitophagy. Disruptions in these processes are linked to cancer and neurodegenerative diseases, with mitophagy playing a key role in stress responses. Naringenin, a citrus flavonoid, exhibits antioxidant, anti-inflammatory, and anticancer effects. While it influences mitochondrial dynamics, its impact on mitophagy remains unclear. This study examines its role in mitophagy and apoptosis, particularly in the PINK1-Parkin pathway and mitochondrial fission.

Material and method

HepG2 and HDF were maintained under standard culture conditions. The cytotoxic effects of naringenin were assessed using MTT assays to determine IC₅₀ values, while colony formation assays were conducted to evaluate long-term viability and proliferation.

Additionally, a wound healing assay was performed to investigate the effects of naringenin on cell migration. A sterile tip was used to create a scratch in the cell monolayer, and wound closure was monitored over time to assess its influence on motility. Mitochondrial membrane potential changes following naringenin treatment were examined using JC-1 staining. Apoptotic activity was further investigated by analyzing cytochrome c release and Bax expression through Western blotting.

Additionally, DAPI staining was used to observe nuclear integrity and morphological alterations in treated cells. Mitophagy levels were assessed via real-time PCR and Western blotting, focusing on key markers in the PINK1-

Parkin pathway. Mitochondrial fission was evaluated by monitoring DRP1 protein expression.

Result and discussion

Naringenin treatment led to increased mitophagy, with PINK1 and Parkin expression being more pronounced in HepG2 cells compared to HDF cells. This suggests that naringenin promotes mitophagy more effectively in cancerous cells, possibly as a stress response mechanism. Mitochondrial fission, indicated by DRP1 expression, was higher in HepG2 cells, whereas it was less evident in HDF cells. This suggests a differential regulatory effect of naringenin on mitochondrial dynamics depending on cell type. Apoptotic markers showed that cytochrome c release was significantly elevated in HepG2 cells, while Bax was expressed in both cell types. This indicates that naringenin promotes apoptosis in both cancerous and healthy cells but has a stronger effect on cancer cells. These findings highlight naringenin's dual role in mitochondrial quality control and apoptosis regulation.

Conclusion

This study provides new insights into naringenin's effects on mitophagy and apoptosis. Its ability to modulate the PINK1-Parkin pathway and mitochondrial fission suggests a potential role in cancer therapy. While further research is needed, these results indicate that naringenin may serve as a promising compound for targeting mitochondrial dysfunction in cancer.

EACR25-2445

tRNA Optimality Shapes Colorectal Cancer Stem Cell Identity Through Regulation of mRNA Translation

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Introduction

Colorectal cancer stem cells (CSCs) expressing Lgr5 exhibit remarkable plasticity and are key drivers of tumor initiation, metastasis and therapy resistance. Recent studies suggest that Lgr5+ CSCs can establish themselves in a niche-independent manner during metastasis, indicating that these cells possess the intrinsic ability to sustain multipotency and self-renewal. However, the molecular mechanisms governing this plasticity remain poorly understood. Transfer RNAs (tRNAs) are essential for accurate mRNA translation and undergo extensive chemical modifications that regulate protein synthesis. While traditionally seen as passive translation molecules, growing evidence highlights their active role in cancer progression, including tumor initiation, invasion, and therapy resistance. Nonetheless, whether tRNA modifications influence CSC plasticity and survival remains unexplored.

Material and method

A conditional Apc knock-out mouse model was used to induce Wnt-driven transformation specifically in Lgr5+ intestinal stem cells, simulating colorectal cancer. Intestinal crypts were cultured as organoids, and Lgr5+ healthy and cancer stem cells were isolated using an Lgr5-GFP reporter. Multi-omics profiling (RNA sequencing, proteomics and tRNA analysis) was performed to characterize the differences between healthy and cancerous organoids (whole population) and healthy and cancer sorted stem cells. A CRISPR-Cas9 screen targeting genes involved in translation was then conducted on these organoids, and the role of a selected tRNA-modifying enzyme in CSCs was assessed through its depletion and chemical inhibition in organoid cultures, followed by proteome analysis and ribosome profiling.

Result and discussion

Our multi-omics analysis revealed an upregulation of protein synthesis in CSCs, independent of transcription. CSCs also showed enhanced tRNA adaptation, suggesting they rely on an optimized tRNA pool to support the increased translation observed, unlike healthy stem cells. Consistent with this, both tRNA availability and tRNA modifications were able to discriminate CSCs from healthy stem cells. Notably, the CRISPR-Cas9 screen identified tRNA aspartic acid methyltransferase 1 (Trdm1) as an essential tRNA-modifying enzyme for Lgr5+ CSC functionality, while being dispensable in normal stem cells. Further experiments showed that genetic depletion and chemical inhibition of Trdm1 resulted in the failure of CSC-derived intestinal organoid formation, which was linked to a disruption in the translation of key regulators of Lgr5+ CSC identity. This suggests that Trdm1-dependent translation regulation is vital for sustaining the plasticity and self-renewal of colorectal CSCs.

Conclusion

Together, these findings highlight the key role that tRNA biology plays in sustaining the intrinsic plasticity of Lgr5+ CSCs and pave the way for the development of targeted therapies against colorectal cancer stem cells.

EACR25-2447

AMPK as a regulator of glioblastoma invasion and cytoskeleton dynamics

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Introduction

Glioblastoma is the most prevalent and aggressive primary brain tumor. AMP-activated kinase (AMPK), the main energy sensor of the cell, has been previously described by our group as a key factor in glioblastoma oncogenesis and proliferation and is known for taking part in tumoral metabolic transformation. Our lab has also formerly reported how AMPK regulates lipid

reliance of glioblastoma cells. Overall, this evidence positions AMPK inhibition as a promising antitumoural therapy for glioblastoma. Furthermore, AMPK has been reported to regulate cell invasion and migration, suggesting a potential role in orchestrating glioblastoma spreading in the brain.

Material and method

To study the role of AMPK in glioblastoma cytoskeleton dynamics, we used human glioblastoma cell lines U87 and U373 and suppressed AMPK activity by transfection with an siRNA for the catalytic subunits AMPK α 1/2. Cell migration was assessed by wound healing assays, and invasion was studied using spheroids in 2D and 3D experiments. Also, cytoskeleton morphology was studied by confocal microscopy, and cytoskeleton-associated protein levels were determined by western blot.

Result and discussion

Our data show that the silencing of AMPK reduces three-dimensional cell invasion. We also found that AMPK plays an important role in organizing the actin cytoskeleton and forming focal adhesions in the cell lines studied, probably through myosin regulation.

Conclusion

AMPK holds a relevant part in regulating glioblastoma cytoskeleton dynamics, affecting its invasion *in vitro*. This positions the inhibition of AMPK as a promising therapeutic strategy in glioblastoma, potentially decreasing or slowing down tumor expansion in the brain.

EACR25-2459

The Transcription Factor SNAI2 as a Novel Mediator of EWS-WT1 (+KTS) Oncogenic Activity in Desmoplastic Small Round Cell Tumour

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Introduction

Desmoplastic small round cell tumour (DSRCT) is a rare and aggressive sarcoma with a poor prognosis, highlighting the urgent need for improved therapeutic strategies. The driver of tumour malignancy is the chromosomal translocation between EWSR1 and WT1 genes. WT1 function is regulated by alternative splicing in exon 9, generating two isoforms depending on the presence of three amino acids (KTS), which alter the DNA-binding properties of EWS-WT1. The impact of both variants on tumour regulatory networks and their interactions with other transcription factors (TFs) remains poorly understood. This study identified SNAI2 as a potential target of EWS-WT1 +KTS.

Material and method

JN-DSRCT-1/Cas9-GFP cells were infected with a lentiviral gRNA library targeting 18479 TFs for CRISPR-based screening. NGS data were analysed using Galaxy platform. Transformed Human Mesenchymal Stem Cells (hMSC) were transfected with plasmids harbouring the two most prevalent gene fusion (GF) variants with KTS, fused to an HA-tag. RNA from these cells was used for a ClarionS expression array and analysed with TAC4.0 software. Integration of both models with a shRNA model targeting the GF (GSE180031) identified SNAI2 and its expression pattern was analysed in a set of public microarrays and validated at the protein level by IHC, in a subset of sarcomas samples including DSRCT. SNAI2 dependant expression of the GF was studied by targeting WT1 by siRNAs and analysed by qPCR and WB. Moreover, the binding of the GF to SNAI2 promoter was evaluated by a dual-luciferase reporter assay in JN, BER and SK-DSRCT cell lines. Finally, to investigate the dependence on the presence of KTS for the binding of the GF to SNAI2 promoter, a ChIP assay was developed in hMSC cells transfected with the GF with KTS. To assess the relevance of SNAI2 in the tumour biology, functional assays such as MTT assay, clonogenic, migration (wound healing), and invasion were conducted in the three DSRCT cell lines using siRNAs targeting SNAI2.

Result and discussion

Public microarray analysis revealed that DSRCT exhibits one of the highest expressions of SNAI2 among the studied sarcomas subtypes. These findings were validated by IHC, demonstrating moderate to high SNAI2 expression in DSRCT, compared to weak or absent expression in healthy tissues and other sarcomas. SNAI2 downregulation after the silencing of the GF verified its expression-dependency. Direct binding of EWS-WT1 +KTS to SNAI2 promoter was confirmed with dual-luciferase reporter assays, and validated by ChIP, showing significant binding of EWS-WT1 +KTS to the identified target sites. SNAI2 silencing significantly impaired cell proliferation, clonogenic capacity, migration, and invasion, underscoring its key role in DSRCT cell biology.

Conclusion

This study demonstrates that SNAI2 is a direct target of EWS-WT1 +KTS and a key driver of DSRCT malignancy, positioning it as a potential therapeutic target for further investigation.

EACR25-2460

Purine Synthesis as a Metabolic Bottleneck in TP53/KRAS Double-Mutant Lung Adenocarcinoma

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Introduction

Non-small cell lung cancers (NSCLC) harboring concurrent KRAS and TP53 mutations account for approximately 15% of all NSCLC cases and are associated with an aggressive clinical phenotype. While both TP53 and KRAS mutations are known to influence cancer metabolism, the specific metabolic adaptations in

KRAS/TP53 double-mutant tumors remain poorly understood. Given that metabolic reprogramming is a hallmark of cancer, we hypothesize that KRAS/TP53 double-mutant tumors undergo distinct metabolic remodeling to support their proliferative demands.

Material and method

We performed genomics screens, using human KRAS and KRAS/TP53 double-mutant lung adenocarcinoma cell lines with metabolism-focused CRISPR KO libraries targeting ~3,000 metabolic genes per species. To validate findings, we integrated TCGA and CCLE datasets, performed drug response assays, shRNA experiments, and analyzed DNA methylation and DNA damage. In preliminary *in vivo* studies, mice were injected with tumor cells expressing KRAS alone or KRAS/TP53 mutations, followed by treatment evaluation.

Metabolomics profiling and WGS were used to assess metabolic reprogramming and potential increases in mutational burden, respectively.

Result and discussion

Our functional genomics screening identified purine synthesis as a critical metabolic vulnerability in TP53/KRAS double-mutant lung adenocarcinoma. The metabolic essentiality of purine biosynthesis was further supported by data from the Cancer Cell Line Encyclopedia (CCLE), where cell lines with low p53 activity exhibited a similar dependency on purine metabolism. To validate these findings, we pharmacologically inhibited purine synthesis, revealing a higher sensitivity in TP53/KRAS double-mutant cell lines compared to their single-mutant or wild-type counterparts. Among purine metabolites, adenosine emerged as a key factor, as supplementation with adenosine rescued cell growth in both 2D and 3D models, suggesting that adenosine depletion plays a central role in the observed growth impairment upon purine inhibition. Furthermore, knockdown (KD) of key enzymes in the de novo purine synthesis pathway significantly increased sensitivity in TP53/KRAS double-mutant cells, reinforcing the notion that these tumors rely on purine biosynthesis for survival. Notably, inhibition of this pathway led to an accumulation of DNA damage and alterations in DNA methylation profiles, affecting specific epigenetic markers. These findings indicate that purine metabolism not only fuels tumor growth but also plays a role in maintaining genomic stability in TP53/KRAS double-mutant tumors.

Conclusion

Overall, our results highlight purine synthesis as a metabolic bottleneck in TP53/KRAS double-mutant lung adenocarcinoma, offering a potential therapeutic target for disrupting tumor progression while uncovering a link between purine metabolism and DNA integrity.

EACR25-2469

Asparagine-driven transsulfuration compensates for malate-aspartate shuttle deficiency in tumor growth

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Introduction

The malate-aspartate shuttle is a metabolic system with two major roles: (i) transferring reducing equivalents from the cytosol into the mitochondria to normalize the cytosolic NADH/NAD⁺ ratio and (ii) generating aspartate. Aspartate serves as a metabolic precursor for asparagine, arginine, and nucleotides. Both aspartate availability and a properly regulated cytoplasmic redox state are essential for biosynthesis and tumor progression. However, the combined effects of malate-aspartate shuttle disruption and aspartate synthesis deficiency on tumorigenesis remain unaddressed.

Material and method

We ablated GOT1 and GOT2, the malate-aspartate shuttle transaminases and the only two enzymes responsible for canonical aspartate synthesis in mammalian cells, in B16 mouse melanoma (GOT1/2 dKO) cells. Syngeneic subcutaneous tumors were generated in C57BL/6 mice for analysis by scRNA-seq and metabolomics. The substrate dependency of GOT1/2 dKO cells was assessed by measuring proliferation in culture media supplemented or depleted for specific nutrients. Metabolic tracing was performed to measure aspartate synthesis, and a loss-of-function CRISPR screen was used to identify dependencies in GOT1/2 dKO cells.

Result and discussion

Surprisingly, GOT1/2 dKO cells formed tumors normally. Integrated analysis revealed increased pyruvate utilization and decreased aspartate levels in GOT1/2 dKO tumors, consistent with disrupted aspartate synthesis and cytosolic redox imbalance. The growth of GOT1/2 dKO cells was constrained by an elevated cytosolic NADH/NAD⁺ ratio rather than by aspartate availability. CRISPR screening demonstrated that GOT1/2 dKO cells depend on the transsulfuration pathway. When transsulfuration is active, the redox imbalance in GOT1/2 dKO cells can be rescued by asparagine, which is exchanged for serine, a key substrate in transsulfuration.

Conclusion

We identified a new metabolic pathway wherein asparagine-stimulated transsulfuration corrects the cytoplasmic redox imbalance and supports tumor growth in the absence of functional malate-aspartate shuttle.

EACR25-2474

Androgen receptor, Filamin A, and CXCR4: partners in crime in TNBC

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Introduction

Triple-negative breast cancer (TNBC) represents 15–20% of all breast cancer subtypes and is characterized by the lack of the three classical BC markers: estrogen receptor (ER), progesterone receptor (PR), and type-2 epidermal growth factor receptor (HER-2). To date, chemotherapy remains the mainstay of systemic treatment, since no relevant druggable targets have been identified. Many studies have tried to characterize TNBC to find new targetable proteins that can cure this BC subgroup. AR has gained a promising position during the past few years. This receptor is expressed in 20–50% of TNBCs and regulates different downstream pathways thus affecting the progression of this incurable disease.

Material and method

Using *in vitro* and *in vivo* experiments, we analyzed how AR and its partners, FLNA and CXCR4, promote TNBC cell proliferation and growth and influence the behavior of the tumor microenvironment (TME). In 2D and 3D models of TNBC-derived MDA MB 231 and 453 cell lines, and stroma NIH3T3 cell line, we analyzed androgen-induced cell proliferation by measuring 5-bromo2'-deoxyuridine incorporation and spheroid growth. We performed co-immunoprecipitation experiments, immunoblots, and immunofluorescence analysis to evaluate how and where AR interacts and co-works with FilA and CXCR4 and which pathways they control. Trans-well assays allow us to define how the androgen treatment of cancer cells conditions the stroma cells' activity. Using specific inhibitors in both *in vitro* experiments and MDA231-BALBc xenografts shows that AR, in collaboration with FilA and CXCR4, is responsible for TNBC progression. Immunohistochemical assays confirmed that AR action influences cancer cell proliferation and TME composition.

Result and discussion

Our results show that androgen treatment triggers cell proliferation in TNBC cells and, indirectly, in stroma cells. Furthermore, androgen-elicited cancer cells can influence TME. These effects are specifically due to the association between AR and Filamin A that controls, on one side, the chemokine receptor CXCR4 and on the other, the downstream activation of Ras and PKC. The use of the specific AR antagonist, the bicalutamide or Casodex (Cx), and of two peptides, the Rh2025u, specifically designed to mimic the AR sequences responsible for its interaction with Filamin A, and the R54, able to inhibit the CXCR4 activity, prevents cancer and stroma cell proliferation and strongly reduces cancer growth in MDA231-BALBc xenografts. In sum, our data demonstrates the pivotal role of AR in TNBC progression and how this protein can control cancer growth by acting directly on cancer cells and indirectly on TME.

Conclusion

These results give high importance to AR as a molecular target acting on cancer *in toto*. The use of combined therapy inhibiting AR and its partners in crime (FilA and CXCR4) can represent a winning proposal to develop effective therapies for fighting TNBC.

EACR25-2489

Divergent Effects of Tet2- and Dnmt3a-mutant Clonal Hematopoiesis on Breast Cancer Progression Upon Aging

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Introduction

Clonal hematopoiesis of indeterminate potential (CHIP) is a hematological condition defined by the presence of an expanded somatic blood cell clone harboring mutations in leukemia driver genes, most commonly DNMT3A and TET2, at a variant allele frequency more than 2% in the absence of other hematological abnormalities. CHIP incidence increases with age and is linked to higher risk of developing blood cancer, cardiovascular disease and all-cause mortality which associates mechanistically with increased pro-inflammatory responses by mutant myeloid cells. Recent findings suggest higher CHIP incidence in solid tumor patients, with carriers exhibiting a higher risk of developing breast cancer. However, whether hematopoietic cells carrying CHIP mutations directly impact solid tumor progression, and what are the underlying mechanisms, remain unclear. Thus, we hypothesize that Tet2/Dnmt3a-driven clonal hematopoiesis contributes to breast cancer incidence, growth, and/or metastasis by altering the inflammatory tumor microenvironment and immune compartments.

Material and method

To test our hypothesis, we established a transgenic mouse model of CHIP, where floxed Tet2 or Dnmt3a fl-R878H genes and a reporter gene are controlled by a hematopoietic-specific, tamoxifen-inducible CreER enzyme. Combining this CHIP model with a syngeneic, orthotopic and traceable breast cancer model using E0771 cell line, we aim to assess the contribution of CHIP-bearing hematopoietic cells to breast tumor progression in young (Y, 3–4 months old) and middle-aged (MA, 12–14 months) animal cohorts, carrying high (~70% of leukocytes) clonal fractions. Using longitudinal tumor size and metastasis quantification combined with histology, flow cytometry and scRNA-sequencing data, we further aim at revealing the potential underlying molecular mechanisms.

Result and discussion

We observe that Y Tet2^{+/−} CHIP mice showed comparable primary tumor growth, but increased lung metastasis burden compared to WT animals. In contrast, MA Tet2^{+/−} CHIP mice exhibited significantly enhanced tumor growth and elevated lung metastasis. Notably, we observed reduced numbers of Tet2^{+/−} tumor infiltrating T cells, particularly CD8+ and NKT populations, in MA animals. Conversely, both Y and MA Dnmt3a fl-R878H CHIP mice had slower breast tumor growth than WT littermates, with comparable metastatic burden in the lungs. Preliminary scRNA sequencing analysis of the tumor-infiltrating leukocytes revealed a suppressed T-cell response in MA Tet2^{+/−} CHIP animals, which contrast with increased type I interferon signatures in MA Dnmt3a fl-R878H CHIP mice.

Conclusion

These findings suggest that age and CHIP-mutations have a synergistic impact on breast cancer progression potentially by shaping the tumor infiltrating immune compartment. However, the precise molecular mechanisms linking CHIP and breast cancer, and its potential therapeutic implications remain to be explored.

EACR25-2512

Anti-Cancer Effects of *Haplophyllum tuberculatum* Extract Against Triple Negative Breast Cancer

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Introduction

Breast cancer (BC) is the most commonly diagnosed cancer in women, with over 2 million new cases reported globally in 2020. In the United Arab Emirates (UAE), BC has the highest incidence rate among women, accounting for 32.16% of cases in UAE citizens and 41.41% in non-UAE citizens. Although several treatment options, including surgery, radiotherapy, and chemotherapy, are available for BC, these approaches often affect surrounding tissues and lead to various side effects. As a result, there is a growing interest in alternative treatments that offer high efficacy with reduced cytotoxicity. Medicinal plants present a promising alternative for cancer therapy due to their accessibility, efficacy, and lower toxicity. *Haplophyllum tuberculatum* is a widely recognized medicinal plant traditionally used by local communities to treat various ailments. This study aims to conduct a phytochemical screening of the ethanolic extract of *Haplophyllum tuberculatum* (HTEE) and its fractions to identify secondary metabolites, evaluate their antioxidant activity, and assess their anticancer potential on human breast cancer cell lines.

Material and method

MDA-MB-231 cell line was used in this study. CellTiter-Glo Luminescent cell viability assay was used to measure cell viability after the treatment following the manufacturer's protocol. Cell Morphology of treated and control MDA-MB-231 cells was examined using a light microscope with 40X magnification after 24 hours. Caspase 3/7 activity was examined using a luminescent Caspase-Glo® 3/7 assay kit following the manufacturer's procedures. The effect of HTEE on the migration of MDA-MB-231 cells was assessed using a wound-healing assay. Protein expression of the target proteins was measured using Western blotting.

Result and discussion

Our results demonstrated that HTEE inhibits the proliferation of MDA-MB-231 cells in a time- and concentration-dependent manner. Treatment with HTEE led to the downregulation of key proteins associated with cell proliferation, including cyclin D1, c-Myc, phospho-RB, and PCNA. The activation of caspase 3/7 and PARP cleavage indicated that HTEE induced apoptosis in MDA-MB-231 cells. Furthermore, the downregulation of p62 and accumulation of LC3II suggested the involvement of autophagic cell death in the observed cytotoxic effects. HTEE also effectively inhibited the migration of

MDA-MB-231 cells and decreased the protein expression of β-catenin, which is involved in both cell proliferation and migration. Additionally, HTEE increased phospho-H2A.X, a marker for DNA breaks, suggesting DNA damage induction.

Conclusion

Overall, our findings highlight the therapeutic potential of HTEE in targeting breast cancer cells.

EACR25-2520

Exploiting inhibitors of histone deacetylase and metabolism for pancreatic cancer therapy

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Introduction

Pancreatic ductal adenocarcinoma (PDAC) is a lethal cancer, largely driven by oncogenic KRAS and has limited therapeutic options. Although histone deacetylase inhibitors (HDACi) have been approved for various cancer treatment, these inhibitors showed limited efficacy in PDAC for unclear reasons.

Material and method

We used CRISPR/Cas9 gene editing to delete histone deacetylase in human and mouse pancreatic cancer cell lines, followed by metabolomics, molecular characterization, drug screening, and mouse subcutaneous xenograft studies. In addition, we performed bioinformatic analysis of human and mouse transcriptomics data of cells treated with HDACi.

Result and discussion

Here we show that, independent of KRAS activity, a subset of PDAC cell lines undergo cell death upon treatment with HDACi. Integrative metabolomics and transcriptomics analyses revealed that HDACi interfere with multiple metabolic pathways, notably disrupting nucleotide metabolism and amino acid pathways. These cataclysmic events are actively counteracted in the HDACi-resistant cells, which amplify de novo metabolism to sustain growth.

Conclusion

Our data identify new compensatory mechanisms enabling HDACi resistance and suggest that nucleotide biochemical axis is a major metabolic hub that could be exploited to improve response to HDACi in pancreatic cancer.

EACR25-2522

Seamless Capture Integration for Accurate NGS-Based Applications

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Introduction

Capture-based NGS assays enable the identification of actionable biomarkers across hundreds of cancer-

associated genes in a single experiment. However, sample preparation for such assays can be time-consuming and technology demanding. The Trinity technology streamline the capture workflow by enabling directly library loading onto the sequencer after hybridization, eliminating the need for hands-on capture and wash steps. This study evaluates the compatibility of Trinity with established end-to-end solutions. The results suggest that the benefits of Trinity can be combined with the proven accuracy of the SOPHiA GENETICS™ applications.

Material and method

Reference samples were processed using the Trinity workflow on the AVITI™ sequencer with the target enrichment panels from two SOPHiA GENETICS™ applications; MSK-IMPACT® powered with SOPHiA DDM™ and SOPHiA DDM™ Whole Exome Solution v2. The same samples were processed in parallel using the end-to-end solutions from SOPHiA GENETICS, with sequencing on either the Illumina or Element instrument. Capture quality metrics and variants calling performance were assessed using the SOPHiA DDM™ Platform through its dedicated bioinformatics pipelines. The performance of the Trinity workflow with each target enrichment panel was compared to that of the corresponding end-to-end solution.

Result and discussion

Both capture assays displayed highly similar quality metrics, with 94% on-target rates and >99% coverage uniformity for traditional captures as well as Trinity captures. Whole exome sequencing using the Trinity workflow with SOPHiA GENETICS™ applications and pipelines showed excellent analytical performance with 99.3% sensitivity and 98.7% precision.

Conclusion

The findings demonstrate compatibility of the Trinity workflow that includes on-sequencer capture with existing end-to-end NGS solutions, such as MSK-IMPACT® powered with SOPHiA DDM™ and SOPHiA DDM™ Whole Exome Solution. The high accuracy of the SOPHiA GENETICS™ applications is preserved with the simplified workflow, indicating that solutions with a proven reliability can benefit from the technology. Further studies are planned to explore in more details the analytical performance and evaluate the utility in routine practice.

EACR25-2536

Dissecting the molecular mechanisms underlying the ERKi-Entrectinib combination in LKB1-mutated NSCLC

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Introduction

LKB1-mutated NSCLCs represent a highly aggressive subtype lacking targeted therapeutic options. Previous preclinical studies demonstrated that these tumors are particularly sensitive to ERK inhibitors (ERKi). However, co-occurring alterations in the PI3K/Akt/mTOR pathway or other signaling networks may reduce ERKi efficacy. The project aimed to identify the most

effective ERKi-based drug combinations for LKB1-deficient NSCLCs using advanced preclinical models, including patient-derived xenografts (PDXs) and organoids (PDXOs).

Material and method

PDXs and PDXOs were generated from fresh tumor specimens and characterized histologically and molecularly. Their growth patterns and genetic profiles were analyzed by STR and Whole exome sequencing (WES) to confirm fidelity to the original tumor. A comprehensive drug screening of FDA-approved compounds was performed to identify candidates that enhance ERKi sensitivity. Key molecular mechanisms were investigated by western blot analyses and CRISPR/Cas9-mediated gene deletion to understand ERKi synthetic lethality in LKB1-mutated models. Drug efficacy was validated in 2D and 3D cultures by carrying out distinct cell viability and cell death assays, with selected combinations tested in vivo.

Result and discussion

From the drug-library screening, the ALK, NTRK, ROS1 inhibitor Entrectinib emerged as the best promising ERKi partner, showing the most significant impact in LKB1-deficient cells. This combination induced apoptosis, and demonstrated efficacy both in 2D and 3D models. To further clarify the specific mechanisms involved in Entrectinib activity, a pharmacological approach was applied by using Entrectinib analogues targeting only one or two receptors (ALK, NTRK, ROS1) and combined them with the ERK inhibitors. This approach revealed that inhibiting ALK is necessary, but probably not sufficient, to sensitize LKB1-mutated cells to ERKi. In vivo, the ERKi-Entrectinib combination reduced tumor growth, and improved survival rates, supporting its clinical potential. Further experiments are ongoing on patients-derived models to corroborate these results.

Conclusion

These findings highlight the therapeutic potential of ERKi-based combinations for LKB1-mutated NSCLCs, particularly with Entrectinib. The study provides a strong rationale for further preclinical and clinical evaluations to refine combination strategies, enhance efficacy, and minimize toxicity. This offers a new avenue for treating LKB1-deficient NSCLCs, a subtype with limited targeted therapies.

EACR25-2548

Mitochondrial dynamics control cancer cell migration

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Introduction

Tumour metastasis remains the leading cause of mortality in breast cancer patients, with cell migration playing a pivotal role in this process. Our study reveals that mitochondria play a central role in orchestrating the directional migration of metastatic breast cancer cells.

Material and method

Utilizing high-resolution live cell imaging and micro patterns, we analysed the migratory behaviour of MDA-

MB-231, MDA-MB-468, and MCF7 cells, which have different metastatic potentials.

Result and discussion

We identified a distinct phenotype in MDA-MB-231 cells, which exhibited sustained actin cytoskeletal coordination that favoured directed migration in different levels of confinement. MDA-MB-231 cells presented a highly polarised distribution of mitochondria towards their leading edge as well as in protrusions where mitochondria maintained an elevated membrane potential ($\Delta\Psi_m$). In contrast, perinuclear mitochondria exhibited changes in the membrane potential. Multi-omics analyses revealed that the voltage-dependent anion channel (VDAC) family is overexpressed in the metastatic breast tumours from 1100 patient samples and more than 55 cell lines, with VDAC1 levels correlating with poor patient survival. Pharmacological disruption of mitochondrial function or inhibition of the VDAC1 channel significantly reduced migration speed and the number of actin stress fibres.

Conclusion

Together, these findings deepen our mechanistic understanding of how the precise spatiotemporal positioning of mitochondria-mediated by VDAC1 channel oligomerization contributes to the migratory behaviour of metastatic breast cancer cells. This insight encourages further exploration of mitochondrial dynamics as a potential component of future therapeutic strategies for aggressive breast cancer.

EACR25-2578

Type I interferons suppress pancreatic cancer initiation

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Introduction

Pancreatic ductal adenocarcinoma (PDAC) is characterized by a highly immunosuppressive tumor microenvironment (TME). The evolution of the TME during the early stages of PDAC development is poorly understood. Loss of chromosome 9p21.3 is the most common homozygous deletion across cancers; these deletions invariably affect the tumor suppressor genes CDKN2A/B and commonly extend to include a linked cluster of type I interferon (IFN) genes. Our laboratory recently found that IFN co-deletion contributed to an immunosuppressive TME and increased metastasis and resistance to immune checkpoint blockade in orthotopic mouse models of advanced PDAC. However, the role of type I IFN signaling in shaping the tumor immune microenvironment to suppress early stages of tumor initiation is not well characterized.

Material and method

In this study, we used autochthonous mouse models of PDAC driven by oncogenic Kras and a doxycycline-inducible shRNA targeting Trp53 restricted to the pancreatic epithelium. Mice were treated with an interferon alpha receptor 1 (IFNAR1) blocking antibody to induce systemic blockade of type I IFN signaling or an IgG control for six weeks. Tissue was collected for

histological analysis and Kras-mutant epithelial cells, immune cells, and stromal cells were isolated for single-cell RNA-seq.

Result and discussion

Using mouse models of PDAC driven by oncogenic Kras, we found that systemic blockade of type I IFN signaling increases the frequency of cancer initiating epithelial cells in a p53-dependent manner during tumor initiation. These changes in the epithelial compartment are coupled with an increased frequency of pro-inflammatory and pro-tumorigenic myeloid cell populations. We observed that type I IFNs are induced upon mutant Kras activation, and loss of type I IFN signaling also accelerated the progression of premalignant lesions to adenocarcinoma.

Conclusion

Our work implies that type I IFNs function as tumor suppressors during PDAC initiation through crosstalk with the immune microenvironment, and loss of this program accelerates tumor development.

EACR25-2579

IL-2/STAT5 signalling drives metabolic reprogramming to support cervical cancer cell proliferation

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Introduction

A relevant hallmark of human cancer is the ability to reprogram energy metabolism to fuel cell growth and proliferation. Cancer cells show enhanced glucose uptake and glycolysis and a decreased oxidative metabolism, mainly by upregulating glucose transporters and multiple enzymes of the glycolytic pathway. On the other hand, in normal T cells, this metabolic switch is maintained by continuous signalling of IL-2 through the JAK/STAT pathway. We have shown that the IL-2 receptor is present in cervical cancer cells and that the JAK/STAT pathway is constitutively active. However, little is known about the role of the IL-2/STAT signalling in metabolic reprogramming in cervical cancer cells.

Material and method

To elucidate the role of the IL-2/STAT5 signalling in metabolic reprogramming, cervical cancer cell lines HeLa and SiHa were treated with 10UI/mL of IL-2 to measure lactate secretion and the expression of HIF-1a, PDK1, GLUT1, and UQCRC1. The role of STAT5 was evaluated by using a shRNA to silence the STAT5 gene in cervical cancer cells. Cell proliferation was evaluated by Cristal violet.

Result and discussion

Our results show that the treatment with IL-2 induces an increase in lactate secretion and the expression of HIF1a, PDK1, GLUT1 and UQCRC1. When STAT5 was silenced, the lactate secretion decreased. Also, the expression of HIF1a, PDK1 and GLUT1 decreased, but the expression of UQCRC1 was not affected. Cervical

cancer cell proliferation was reduced by STAT5 silencing. These results indicate that STAT5 regulates metabolic reprogramming to enhance aerobic glycolysis by regulating genes related to energy metabolism which is important for cell proliferation.

Conclusion

These results suggest that IL-2 regulates glucose conversion into lactate to increase its concentration for intracellular acidification giving cervical cancer cells an advantage in proliferating and migrating. Also, STAT proteins modulate the metabolic switch in cervical cancer cells underlying the enhanced proliferation of these cells. A detailed understanding of the adaptations required downstream of the IL-2/STAT signalling could reveal targetable metabolic vulnerabilities.

Research supported by a grant from DGAPA, UNAM (PAPIIT IN225324).

EACR25-2587

Cancer associated fibroblasts regulate breast cancer migration through Laminin-511

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Introduction

Although under active investigation, alterations within the tumor microenvironment both causative to and a consequence of tumor progression are only beginning to be better appreciated. Within the tumor microenvironment, fibroblasts play complex multidimensional roles in modulating cancer cell migration (Sahai et al, Nat Rev Cancer, 2020; Labernadie et al, Nat Cell Biol, 2017; Barbazan et al, Nat Commun, 2023). In addition, computational and experimental studies suggest the requirement of a laminin-rich ECM in driving robust multimodal invasion of cancer cells rather than dispersed migration as seen in laminin-depleted setups (Pally et al, Front Physiol, 2019; Chang J et al, Nat Mater, 2024).

Material and method

This led us to hypothesize if cancer associated fibroblasts (CAFs) may remodel the tumor microenvironment through synthesizing an ECM that has biochemical and/or biophysical properties appropriate to facilitating more collective invasive migratory behaviors of cancer cells. To do so, we first standardized protocols for isolating CAFs from breast cancer patient tissues. We began by integrating immortalized and patient-derived primary fibroblasts within histopathologically complex 3D pathotypic models.

Result and discussion

demonstrated that CAFs enhance multimodal breast cancer migration through Collagen I-rich stromal-like spaces to a greater extent than non-cancerous fibroblasts (NFs). We further validated our results in a murine model. This was supported by our observations that the conditioned medium of CAFs could also spur the migration of cancer cells to a greater extent than the medium from normal fibroblasts (NFs). We performed a multilayered screen consisting of bioinformatics, immunocytochemistry and PCR to identify specific laminins that are expressed to greater extents in CAFs: laminin- β 1 and laminin- γ 1. Further investigations also

revealed an increase in breast cancer cells adhesion and migration on laminin-10 (α 5 β 1 γ 1) compared to laminin-1 (α 1 β 1 γ 1), laminin-2 (α 2 β 1 γ 1), and laminin-4 (α 2 β 2 γ 1). Furthermore, genetic perturbations and pharmacological screen revealed laminin-10 mediated cancer cell migration through Integrin α 6 β 1-FAK- Arp2/3 signaling axis.

Conclusion

Our findings reveal a specific correlation between CAFs secreted laminins and breast cancer migration, which we will investigate further for deciphering specific molecular mechanisms in the future.

EACR25-2589

PPP2R2B-derived circRNA in the suppression of ferroptosis in Pancreatic cancer cells

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Introduction

The limited availability of early-detection biomarkers and the poor response to current treatments have led to a low five-year survival rate of pancreatic ductal adenocarcinoma (PDAC) patients. Recent studies suggest that cancer cells can be susceptible to ferroptosis. However, the role of circular RNAs (circRNAs) in ferroptosis remains largely unexplored in PDAC. Hence, this project aims to identify circRNAs in ferroptosis to uncover further mechanisms and provide potentially effective targets for anti-cancer therapies.

Material and method

CircRNA-sequencing of pancreatic cancer cells after ferroptosis induction was performed, from which circPPP2R2B was selected for further analysis. Several ferroptosis markers were measured after circPPP2R2B knockdown. CircPPP2R2B expression was measured in PDAC cell lines, tumours, and adjacent tissue samples. The potential oncogenic activity was assessed by performing cell viability, migration, and invasion assays after knockdown and overexpression. CircPPP2R2B interacting proteins were investigated by performing mass spectrometry after a pull-down assay, while its downstream targets were identified by mRNA sequencing after its knockdown.

Result and discussion

The knockdown of circPPP2R2B showed higher levels of positive ferroptosis markers. CircPPP2R2B was up-regulated in PDAC cells and tumour tissues (p-value < 0.01), where patients with higher expression had a median overall survival (OS) of 7 months less than those with lower expression. Upregulation of circPPP2R2B was also observed in the serum of PDAC patients compared to healthy individuals. The loss of circPPP2R2B decreased the proliferation rate, migration, and invasiveness of PDAC cells, while its overexpression increased the IC50 of gemcitabine. The mRNA-sequencing data showed that PD-L1 was downregulated after circPPP2R2B knockdown and that circPPP2R2B interacts with proteins of the cohesin complex to potentially regulate PD-L1 expression.

Conclusion

CircPPP2R2B downregulation in pancreatic cancer cells undergoing ferroptosis indicates that its low levels are required for ferroptosis. The lower viability, migration, and invasion ability after circPPP2R2B knockdown shows it supports PDAC progression. Additionally, the increased IC₅₀ of gemcitabine after circPPP2R2B over-expression suggests its involvement in the resistance to this drug. By regulating the expression of PD-L1, a ligand suppressing the anti-cancer immune response, circPPP2R2B could be playing a critical role in the regulation of the tumour immune microenvironment.

EACR25-2598

Age-specific factors determine brain metastasis growth and immunotherapy response

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Introduction

Cutaneous melanoma is one of the most common cancers to metastasise to the brain. Melanoma cells that seed the brain and establish brain metastases (MBM) are characterised by a high OXPHOS metabolism and a highly invasive phenotype that destroys tissue locally and leads to mortality, primarily due to local symptoms and complications. One striking epidemiological hallmark is the median age of stage IV metastatic melanoma patients who develop MBM, which is 55 years. In contrast, the median age of patients with extracranial metastases is significantly higher at 78 years.

Material and method

We used metabolomics, lipidomics, bulk and spatial transcriptomics to discover key differences between young and aged brains that support distinct MBM behaviour and IT response by age.

Result and discussion

We studied whether factors in the primary cutaneous melanoma tumour microenvironment (TME) differ by age; and whether local cutaneous TME factors promote intracranial vs extracranial metastatic spread. We found ceramides, a lipid family that accumulates in aged skin, delayed brain metastatic seeding and promoted the rapid progression to liver metastasis. In contrast, lipids in young skin are permissive of MBM development. We next investigated whether brain-specific factors differ by age and contribute to greater melanoma cell growth. We used orthotopic brain melanoma models in immuno-competent animals to show that melanoma cells have distinct growth patterns in brains of different aged hosts. Melanoma cells that seed the young brain develop the classic hallmarks of MBM: a highly invasive phenotype, rapid growth, a switch to OXPHOS metabolism and activation of the PI3K/AKT signalling pathway. In contrast, the aged brain is less fertile for MBM to grow and presents a unique tumour immune cell landscape. Furthermore, we found the age of the brain impacts the

response to immunotherapy. Critically, we show that age-specific factors similarly impact the development and immunotherapy response of triple negative breast cancer and glioblastoma, which are also more likely to metastasise or develop in the brain of younger patients.

Conclusion

Our study shows the soil-specific brain factors that impact metastatic tumour growth and immunotherapy response by age.

Cancer Genomics

EACR25-0001

Genomic and single-cell characterization of patient-derived tumor organoid models of head and neck squamous cell carcinoma

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Introduction

Head and Neck Squamous Cell Carcinoma (HNSCC) remains a significant health burden due to tumor heterogeneity and treatment resistance, emphasizing the need for improved biological understanding and tailored therapies.

Material and method

While these recent important efforts in PDOs have greatly advanced the investigation of HNSCC precision medicine and predictive biomarker development, several key questions remain to be addressed: to what extent can PDOs model histopathology, drug responsiveness and mutational profiles of parental tumor specimens?

Relatedly, how does long term culture affect the biology and genomic stability of PDOs? Moreover, considering the high intratumor heterogeneity of HNSCC, single-cell transcriptomic profiling is required to further delineate gene regulatory network and cellular states within the PDOs. This study is designed to address these important questions by multi-omic, single-cell genomic and functional analyses of both primary tumors and matched PDO models maintained for various durations ex vivo

Result and discussion

This study enrolled 31 HNSCC patients for the establishment of patient-derived tumor organoids (PDOs), which faithfully maintained genomic features and histopathological traits of primary tumors. Long-term culture preserved key characteristics, affirming PDOs as robust representative models. PDOs demonstrated predictive capability for cisplatin treatment responses, correlating ex vivo drug sensitivity with patient outcomes. Bulk and single-cell RNA sequencing unveiled molecular subtypes and intratumor heterogeneity (ITH) in PDOs, paralleling patient tumors. We performed non-negative matrix factorization (NMF) to identify gene modules as described previously. This integrative computational approach attempts to alleviate the impact of both technical confounders (e.g., batch effects) and patient-specific characteristics, which often hinder the goal of

identifying common expression programs across different patient tumors. As a result, we identified eight clusters of recurrent gene modules across individual PDOs. Notably, a hybrid epithelial-mesenchymal transition (hEMT)-like ITH program is associated with cisplatin resistance and poor patient survival. Functional analyses identified amphiregulin (AREG) as a potential regulator of the hybrid epithelial/mesenchymal state. Moreover, AREG contributes to cisplatin resistance via EGFR pathway activation, corroborated by clinical samples.

Conclusion

In summary, HNSCC PDOs serve as reliable and versatile models, offer predictive insights into ITH programs and treatment responses, and uncover potential therapeutic targets for personalized medicine.

EACR25-0013

Genes that affect DNA repair and calcium metabolism predispose to Malignant Mesothelioma

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Introduction

Mesothelioma, an aggressive cancer often caused by exposure to asbestos, is one of the malignancies currently seen with increasing frequency in younger patients. We have sequenced and analyzed the DNA of 61 patients who developed mesothelioma at young age (<55 y old) and who had no evidence of asbestos exposure. We discovered that ~1.8% of all mesothelioma patients and 4.9% of those younger than 55, carry rare pathogenic germline variants of the BRCA1 associated RING domain 1 (BARD1) gene.

Material and method

Cell cultures Human dermal skin fibroblasts from explants of skin biopsies were collected, as previously described (Bononi et al 2017) from BARD1WT and BARD1 mutant carriers. Fibroblasts were cultured in Dulbecco modified Eagle's medium (DMEM) (Corning Cellgro, #10-017-CV), supplemented with 10% fetal bovine serum (FBS) (Seradigm, #1500-500), and 1% penicillin-streptomycin (PS) (Life Technologies, #15140-163). Primary human mesothelial cells (HM) were derived from pleural fluids accumulated in individuals with congestive heart failure and cultured in DMEM with 20% FBS and 1% penicillin-streptomycin (PS). PBMCs were collected from BARD1WT and BARD1 mutant carriers. Gene Silencing with siRNA siRNA oligonucleotides were obtained from Qiagen. GeneSoluton siRNAs targeting four different BARD1 mRNAs were as follows: Hs_BARD1_2, (cat.no. 1027415, D: SI00010136); Hs_BARD1_3, (cat.no. 1027415, ID: SI00010143); Hs_BAP1_9, (cat.no. 1027415, ID: SI05103847); Hs_BAP1_10, (cat.no. 1027415, ID: SI0-5103854). A nonspecific control siRNA was used as a negative control (cat. no. 1027280). Transfection was performed with HiPerfect (Qiagen) for

24 hours. Western Blot: Total cell lysates were prepared in M-PER (Thermo Scientific, cat.no. 78501) reagent supplemented with proteases and phosphatases inhibitors (2mM Na3VO4, 2mM NaF, 50nM okadaic acid, 1mM PMSF and protease inhibitor cocktail) and 1mM DTT. 10 µg of protein were loaded and separated on NuPAGE Novex 4–12% Bis-Tris Gel (Life Technologies), and electron-transferred to PVDF according to standard procedures.

Result and discussion

We performed functional assays in primary fibroblasts obtained from a patient carrying a heterozygous BARD1 mutation. We found that these cells had genomic instability, reduced DNA repair, and impaired apoptosis. Studying underlying signaling pathways, we found that BARD1 interacts with p53 and SERCA2 modulating calcium signaling and apoptosis.

Conclusion

Our study elucidated mechanisms of BARD1 activity and revealed that heterozygous germline BARD1 mutations favor mesothelioma development in young patients and increase the susceptibility to asbestos carcinogenesis. These mesotheliomas are significantly less aggressive compared to asbestos-caused mesotheliomas. These patients experience significantly prolonged survival up to 20+ y and they require tailored screening and personalized therapeutic approaches.

EACR25-0024

The expression and clinical significance of serum exosomes miR-122 and miR-874 in patients with colorectal cancer

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Introduction

To investigate the expression levels of miR-122 and miR-874 in serum exosomes of patients with colorectal cancer (CRC) and their clinical significance.

Material and method

140 patients with colorectal cancer treated in our hospital from January 2020 to January 2022 were selected as observation group, and 90 healthy subjects were selected as control group. The expression of serum exosomes miR-122 and miR-874 was detected by qRT-PCR, and the relationship between the expression and clinicopathological features of CRC was analyzed. The optimal cut-off value of serum exosome miR-122 and miR-874 in predicting the prognosis of CRC was determined by receiver operating characteristic (ROC) curve. According to this value, patients in the observation group were divided into low expression group and high expression group. After 2 years of follow-up, Kaplan-Meier curves were drawn to analyze the relationship between the expression of serum exosomes miR-122 and miR-874 and overall survival (OS) and disease-free survival (DFS).

Result and discussion

The expression of miR-122 in serum exosomes in observation group was higher than that in control group, and the expression of miR-874 was lower than that in healthy group ($P < 0.05$). The expression of serum

exosome miR-122 was positively correlated with infiltration depth, TNM stage, lesion diameter, differentiation degree, and lymph node metastasis status in colorectal cancer patients ($r > 0$, $P < 0.05$), while the expression of miR-874 was negatively correlated with the above pathological characteristics ($r < 0$, $P < 0.05$). In the observation group, the 2-year OS was 72.86% and DFS was 61.43%. In the miR-122 overexpression group, the OS and DFS were 15.39 months (95% CI: 13.078-17.941) and 17.09 months (95% CI: 14.743-18.012) respectively. The results were lower than those in the low-expression group at 17.78 months (95% CI: 15.78-18.46) and 20.09 months (95% CI: 17.378-21.098) ($P < 0.05$). In the group with low expression of miR-874, the OS was 16.209 months (95% CI: 14.189-18.067) and DFS was 18.026 months (95% CI: 16.801-20.109). The results were lower than those in the highly expressed miR-874 group at 19.230 months (95% CI: 17.902-21.026) and 22.035 months (95% CI: 19.830-23.089), respectively ($P < 0.05$).

Conclusion

The expression of serum exosome miR-122 is high and miR-874 is low in patients with colorectal cancer, and the expression levels of both are related to the clinicopathological features and survival time of patients.

EACR25-0190

Expression of APOC3 in human colon cancer, the clinical and therapeutic value

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Introduction

The APOC3 gene is located on 11q23.3 which is close to a region that frequently aberrant in breast cancer and encodes apolipoprotein-CIII (APOC3), a protein known to regulate the metabolism of triglycerides and involved in coronary heart diseases. APOC3 is highly expressed in the liver and in small intestines. Loss of heterozygosity (LOH) of the APOC3 gene has been found to have a possible association with disease progression in certain cancers including liver hepatocellular carcinoma. The present study aimed to determine the clinical value of the APOC3 gene transcript expression in human colon cancer.

Material and method

Expression of The APOC3 gene transcript was quantitatively analysed in a cohort of human colon cancer with matched normal colon tissues. Survival analysis was carried out using the Kaplan-Meier survival model for overall, disease free and distant metastasis outcomes. Comparisons were made against clinical and pathological factors and status of the ERBB family members.

Result and discussion

Colon cancer tissues expressed markedly higher levels of APOC3 transcript than normal tissues ($p < 0.00001$). High levels of APOC3 in colon cancer resulted in significantly shortened overall survival (OS) ($p = 0.031$, Hazard ratio (HR = 2.718 (95% CI 1.056-6.997)),

significantly shortened disease free survival (DFS) ($p = 0.011$, HR = 3.171 (95% CI. 1.243-8.086)). It was very interesting to note that the connection between APOC3 expression and OS and DFS largely existed in patients with Her2 positive tumours, namely DFS in Her2 negative ($p = 0.331$) and Her2 positive ($p = 0.012$) when comparing APOC3 high and low expressing groups. The same observation was seen when EGFR (ERBB1) and Her4 (ERBB4) were taken into consideration. In the multifactorial analysis against clinical factors including tumour grade, TNM staging, Dukes staging, ERBB family members and APOC3 expression, APOC3 was the only significant independent factor correlated with DFS. To further establish the clinical value of APOC3 in colon cancer, we also explored the TCGA database and found that APOC3 high levels were associated with resistance to chemotherapies ($p = 0.0016$ when comparing patients who were sensitive and who were resisted to the treatment). This connection was particularly strong for those receiving Capecitabine and Fluoropyrimidines, but not for 5-FU, Oxaliplatin nor Irinotecan.

Conclusion

The present study is the first report of the clinical connection between APOC3 and clinical colon cancer, which shows that high expression levels of APOC3 is an indicator for patient survival and response to chemotherapies. APOC3 thus has an important clinical and therapeutic value in colon cancer and constitutes a valuable therapeutic target.

EACR25-0193

Paired scRNA/TCRseq meta-analysis of T cells reveals clonal dynamics of response to checkpoint immunotherapy

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Introduction

T-cell clones play a crucial role in the anti-tumor activity of cancer patients treated with checkpoint immunotherapy. However, their characterization and association with clinical outcome remain insufficiently understood. Studying response mechanisms of T-cell clones could therefore reveal important factors driving therapy effectiveness or resistance.

Material and method

We performed a single-cell meta-analysis using paired RNA-sequencing/T-cell receptor sequencing of 767,606 T cells from 460 samples spanning 6 cancer types. This approach enabled us to analyze transcriptional and clonal dynamics in patients undergoing checkpoint immunotherapy.

Result and discussion

We identified a robust signature of expanded CD8+ clones that differentiates responders from non-responders. Analysis of persistent clones revealed therapy-induced transcriptional changes, suggesting an improved reinvigoration capacity in responding patients. A gene-trajectory analysis demonstrated changes in the pseudo-temporal state of de-novo clones that are associated with clinical outcome. Lastly, we found that clones shared between tumor and blood are more

abundant in non-responders and execute distinct transcriptional programs.

Conclusion

Our results highlight differences in clonal transcriptional states that are linked to patient response. They provide insights into cellular factors associated with effective anti-tumor immunity, suggesting biomarkers and potential therapeutic targets for monitoring and enhancing therapy efficacy.

EACR25-0241

Genome-wide CRISPR Screening in Embryonic Stem Cells Identifies Synthetic Lethality Interactions in MLH1 and TP53 Deficient Tumors

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Introduction

The genetic complexity and clonal heterogeneity of malignant tumors is a major challenge in the field of cancer therapeutics. A potentially useful tool in personalized oncology is synthetic lethality, a phenomenon where the combination of two genetic alterations leads to cell death, while each of them separately does not.

Material and method

We aimed to identify genetic mutations that have synthetic lethal interactions with either MLH1, involved in DNA mismatch-repair and is commonly mutated in certain types of cancers, or TP53, the most frequently mutated gene in cancer. We thus performed genome-wide CRISPR-Cas9 loss-of-function (LoF) screens in haploid human embryonic stem cells (hESCs) with or without a mutation in the MLH1 or TP53 genes. This screen, targeting all protein-coding genes, allowed us to identify genetic interactions that can lead to cell death in tumors harboring a mutation in MLH1 or TP53.

Result and discussion

The analysis revealed a list of potential hits with EXO1, NR5A2, and PLK2 genes for MLH1, and MYH10 gene for TP53 emerging as the most promising candidate genes. The synthetic lethality effects of these genes were validated either genetically or chemically using small molecule inhibitors. We further validated the effects of SR1848 (which inhibits NR5A2), ON1231320 or BI2536 (which inhibit PLK2), and blebbistatin (which inhibits MYH10) in cancer cell lines with and without LoF mutations in MLH1 or TP53. The selective effect of the small molecule BI2536 on MLH1-null tumors and of blebbistatin on TP53-mutated tumors was further validated in-vivo using animal studies with cancer cell lines xenografts.

Conclusion

Overall, our findings show the potential of genetic screening in haploid hESCs in identifying selective inhibitors and therefore advancing the realm of cancer precision medicine.

EACR25-0270

Characterizing Circulating Tumor Cell and containing Cancer Stem Cell Heterogeneity in Pancreatic Adenocarcinoma: A Single Cell Perspective

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Introduction

Pancreatic ductal adenocarcinoma (PDAC) is a highly aggressive cancer characterized by extensive inter and intratumoral heterogeneity, driving therapy resistance and metastasis. Among the key subpopulations contributing to tumor progression and relapse are circulating tumor cells (CTCs) and contained cancer stem cells (CSCs). CTCs, which detach from primary and metastatic lesions into the bloodstream, serve as a valuable liquid biopsy tool, providing real time insights into tumor diversity and evolution. As intact tumor derived cells, they offer a unique opportunity for comprehensive molecular profiling. CSCs, another rare but critical subpopulation, play a pivotal role in tumor initiation and metastasis, further contributing to PDAC's refractory nature. Recent advances in single cell transcriptomics and microfluidic technologies for isolation have enabled high resolution characterization of these rare cells, paving the way for novel biomarker discovery and targeted therapeutic strategies.

Material and method

CTCs and CSCs were isolated from PDAC patient blood samples using the Parsortix microfluidic platform. Cells were stained with antibodies against CAPRIN1 (cancer marker), CXCR4 (stemness marker), CD45 (leukocyte exclusion), and DAPI (nuclear stain). Single cells were selected via CellCeptor and subjected to transcriptomic profiling. Libraries were prepared using FLASHseq and sequenced on an Illumina NovaSeq X platform (150 bp paired end).

Result and discussion

Single cell transcriptomic analysis identified genes enriched in rare subpopulations with stemness features. Candidate genes were prioritized based on expression levels, public datasets, and literature evidence of involvement in PDAC progression, metastasis, and therapy resistance. qPCR validated candidate gene expression in CTC derived cultures under adherent and spheroid conditions. Functional characterization via siRNA mediated silencing revealed their impact on migration, invasiveness, and proliferation. The most promising candidates will undergo in vivo validation.

Conclusion

We established an effective approach for isolating and profiling of CTCs and CSCs at the single cell level, unveiling distinct molecular features associated with PDAC aggressiveness. Identified candidates may serve as novel biomarkers or therapeutic targets, paving the way for improved precision medicine strategies in PDAC.

EACR25-0314**Comparative analysis of genomic and clinicopathologic characteristics between multifocal and unifocal breast cancer patients by whole-exome sequencing***C. Kim¹, T. Jeon¹*¹*Korea University College of Medicine, Pathology, Seoul, Korea (Republic of)***Introduction**

Multifocal breast cancers (MFBCs) are associated with a higher risk of lymph node metastasis and poorer prognosis compared to unifocal breast cancers (UFBCs). However, there is a lack of comprehensive studies comparing their clinicopathologic and genomic characteristics. This study aims to analyze the differences between MFBCs and UFBCs using whole-exome sequencing (WES) to identify genetic mutations related to tumor invasiveness and progression.

Material and method

A total of 47 breast cancer patients (30 UFBC, 17 MFBC) who underwent surgery in 2018 were selected. Histologic and immunohistochemical characteristics (ER, PR, HER2, Ki-67) were compared between the two groups. WES was performed on tumor tissues, and genetic mutations were identified using variant calling and annotation tools. Clinicopathologic variables, including tumor grade, lymph node metastasis, and hormone receptor status, were statistically analyzed.

Result and discussion

MFBCs exhibited a higher histologic grade, increased HER2 positivity, and a higher proliferation rate than UFBCs. The average age at diagnosis was lower in MFBC patients, and they were more frequently associated with HER2 and triple-negative (TN) subtypes. WES analysis revealed that MFBCs had mutations commonly found in aggressive subtypes, including ERBB2, ERBB3, FGFR4, ZFHX3, and ROS1, which are associated with tumor proliferation and migration. In contrast, UFBCs showed mutations in PIK3CA, FOXA1, and NCOR2, which are more common in hormone receptor-positive tumors. Copy number alterations in MFBCs were more homogeneous within individual patients, suggesting a common clonal origin for multifocal lesions.

Conclusion

This study highlights the distinct clinicopathologic and genomic profiles of MFBCs compared to UFBCs. The findings suggest that MFBCs exhibit genetic alterations that contribute to aggressive tumor behavior and a higher risk of metastasis. The results emphasize the need for personalized treatment strategies that account for the unique molecular characteristics of MFBCs. Further studies with larger cohorts are necessary to validate these findings and improve clinical decision-making for breast cancer management.

EACR25-0317**The use of ultra-low-pass sequencing data to classify tumors' molecular deficiencies***S. Naky¹, Y. Maruvka¹, C. Hawkins², U. Tabori³, L. Negm⁴, R. Yuditskiy⁴*¹*Technion, Biotechnology and Food Engineering, Haifa, Israel*²*SickKids Research Institute, Laboratory Medicine and Pathobiology, Toronto, Canada*³*SickKids Research Institute, Haematology/Oncology, Toronto, Canada*⁴*SickKids Research Institute, Arthur and Sonia Labatt Brain Tumour Research Centre, Toronto, Canada***Introduction**

Genomic instability and hypermutation play a crucial role in tumor development and response to therapy. Two key drivers of these traits are Polymerase Proofreading Deficiency (PPD) and Homologous Recombination Deficiency (HRD), respectively. PPD arises from mutations in DNA polymerase genes, while HRD results from defects in DNA repair pathways, particularly in BRCA1/2 and associated genes. Detecting these deficiencies is essential for patient stratification and targeted treatment. The Low-Pass Genomic Instability Consortium (LOGIC) assembly was employed to develop a computational framework for identifying PPD and HRD from genomic data, leveraging mutational patterns and genome instability scores.

Material and method

To detect PPD, we analyzed somatic mutation profiles using whole-genome sequencing data. We looked into the known distinct mutational patterns associated with POLE/POLD1 deficiency. Our approach utilized meticulous noise filtration and normalization of each pattern by the frequency of each trinucleotide. For HRD detection, we implemented a workflow incorporating loss-of-heterozygosity (LOH), telomeric allelic imbalance (TAI), and large-scale state transitions (LST) scores. These features were derived from Sequenza-based copy number segmentation. The LOGIC assembly was optimized to compute these scores efficiently from low-pass sequencing data, ensuring applicability in clinical and research settings. We validated our approach using known BRCA-deficient tumor samples and compared the performance with existing HRD detection tools.

Result and discussion

Our analysis successfully identified PPD tumors exhibiting high mutational burden and distinct mutational patterns. Our method achieved high sensitivity and specificity in distinguishing PPD tumors from PPD normals and PP-proficient tumor/normal samples (AUROC = 0.9985). For HRD detection, our genome instability scores showed strong concordance with established HRD classifiers, effectively separating BRCA-mutant from BRCA-wildtype tumors (AUROC = 0.874). The integration of TAI, LOH, and LST provided a robust measure of HRD-associated genomic instability. Additionally, LOGIC's low-pass sequencing adaptation retained predictive power while reducing sequencing costs, making HRD testing more accessible.

Conclusion

We present a computational approach for detecting PPD and HRD using the LOGIC assembly. By leveraging mutational patterns and genome-wide instability metrics, our method provides a reliable framework for identifying these genomic deficiencies. The ability to infer HRD and

PPD from low-pass sequencing enhances their clinical applicability. Future work will focus on expanding validation datasets and refining models for lower quality samples (Formalin-Fixed, Paraffin-Embedded, or FFPE).

EACR25-0336

Spatiotemporal multi-omics uncover tumor ecosystem dynamics driving metastatic colonization

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Introduction

The mechanisms underlying the interaction between disseminated tumor cells (DTCs) and their tissue microenvironment during metastasis colonization are poorly understood.

Material and method

We integrated multimodal single-cell and spatial profiling from liver cancer mouse models and human metastases to track the spatiotemporal dynamics of DTCs and their microenvironments across nine sequential time points from single-cell seeding until overt lung metastasis.

Result and discussion

We identified a residual population of quiescent Phgdhhigh DTCs that survive initial innate immune clearance and become transiently enriched in micro-metastases. These cells shape an immune-scarce microenvironment through PHGDH-dependent, H3K27me3-mediated epigenetic silencing of chemokine transcription, promoting metastatic expansion. Cx3cr1high interstitial macrophages were also found to be transiently enriched before DTCs expansion, creating an immune-privileged niche for metastatic outgrowth by recruiting immunosuppressive cells. Inactivating the Phgdh-H3K27me3 axis in DTCs or depleting interstitial macrophages restored immune surveillance and inhibited metastatic colonization. These findings provide new therapeutic concepts for developing micrometastasis-targeting regimens.

Conclusion

In summary, an integrated analysis of this atlas coupled with additional multi-omics data from human patients and *in vitro*, *ex vivo*, *in vivo* validation assays identified key cellular programs transiently activated in disseminated tumor cells (DTCs) or in immune cells that promote metastatic seeding by building an immune-compromised microenvironment. Our results shed light on the spatial and temporal complexity of metastatic colonization, laying a foundation for elucidation of the pathogenic mechanisms and therapeutic vulnerabilities of cancer metastases.

EACR25-0382

Machine learning-derived mutational signatures reveal distinct DNA repair pathway dysregulation and clinical outcomes in BRCA1/2 ovarian cancer

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Introduction

DNA signatures reflecting underlying mutational processes are key for precision medicine, especially in chromosomally unstable cancers (CIN). Cancers with homologous recombination deficiency (HRD) utilize diverse DNA double-strand break (DSB) repair pathways with specific genomic features, including micro-homologies (MH). However supervised methods (e.g. non-negative matrix factorisation) are insensitive to these patterns and may miss clinically important associations between mutation contexts and active DSB pathways and do not predict treatment. We reasoned that explainable cross-modal factor analysis (MOFA) could improve mechanistic associations from WGS data to develop improved clinical biomarkers.

Material and method

Cross-mutational process factor analysis was applied to 483 high-grade ovarian carcinoma (HGOC) deep whole-genome sequencing (WGS) samples from the National Research Genomic Library. Mutation contexts for single base substitutions (SBS), double base substitutions (DBS), small indels, structural variants (SVs) were extracted. A MOFA model with 15 factors was fitted. Factors were compared with clinical parameters (survival status, histotypes [high-grade serous carcinoma [HGSC], clear-cell carcinoma [OCCC], platinum response, sample type], key gene statuses and key COSMIC version 3.4 signatures.

Result and discussion

MOFA uncovered latent factors for multiple clinical and biological features. We identified a factor that distinguishes between Polθ-mediated end-joining (TMEJ) and classical non-homologous end-joining (NHEJ) based on deletion patterns with MH (positive weights for deletions with ≥ 2 bp MH). This factor strongly correlated with alterations in BRCA2, RB1, and MYC, moderately correlated with BRCA1 mutations, and significantly predicted improved survival outcomes ($HR < 1$, $p < 0.05$). In contrast, another factor identified samples with NHEJ/microhomology-mediated break-induced replication (MMBIR) activities and was the composite of small/medium tandem duplications with shorter MH lengths. It positively correlated with BRCA1 deficiency and negatively with BRCA2 deficiency, displaying a trend towards poorer overall survival ($HR > 1$, $p > 0.05$).

Conclusion

MOFA frameworks provide high resolving power for technical, biological, and clinical variability from clinically available WGS data. Cross-modal integration confirmed two distinct error-prone repair phenotypes in HGSC. TMEJ-like correlates with BRCA2 deficiency and better prognosis, whereas NHEJ/MMBIR-like is tied to BRCA1 deficiency and worse outcomes. We are now designing protocols to test how these findings predict treatment efficacy and outcomes in patients in the DENOVA trials framework.

EACR25-0388**Single-cell analysis reveals tumor-driven immune microenvironment reprogramming that promotes lung adenocarcinoma metastasis**

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Introduction

As the leading cause of cancer-related mortality worldwide, the dismal prognosis of lung adenocarcinoma (LUAD) primarily stems from tumor metastasis and recurrence. To address this clinical challenge, our study systematically investigated the molecular mechanisms underlying LUAD invasion and metastasis through single-cell multiomics approaches, with particular emphasis on deciphering the composition and function of different cell populations in the tumor immune microenvironment.

Material and method

Based on data from the GEO database and the Human Cancer Atlas Network database, LUAD single-cell RNA sequencing data was downloaded and standardized for quality control. Various bioinformatics approaches, including cell clustering analysis, survival analysis, enrichment analysis, single-cell trajectory analysis, and cell-cell communication analysis, were employed to investigate how tumor cells in primary and metastatic LUAD lesions influence the tumor immune microenvironment. These analyses provide insights into the mechanisms by which tumor cells create a favorable niche for survival and progression during LUAD metastasis.

Result and discussion

This study constructs a comprehensive single-cell landscape of primary LUAD and metastatic LUAD primary lesions, identifying key cellular subsets involved in tumor progression. Notably, the IGF2BP1⁺ HOXA7⁺ double-positive LUAD subset was found to activate tight junction signaling pathways, promoting LUAD cell migration. Additionally, analysis of immune cell populations revealed that CD8⁺ T cell subsets exhibited an exhausted phenotype, characterized by the expression of exhaustion-related genes and a significant reduction in immunocytotoxic function. Furthermore, abnormal immunosuppressive phenotypes were observed in CD4⁺ T cells and natural killer T cell subsets. These findings suggest that LUAD cells actively reprogram the tumor immune microenvironment within the primary metastatic site, establishing conditions that facilitate tumor survival and metastatic progression.

Conclusion

This study offers a comprehensive perspective on LUAD and its immune landscape, uncovering novel mechanisms of LUAD progression from a unique standpoint. By providing a valuable resource for identifying actionable targets in LUAD research and treatment, this work lays a

strong theoretical foundation for the clinical diagnosis and therapeutic strategies of LUAD.

EACR25-0429**Whole-genome CRISPR-Cas9 screens identify SHOC2 as a genetic dependency in NRAS-mutant melanoma**

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Introduction

Melanoma is the most lethal form of skin cancer, with over 300,000 new cases worldwide every year. In particular, melanoma with NRAS mutations presents a significant clinical challenge due to its association with poor prognosis and the lack of specific treatment options. This highlights the urgent need for novel therapeutic strategies to address NRAS-mutant melanoma. A current approach to identify novel drug targets is based on the genetic concept of induced essentiality, where functional interactions that occur in response to oncogene addiction create a dependency on another gene.

Material and method

Whole-genome CRISPR-Cas9 knockout screens were conducted in 6 NRAS-mutant and 7 NRAS-wildtype melanoma cell lines that were derived from New Zealand melanoma (NZM) patients. These NZM cell lines were stably transduced with the whole-genome Brunello lentiviral single guide (sg) RNA library and screened for up to 35 days. In addition to the NZM whole-genome knockout screens, CRISPR-Cas9 screening data using the Avana sgRNA library from an additional 54 melanoma cell lines, available on the Cancer Cell Line Encyclopaedia (CCLE) database, were analysed using the BAGEL (Bayesian Analysis of Gene Essentiality) program.

Result and discussion

Comparative analysis between the NRAS-mutant and NRAS-wildtype cell lines revealed NRAS and SHOC2 as the top candidates which exhibit greater detrimental effects on the fitness of NRAS-mutant cell lines compared to the NRAS-wildtype cell lines. Further validation through in vitro individual gene knockout studies demonstrated that knockout of SHOC2, a scaffold protein essential for activation of the MAPK signalling pathway, results in the prevention of ERK phosphorylation and a more substantial reduction in cell proliferation in NRAS-mutant NZM cell lines when compared to NRAS-wildtype cell lines.

Conclusion

Our findings support previous studies that have identified SHOC2 as a potential therapeutic target for RAS-driven cancers and suggest that targeting of SHOC2 may have

utility in NRAS-mutant melanoma, where greater treatment options are urgently needed.

EACR25-0437

Molecular Evolution of Driver Mutations in Cancer with Microsatellite Instability and Their Impact on Tumor Progression: Implications for Precision Medicine in Patients with UCEC

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Introduction

Cancer development involves genetic alterations, particularly cancer driver mutations (CDMs), linked to aggressive phenotypes and shorter survival. In contrast, higher mutation loads from mismatch repair deficiency (MMRD) can enhance anti-cancer immunity, leading to tumor shrinkage and improved responses to checkpoint blockade therapies. However, understanding how CDMs and MMRD, influence cancer evolution remains limited.

Material and method

Datasets for ovarian serous cystadenocarcinoma (OVCA), cervical squamous cell carcinoma (CSCC), and uterine corpus endometrial carcinoma (UCEC) were sourced from The Cancer Genome Atlas (TCGA) and the Clinical Proteomic Tumor Analysis Consortium (CPTAC). As CSCC data was unavailable in CPTAC, its analysis relied solely on TCGA. From the OncoKB database, 682 genes were evaluated, of which 297 were identified as driver oncogenes. The analysis was restricted to oncogenes with mutation frequencies greater than 10% in OVCA, CSCC, and UCEC. Mutation counts per patient were calculated, and UCEC patients were categorized into two groups: those with mutation counts above 200 and those with fewer than 200. We selected a panel of UCEC cell lines with different microsatellite instability (MSI) statuses for drug efficacy testing using data from GDSC, CCLE, gCSI, and CTRPv2 databases. Drug efficacy metrics included Area Under the Curve (AAC), Half Maximal Inhibitory Concentration (IC50), and Drug Sensitivity Score (DSS1).

Result and discussion

We opted uterine corpus endometrial carcinoma (UCEC) due to its microsatellite instability (MSI) classification. Somatic mutation screening revealed that UCEC has a significantly higher rate of CDMs compared to ovarian cancer (OVCA) and cervical squamous cell carcinoma (CSCC), despite these cancers arising from histologically related organs in the reproductive tract. Interestingly, these CDMs did not necessarily drive progression. Using a mutation count (MC) cutoff of 200, we classified UCEC patients into two groups with distinct clinical features, genetic profiles, and drug sensitivities. Among the known CDMs, TP53 mutations and their functional networks emerged as key drivers in UCEC progression, while mutations in CTNNB1, PTEN, and ARID1A, may enhance anti-tumor immunity, correlating with longer overall survival. Drug screening using GDSC and

CTRPv2 databases suggested that GSK-3 inhibitor IX might be effective for aggressive UCEC patients with a non-MSI phenotype. Curcumin showed efficacy for MSI-UCEC patients, especially with immune checkpoint blockade therapy.

Conclusion

Our study highlights the importance of immune regulation and tolerance over CDMs in cancer development, particularly in those with an MSI phenotype. We propose that mutation count (MC) could serve as a valuable screening method alongside molecular and histopathological classifications to guide treatment strategies for UCEC patients.

EACR25-0489

Spatial proteomic profiling of tumor microenvironment dynamics in ER-positive breast cancer

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Introduction

Patients with large and aggressive ER-positive/HER2 negative breast tumors are currently treated with neoadjuvant chemotherapy, but the optimal therapeutic regimen is not known. The NeoAva clinical trial investigated the efficacy of combining anti-angiogenic treatment using bevacizumab with neoadjuvant chemotherapy in this patient group. The addition of bevacizumab significantly increased the response rate, but the impact of such treatment on the tumor microenvironment remains largely unknown. This study aimed to identify factors that are important for treatment response and resistance through analysis of the tumor landscape and interplay between cancer cells and surrounding immune and stromal cells using spatial proteomic profiling.

Material and method

Needle tumor biopsies from 13 patients collected at baseline ($n = 13$) and at week 12 ($n = 11$) in the NeoAva clinical trial were profiled using cyclic immunofluorescence (cycIF). Full tissue sections were stained in nine cycles, each with four antibodies and DAPI. The images were processed on the Galaxy platform [1] using the MCMICRO pipeline with Mesmer for single cell segmentation. Epithelial, stromal, endothelial and immune cells were phenotyped by hierarchical gating of functional markers. The immune cells were further

subtyped into CD8+ T-cells, CD4+ T-cells, T-regulatory cells, macrophages, and dendritic cells. A total of 1.1 million cells were identified for which downstream spatial statistics were performed using vector and raster-based analysis.

Result and discussion

At baseline, the dominant cell type across all samples was epithelial cells. Notably, there was no significant difference in CD8+ T-cell fraction or density between responders and non-responders. In responding patients, macrophages exhibited a higher overall fraction ($p = 0.014$) and density ($p = 0.022$), and average minimum distance (AMD) revealed higher co-localization with epithelial cells ($p = 0.035$) compared to those with a poor response. On-treatment samples revealed increased fractions of stromal, endothelial, and immune cells. The density of epithelial cells was significantly higher in non-responders than in responders among these samples ($p < 0.01$), although the treatment did not significantly affect the average density of stromal, endothelial, or immune cells. Furthermore, AMD indicated that CD8+ T-cells were highly co-localized with CD4+ T-cells, macrophages, and dendritic cells in responding patients, suggesting a link between spatial immune cell proximity and therapeutic efficacy.

Conclusion

This study shows that spatial proteomic profiling provides a valuable opportunity to study tumor ecosystem dynamics in the neoadjuvant setting. Our findings demonstrate that the tumor-immune cell spatial structure is an important factor for response to treatment with bevacizumab added to neoadjuvant chemotherapy.

[1] URL: cancer.usegalaxy.org

EACR25-0494

Studying genomic evolution in colonic cell line models of mismatch repair deficiency

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Introduction

Mismatch repair deficiency (MMRD) occurs in ~15% of colorectal cancers (CRC). Its causes can be sporadic or familial (Lynch syndrome). MMRD results in a high burden of single nucleotide variants and indels and microsatellite instability (MSI). MMRD CRC demonstrate profound clonal complexity and evolvability under immune selection. High mutational load translates into elevated neoantigen numbers which is thought to be

the basis of their excellent response to immune checkpoint inhibitors (ICIs). However, for reasons yet unclear, ~50% of MMRD CRC do not respond to ICIs. Thus, improved biomarkers for patient stratification are required. The aim of my project is to understand genomic differences between MMRD genotypes that likely contribute to the variable clinical outcomes.

Material and method

CRISPR-Cas9 was used to knock out four genes implicated in MMR (MLH1, MSH6, MSH3, MBD4) in different combinations in a human colonic epithelium cell line (HCEC). The gene knockouts were validated through DNA sequencing (Sanger and Illumina MiSeq) and Western blotting. The cell lines were subjected to two rounds of subcloning and 3–4 weeks of mutation accumulation, followed by WGS at 30x. The cell doubling time was investigated using live-cell fluorescent imaging and flow cytometry. The WGS dataset was processed using an established bioinformatic pipeline for variant calling and mutational signature discovery. CUT & TAG to profile the distribution of H3K36me3 was conducted on select cell lines and analysed with a published protocol (Zheng Y et al (2020). Protocol.io).

Result and discussion

29 colonic epithelial cell lines were produced that model eight MMRD genotypes observed in clinical setting. Cell cycle profiles and cell doubling times were demonstrated to be similar, irrespective of the genotype. Evidence from Western blot experiments suggested that knockout of one MMR gene in cells does not significantly affect expression levels of other MMR genes. WGS data analysis (in progress) yielded reliable variant counts proving that the cell lines exhibit hypermutation, reproduced characteristic MMRD mutational signatures and provided novel insight into the mutational profile of MSH3. Preliminary CUT&TAG data processing revealed similar H3K36me3 distributions in WT and MSH6 -/- lines and identified peaks of marker abundance that will be used to assess mutations in transcribed genomic regions.

Conclusion

A suite of valuable cell models was established to study genomic impact of MMRD. Further analysis of the WGS dataset obtained through carefully planned rounds of cell line subcloning, as well as the complementary CUT&TAG data, will provide detailed insights into the burden, rate and types of mutation caused by various forms of MMRD. Overall, this work adds to the view of MMRD as a multifaceted genetic phenomenon and the catalogue of mutational signatures which carries potential as future biomarkers.

EACR25-0498

CRISPR-Cas9 knock-out screening of lncRNAs highlights new therapeutic targets for cancer treatment

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Introduction

The genomic landscape of cancer hides numerous alterations in coding and non-coding genes. The molecular dissection of tumors revealed the key role of long-non coding RNAs (lncRNAs) in cancer progression and drug response. lncRNAs are non-coding transcripts longer than 200 nucleotides, which control gene expression at multi-omic levels. They are considered promising biomarkers and therapeutic targets but most of them remain unexplored.

Material and method

We i) performed a CRISPR-Cas9 screen to knock-out (KO) 671 lncRNAs in Multiple Myeloma (MM) cell lines sensitive and resistant to the conventional drug Bortezomib (BZB); ii) developed a bioinformatic prioritization pipeline able to integrate functional data from in vitro screens with patients-derived prognostic and genomics data from public datasets; iii) functionally validated the role of the most promising target RP11-350G8.5 via cellular and molecular studies in vitro and in vivo; iv) structurally characterized RP11-350G8.5 via biophysical assays including Thioflavin T, 1H-NMR spectroscopy and circular dichroism v) applied computational tools for predicting the putative binding regions of selective therapeutics vi) performed pan cancer bioinformatic analyses to identify biomarkers associated with the expression of our candidate lncRNA, and to evaluate its differential expression and prognostic value in other cancer types.

Result and discussion

Our CRISPR-screen revealed 8 lncRNAs whose KO reduced cell viability in MM cells, and that are associated with high expression and poor prognosis in MM patients'. Among them, RP11-350G8.5 emerged as the most promising target for MM also in BZB-resistant models. We highlighted that the loss of RP11-350G8.5 i) reduces tumour cell viability, proliferation and 3D colony-formation in vitro and impairs tumour growth in vivo, ii) induces apoptosis and sensitizes cells to BZB, iii) modulates the unfolded protein response system and triggers an Immunogenic Cell Death. We identified 2 G-quadruplex and 3 hairpin-forming regions and focused on specific 3D conformations for the development of selective drugs. In addition, we revealed RP11-350G8.5 overexpression and correlation with poor prognosis across different cancer types in addition to MM, including kidney renal papillary cell carcinoma, and predicted a genetic signature related to the expression of our candidate lncRNA that may help the choose of anti-tumoral therapeutic regimens.

Conclusion

Our study (Grillone K. et al., Blood 2024) highlighted novel genetic vulnerabilities never associated before to MM, among which the previously uncharacterized RP11-350G8.5 emerged as the most promising therapeutic target. We then moved forward the design of innovative anti-cancer compounds and to the investigation of the oncogenic role of our candidate lncRNA in a pan cancer setting.

EACR25-0519

Advancing Biobanking in Europe to foster Cancer Research

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Introduction

BBMRI-ERIC is the European research infrastructure for biobanking and biomolecular resources in health and life sciences. BBMRI-ERIC encompasses 24 Members and Observer countries and IARC/WHO; comprising 25 National Nodes, 473 biobanks and affiliated partners. BBMRI-ERIC enables the development of innovative technology and processes as transnational European infrastructure that facilitates responsible access to high quality samples, data and biomolecular resources.

Material and method

This is facilitated through services and advancements in the areas of Ethical, Legal and Societal Issues (ELSI), Quality Management (QM), Information Technology (IT), Biobank Development (BBD), Public Affairs (PA), Outreach, Education & Communications (OEC), and Finance- and Project Management (FPM).

Result and discussion

Hereby, BBMRI-ERIC engages its community and partners in more than 23 active EU projects, including various projects for advancing cancer research that are strongly aligned with Cancer Mission and the EU Cancer beating plan namely, canSERV, EOSC4Cancer, EUCAIM, and the newly granted UNCAN projects. Our federated access and analysis platform has become a strong asset and reference point for major EU initiatives (e.g., EHDS, 1+Mio, GDI, EUCAIM) and helps scientists and clinicians to accelerate their personalised medicine research: BBMRI's fully GDPR-compliant pipeline enables access to samples and data of over 7 Mio. patients facilitated across 32 countries. The data comprise 7,834 distinct OMOP variables with biochemical measurements plus different types of OMICs data including e.g., over 150,000 genomes.

Conclusion

We will showcase how to access and make use of the richness of BBMRI-ERIC's resources and how to collaborate with our wider community on different topics through in-house or EU funding – locally, nationally or EU wide to foster cancer research.

EACR25-0522

Functional exploration of the molecular mechanism of the regulation factors which are related to the tumorigenesis and prognosis of colorectal cancer

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Introduction

Colorectal cancer (CRC) is the third most prevalent malignancy worldwide and holds the second highest mortality rate among cancers, as reported by the International Agency for Research on Cancer in 2022. Our laboratory has developed a comprehensive functional genomics database from 104 Taiwanese CRC patients using next-generation sequencing (NGS) technology. This database has facilitated the identification of a 13-gene signature that is predictive for both diagnostic and prognostic applications in CRC. Notably, one gene, DLG5, exhibited a positive correlation with tumor progression and distant metastasis. Elevated levels of DLG5 were associated with reduced survival and increased recurrence rates, suggesting its potential role as an oncogene and a biomarker for CRC prognosis and diagnosis. DLG5 is a member of the MAGUK family, which is crucial for maintaining cell polarity through the formation of the SCRIB-LGL-DLG complex. However, the role of DLG5 in CRC remains unexplored.

Material and method

Employed siRNA to downregulate DLG5 expression in HCT116 CRC cells. To perform proliferative test, we conduct colony formation assay. To perform cell migratory test, we conduct migration and invasion assay. Further investigations were conducted using NGS, quantitative PCR, and Western blot analyses to delve into the potential regulatory mechanisms.

Result and discussion

Downregulate DLG5 reduced the proliferative and migratory abilities, which supports its oncogenic function. Preliminary analyses suggest that DLG5 may influence several pathways including epithelial-mesenchymal transition (EMT), extracellular matrix organization, and immune-related pathways. These findings indicate a multifaceted role of DLG5 in CRC pathogenesis. Further experimental studies are necessary to elucidate the detailed mechanisms by which DLG5 contributes to CRC progression and to assess its viability as a therapeutic target. This study underscores the potential of DLG5 as an oncogenic factor in CRC and as a candidate for clinical biomarker development. By investigating its regulatory pathways, we aim to highlight new therapeutic avenues for CRC treatment.

Conclusion

1. Through the integration of functional genomic data and clinical analysis from a Taiwan-based CRC cohort, our study identifies DLG5 as a significant biomarker for poor prognosis and distant metastasis.
2. Inhibition of DLG5 expression significantly reduces the proliferative and migratory abilities of CRC cells.
3. Transcriptome profiling reveals that DLG5 may participate in several pathways, including EMT, extracellular matrix organization, and immune-related pathways.
4. Validation of DLG5's potential downstream targets was performed through Western blotting and quantitative

PCR.

5. Further experimental studies are necessary to elucidate the detailed mechanisms by which DLG5 contributes to CRC progression and to assess its viability as a therapeutic target.

EACR25-0576

Non-canonical ORF Translation in Neuroblastoma: A New Source of Therapeutic Targets?

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Introduction

Neuroblastoma (NBL) remains one of the most devastating childhood cancers, with high-risk patients facing survival rates below 50% despite aggressive therapy. The biological complexity of NBL characterized by low mutation burden and frequent MYCN amplification severely limits conventional immunotherapy approaches. Despite advances with anti-GD2 antibody therapy, resistance mechanisms and toxicity concerns underscore the urgent need for alternative therapeutic targets. The translation of non-canonical open reading frames (ncORFs) represents an untapped reservoir of putative intracellular proteins that may play roles in neuroblastoma biology or may arise from cancer-specific translation mechanisms, offering unique advantages for targeted therapy.

Material and method

We conducted comprehensive transcriptomic and translational profiling across patient tumors, cell lines, and organoid models. Our integrated approach combining RNA-seq with Ribosome profiling (Ribo-seq) allowed us to distinguish actively translated regions from merely transcribed sequences. Differential expression analysis between NBL and normal tissue samples enabled the identification of NBL-enriched ncORFs, whereas separation of patient samples or models by driver mutations, such as MYCN, helped nominate ncORFs possibly important for specific NBL subtypes.

Result and discussion

Our analysis revealed substantial translation of non-canonical ORFs in neuroblastoma, nearly doubling the total number of ORFs presented in NBL-enriched genes. These might represent promising (immuno)therapeutic targets due to their tumor-specific expression patterns. The integration of RNA-seq and Ribo-seq highlighted that many non-canonical ORFs would remain undetected using conventional transcriptome analysis alone.

Conclusion

By expanding the targetable proteome beyond conventional protein-coding genes, this approach opens new avenues for therapy development against an unexplored class of coding sequences. Ongoing functional characterization of promising candidates is further validating their potential, potentially revolutionizing treatment strategies for children with this aggressive malignancy.

EACR25-0610

A Cost-Effective Droplet-Based Single-Cell Total RNA Sequencing Technology Reveals Transcriptional Heterogeneity in Drug-Tolerant Cancer Cells

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Introduction

Drug resistance remains a major hurdle in non-small-cell lung cancer, driven by mechanisms such as cellular plasticity, transcriptional heterogeneity, and genetic mutations that enable tumor cells to evade targeted therapies. Recent studies highlight the importance of cell-to-cell transcriptional heterogeneity in drug-tolerant persistor (DTP) cells, yet conventional scRNA-seq methods capture only polyadenylated RNAs, overlooking crucial non-polyA transcripts. To address this, we developed sc-rDSeq, a cost-effective, droplet-based full-length total RNA sequencing method that profiles both polyA+ and polyA- RNAs, enabling a comprehensive view of transcriptomic changes in DTP cells.

Material and method

We applied sc-rDSeq to profile 7,051 PC9 cells, subjected to 9-day high-dosage (10X IC50) treatments with EGFR inhibitors Gefitinib or Osimertinib, or DMSO controls. Cells were co-encapsulated with barcoded hydrogel beads for strand-specific total RNA capture. Differential gene expression, cell cycle analysis, and alternative splicing events, chromosomal aberration (CNV) and single nucleotide variants (SNVs) were analyzed.

Result and discussion

In benchmarking against polyT-based inDrops, sc-rDSeq demonstrated a tenfold increase in unique molecules detected per cell, capturing polyA- RNAs. This enhanced sensitivity revealed four distinct clusters within naïve PC9 cells, usually considered homogeneous by conventional scRNAseq. sc-rDSeq of DTP cells showed G1 arrest, confirmed by cell cycle markers and elevated non-polyA histone mRNAs. Despite similar CNV backgrounds, we uncovered divergent resistance pathways in seven clusters, including metabolic reprogramming, epithelial-mesenchymal transition, and extracellular matrix remodeling. Differential expression of miRNA and enhancer RNA correlated with these clusters. Notably, sc-rDSeq detected isoform switching, such as MDK-204 (truncated midkine) linked to aggressive phenotypes. SNVs played a minor role in transcriptional heterogeneity, suggesting non-mutational mechanisms drive early drug persistence.

Conclusion

Our study highlights the power of sc-rDSeq in dissecting the full transcriptomic landscape of drug persistor cells, revealing novel non-polyA RNA signatures, alternative splicing and SNVs linked to drug resistance. These findings provide new insights into adaptive responses beyond genetic mutations, offering potential targets for combination therapies. The high-throughput scalability, high sensitivity and cost-effectiveness, with a library preparation cost of \$0.08 per cell, makes sc-rDSeq as a

valuable tool for basic cancer research and personalized cancer medicine.

EACR25-0617

Integrative Analysis Reveals RNF135 as a Prognostic and Immunological Biomarker in Glioblastoma

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Introduction

Glioblastoma (GBM) is among the most aggressive primary brain tumors, despite advances in surgical and adjuvant therapies, GBM patients often surviving less than two years after diagnosis. The exploration of new therapeutic targets and novel genes to alter the prognosis of GBM patients holds significant importance. Here, we identified RNF135, a ring-finger protein-encoding gene, as a potential immune-related biomarker and therapeutic target in GBM.

Material and method

We integrated high-throughput RNA-sequencing data from multiple public databases, including The Cancer Genome Atlas (TCGA), Chinese Glioma Genome Atlas (CGGA), and the IVY Glioblastoma Atlas Project. Bulk transcriptomic data were used for survival analyses and gene function analyses. Immune infiltration was assessed using MCP-counter, xCell and ssGSEA. Single-cell RNA-sequencing data from GSE131928 were utilized to provide cellular resolution of RNF135 expression among tumor-associated macrophage (TAM) subtypes, while bulk RNA-sequencing data from the IVY Glioblastoma Atlas were used to validate its spatial distribution within distinct anatomical regions of GBM. Drug sensitivity analysis was conducted to explore effective treatment options for the RNF135-high patient group.

Result and discussion

High RNF135 expression was strongly associated with poor overall survival. Immune deconvolution analyses revealed that RNF135-high tumors exhibit increased infiltration of T cells, NK cells, monocyte-derived macrophages, and myeloid-derived suppressor cells (MDSCs). Notably, single-cell RNA sequencing data showed that RNF135 is enriched in aggressive TAM subsets, specifically monocyte-derived TAM and proliferative TAM, suggesting a role in immuno-suppression and tumor angiogenesis. Validation using the IVY Glioblastoma Atlas confirmed that RNF135 is predominantly expressed in highly vascularized tumor niches, particularly in microvascular proliferation (CT.mvp) and highly vascularized (CT.hbv) regions. Gene set enrichment analysis (GSEA) demonstrated significant enrichment of inflammatory and oncogenic pathways, including KRAS, IL6-JAK-STAT3, and TNFalpha-NFKappaB, which support RNF135's involvement in tumor progression. Finally, drug sensitivity analyses indicated that RNF135-high tumors may be more responsive to MEK inhibition (e.g., selumetinib), aligning with the observed enrichment of KRAS-driven pathways.

Conclusion

Our study identifies high RNF135 expression in GBM is strongly associated with poor prognosis, increased

immune infiltration, and an immunosuppressive tumor microenvironment. RNF135 could serve as both prognostic marker and potential target for precision therapy. Further comprehensive experimental validation and clinical investigations are warranted to explore RNF135-targeted therapeutic strategies in GBM.

EACR25-0638

Spatial transcriptomics in precancerous lesions of the colorectum

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Introduction

Colorectal cancer (CRC) develops through two main pathways: the traditional pathway, which contributes to 70% of colorectal cancers, and the serrated pathway, which accounts for 15% to 30% of CRCs. The lesions in the serrated pathway are mostly hyperplastic polyps (HP), followed by sessile serrated lesions (SSLs), and few traditional serrated adenomas (TSA). TSAs and SSLs have a higher risk of progressing to cancer.

Distinguishing the serrated lesions can be difficult, although very important to handle the patient in the most appropriate way. Another defy is the identification of dysplasia in SSL, which can be difficult to distinguish, yet of great diagnostic importance. A better understanding of the developmental processes in the serrated lesions of the colorectum is necessary to develop better clinically applicable diagnostic tools.

Material and method

Spatial transcriptomic analyses were performed on formalin-fixed paraffin-embedded tissue sections from selected lesions using the GeoMx® Digital Spatial Profiler from NanoString Technologies. Here, we analysed 8 SSLs, 3 TSAs, 2 HPs, 4 conventional adenomas and 2 normal tissues. Regions of interest were selected from both the base and surface of colonic crypts.

Result and discussion

When comparing gene expression between conventional and serrated lesions, the later showed significant higher expression of genes involved in colorectal epithelial cell differentiation, such as CLCA4, GUCA2A, GCG, CA1 and AQP8. In parallel, we found ADAM15 to be significantly overexpressed in the serrated lesions – independently of the presence of dysplasia, compared to the conventional lesions. ADAM15 – coding for an enzyme that is part of the disintegrin-like and metallo-proteinase family, has been shown to be overexpressed in colon cancer tissues and its overexpression led to a worse overall survival in CRC patients. We also found genes that were differently expressed between the different serrated lesions. We next focused on the SSLs, comparing lesions with and without dysplasia. Dysplastic SSLs were found associated with overexpression of genes involved in cell proliferation (e.g. TIMP1, S100A4, ID1, TM4SF4), and anti-microbial responses (e.g. LCN2, PI3, SLP1). Furthermore, we found differences in gene expression between the base and surface of the crypts.

Conclusion

Using spatial transcriptomics, we were able to identify unique genes that characterize different types of pre-cancerous serrated colorectal lesions. Further studies are needed to explore the potential of these candidate genes as clinical biomarkers.

EACR25-0673

Functional diversity of the TP53 mutational landscape revealed by saturating genome editing

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Introduction

The tumor suppressor gene TP53 is the most frequently mutated gene in cancer, with mutations present in approximately half of all tumors. Unlike other tumor suppressors, TP53 is primarily affected by missense mutations, leading to the production of a full-length protein with single amino acid substitutions. The mutational landscape is highly diverse, with over 2,000 distinct missense mutations with varying effects on the functionality of the protein identified in patients. This functional heterogeneity complicates precision oncology, where a detailed understanding of mutation-specific consequences is essential for patient stratification and targeted therapy.

Material and method

To systematically map the functional impact of TP53 mutations, we performed a deep mutational scan using saturation genome editing (SGE) with CRISPR-mediated homology-directed repair. This approach allowed the precise engineering of 9,225 variants in the endogenous TP53 locus of cancer cells, preserving native regulatory elements and physiological expression levels. We assessed variant abundance in response to specific p53-pathway stimulation, generating high-resolution functional activity maps covering approximately 94.5 % of all cancer-associated missense mutations.

Result and discussion

Our approach provides an unprecedented resolution in differentiating benign from pathogenic TP53 variants. By directly introducing mutations in the endogenous TP53 locus we identified previously misclassified loss-of-function variants with subtle phenotypic effects, some of which may be promising candidates for pharmacological reactivation. Additionally, we uncovered the roles of splicing alterations and nonsense-mediated mRNA decay in TP53 dysfunction, offering new insights into mutation-driven regulatory mechanisms.

Conclusion

The CRISPR-based SGE approach significantly improves the clinical interpretation of TP53 variants, enabling improved discrimination between benign and pathogenic mutations. By providing a comprehensive functional dataset with high predictive value, sensitivity, and specificity, this study supports the refinement of variant classification. These findings highlight the potential of SGE to advance genetic counseling and personalized cancer therapy by enabling more accurate TP53 variant assessment.

EACR25-0674

Cardiac metastasis: From spatial transcriptomics to targeted therapy

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Introduction

Cardiac metastases are currently rare, but their incidence is expected to increase in the upcoming years due to improved survival of cancer patients. Unspecific cardiac symptoms and anatomical location prevent timely diagnosis and biopsy collection for molecular studies. Thus, cardiac metastases remain poorly characterized and current therapies are limited to surgery and adjuvant chemo- and radiotherapy.

Material and method

Spatial transcriptomics was performed on formalin-fixed paraffin-embedded sections from samples of primary tumor, cardiac metastasis and extra-cardiac metastasis, collected from three patients affected by different primary tumors. Candidate genes were silenced/over-expressed in human cancer cell lines for functional characterization, including mechanical stimulation with cell confiner and stretcher to mimic compressive and tensile cardiac forces. In addition, Engineered Heart Tissues (EHT) were used to reproduce the 3D structure of cardiac tissue, casting iPSC-derived cardiomyocytes together with tumor cells.

Result and discussion

Differentially expressed gene (DEG) analysis between tumor samples revealed 3095 dysregulated genes in

tumor cells of cardiac metastases. Unsupervised hierarchical clustering showed sample clusterization based on the tumor location (cardiac vs extra-cardiac tumors), regardless of the origin of the primary tumor, suggesting that various types of cancer cells share a common transcriptional signature once they have reached the cardiac tissue. Several of the upregulated genes encode for cytoskeletal proteins, including keratins, tubulin and actin subunits. Keratin-36 (KRT-36), a major constituent of intermediate filaments of hair and nails, stands out as one of the most upregulated genes. Lung and breast cancer cells overexpressing KRT-36 proliferate more than their wild-type control in basal conditions. Whereas wild-type cells display reduced proliferation in response to mechanical stimuli that mimic cardiac load, KRT-36-overexpressing cells retain their proliferative capability. This suggests that high levels of KRT-36 create a cytoskeletal shield that protects the nucleus and confers resistance to mechanical stimuli, eventually allowing tumor cell proliferation in the beating heart.

Conclusion

This study points to KRT-36 as a potential therapeutic target for cardiac metastases. In addition, we propose the repurposing of existing chemotherapies that target cytoskeletal filaments for tumor cell re-sensitization to the anti-proliferative effect of cardiac mechanical load, thus offering the first targeted therapy for cardiac metastasis.

EACR25-0742

Transcriptomic Signatures and Fusion Landscape in Relapsed B-ALL: Insights from RNA-Sequencing Analysis

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Introduction

B-cell acute lymphoblastic leukemia (B-ALL) is a genetically heterogeneous disease characterized by various genetic aberrations including fusions, mutations, and gene expression profiles. Despite significant advancements in therapy, nearly 20% of B-ALL cases relapse or develop resistance, underscoring the need for novel biomarkers to refine risk stratification. Indian B-ALL patients exhibit an aggressive disease phenotype, with a higher prevalence of high-risk genetic features and a lower frequency of favorable-risk markers. This study aims to characterize differentially expressed genes (DEGs), pathway dysregulation, and fusion events associated with B-ALL relapse.

Material and method

RNA sequencing was performed on 56 B-ALL samples (21 relapsed, 35 non-relapsed). Differential expression analysis was conducted using DESeq2 ($|\log_{2}FC| > 1.5$, $FDR < 0.05$) to identify DEGs. Gene fusions were detected via STAR-Fusion and confirmed by RT-PCR followed by Sanger Sequencing. Functional enrichment

analysis was performed using clusterProfiler (KEGG database).

Result and discussion

We identified 197 differentially expressed (DE) RNAs (43 upregulated, 154 downregulated) in relapsed B-ALL. Key upregulated genes included PRAME ($\log_{2}FC = 3.1$, $FDR = 0.02$), a cancer/testis antigen linked to immune evasion, SPP1 ($\log_{2}FC = 3.1$, $FDR = 0.002$), involved in chemoresistance/tumor progression, LAMB4 ($\log_{2}FC = 3.5$, $FDR = 0.001$; extracellular matrix) and LMTK3 ($\log_{2}FC = 2$, $FDR = 0.02$), a kinase involved in tumor progression/chemoresistance. IFI27 (associated interferon response) and ESAM (angiogenesis) were also elevated. KEGG pathway analysis revealed enrichment in PI3K-Akt signaling and focal adhesion, implicating these pathways in relapse-driven mechanisms. A total of 61 long non-coding RNAs (lncRNAs) were differentially expressed, notably LINC00922 ($\log_{2}FC = 3.3$, $FDR = 0.014$), which is associated with cell proliferation and invasion and was observed to be significantly upregulated in relapsed cases. Additionally, three microRNAs (miRNAs) were downregulated, including miR-26a-2 ($\log_{2}FC = -1.7$, $FDR = 0.02$), a tumor suppressor implicated in cancer progression. Fusion analysis identified ETV6::RUNX1, KMT2A::AFF1, DUX4::IGH, FOCAD::EBF1 (novel fusion), IKZF1::CIITA, TCF3::ZNF384, and PAX5::ZNF521 in relapsed cases. In contrast, non-relapsed cases harbored BCR::ABL1, EPOR::IGH, and PAX5::ETV6 fusions.

Conclusion

Our study reveals distinct transcriptomic signatures and fusion landscapes in relapsed B-ALL. Upregulation of PRAME, SPP1, and LAMB4, alongside dysregulated PI3K-Akt and focal adhesion pathways, suggests potential mechanisms driving therapy resistance. The altered expression of lncRNAs and miRNAs further highlights regulation mechanisms in relapse. These findings underscore the need for transcriptome-based biomarkers, in addition to characteristic fusions, to enhance relapse prediction in B-ALL.

EACR25-0768

Identification of therapeutic targets and dependencies in ZNF703-amplified breast cancer

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Introduction

Focal amplifications of chromosome 8p11.23, likely precipitated by oestrogen-induced chromosomal instability, are recurrent in ~15% of breast cancer,

defines the IntClust6 subtype, and portends poor prognosis with high risk of late relapse and chemotherapy resistance. ZNF703 is the most frequently amplified gene and the putative driver oncogene within this locus, though mechanisms are poorly understood and targeted therapies are unavailable.

Material and method

To identify therapeutic vulnerabilities for ZNF703-amplified breast cancers, whole-genome CRISPR-Cas9 screens were performed in isogenic cell lines with modulated ZNF703 expression as well as ZNF703-amplified breast cancer cells. Top hits were further validated in targeted CRISPR-Cas9 competition assays using orthogonal guide RNAs. To further understand the mechanisms of ZNF703-driven growth, the ZNF703-dependent interactome and transcriptome were characterised using rapid immunoprecipitation mass spectrometry of endogenous proteins (RIME) and RNA sequencing of the aforementioned cell line models.

Result and discussion

We report that ZNF703 amplification is synthetic lethal with loss of OTUD5, a regulator of DNA double strand break repair and interferon signalling. Furthermore, ZNF703 overexpression favours error-free repair of CRISPR-Cas9-induced double strand breaks and confers increased dependency on homologous recombination. Interactomic and transcriptomic profiling suggests ZNF703 binds to transcriptional repressors and interferon-related transcription factors, resulting in down-regulation of interferon response. Notably, under-expression of interferon response genes is associated with dependency on OTUD5 and BRCA1 in a tumour agnostic dataset of 1066 cancer cell lines (DepMap 24Q2).

Conclusion

This is the most comprehensive study to date of ZNF703-driven tumorigenesis, where we report the first characterisation of the ZNF703 interactome and associated dependencies. Together, the findings propose that ZNF703 facilitates cell growth under chromosomal instability, and antagonism of this function by OTUD5 loss represents a promising therapeutic strategy.

EACR25-0803

Genetic Profiling of Homologous Recombination Repair Pathway Alterations in Ovarian Cancer patients' cohort from Serbia

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Introduction

Among all gynecological cancers, ovarian cancer (OVCA) has the highest mortality rate. It develops and evolves as a result of acquiring genetic and epigenetic changes, and it is frequently identified when it is too late to receive an effective treatment. The vast majority of ovarian cancer cases is due to mutations in BRCA genes, involved in homologous recombination repair pathway. HR-deficient (HRD) cells usually rely on poly (ADP-ribose) polymerase (PARP) single-strand repair pathway.

The clinical usage of PARP inhibitors that cause synthetic lethality in HRD cancer cells has a significant impact on ovarian cancer therapy techniques. Therefore, identification of a predictive biomarker, other than BRCA mutation, is critical for the selection of patients who are most likely to benefit from these agents. The aim of this study was to obtain information about genetic alterations in genes involved in HRR pathway, beside BRCA, as it is thought that patients harbouring mutations in those genes can benefit from the same type of therapy.

Material and method

The study cohort included ovarian cancer patients who were tested on BRCA mutations at IORS from 2016 until June 2023., as a part of routine diagnostic procedure, as well as on BRCA promoter methylation status. One subgroup had a result of genetic screening from blood with panel that included other genes beside BRCA. Genetic material is extracted using commercial kits for isolation (QIAGENE®). For screening of HRR genes and library preparation we used QIASeq Targeted HRR Panel (QIAGENE®), we used 100-200ng of extracted DNA for library construction. Panel consisted of 2303 primers covering 15 HRR genes. Sequencing was carried out on Illumina NextSeq550Dx instrument using MidOutput sequencing kit in 150bp PE mode. We applied 10% Variant Allele Frequency and 500x coverage as cut-off.

Result and discussion

Tested cohort consisted of 38 newly diagnosed and 58 relapsed cases of OVCA. Of 96 tested samples, in 21 BRCA1/2 pathogenic mutations were found, 3 BRCA1/2 VUS alterations, and 72 were characterized as BRCA wild type. In germline testing, beside BRCA alterations, we found 3 ATM VUS, 1 CHEK2 likely pathogenic and 1 PALB2 pathogenic variant. Germline testing results were used as a control for Targeted HRR panel. Results of BRCA methylation status were used for potential discovering of co-existing epigenetic and genetic alterations that can possibly cause HRD when combined in cell. By testing other HRR genes we got additional information on OVCA etiology, needed for proper understanding of its origin and, in future, potential expanding of group of patients who can take PARPi treatment.

Conclusion

Genetic sequencing extended to other HRR genes, in combination of previously gained genetic and epigenetic screening, gave us additional picture about ovarian cancer etiology important for broadening the group of patients who can gain PARPi therapy.

EACR25-0832

Recurrent endocervical polyps harbor UBE2A p.(Arg6Trp) mutation

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Introduction

Endocervical polyps are overgrowths of cervical glands and stroma that protrude into the cervix. These common tumor-like-lesions affect 2-5% of adult women and exhibit 3-11% recurrence rate. Although endocervical polyps are generally benign, 0.1-1.4% may undergo

malignant transformation. Typically, these polyps are less than 20mm in size and often asymptomatic; however, they can cause intermenstrual, postcoital, and postmenopausal bleeding. Despite their high prevalence, the etiology of endocervical polyps remains poorly understood.

Material and method

In this study, we present a 43-year-old woman with recurrent endocervical polyps. Over a period of nine years, five endocervical polyps were removed from the patient during five separate gynecological visits. The histology of all five polyps was characteristic of endocervical polyps and no endometrioid components were present. As there are no known drivers in endocervical polyps, we analyzed the known endometrial polyp alterations: HMGA1 and HMGA2 expression levels were evaluated with immunohistochemistry, and alterations in KRAS and UBE2A with Sanger sequencing.

Result and discussion

Sanger sequencing revealed that all polyps harbored the same UBE2A c.16C>T, p.(Arg6Trp) missense mutation. Analysis of the blood sample confirmed the somatic origin of the mutations. While UBE2A mutations have not been reported in endocervical polyps previously, the same UBE2A p.(Arg6Trp) mutation has been identified recurrently in endometrial polyps. Mutation was predicted likely pathogenic based on REVEL (score 0.56) and CADD (score 24.4) assessments. The presence of the exact same mutation in both endometrial and endocervical polyps supports UBE2A as a novel driver gene in gynecological polyps. None of the polyp samples harbored KRAS or HMGA1/2 alterations.

Conclusion

We identified UBE2A c.16C>T, p.(Arg6Trp) mutation in five consecutive endocervical polyps. This finding suggests that UBE2A is a new driver gene in gynecological polyps. Further studies are required to determine the prevalence of UBE2A mutation in endocervical polyps, and to elucidate the tumorigenic mechanisms by which these mutations contribute to polyp development. Systematic, large-scale molecular analyses are needed to define the complete molecular background of endocervical polyps.

EACR25-0848

Core2Edge: A Human Glioblastoma Organoid and Brain Slice Co-Culture Model Capturing Tumor Infiltration and Transcriptional Heterogeneity from Core to Single-Cell Dispersion

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Introduction

Glioblastoma functions as intricate cellular networks that extend into the surrounding brain tissue enabling long distance communication. This malignant connectivity spans from the tumor core to remote infiltration zones, supporting the concept of glioblastoma as a whole-brain disease. With growing ethical considerations in biomedical research and the limitations inherent in cross-species comparisons between animal studies and human glioblastoma models, human tumor platforms remain constrained in their ability to replicate the full infiltration spectrum from the tumor core to single-cell dispersion.

Material and method

To address these challenges, we have developed Core2Edge, a three-dimensional, fully human ex vivo glioblastoma model that replicates extensive tumor infiltration while maintaining the intratumoral heterogeneity of patient-derived tumors. The model is established by implanting human glioblastoma organoids (GBOs) into organotypic human brain slices, preserving the genetic integrity and cytoarchitecture of both brain tissue and tumor. Once glioblastoma organoids are prepared, the protocol takes approximately 7–12 days. Key steps include brain slice preparation (~4–6 hours, depending on quantity), one day for initial culture before GBO staining and implanted, a variable culture period (up to ten days), and fixation (~2 hours). Additional time is required for downstream analyses, including imaging, sequencing, and proteomics.

Result and discussion

The Core2Edge model successfully recapitulates the full infiltration range of glioblastoma, from densely packed tumor regions to dispersed single-cell infiltration, addressing a critical limitation of previous human glioblastoma models. By preserving tumor heterogeneity, the model enables in-depth analysis of glioblastoma subpopulations, cell-cell interactions, and the role of the tumor microenvironment (TME) in supporting tumor invasion. The combining of light-sheet fluorescence and expansion microscopy allows for the visualization of tumor cell behavior, invasive front dynamics, and glioblastoma-stroma interactions in high detail.

Furthermore, this system serves as a drug screening and therapeutic testing platform, reducing the reliance on animal models while providing a human-relevant environment for preclinical investigations.

Conclusion

The Core2Edge model represents a significant advancement in glioblastoma research, bridging the gap between traditional *in vitro* models and *in vivo* studies. By maintaining the full spectrum of glioblastoma infiltration and preserving tumor heterogeneity, this model provides a powerful tool for studying tumor invasion, and micro-environment interactions. Additionally, the ability to conduct high-resolution imaging and molecular analyses makes Core2Edge a valuable platform for personalized medicine and drug development.

EACR25-0856

Deciphering the Timing of Somatic Driver Mutations in Early-Onset Breast Cancer

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Introduction

Breast cancer is a heterogeneous disease characterized by distinct molecular subtypes, genetic alterations, and varying clinical outcomes. Among these, early-onset breast cancer (EOBC), typically defined as breast cancer diagnosed before the age of 40, presents unique biological and clinical challenges compared to late-onset cases. EOBC is often associated with more aggressive tumor subtypes, higher genomic instability, and an increased likelihood of hereditary predisposition, particularly involving germline mutations in BRCA1 and BRCA2. However, beyond hereditary factors, the precise evolutionary trajectory of somatic driver mutations in EOBC remains poorly understood.

Material and method

We performed whole genome sequencing (WGS) analysis on 100 early-onset breast cancer (EOBC) patients and 76 conventional breast cancer (CBC) patients diagnosed after the age of 45. Somatic alterations, including single nucleotide variants, insertions/deletions, and structural variants, were identified using the Hartwig Medical Foundation WGS pipeline. Tumors were classified as whole genome duplication (WGD)-positive if more than 10 autosomes had a major allele copy number greater than 1.5. Sub-clonal tumor structures were analyzed using DPclust, and copy number (CN) segment timing was determined using GRITIC and AmplificationTimeR. Mutation timing analysis was conducted with MutationTimeR.

Result and discussion

We observed that the prevalence of whole-genome duplication (WGD) is significantly higher in CBC compared to EOBC, as supported by CN signature analysis. Additionally, EOBC exhibited a higher proportion of early clonal mutations. While differences in the somatic mutation spectrum were relatively minor, the timing of somatic driver mutations varied significantly between EOBC and CBC. Notably, key driver alterations in genes such as TP53, CCND1, ERBB2, and MYC occurred earlier in EOBC. Interestingly, we also found that WGD occurred earlier in EOBC. Finally, the time interval between the most recent common ancestor (MRCA) and WGD was significantly shorter in CBC (3.7 years) compared to EOBC (6.3 years), suggesting that WGD may play a role in promoting oncogenesis.

Conclusion

Our findings highlight distinct evolutionary trajectories between early-onset breast cancer (EOBC) and conventional breast cancer (CBC). EOBC is characterized by earlier acquisition of key driver mutations and WGD, whereas CBC exhibits a higher prevalence of WGD and a shorter interval between the most recent common ancestor (MRCA) and WGD. These differences suggest that WGD may contribute differently to tumor progression in EOBC and CBC, providing insights into

the underlying biology of breast cancer across age groups.

EACR25-0907

Single-Cell Multi-omics with InTraSeq Uncovers Novel Insights into Th17 Differentiation

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Introduction

Single-cell RNA sequencing (scRNA-seq) has advanced our understanding of cellular heterogeneity, but it offers an incomplete view of function by focusing on mRNA. Cellular phenotypes are determined by biomolecule interplay, including proteins, which are often modified and crucial in signaling. We developed InTraSeqTM, a multimodal assay measuring mRNA and proteins in single cells. This integrated approach allows comprehensive characterization of cellular states and regulatory mechanisms.

Material and method

The InTraSeqTM 3' assay profiles the transcriptome and proteome in single cells. Using a buffer kit (CST #82906) and cocktail (CST #48167), InTraSeqTM processing requires one hour of benchwork over three days (Figure 1A, Supplementary Protocol 1). Briefly, cells are fixed in Methanol, stored at -20°C for up to seven days. Following fixation, cells are incubated with "scBlock" buffer and immunostained with antibody-oligo conjugates for 16 hours. On the third day, cells are washed before scRNA-seq using the 10X Genomics 3' assay. For this study, naïve CD4+ T cells from mouse spleens and lymph nodes were cultured under Th0, non-pathogenic Th17, pathogenic Th17, or stimulated with PMA and Ionomycin (PMA/IO). Cells were collected at 0 minutes (naïve), 10 minutes, 45 minutes, 6 hours, and 24 hours post-stimulation (Figure 3A). The InTraSeq Th17 dataset profiled RNA and protein in 83,772 cells across 16 samples.

Result and discussion

InTraSeqTM profiled mRNA and proteins, including surface markers, signaling molecules, and post-translational modifications, in single cells. Applying InTraSeqTM to Th17 cell differentiation revealed insights into this process. We identified regulatory factors and target genes, observed protein expression changes correlated with differentiation, and uncovered post-translational modifications associated with cell states. Our results demonstrate InTraSeqTM's power to dissect cellular processes and uncover regulatory mechanisms by providing a comprehensive view of cellular function compared to scRNA-seq.

Conclusion

InTraSeqTM is a multimodal assay that expands single-cell analysis by enabling simultaneous measurement of mRNA and proteins. This integrated approach provides a holistic understanding of cellular function and allows for the identification of regulatory mechanisms. By combining scRNA-seq with protein profiling, InTra-

SeqTM has the potential to advance our understanding of cellular heterogeneity and its role in health and disease.

EACR25-0921

Distinct Mutational Profiles in Primary Sclerosing Cholangitis-Associated Cholangiocarcinoma Compared to de novo Cholangiocarcinoma

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Introduction

Cholangiocarcinoma (CCA) is a rare and aggressive malignancy characterized by etiologic heterogeneity and poor survival. Primary sclerosing cholangitis (PSC) is the most recognized risk factor for CCA in Western countries. PSC-associated CCA (PSC-CCA) is a leading cause of morbidity and mortality in PSC patients and exhibits distinct clinical features compared to those in de novo CCA. However, the molecular mechanisms driving these two subtypes of CCA remain largely unexplored. This study aimed to characterize the spectrum and prevalence of germline genetic variants in pathologically confirmed PSC-CCA, and de novo CCA as well as PSC patients without CCA (PSC-w/o CCA).

Material and method

This retrospective cross-sectional study included 301 patients with PSC-w/o CCA and 170 patients with CCA (PSC-CCA, n = 88; de novo CCA, n = 82) identified from two population genomics studies conducted at Mayo Clinic between 2016 and 2023. Their diagnoses, phenotypes, outcomes, as well as medical and family histories were obtained from electronic health records (EHRs) and self-reported questionnaires. Exome sequencing of these patients was conducted with genomic DNA, and genetic variants were identified using bio-informatics workflow following the Genome Analysis Toolkit (GATK) best practices. A comprehensive list of cancer susceptibility genes was compiled from prior cancer studies. Functional annotation and pathogenicity assessment of cancer-associated genetic variants were performed according to current American College of Medical Genetics and Genomics and the Association for Molecular Pathology (ACMG/AMP) guidelines.

Result and discussion

Analysis of exome sequencing data from 471 patients identified 53 pathogenic/likely pathogenic (P/LP) germline variants across 25 cancer susceptibility genes (CSGs) in 10.8% (51/471) of patients. The highest prevalence of P/LP germline variants was observed in PSC-CCA patients (13.6%, 12/88), followed by PSC-w/o CCA (10.0%, 31/301) and de novo CCA (9.76%, 8/82). Interestingly, PSC-CCA patients exhibited P/LP germline

variants mainly in moderate-, low-penetrance, and/or autosomal recessive genes, with significant enrichment in the Fanconi anemia DNA repair pathway. In contrast, patients with de novo CCA predominantly carried P/LP germline variants in the tumor suppressor genes that are key players in homologous recombination repair pathway. Similarly, germline variants led to differentially altered metabolic and signal pathways observed between PSC-CCA and de novo CCA patients.

Conclusion

These findings provide key insights into distinct CCA subtypes and call for an effort to systematically study germline testing of patients with PSC-CCA and de novo CCA as an approach to inform personalized approaches to screening, clinical management and targeted therapy of CCA in these patients.

EACR25-0950

Metabolic relevance of p53 hotspot mutations in hepatocellular carcinoma

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Introduction

Several systemic treatments are approved for advanced stage hepatocellular carcinoma (HCC) (e.g., sorafenib, a multi kinase inhibitor) with limited improvement in disease outcome, thus making further treatment options urgently needed. By blocking two central energy-producing pathways, glycolysis and oxidative phosphorylation (OxPhos), in wild-type and p53 knockout (KO) models, we showed that adjacent nutrient deprivation (ND) improves the efficacy of sorafenib in HCC and is dependent on the p53 status. Six different human TP53 hotspot mutations are known to account for up to 25% of all TP53 missense or nonsense mutations in HCC. Here, we aim to address how three most common TP53 hotspot mutations in HCC (mut: R175H, R249S, and R273H) affect its response to combination therapy (sorafenib/ND) *in vitro*.

Material and method

To demonstrate the involvement of p53 in cell metabolism, we analysed gene expression profiling datasets (TargetGeneReg 2.0). RNAseq was performed in p53 knockout and wild type HepG2 cell line upon ND and sorafenib treatment. Vectors carrying TP53 hotspot mutations were overexpressed in p53-deficient HepG2 cells. We further created traceable isogenic Trp53-mutant models combined with wild type or knockout alleles utilizing a CRISPR/Cas9 knock-in strategy to analyse the metabolic phenotypes and potential metabolic vulnerabilities resulting from HCC-specific mutations in Trp53. Metabolic phenotyping was performed using a

Seahorse analyser and viability assays were performed to test response to treatment.

Result and discussion

The analysis of gene expression profiling datasets demonstrated that p53 binds to either transcription start sites and/or enhancers of metabolism-relevant genes. Also, p53 can have indirect effects on metabolic gene expression without occupying a direct binding site. Our RNAseq results in HepG2 p53KO cells, and upon p53 re-expression show p53-dependent regulation of metabolic genes after ND. Next, we overexpressed TP53 harbouring one of the three hotspot mutations in TP53KO HepG2 cells and then analysed the cells' metabolic capacity in energy-producing pathways. The cells expressing mutTP53 had different capacity for mitochondrial respiration and glycolytic function only upon ND, while no difference was observed in standard growth conditions. We observed loss of transactivation potential of canonical p53 targets CDKN1A and MDM2, as well as differential mutTP53 transactivation potential at metabolic gene loci under ND. The metabolic profiles and responses to ND and sorafenib treatment were dependent on the hotspot mutations and their combination with p53 knockout alleles.

Conclusion

We show that p53 with hotspot mutations modifies the metabolic phenotype of HCC-derived cells and subsequently their response to the combination therapy.

EACR25-0959

Massively scalable direct guide RNA capture for pooled CRISPR screening

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Introduction

Single-cell direct capture CRISPR screens have the potential to uncover critical biological insight across a broad range of research fields such as oncology, immunology, and more. However, their utility to date has been limited by the availability of a truly high-throughput, economical, and accessible single-cell direct CRISPR capture technology. The number of cells processed per experiment scales with the number of perturbations; this can result in experiments requiring the processing of tens to hundreds of thousands of cells to ensure sufficient representation of each perturbation. In response to this challenge, we present a novel method enabling simultaneous capture of single-cell transcriptomes and CRISPR-Cas9 guide RNAs from individual cells facilitated by dual-oligo functionalized polymer particles called particle-templated instant partitions (PIPs).

Material and method

With the potential for multimodal profiling of up to one million cells in a single reaction, this method makes possible the profiling of pooled screens with tens of thousands of guides, a significant advance in direct capture CRISPR screening. To demonstrate the utility and scalability of this technology, millions of CRISPR-

Cas9 perturbed mammalian cells were combined with dual-oligo PIPs, emulsified by vortexing, and processed to distinct gene expression and guide libraries.

Result and discussion

We demonstrate the unique ability to archive cDNA on PIPs to enable segregated amplification of gene expression and sgRNA libraries, enhancing specificity and streamlining the workflow. We observed robust direct guide capture among a diversity of samples and confirmed that whole transcriptome mRNA sensitivity was comparable between uni-linker (mRNA only) and dual linker PIPs (mRNA and Cas9).

Conclusion

The combination of simple, high throughput single-cell analysis with emerging low cost high-capacity sequencing capabilities will enable routine genome-wide perturbational assays across many biological fields.

EACR25-0971

Different mutational patterns in primary colorectal cancer and its paired synchronous or metachronous metastasis and their association with patient outcomes

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Introduction

Colorectal Cancer (CRC) is the second most deadly cancerous disease worldwide. Early diagnosis is vital for successful treatment as survival gradually drops from 90 % at stage I below 20% for stage IV. Most frequently CRC metastasizes to the liver, and it can be diagnosed simultaneously with primary tumor (synchronous metastasis) or after primary tumor (pCRC) removal (metachronous metastasis). We performed whole exome sequencing to depict genetic changes in primary tumors and their paired synchronous or metachronous liver metastases.

Material and method

Whole exome sequencing was performed on 87 samples of pCRC and its paired liver metastasis (LM) and 36 singletons (where only one tissue type was available). Firstly, we examined differential mutated genes and type of mutations and CNV. Secondly, we focused on the tumor mutation burden (TMB) in pCRC and LM. We evaluated its association with overall survival (OS) and disease-free survival (DFS) from colon and liver surgery by Cox regression and Kaplan-Meier method. For all analysis, different chronicity of metastases was considered.

Result and discussion

APC and TP53 were identified as the most commonly mutated genes in pCRC (both ~50%) and its metachronous metastasis (both 64%). For synchronous metastasis we observed much lower frequencies of

mutations in APC gene (40%). MPDZ gene mutations were characteristic to metachronous pCRC only, mutations in VCAN, MTCL1, MDN1, SHROOM2, SPEG and GLI2 were more prevalent in synchronous pCRC. FBN1 mutations were unique to synchronous LM. We observed significantly higher TMB ($p = 0.03$) in metachronous group when comparing pCRC and LM. On the other hand, in the synchronous group, we observed a significant positive correlation between TMB in pCRC and LM. We observed longer OS (HR = 0.3, $p = 0.01$) for patients with higher TMB in pCRC leading to synchronous metastasis. On the other hand, higher TMB in Metachronous metastasis was associated with shorter DFS (HR = 2.01, $p = 0.03$).

Conclusion

The results suggest that distinct tumor progression pathways account for different chronicity and may have ultimate impact on patient's prognosis.

EACR25-0979

Saturation mutagenesis of the FGFR kinase domains reveals all activating point mutations and their drug response

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Introduction

To overcome the major challenge of variants of unknown significance in precision oncology, high-throughput functional genomics is essential. Only a comprehensive characterization of the vast landscape of cancer mutations enables optimized clinical treatment targeting molecular aberrations. Aberrant activation of fibroblast growth factor receptors (FGFRs) is a common oncogenic driver across multiple cancer types, with selective FGFR inhibitors (FGFRis) already approved for clinical use. However, the therapeutic potential of these inhibitors is constrained by the unknown characteristics of point mutations in FGFR1, FGFR2, FGFR3 and FGFR4, which can drive signaling and/or confer resistance. Here, we established a saturation mutagenesis scanning platform to assess all 11520 possible single nucleotide variants in the kinase domains of FGFR1-4 through pooled positive selection screens.

Material and method

We generated lentiviral libraries of all possible 11520 SNVs in the kinase domains of FGFR1-4 and tested their impact on FGFR activation as well as their capacity to mediate resistance to the FDA-approved FGFR inhibitors pemigatinib and futibatinib in positive selection screens. Selected activating and resistance mutations were individually cloned and their impact validated in a second cell line model.

Result and discussion

We classified 474 activating mutations and 738 resistance-conferring mutations to pemigatinib and/or futibatinib – many of which clustered within specific structural elements. Merging these two datasets we identified 301 targetable FGFR point mutations analogous to a strong PS3/BS3 evidence level. Notably, mutations at the same codon or the same mutations in different FGFRs could exhibit strikingly diverse effects, highlighting the necessity for a saturation mutagenesis approach. The screens also identified loss-of-function mutations and FGFR2-specific activating nonsense mutations. Importantly, the functional screens detected 97% of acquired resistance mutations in a clinical trial. To enhance experimental robustness and universality, the screening protocol was also established using a urothelial cancer cell line - a common FGFR-altered cancer entity. This approach revealed a significant overlap in identified resistance mutations, contributing to a more comprehensive understanding of the utility of model cell systems in saturation mutagenesis screening.

Conclusion

In summary, we present a thorough and clinically valuable catalog of all druggable point mutations within the FGFR kinase domains which is readily accessible for clinical decision making to select the optimal cancer therapy and expanding the potential application of the approved inhibitors.

EACR25-0986

Exploring EGLN1 as a therapeutic target for KRAS mutated lung cancer: insights into mitochondrial modulation

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Introduction

Lung cancer is the leading cause of cancer-related deaths worldwide. KRAS mutations, present in ~30% of lung adenocarcinomas, are associated with poor prognosis and limited treatment options. We identified EGLN1 as a novel druggable dependency in KRAS- mutated lung cancer. EGLN1 encodes PHD2 prolyl-hydroxylase, known for degrading HIF1α under normoxia. We previously showed that EGLN1 supports tumor progression via HIF1α-dependent and independent mechanisms.

Material and method

Using CRISPR/Cas9 system we silenced EGLN1 in several NSCLC cell lines. On these cells, we performed functional assays, including proliferation, migration and invasion. In order to investigate the HIF1alpha independent role of EGLN1, we have generated knockout (KO) cell

lines for EGLN1, HIF1alpha or both genes and performed proteomic and transcriptomic profiling. To characterize EGLN1 role in regulating mitochondria biology, we performed seahorse mitostress test, confocal microscopy analysis and TEM analysis.

Result and discussion

We identified and validated EGLN1 as a novel druggable dependency gene, associated with KRAS- mutated lung cancer. EGLN1 is overexpressed in tumor tissue and its high expression correlates with worse prognosis in lung cancer patients. In lung cancer cell lines, EGLN1 supports proliferation, migration, colony formation and 3D growth. Pharmacological inhibition of EGLN1 exerts anti-proliferative effects both in cell lines and patient-derived organoids. EGLN1 acts at least through two different molecular mechanisms, one HIF1α dependent and one HIF1α independent. Through integrative analysis of transcriptomic and proteomic profiles of EGLN1 KO, HIF1α KO and double KO cells, we identified a panel of mitochondrial genes deregulated independently on HIF1α transcriptional activity. Strikingly, EGLN1 KO leads to aberrant mitochondria morphology and profound impairment of mitochondrial respiration.

Conclusion

Overall, we identified EGLN1 as a novel therapeutic target in KRAS-mutated lung cancer. EGLN1 has a pro-oncogenic function in lung cancer, in part because of its capacity to control mitochondria through an HIF-independent mechanism.

EACR25-1010

Decoding the Molecular Complexity of HCC: Clonal Diversity, Functional States, and Tumor-Stroma Crosstalk

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Introduction

Hepatocellular carcinoma (HCC) is the third leading cause of cancer-related mortality worldwide, with surgery remaining the only curative option due to limited effectiveness of alternative treatments. Two key hallmarks of HCC, intra-tumoral heterogeneity (ITH) and a complex tumor microenvironment (TME), play crucial roles in tumor progression and therapeutic resistance, yet their molecular features remain poorly understood.

Material and method

To address this gap, single-cell RNA sequencing was performed on 15 samples (13 treatment-naïve HCC patients and 2 normal livers) to map the transcriptional

landscape of HCC tumors and their surrounding TME. Copy-number analysis inferred the clonal architecture of the tumor cells. By applying the non-negative matrix factorization, heterogeneous cancer cells were classified into distinct functional metaprograms (MPs). Spatial transcriptomics, combined with cell-cell interaction analysis, provided insights into the dynamic interactions between malignant cells and TME components.

Result and discussion

We identified seven distinct transcriptional metaprograms, along with their potential master regulators, within malignant hepatocytes, reflecting a broad spectrum of biological processes, including stress responses, metabolism, and epithelial-to-mesenchymal transition (EMT). By examining the genetic regulation of these inferred MPs, we found most HCCs are polyclonal at the copy number level. Some tumors exhibited homogeneous expression patterns across all CNV-defined malignant subclones, while others displayed multiple distinct transcriptional states, highlighting how clonal diversity contributes to the complex transcriptional landscape of HCC, yet only partially accounts for its functional heterogeneity. Moreover, integrating spatial transcriptomics with ligand-receptor analysis, we uncovered specific tumor-stroma interactions involving angiogenic endothelial cells, mediated by type IV collagen (COL4A1/2) and fibronectin (FN1), which engage integrins (ITGA1/B1) and syndecans (SDC1/4) on tumor cells. We also observed that some tumor subclones in distinct spatial regions exhibit different interaction patterns with the TME, suggesting their location may influence cell communication and contribute to functional heterogeneity.

Conclusion

Our study offers a high-resolution spatial map of ITH in HCC. We identified distinct tumoral cell states showing that transcriptional diversity extends beyond genetic variability and may result from tumor plasticity. Spatial interactions between tumor and angiogenic endothelial cells suggest mechanisms reshaping the TME and influencing tumor behavior. These findings provide new insights into the complex relationship between subclonal structure and the TME, highlighting potential therapeutic targets for HCC.

EACR25-1011

Arm-level somatic copy number alterations in liver metastases of colorectal carcinoma associated with time to relapse after surgical resection

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Introduction

High mortality of colorectal cancer is usually due to the development of distant metastases, the most common site of which is the liver. Unlike in primary tumors, there are no known molecular markers predicting relapse in the metachronous metastatic setting, in which a metastasis develops more than 6 months after primary tumor diagnosis, is then surgically resected, but a recurrence/relapse soon occurs in more than half of patients, while in others it does not for many years. Markers for risk of metastasis relapse could help optimize treatment strategies and minimize over/undertreatment of patients.

Material and method

We selected a cohort of patients with differing time to relapse after resection, treated at the University Hospital in Pilsen ($n = 41$), and analyzed matched pairs of fresh frozen metachronous colorectal cancer liver metastasis (mCLM) and adjacent non-tumor liver ($n = 41$) by whole exome sequencing and mRNA sequencing ($n = 38$, overlap $n = 34$). Detailed clinical data were collected, including relapse-free survival after mCLM resection (RFS) and complete blood work. Associations of somatic short mutations, as well as copy number alterations and gene expression profiles with all available clinical data were tested using a range of statistical methods. Data were also compared to those from primary tumors from TCGA ($n = 613$).

Result and discussion

While short SNV and indel and gene-level CNA analysis observed features known from primary tumors, no individual genes were associated with RFS. However, arm-level loss of chr3p, chr5q and gain of chr16p were associated with poor RFS (FDR-adjusted P values 0.029, 0.017 and 0.042, resp.). TCGA samples showed a similar association of loss of chr3p and chr5q with progression-free interval (unadjusted $p = 0.015$ and $p = 0.0015$, resp.), but not for gain of chr16p. The frequencies of these CNAs were approx. 2x higher in early relapse mCLMs than in the rest of mCLMs and TCGA, despite the overall CNA load not being significantly different. Gene set enrichment analyses showed that these CNAs led to expected changes in mRNA levels for their respective arms, but the dysregulated pathways differed between mCLMs and primary tumors with the same alterations, depending on the subset of the TCGA cohort. The general pathway signature of early relapse mCLMs was similar to that of more advanced primary tumors and less advanced tumors with early progression.

Conclusion

Arm-level CNAs in chr3p, chr5q (contain major tumor suppressor genes), and chr16p (host to several oncogenes) should be the subject for further study as potential predictors of early relapse in mCLMs. In general, early relapse mCLMs show transcriptomic signatures similar to those of more aggressive/advanced primary tumors.

Supported by the National Institute for Cancer Research (Program EXCELES, ID Project No. LX22NPO5102) – funded by the European Union – Next Generation EU, and the Charles University Grant Agency (project no. PRIMUS/25/MED/007).

EACR25-1073

Genomic instability and mitochondrial dysfunction drive high grade serous ovarian cancer

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Introduction

Deciphering the complex somatic variation present in tumour genomes is crucial to determine the events that drive tumour progression and fully understand the evolution of tumours. High grade serous ovarian cancer (HGSOC) is often considered an archetypal example of a tumour type that is driven by frequent structural variants and genomic instability, while the roles of somatic mtDNA variants in this tumour type are poorly studied. We have comprehensively studied the mutational landscape driving HGSOC, exploiting a large, deeply whole genome sequenced dataset with matched RNA-seq expression data.

Material and method

We constructed a combined WGS cohort ($N = 324$) of primary HGSOC tumours, to generate a harmonised dataset of consensus somatic mutation calls and gene expression for five HGSOC WGS sub-cohorts, including our own cohort ($N = 115$) and four previously published WGS cohorts. We also used state-of-the-art algorithms to predict complex structural variants, which are prevalent in this tumour type, including chromoplexy (55% of samples), chromothripsis (31%), pyrgo (28%) and breakage-fusion-bridge events (BFB) (27%) and ecDNA (16%).

Result and discussion

We show that the genomic chaos seen in these tumours obscures meaningful underlying patterns, namely two divergent evolutionary trajectories, affecting patient survival and causing different genomic aberrations. One involves homologous recombination repair deficiency while the other is dominated by whole genome duplication with frequent chromothripsis, breakage-fusion-bridges and ecDNA. Remarkably, these heavily

disrupted nuclear genomes are also frequently accompanied by alterations to the mitochondrial genome, impacting patient survival. The magnitude of the impact on survival is directly related to the types and frequencies of the mtDNA variants involved. There is also evidence that mtDNA mutation loads interact with some structural alterations in the nuclear genome to affect survival.

Conclusion

These new layers of driver mutations, mediated by genomic instability and mitochondrial dysfunction, suggests new directions for research into tumour biology and potential therapeutic targets.

EACR25-1076

Single-cell sequencing analysis highlights the transdifferentiation potential of cancer cells in a neuroblastoma cell line

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Introduction

Neuroblastoma is the most commonly diagnosed extracranial tumor in infants and originates from neural crest cells during development. Our laboratory identified in the past a chromosomal region encoding a long non-coding RNA, (namely 29A) within the first intron of ASCL3 gene. Several studies characterized the role of this 29A nc-RNA in neuroblastoma, showing that its expression leads to tumor differentiation, thereby reducing malignancy. Genetically modified cell clones over-expressing this non-coding RNA were generated from the SK-N-BE(2) neuroblastoma cell line. High levels of 29A lead to neuronal differentiation of the culture, as evidenced by the expression of specific neuronal markers and the generation of action potentials and specific currents by these cells. Alongside these observations, molecular markers and microscopical analyses showed the presence of different cell types within the culture. This finding was particularly intriguing because these clones originated from a single cell, thus suggesting that the observed heterogeneity is reached by trans-differentiation processes occurring within the culture, a key aspect to be investigated in tumor biology.

Material and method

These findings were further validated through a single-cell RNA sequencing analysis which identified nine distinct cell populations within the culture. The single-cell RNA sequencing analysis allowed us to identify specific membrane markers expressed by individual subpopulations, thus enabling us to attempt their separation via Fluorescence-Activated Cell Sorting (FACS) for further subpopulation-specific studies.

Result and discussion

The study aimed to investigate these nine NB cell subpopulations by analyzing the gene expression of specific pathways relevant to tumorigenesis. In particular,

we identified subpopulations producing vascular endothelial growth factor (VEGF), as well as a single population capable of receiving this signal expressing VEGFR. This suggests the relevance of this cell-to-cell communication in coordinating angiogenesis. Additional gene expression analyses were conducted to examine pathways involved in DNA repair, cell cycle regulation, invasion and metastasis, and the epithelial-mesenchymal transition process in order to better characterize the behavior of these subpopulations.

Conclusion

In conclusion, we report that the presence of specific subpopulations within the NB tumor mass might be consequent to specific transdifferentiation processes whose appropriate understanding could lead to more targeted therapies. The use of specific antibody panels to assess the presence identify specific cell subpopulations within the tumor nodule might help to increase the accuracy of therapies.

EACR25-1081

"Hello from the other side" – Exploring the interplay between cancer cell metabolism and immune cells in Ductal Carcinoma In Situ (DCIS)

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Introduction

Ductal carcinoma in situ (DCIS) is an early form of breast cancer where cancer cells grow within the milk ducts, separated from the surrounding stroma by the myoepithelium and the basement membrane. DCIS itself is indolent, and as many as 50% of DCIS will never progress to invasive breast cancer (IBC), even if untreated. However, there exist no reliable diagnostic tools to stratify DCIS lesions according to risk of progression. The escape of cancer cells from the milk ducts is influenced by the cancer cells themselves, the microenvironment surrounding the ducts and the interplay between the two. Exploring this interplay could help us increase our understanding of the invasive potential of DCIS.

Material and method

We performed GeoMx spatial transcriptomics of 16 cases of pure DCIS (Luminal A: n = 8, Triple Negative (TN): n = 8) to characterize cancer cell and stromal cell compartments separately. Differential gene expression analyses and gene set enrichment analyses were used to compare cancer cell expression profiles between the subtypes. To characterize the stromal immune cell composition, we performed *in silico* immune cell deconvolution and multiplex immunofluorescence for immune cell protein

markers. For validation, we utilized a gene expression dataset from bulk tumor tissue comprising pure DCIS (n = 57) and IBC (n = 131).

Result and discussion

Compared to Luminal A, we found enrichment of genes involved in energy metabolism in TN DCIS cancer cells with enhanced glucose metabolism, increased pentose phosphate pathway activity, increased glutamine uptake, altered mitochondrial metabolism and increased iron uptake, indicating extensive metabolic reprogramming in TN DCIS. In addition, we found higher expression of genes encoding secreted proteins involved in extracellular matrix reorganization in Luminal A DCIS. The relative composition of immune cells surrounding ducts with DCIS cancer cells was similar between the subtypes, however, the immune cell abundance was much higher in TN DCIS compared to Luminal A. In TN DCIS, immune cells were commonly organized as immune cell clusters, while in Luminal A DCIS, immune cells were more often dispersed throughout the periductal stroma. Validation performed in a bulk gene expression dataset confirmed metabolic differences between the TN and Luminal A subtypes in DCIS. These subtype differences were also found in IBC.

Conclusion

This study revealed a correlation between cancer cell metabolic reprogramming and immune cell abundance in DCIS which was linked to subtype. These findings provide insights into the breast cancer invasion process, potentially contributing to improved understanding of DCIS progression and future treatment strategies.

EACR25-1135

Investigating the molecular diversity of luminal breast cancer arising in young women

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Introduction

Breast cancer (BC) arising in young women (Y) is uncommon, yet clinically relevant. Even when corrected for tumor stage, grade and subtype, BC in Y display worse response to standard therapies, higher risk of relapse and lower overall survival compared to older patients (nY), particularly evident in the Luminal subtype (LBC). LBC biology in Y remains poorly understood and models that accurately reflect its molecular characteristics are still lacking. Therefore, this study aims to uncover the genetic, molecular and biological bases of LBC in Y, in order to identify mutational signatures, novel biomarkers and, possibly, therapeutic targets for LBC Y patients.

Material and method

Next-Generation Sequencing (NGS) approaches were used to perform a comprehensive mutational profiling analysis on a cohort of LBC samples from Y (<45 y) (n = 119) and nY (>55 y) (n = 92) patients. Samples were first sequenced using a custom-made amplicon-based panel,

targeting the most frequently mutated genes in LBC (PIK3CA, AKT1, TP53, GATA3) at high coverage, then by whole exome sequencing (WES). Next, using lentiviral approaches, we generated primary luminal breast epithelial (BPE) cells carrying PIK3CAE545K, PIK3CAH1047H, TP53R175H mutants, either alone or in combination, and tested them in proliferation, survival, 3D-morphogenesis and response to therapies.

Result and discussion

Targeted NGS analyses revealed that mutations in PIK3CA/AKT1 and GATA3 were significantly enriched in Y, who also harboured higher frequency of PIK3CA/AKT1 co-mutations with GATA3 or TP53, especially in the luminal B subtype. WES analyses confirmed these results revealing also other gene mutations enriched in Y (ERBB2, FAT3, CBFB and MAP3K1) and in nY (LRP1B, EPHA3 and CSMD3). Copy number variation analysis also revealed that Y women displayed TP53 deletions and GATA3 amplification at higher frequency compared to nY ones. To test the biological implications of these alterations, we characterized BPE cells carrying these mutants. As expected, PIK3CA mutations, especially in combination with TP53 mutations, altered the growth and led these normal cells to a transformed phenotype. More interestingly, combining PIK3CA and TP53 mutations made these cells resistant to endocrine therapy and CDK4/6 inhibitors. Synthetic lethality approach revealed vulnerability by inhibiting EZH2.

Conclusion

Overall, our results provide new insights into the molecular fingerprint of LBC patients supporting the hypothesis that BC arise in Y may represent a unique biological entity. Moreover, we shed new light on the biological impact of PIK3CA and TP53 co-mutations on tumorigenesis and drug resistance in LBC context, suggesting that they might represent novel predictive biomarkers for epigenetic therapies, such as EZH2i, possibly not just in Y but across all LBC patients carrying these mutations.

EACR25-1164

Same-cell spatial transcriptomics and proteomics expression at single-cell resolution to decipher melanoma cell state heterogeneity and relationships with the inflammatory microenvironment in metastasis

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Introduction

Melanoma exhibits significant tumoral heterogeneity, with several melanoma cell states (mCS) identified through single-cell RNA sequencing (scRNA-seq). Although some mCS have been linked to poor immunotherapy response, their role in metastasis is still hypothetical. Immunohistochemistry, the most widely used diagnostic technique, identifies cellular phenotypes

affecting diagnosis and treatment. However, since mCS signatures were discovered at the transcriptomic level, validating their protein expression is crucial. Moreover, while most early stage melanomas (ESM) cases have favorable prognoses and are not followed after excision, 10% of patients develop metastatic disease. Identifying biomarkers to predict progression is therefore essential. This study aims to (1) validate RNA-protein correlation at the single-cell level using an innovative spatial technology enabling simultaneous RNA and protein detection in the same cell; (2) investigate the presence of mCS in ESM; (3) compare mCS enrichment and spatial distribution in metastasising (M+) vs. non-metastasising (M-) ESM.

Material and method

We assembled a cohort of 10 ESM cases (5 M+ vs. 5 M-), matched for key clinicopathological prognostic factors to minimize bias. A single tissue section per case was analyzed using the hyperplex multiomics technology on Lunaphore's COMET instrument. A panel of 24 proteins (MITF, MelanA, AQP1, S100B, SOX10, SOX9, NGFR, TCF4, PDGFRb, BRAF, FAP, CD3, CD4, CD8, CD20, FOXP3, CD68, CD163, podoplanin, MECA79/pNA, α SMA, CK, CD31, and CD45) and 12 RNAs (THY1, MLANA, S100A1, S100B, SOX10, TCF4, ID3, PRAME, TYR, COL5A1, COL1A1, and PMEPA1) was selected to assess RNA-protein correlation for mCS markers and explore their spatial relationship with the inflammatory microenvironment. RNAs were chosen based on minimal signatures from relevant studies. Evaluating protein and RNA markers simultaneously was also evaluated. For data processing and integration, we developed a novel bioinformatics software, DISCOVERY, specifically designed for the integrated analysis of high-dimensional multiplex IF and spatial transcriptomics data.

Result and discussion

MelanA showed strong RNA-protein correlation, whereas SOX10, S100A1, and TCF4 protein expression did not align with their RNA levels. Significant heterogeneity in mCS was observed across samples. Trajectory analyses were performed using protein, RNA, and hybrid approaches. M+ ESM showed enrichment in undifferentiated mCS subtypes, whereas M- cases, despite heterogeneous melanoma marker expression, had minimal undifferentiated mCS content.

Conclusion

This study underscores the importance of multiomics approaches in translating RNA-based signatures into protein-level markers. Unique melanoma cell subtypes identified in this study may serve as potential prognostic markers for high-risk ESM.

EACR25-1195

KRAS mutation derived PSMB8 activates MEK/ERK signaling to promote chemoresistance in pancreatic adenocarcinoma

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Introduction

Pancreatic adenocarcinoma (PAAD) harbors KRAS mutations in >90% of cases, yet KRAS-targeted therapies face limited efficacy and rapid resistance. The ubiquitin-proteasome system (UPS), a master regulator of proteostasis, is frequently hijacked in tumors to drive progression and therapy resistance. Here, we interrogated the clinical and mechanistic roles of KRAS mutation-associated UPS-related genes (KMUPSGs) in PAAD, with a focus on their potential as predictive biomarkers and therapeutic targets to overcome chemoresistance.

Material and method

KMUPSGs were systematically identified via univariate Cox regression and LASSO-penalized regression to construct a prognostic risk signature. Independent prognostic value was validated using multivariate Cox models. Survival differences were assessed by log-rank tests, while Spearman correlation and t-tests evaluated associations with immune infiltration, tumor mutational burden (TMB), and pathway activity. Functional validation included PSMB8 knockdown (siRNA) and pharmacological inhibition (oprozomib) in PAAD cell lines, with proliferation (CCK-8), metastasis (transwell), apoptosis (flow cytometry), and drug sensitivity assays (IC50). Mechanistic studies focused on MEK/ERK pathway activation (Western blot). Experiment in vivo was performed using a cell line-derived xenograft (CDX) model.

Result and discussion

A 7-gene KMUPSG signature stratified PAAD patients into high- and low-risk groups, with high-risk patients exhibiting shorter overall survival, immunosuppressive microenvironments, elevated TMB, and gemcitabine resistance. PSMB8, the top-ranked KMUPSG, was overexpressed in KRAS-mutant tumors and correlated with advanced stages. PSMB8 silencing suppressed proliferation and invasion, and enhanced sensitivity to gemcitabine treatment. Oprozomib, a PSMB8 inhibitor, also exhibited favorable anti-tumor effect.

Mechanistically, PSMB8 drove chemoresistance via MEK/ERK hyperactivation, evidenced by MEK1/2 and ERK1/2 phosphorylation and rescued sensitivity upon its silence. Finally, the PSMB8 knockdown CDX models illustrated better response to gemcitabine treatment.

Conclusion

We established a robust KMUPSG signature for prognostic stratification and chemotherapy response prediction in PAAD. PSMB8 emerged as a central effector of KRAS-mediated chemoresistance, operating through MEK/ERK signaling. Targeting PSMB8 represents a promising strategy to overcome gemcitabine resistance, offering actionable insights for precision therapy in KRAS-mutant PAAD.

EACR25-1200

Integrated multi-omics analysis identifies sex differences in muscle-invasive bladder cancer

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Introduction

Bladder cancer (BC) exhibits distinct sex disparities, with men having a fourfold higher incidence rate than women, while women are more likely to present with aggressive tumors and worse outcomes. Despite these disparities, research on sex-based differences in BC remains limited, and sex-specific therapeutic strategies are lacking. Studies in other cancers, such as non-small cell lung cancer (NSCLC), have demonstrated sex-based differences in oncogene expression, mutation profiles, and treatment response, underscoring the importance of investigating sex-based differences in cancer research. In this study, we aim to elucidate sex-based molecular differences in muscle-invasive bladder cancer (MIBC) through a multi-omics analysis, identifying potential biomarkers and therapeutic targets to facilitate precision medicine approaches.

Material and method

We analyzed the MIBC cohort from publicly available dataset, by integrating genomic, transcriptomic, and proteomic data. In genomics, somatic mutations and copy number variations (CNV) were identified from whole-exome sequencing (WES) data. Differential gene expression was analyzed using mRNA sequencing data, while protein abundance was assessed with reverse-phase protein array (RPPA) data. Statistical analyses and data visualization were conducted using R.

Result and discussion

Our analysis revealed significant sex-based differences in BC at multiple molecular levels. Genomic analysis identified distinct mutational profiles between sexes, with specific oncogenic alterations enriched in each group. Transcriptomic profiling uncovered differentially expressed genes related to immune response and cell cycle regulation, which may contribute to the observed disparities in tumor progression and patient outcomes. By performing consensus clustering using immune-related genes, we identified distinct immune microenvironment differences between sexes and uncovered key genes contributing to these variations. Proteomic analysis further supported these findings, revealing sex-specific variations in protein expression patterns and identifying key signaling pathways associated with tumor aggressiveness and therapy response. By integrating multi-omics data, we identified key molecular interactions that contribute to sex-based disparities in BC progression.

Conclusion

This study provides a comprehensive molecular characterization of sex-based differences in BC through an integrative multi-omics approach. By delineating distinct molecular signatures, we identify potential biomarkers and therapeutic targets that could inform the development of sex-specific precision medicine strategies for BC. These findings provide an approach for

personalized patient management and survival prediction in future clinical studies.

EACR25-1254

Comprehensive genomic profiling and RNA-seq analysis reveal distinct genomic and transcriptomic signatures associated with treatment response in metastatic colorectal cancer (mCRC) patients (pts) enrolled in the NIVACOR trial

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Introduction

In the phase II NIVACOR trial, pts with RAS/BRAF mutated (mut) mCRC received FOLFOXIRI/bevacizumab (bev) combined with nivolumab (niv) as first-line therapy. An objective response rate (ORR) of 76.7% and a median progression-free survival (mPFS) of 10.1 months was reached. Here, we describe data from comprehensive genomic profiling (CGP) and RNA-seq of tumor samples from NIVACOR pts, focusing on the microsatellite stable (MSS) cohort.

Material and method

Pts with untreated RAS/BRAF mut mCRC received FOLFOXIRI for 8 cycles followed by bev and niv as maintenance. DNA and RNA were obtained from formalin fixed paraffin embedded (FFPE) specimens of primary tumor samples. Translational analyses were performed by the Oncomine Comprehensive Assay Plus® and the Ion AmpliSeq™ Transcriptome Human Gene Expression® assays.

Result and discussion

CGP on DNA was successful for 49 MSS tumors. The tumor mutation burden (TMB) was >10.4 mutations/Mb in 9 pts with a mPFS of 18 months, while the 40 TMB-low showed a mPFS of 9 months ($p = 0.06$). In order to identify a genomic signature, we compared the mutational landscape exclusively present in the 25th or 75th PFS percentile MSS cases. We identified two distinct signatures: a poor prognosis signature (RES) of 53 genes and a good prognosis signature (SENS) of 98 genes. When the two signatures were applied to the MSS cohort, pts with at least one genomic alteration (GA) in RES showed a statistically significant shorter mPFS ($p < 0.0001$), while pts carrying at least one GA in SENS showed a longer mPFS ($p = 0.0003$). Furthermore,

RNAseq analysis between the 75th vs 25th percentile cohorts revealed 219 differentially expressed genes involved in immune system, PI3K cascade and DNA Replication-Dependent Chromatin Assembly.

Interestingly, from both DNA and RNA analysis we found an involvement of the PI3K/AKT pathway (PIKpath). For this reason, we performed a survival analysis considering as mut pts with at least one GA in this pathway. The results showed a mPFS of 10 months for the mutant versus 8 months for the wild type cases in the MSS subgroup ($p = 0.12$). Remarkably, the fraction TMB-high patients was higher in pts mut in PIKpath in the MSS cohort (25% vs 5,5%).

Conclusion

The NIVACOR trial demonstrates the potential of combining ICIs with intensive chemotherapy and anti-angiogenic therapy in a challenging mCRC population, and suggests the utility of molecular profiling that might improve patients' selection and optimize treatment efficacy. These results are particularly relevant because they were obtained in the subgroup of RAS/BRAF mut mCRC with worst prognosis.

EACR25-1261

Analysis and machine-learning based prediction of cellular adaptation during ovarian cancer treatment

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Introduction

In cancer, the inherent diversity in cell states lays the ground for evolutionary selection. Furthermore, plasticity allows for adaptation during treatment. Recently, the lab identified a stress-related cellular state that contributes to both inherent and acquired resistance in high-grade serous ovarian cancer (HGSC) metastases, suggesting that prior stress exposure enhances cancer cell resilience during chemotherapy. To address the role of stress in pre-existing and adaptive resistance, we are developing a single-cell stress recording system combined with lineage tracing methodology, in addition to a data-analysis pipeline to process the integrated information. The unique generated data will then be used to build a machine learning model that predicts past and future stress responses from gene expression profiles alone, allowing stress history estimation from existing scRNA-seq data to reveal how adaptive stress is associated with cancer cell resilience.

Material and method

The experimental design of the project is based on two particular methodologies, which lately are analyzed by a collection of in-house scripts, combined with public software. We use a (1) CRISPR-based recording system (Cassiopeia, by Jones et al. 2020.) to study the stress activity in individual cells at the level of transcription factors (TFs). Briefly, the stress activity is measured by a system of "scars" (indels), that will be used to reconstruct cellular evolution to explore treatment adaptation at the level of cells and clones. To determine the sisterhood relationship between cells, we use a (2) barcode-labeling

system, performing pairwise comparisons of similarity and determining the “strength” of the connection by analyzing the evidence supporting the barcodes. After testing our pipeline and validating our data, in the future, we aim to test neural network models, to predict future and past adaptations.

Result and discussion

For the sisterhood-relation pipeline, recent in-house results suggest recovering a couple of thousands of cells, allowing us to use them to study their transcriptomic profiles. Preliminary conclusions on the stress recording system show consistent results compared with the literature, suggesting a successful induction of the CRISPR scarring machinery.

Conclusion

Our preliminary results support that our experimental methodology in combination with custom data analyses enables us to explore the programs driving inherent and acquired resistance, using sister cells. We now intend to refine our approach by testing variations in our parameters during the analysis, to then integrate the whole pipeline, experimental validation, and laying the foundation for our deep learning prediction.

EACR25-1262

Novel replication stress signature predicts non-homologous end-joining association mediated by the MutS α complex

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Introduction

Cancer development is closely linked to genomic instability, with chromosomal instability (CIN), characterized by the continuous gain of aneuploidies, as its most common form. One major contributor to CIN is replication stress (RS), which refers to disruptions in DNA replication leading to chromosomal missegregation. The impact of CIN and RS on cancer cell proliferation is complex and context-dependent. While some studies suggest that the increased intra-tumor heterogeneity as a result of RS and CIN provides a mechanism for adaptability, others have observed genomic instability to be a cellular burden. Existing signatures aiming to quantify the level of RS are derived by overexpressing oncogenes like MYC, likely introducing a bias toward proliferation.

Material and method

We gathered RNA-sequencing data from five independent studies, using six gene targets to induce replication stress: three methods used oncogene over-expression and the other three relied on gene knockouts disrupting replication integrity. Using these labeled samples, we identified genes consistently deregulated in RS samples. By leveraging large-scale cancer databases, we explored pan-cancer processes associated with the RS signature.

Result and discussion

Our RS signature was the only one that reliably distinguished RS from non-RS samples in unseen samples. Applying the signature to large cancer databases

revealed distinct differences between primary tumors and cancer cell lines. In tumors, RS was associated with an enrichment of cell cycle gene sets, while the expression of their leading-edge genes suggested accelerated cell cycle progression. Conversely, in cell lines, RS samples displayed a slight reduction in cell cycle activity. Additionally, we identified links between the RS signature, the mismatch-sensing MutS α complex, and non-homologous end-joining (NHEJ) repair pathway activity. Interestingly, the RS-NHEJ association was removed when controlling for protein expression of MutS α components, indicating a potential role for mismatch sensing in modulating the double-strand break response upon replication stress.

Conclusion

By reducing bias toward oncogene-related RS, this new RS signature might capture the complex association of genomic instability with proliferation more accurately. The signature may also facilitate the identification of novel processes associated with tumorigenic RS and uncover new potential targets for cancer treatment.

EACR25-1281

Single-Cell Analysis Reveals U1 snRNA Mutation-Driven Tumor Micro-environment Remodeling in Chronic Lymphocytic Leukemia via CD44 Signaling

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Introduction

Chronic lymphocytic leukemia (CLL) is characterized by the accumulation of monoclonal mature B lymphocytes in peripheral blood, bone marrow, lymphoid tissues, and extranodal sites. Genes involved in RNA splicing such as SF3B1 and U1 are frequently mutated in CLL, leading to altered splicing and generation of tumor neoepitopes.

Material and method

To investigate how these mutations shape the tumor microenvironment (TME), we performed an in-depth characterization of CLL using multiple single-cell sequencing technologies, including 5'GEX, single-cell TCR, and CITE-seq. Our study analyzed 26 tumor samples from the bone marrow or lymph nodes of 23 U-CLL patients, with 7 carrying U1 mutations, 8 carrying SF3B1 mutations, and 10 lacking splicing gene mutations.

Result and discussion

We have developed the most comprehensive single-cell atlas of unmutated CLL, encompassing the largest number of cells and patients while integrating multi-layered information. We observed significant intra-tumor heterogeneity, discerning 12 distinct transcriptional programs, one associated with the U1 g.A3>C mutation and characterized by hyperactivation of NF-κB signaling.

The T-cell and NK compartments exhibited site-specific and mutation-specific enrichment, characterized by elevated CD4+ regulatory T cells (Tregs) and CD8+ exhausted cells within lymph nodes. Conversely, U1-mutant tumors showed enhanced CD8+ cytotoxic activity, predominantly mediated by effector-like CD8+ cells. Single-cell T-cell receptor sequencing revealed clonotype expansion in U1-mutated tumors, particularly within CD8+ effector and exhausted cells, indicative of a neoantigen-driven immune response. Cell-to-cell interaction analyses identified CD44 as a key mediator in U1-mutated tumors, highlighting pro-B survival interactions such as those involving the MIF-CD44-CD74 axis. Furthermore, tumorigenic CD44 isoforms characterized by the inclusion of alternative exons were found to be upregulated in U1-mutant tumors. In addition, enhanced interactions between CD80 on CLL cells and CTLA4 on Tregs and CD8+ exhausted cells underscored an immunosuppressive phenotype associated with U1-mutated CLL.

Conclusion

These findings underscore the intricate crosstalk between CLL mutations and the TME, revealing potential therapeutic avenues for U-CLL with U1 mutations. Notably, CD44, a key mediator of microenvironmental communication and intracellular signaling, was upregulated and alternatively spliced in U1-mutant CLL, positioning it as a promising target for therapies such as anti-CD44v6 treatment. Additionally, the heightened CD8+ effector response in U1-mutant tumors suggests neoantigen recognition, highlighting a potential opportunity for immunotherapeutic interventions.

EACR25-1290

Glucocorticoid receptor as a prognostic factor in triple-negative breast cancer correlates with migratory signature at a single-cell level

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Introduction

Introduction. The glucocorticoid receptor (GR) plays a highly context-dependent role in tumorigenesis. The GR agonist dexamethasone is routinely administered as supportive therapy alongside chemotherapy to reduce anaphylactic reactions and nausea. However, recent RNA-based and preclinical studies have suggested caution when using glucocorticoids in patients with breast cancer, as GR activation has been shown to promote metastasis in mouse models. Our aim was to investigate GR mRNA and protein expression at the single-cell level and to correlate it with patient survival.

Material and method

Materials and Methods. GR expression was analyzed at the single-cell level using the following approaches:

- i) First, confocal immunostaining was performed to examine GR expression in different cell types within triple-negative breast cancer (TNBC).
- ii) Findings were validated using single-cell sequencing of TNBC samples and conventional immunohistochemistry on 161 TNBC tissue samples.
- iii) Finally, the effect of GR activation on tumor cell migration was assessed through time-lapse imaging of single-cell tracking in vitro using MDA-MB231 and Hs578T TNBC cell lines treated with dexamethasone.

Result and discussion

Results and Discussion. Confocal staining revealed heterogeneous, medium-to-low GR expression in TNBC tumor cells compared to cells in the tumor microenvironment (TME). High GR expression was detected in immune cells, including infiltrating T cells and macrophages. In our TNBC cohort, cytoplasmic GR staining positively correlated with Ki67 expression. Additionally, patients with high nuclear GR staining had significantly shorter progression-free survival (HR = 0.35 [0.08–1.50]; p = 0.0185). Single-cell tracking time-lapse migration experiments demonstrated that GR activation increased cell motility in a time-dependent manner. Single-cell sequencing showed a significant positive correlation between the GR signature and migration signature ($R = 0.61$, $p < 2.2e-16$). Tumor cells with higher GR encoding NR3C1 expression exhibited an increased migration signature. Fast-migrating cells displayed significantly higher expression levels of NR3C1 and its targets SGK1 and TSC22D3 compared to clusters of slow-migrating cells.

Conclusion

Conclusion. Glucocorticoids are essential supportive therapy in routine chemotherapy. However, our findings suggest that high nuclear GR expression is a negative prognostic marker in TNBC patients. GR activation enhances tumor cell migratory potential, which may contribute to poorer outcomes. Therefore, careful monitoring of TNBC patients with high nuclear GR expression is warranted.

Funding: NRD1 FK135065, Bolyai Research Fellowship of the Hungarian Academy of Sciences, National Laboratories Excellence program (under the National Tumor Biology Laboratory project (NLP17)).

EACR25-1317

Investigating Topoisomerase IIβ Binding Protein 1 as a Potential Prognostic Biomarker and Therapeutic Target in Bladder Cancer

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Introduction

Bladder cancer (BC) is a major global health concern, ranking among the most prevalent cancers worldwide.

Lebanon has one of the highest BC incidence rates, ranking first in 2018 according to the American Institute for Cancer Research (AICR). Despite advances in molecular biology and treatment strategies, BC management remains challenging due to significant side effects and a high rate of recurrence. This is largely attributed to the heterogeneous nature of the tumor and reduced treatment efficacy. Thus, identifying novel targets to improve diagnostic and treatment effectiveness is essential for enhancing patient outcomes. Topoisomerase II β binding protein 1 (TOPBP1) is a key mediator in the DNA damage response (DDR) and genomic integrity. Research has shown that TOPBP1 is overexpressed in several solid tumors and is associated with poor prognosis in cancer patients. However, its role in BC has not yet been investigated. This study aims to evaluate the potential role of TOPBP1 as a prognostic biomarker and therapeutic target in BC.

Material and method

We will assess the expression levels of TOPBP1 in BC tissues and correlate them with clinical outcomes. Additionally, we will assess the therapeutic potential of targeting TOPBP1 in both two-dimensional (2D) and three-dimensional (3D) culture systems using *in vitro* and *ex vivo* BC models. Finally, we will investigate the disrupted protein functions and interactions caused by TOPBP1 aberrant expression in BC.

Result and discussion

Our preliminary analysis of the Cancer Genome Atlas (TCGA) database indicates that TOPBP1 expression is significantly elevated in BC tissues compared to normal tissues. Moreover, patients with high TOPBP1 expression levels exhibit poor overall and progression-free survival probabilities compared to those with low TOPBP1 expression.

Conclusion

This study may identify TOPBP1 as a novel therapeutic and prognostic marker, with the goal of improving BC management and overcoming tumor resistance and recurrence.

EACR25-1323

Origin of chromosomal instability during acinar to ductal metaplasia in pancreatic cancer

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Introduction

Pancreatic ductal adenocarcinoma (PDAC) is among the cancers with the highest mortality rates, with an overall 5-year survival rate of approximately 5%. Similarly to other cancers, PDAC exhibits a wide variety of genetic insults, ranging from point mutations to more intricate events, primarily copy number variations (CNVs) and complex chromosomal rearrangements. However, the mechanisms underlying the generation of this widespread genomic heterogeneity during PDAC tumorigenesis have not yet been elucidated. A proposed pathway originates

from precursor lesions of Pancreatic Intraepithelial Neoplasia (PanIN), characterised by a disruption in the typical architecture of acinar cells. In response to stressors such as inflammation, pancreatic acinar cells transdifferentiate into ductal cells through a reversible process known as acinar to ductal metaplasia (ADM). When sustained, this transformation can result in PanIN lesions and lead to a gradual progression toward PDAC. The present study focuses on the initial phases of PDAC progression and investigates whether ADM constitutes the origin of genomic instability, mainly through ploidy variation in acinar cells, and promoting early tumorigenesis.

Material and method

The study employed an integrative approach combining single-cell RNA sequencing (scRNA-seq) data analysis with experimental validation. Publicly available scRNA-seq datasets were analysed to investigate CNV alterations and differential gene expression in ADM cells. Immunofluorescence staining (IHC) was performed to assess DNA damage and nuclear envelope integrity in samples from PDAC and PanIN mouse models.

Result and discussion

Early bioinformatics analyses revealed variations in CNV profiles across acinar and ADM cells, suggesting potential subpopulations that exhibited distinct patterns of chromosomal alterations. Additionally, functional enrichment analysis of differentially expressed genes indicated a downregulation of genes associated with the nuclear envelope (NE) in ADM cells. These findings were supported by IHC, which identified multiple indicators of genomic instability, such as double-strand DNA breaks, chromatin bridges, micronuclei and NE alterations.

Conclusion

This study provides preliminary evidence suggesting that ADM may play a key role in driving genomic instability during the initial stages of PDAC development. The observed accumulation of genomic damage and ploidy variations, along with potential NE vulnerabilities, highlights potential fragilities in cells undergoing ADM that could contribute to early tumorigenesis. These findings seem to underscore the importance of targeting ADM and its associated mechanisms as potential targets for preventive strategies in PDAC. Further research is required.

EACR25-1328

Spatial profiling of matched adnexal and omental tumors in high-grade serous carcinoma

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Introduction

High-grade serous tubo-ovarian carcinoma (HGSC) is the most common and most lethal ovarian cancer subtype. HGSC is often diagnosed at late stage when it has already metastasised to the abdominal lining. Although the majority of the patients do respond to chemotherapy initially, a relapse is likely to occur shortly after, with eventual treatment resistance. Hence to better treat and

understand HGSC it is essential to investigate both adnexal and extra-pelvic tumours. Moreover, the lack of clinically relevant biomarkers for HGSC remains as a challenge.

Material and method

We collected a retrospective cohort of nine patients which all underwent a primary debulking surgery and received at least six rounds of standard chemotherapy. The cohort consisted of three patients with a long (≥ 18 months) progression-free interval (PFI), two with medium (6–18 months) and four with short (≤ 6 months) PFI. From each patient we used matched adnexal and omental tumor samples. Moreover, all utilized tumor samples were fixed with Paxgene, an alcohol based, preservative. We constructed four tissue microarrays which were used for spatial transcriptomics (10x Genomics Visium Spatial Gene Expression for FFPE). To analyse the data, we applied computational methods including pathway enrichment analysis, spot-wise expression analysis and inferCNV to resolve copy-number alterations.

Result and discussion

We discovered several differences between PFI groups and found also consistently pathways up-regulated in the short PFI groups independent of tumor site when compared to medium- and long PFI group. Interestingly, in tumor epithelium, interferon-related pathways were among the most up-regulated in short PFI group while stromal cell expression was enriched for epithelial-mesenchymal transition. Further, we were able to show that several genes possessing a higher expression levels within the interferon pathway can be explained by copy number gains in tumor cells.

Conclusion

Here we show that spatial transcriptomics can be used as a tool to identify biologically relevant differences between PFI groups as well as tumor sites in treatment-naïve patients. Identifying differentially expressed pathways and copy-number alterations is important as they are recognized to contribute to genomic instability facilitating metastasis and chemotherapy resistance. Therefore, uncovering these alterations may help in the development of better treatments and/or predictive biomarkers for HGSC.

EACR25-1334

Phenotypic and genomic characterisation of single micronuclei

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Introduction

Chromosomal instability (CIN) is an ongoing process involving the gain and loss of whole, or parts of, chromosomes in many cancers. CIN causes structural and numerical abnormalities both within and without the nucleus, the latter of which is exemplified by micronuclei (MN). MN contain either whole lagging chromosomes or

acentric chromosome fragments which were excluded from the main nucleus and encapsulated in their own membrane after an erroneous mitosis. MN initially cause unequal inheritance of genetic material and may be a source of further significant genetic change through accumulation of DNA damage from dysfunctional replication and repair, as well as chromosome shattering and re-ligation events such as chromothripsis. We aim to better understand the contents of micronuclei, their behaviour, and their impact on genomic evolution.

Material and method

Immunofluorescent microscopy was used to characterize frequency of micronuclei, and whether they were actively transcribing or replicating as well as their propensity to rupture. Micronucleus purification was achieved utilizing Qiagen's QProteome Nuclear Protein Kit, and a combination of filtration and centrifugation steps. MN were size-selected using the cellenONE microfluidics platform and a tagmentase-based "single-cell" DNA library protocol was used to sequence micronuclei.

Result and discussion

We have characterised micronuclear behaviour in both a diploid, chromosomally stable cell line with pharmacologically induced CIN and a panel of cancer cell lines to provide a more comprehensive view of MN behaviour, including rupture, DNA damage levels, and their ability to replicate and transcribe their DNA. We demonstrate that, the frequency of rupture and subsequent likelihood of DNA damage varies across cell lines, and that replication of MN DNA occurs at low levels while transcription is more common. To directly examine MN contents, we developed a novel purification method and applied it to both a model of induced MN (confirming known biases in MN content), and to a cancer cell line. We validated this method by single and bulk sequencing of purified micronuclei derived from our induced system. The sequencing data allowed us to clearly distinguish between whole nuclei and micronuclei based on the representation of read counts across chromosome and to estimate copy numbers of chromosomes and chromosome fragments present in MN.

Conclusion

For the first time we have sequenced both single and bulk micronuclei induced by DNA replication stress and by mitotic defects in normal, chromosomally stable cell lines, and are able to apply this method to cancer cell lines. We aim to establish a more holistic view of micronuclei utilizing induced MN in diploid cell lines and spontaneous MN in chromosomally unstable cancer cell lines to establish their contributions to ongoing CIN and tumour evolution.

EACR25-1335

Method Development of Single Cell Readable Stress Signal Recording System

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Introduction

High-grade serous carcinoma (HGSC) is the most common and lethal subtype of ovarian cancer. Although

initially responsive to treatment, most HGSC patients experience recurrence with chemotherapy resistance, leading to poor survival outcomes. This resistance, which develops over time, has been linked to transcription factors (TFs) induced by stress signals. However, the specific stress pathways activated and their role in helping cancer cells adapt and survive chemotherapy remain unclear. To address this, we are developing a single cell-based recording method to track the activation of various stress-related transcription factors. This method employs CRISPR-Cas based editing to introduce genomic scars (insertions and deletions) that can be analyzed using single cell RNA sequencing.

Material and method

This method employs CRISPR-Cas based editing to introduce genomic scars (insertions and deletions) that can be analyzed using single cell RNA sequencing. Our approach involves the integration of single-cell transcriptomics with advanced lineage tracing techniques to map the dynamic transcriptional responses to chemotherapy.

Result and discussion

By capturing the temporal activation patterns of adaptive signaling pathways in individual cells, we hope to discover how pre-existing cellular states influence the emergence of resistance. This will be extended to patient-derived HGSC organoids, providing a clinically relevant model to validate our findings and explore the therapeutic potential of targeting specific stress pathways.

Ultimately, this research aims to uncover novel insights into the mechanisms driving chemoresistance in ovarian cancer, with the potential to identify new therapeutic targets and improve treatment outcomes for patients.

Conclusion

The development of this single-cell readable stress signal recording system represents a significant advancement in our ability to study cellular adaptation mechanisms and could have broad applications in cancer research.

EACR25-1340

Unraveling the influence of risk factors on tumour evolution in clear cell renal cell carcinoma

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Introduction

Clear cell renal cell carcinoma (ccRCC) is the most prevalent and deadliest form of kidney cancer, with more than 434,800 new cases and 155,900 deaths recorded worldwide in 2022, according to the Global Cancer Observatory latest statistics. The incidence of ccRCC

shows wide geographical variation, with Baltic and Eastern European countries, such as Lithuania and the Czech Republic, exhibiting the highest rates (Hsieh et al., 2017, *Nature Reviews Disease Primers*). This has been attributed to differences in exposure to risk factors. Known risk factors for renal cancer include obesity, tobacco smoking and hypertension, although the mechanistic link with tumorigenesis remains unknown. While some risk factors, such as exposure to aristolochic acid, are mutagenic, a recent study (Senkin et al., 2024, *Nature*) concluded that differences in the mutational patterns across geographical regions cannot fully explain the global differences in renal cancer incidence. We therefore asked whether the exposure to some of these risk factors, and the observed regional variation in incidence, are associated with distinct patterns of tumor evolution, such as the age at which key tumorigenic genomic events occur, the types of driver mutations acquired or different degrees of genomic instability.

Material and method

To address this question, we analysed the somatic mutations from whole-genome sequencing and epidemiological data obtained from a cohort of 962 patients diagnosed with primary ccRCC from eleven geographical areas with widely varying risk. This dataset was generated by the Cancer Grand Challenges Kidney Mutograph's project (Senkin et al., 2024, *Nature*).

Result and discussion

By analyzing the clock-like accumulation of mutations that result from mutational processes associated with aging, we estimated the age at which chromosome 3p loss occurred in the cell lineage where the tumor originated. Chromosome 3p loss is a canonical early driver event described in more than 90% of the ccRCC cases (Mitchell et al., 2018, *Cell*). We also estimated the age at which the most recent common ancestor cell of the tumour emerged. Additionally, we examined the landscape of driver events using state-of-the-art driver discovery methods, such as IntOGen, as well as methods to profile the genomic instability of these tumours. With all these genetic evolutionary features, we carried out an association study to uncover potential links with lifestyle ccRCC risk factors.

Conclusion

With this analysis we hope to gain a deeper understanding of how risk factors influence tumour evolution. This is essential to gain a more comprehensive understanding of the global cancer burden, intending to improve prevention strategies and reduce the incidence in the future.

EACR25-1388

Is it the T-cell to Macrophage Ration that matters for treatment in TNBC?

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Introduction

Tumor microenvironment (TME) in breast cancer is a heterogeneous landscape with multiple cell types, each modulating the tumor progression and the disease prognosis. Each cell type in the TME influences clinical outcomes for patients in a good or bad way, depending on the interaction with tumor cells. Our recent study showed that a higher proportion of Indian TNBC patients had intra-tumoral TILs compared to Western cohorts, and these are predictive of better response to NACT and longer disease-free survival in patients, making it a distinct finding from western reports. In the follow-up study, infiltrating immune cells subtypes and the signaling cross-talk are characterized using multiplex IF, GeoMx spatial transcriptomics and whole transcriptomics on FFPE tumor samples comparing responders vs non-responders.

Material and method

A retrospective cohort TNBC patients from a single onco-surgeon breast cancer clinic with a uniform treatment strategy was evaluated for this study. 24 FFPE primary tissue samples with variable TILs scores, taken from patients with a response and no response groups post-NACT, were stained for various immune cell panel; Pan-B cells, Pan-T cells, Pan-macrophages, and CD4+, CD8+, FOXP+, tumor (PanCK) and counterstained with DAPI. Multispectral images of whole slide scans were obtained using the Leica Aperio Versa system.

Parameters such as individual cell types and spatial proximity to tumor cells are determined by HALO Indica software. Total RNA was extracted from the same 24 FFPE samples and was analysed for whole transcriptome. TMA was prepared for a set of responder and non-responder samples and were run through GeoMX for ROI specific transcriptomics between responders and non-responders.

Result and discussion

TNBC samples were stained for immune and T-cells subtypes panel to determine the TiME. Around 50000-300000 cells were evaluated across all samples. Our preliminary analysis shows a higher proportion of T and B cells in stroma of the tissue in patients who show complete response compared to that of macrophages. The cross-talk of the immune infiltrates with each other and with epithelial cells is being evaluated using RNA-seq analysis and GeoMX platform. The analysis results will be presented at the congress.

Conclusion

Immune cell profiles are distinct among responding and non-responding TNBC patients in an Indian cohort. And not just the numbers of different cell types, but the proportions thereof could be influencing tumor response to the treatment. The inter-association of various immune cell types will need to be factored in while determining the clinical relevance of these profiles to identify distinct

prognostic immune subgroups in TNBC in the Indian cohort of breast cancer patients.

EACR25-1392

Investigating the impact of PRUNE2 deregulation on prostate cancer

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Introduction

Prostate cancer (PrCa) remains one of the leading causes of cancer-related morbidity and mortality among men worldwide, with familial susceptibility playing a significant role. Despite this, the underlying genetic mechanisms contributing to PrCa predisposition remain poorly understood. Our research group previously performed whole-exome sequencing in 96 PrCa patients from 45 families and reported, for the first time, pathogenic/likely pathogenic germline variants in the tumor suppressor gene PRUNE2 in patients with hereditary and/or early-onset PrCa. These findings led to the proposal of PRUNE2 as a novel biomarker for PrCa predisposition. Building on these findings, we aimed to further investigate its functional role as a tumor suppressor gene in PrCa.

Material and method

Whole transcriptome sequencing was performed on 12 prostate tissues (tumor and adjacent normal) from two PRUNE2 variant carriers and four non-carriers.

Differential gene expression analysis was conducted to identify deregulated genes and pathways associated with PRUNE2 variants. Additionally, to assess the impact of PRUNE2 depletion on cell viability and apoptosis, we performed siRNA-mediated knockdown in PNT1A cells and evaluated its impacts using Calcein AM and propidium iodide assays.

Result and discussion

Transcriptomic analysis identified 86 differentially expressed genes in PRUNE2 variant carriers (40 upregulated and 46 downregulated), with significant enrichment in extracellular matrix-associated pathways. Key deregulated genes, including LAMC2, IGFBP7, and THBS4, were linked to the PI3K/Akt pathway, a major driver of tumorigenesis. Knockdown of PRUNE2 in PNT1A cells led to increased cell viability and reduced apoptosis, supporting its role in suppressing oncogenic phenotypes.

Conclusion

Our findings provide new insights into PRUNE2's role as a tumor suppressor in PrCa, demonstrating its association with key oncogenic pathways and phenotypic changes upon depletion. These results reinforce PRUNE2's potential as a biomarker for PrCa predisposition and as a target for further functional studies.

EACR25-1403

Isoform Switching in Different Grades of Non-Muscle Invasive Bladder Cancer (NMIBC): Changes in Gene Function without Changes in Gene Expression

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Introduction

Isoform switching refers to differential usage of isoforms of certain genes in different conditions and may play an important role in cancer pathogenesis. Even when total gene expression remains unchanged, changes in isoform composition can significantly impact protein function, influencing cancer initiation and progression. Therefore, identifying and characterizing isoform switching events in non-muscle invasive bladder cancer (NMIBC) is crucial for understanding disease biology. Here, we investigate isoform switching in NMIBC progression, with a focus on the Uracil-DNA Glycosylase (UNG) gene, to determine its potential functional consequences. We also extend our analysis to an independent dataset from UROMOL, a large-scale transcriptomics study of NMIBC, to validate our findings.

Material and method

Bladder tumour samples from the Bladder Cancer Prognosis Program (BCPP) were analyzed [1]. RNA-seq data from 17 G1pTa and 48 G3pT1 tumours were processed using 3D RNA-seq and IsoformSwitch-AnalyzeR tools in R to identify significant isoform switching events. APOBEC mutational activity was quantified using the R package deconstructSigs (v1.8.0) for each sample. To functionally validate key findings, we designed isoform-specific PCR assays to assess the expression levels of the different UNG isoforms across NMIBC samples. Additionally, UROMOL RNA-seq data was analysed to determine whether the observed isoform switching patterns were reproducible in an independent NMIBC cohort.

Result and discussion

Five genes exhibited significant isoform switching events in both computational tools, including UNG, a critical gene in DNA repair and uracil removal from DNA. While total UNG gene expression did not significantly differ between tumour grades, isoform-level analysis revealed a striking switch: ENST00000242576 was highly expressed in G3pT1 tumours but low in G1pTa. ENST00000336865 exhibited the opposite pattern. Structurally, ENST00000242576 harbors both an Intrinsically Disordered Region (IDR) and a Uracil-DNA Glycosylase (UDG) domain, whereas ENST00000-336865 contains only the UDG domain. Our ongoing isoform-specific PCR experiments will further confirm these expression patterns at the RNA level. Additionally, preliminary UROMOL data analysis suggests a similar

isoform switch in an independent NMIBC cohort, supporting the robustness of our findings.

Conclusion

Isoform switching is a key molecular event in NMIBC progression, potentially altering protein function without changes in gene expression levels. In the case of UNG, the presence of an IDR in ENST00000242576 may confer a functional advantage in high-grade tumours (G3pT1) by enhancing DNA repair activity, which we are actively investigating through functional validation. UROMOL cohort and isoform-specific PCR validation will help determine whether UNG isoform switching could serve as a diagnostic and prognostic biomarker in NMIBC.

[1] Ethics: 06/MRE04/65

EACR25-1436

Unveiling plasma cell–tumor cross-talk to boost immunotherapy in microsatellite-stable colon cancer

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Introduction

Microsatellite stability (MSS) tumors represent 80–85% of colon cancer patients, which are poorly infiltrated by lymphocytes and show limited response to immunotherapy. Recent evidence suggests that MSS primary tumors can be classified into high or low evolution subtypes based on the degree of plasma cell infiltration. Tumors with higher plasma cell infiltration exhibit greater genomic diversity, promoting immune-driven tumor adaptation. However, the cross-talk between tumor and plasma cells and their spatial distribution in the tumor microenvironment remain unclear. Unraveling these interactions could reveal new strategies to enhance immune infiltration and improve MSS patients' outcomes.

Material and method

A cohort of 8 MSS colon cancer patients, 3 high and 5 low evolution, classified upon genomic similarity between primary and relapse tumors, was selected. FFPE primary tumor samples underwent spatial transcriptomics at single-cell resolution using Visium HD. Data pre-processing was performed using Spaceranger and Spacexr for cell-type deconvolution with a CRC scRNA-seq reference. Log-normalized counts were tested for differential expression ($|log2FC| > 2$) and functional enrichment with Seurat and clusterProfiler, respectively.

Result and discussion

Spatial transcriptomics data revealed an average of 14,783 normalized spots representing tumor epithelial

cells. Analogously, mean normalized plasma cell spots detected were 3 times higher in high than low evolution samples (718 vs 224). Differential gene expression analysis between high vs low evolution primary samples indicated amplified cytokine/chemokine activity as CXCL10, overexpression of Pattern Recognition Receptors signaling pathways as Nod-like receptors, and activation of JAK/STAT and TNF α signaling via NF κ B pathways ($p < 0.001$) in tumor cells of high evolution samples. This pro-inflammatory microenvironment attracts plasma cells, enhancing their antigen presentation pathways, positively correlating with increased neoepitope presentation by tumor cells ($p = 0.015$). In contrast, tumor cells in low evolution samples over-express mucins as MUC4 or MUC12, creating a barrier for immune cell infiltration. Moreover, spatial analysis exposed that tumor epithelial cells in direct contact with plasma cells undergo epithelial-mesenchymal transition (EMT), trigger an inflammatory response, and secrete extracellular matrix-modifying factors such as metalloproteinases ($p < 0.001$) compared to more distant tumor cells.

Conclusion

High evolution MSS tumors with increased neoepitope presentation foster a pro-inflammatory environment that recruits plasma cells. This positive feedback loop triggers EMT in tumors and stimulates the immune system. These insights suggest that combining plasma cell attractants with immunotherapy could improve immune responses in these patients.

EACR25-1444

Spatial Multiomics Reveals T-cell Activation and Exhaustion States in the Tumor Microenvironment

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Introduction

Spatial Multiomics allows for context-based study of the tumor microenvironment (TME). TME consists of diverse cells that collectively influence tumor progression, therapeutic response, and immune activation. CD8 T-cells, essential for antitumor immunity, often become exhausted in the TME. Recent studies show that PD1+ TCF1+ stem-like CD8 T-cells retain differentiation potential and can be rejuvenated through cytokine signaling and immune checkpoint modulation. This detailed spatial profiling of RNA and protein expression in the TME is crucial to understanding immune cell function, activation, and differentiation.

Material and method

Here we introduce a protease-free workflow for RNAscopeTM Multiplex Fluorescent v2 assay combining RNA in-situ hybridization (ISH) and protein immunofluorescence to study cell marker proteins (CD8, PD1) with RNA targets (TNFA, TCF7, IFNG) in a tumor microarray (TMA). The Manual Multiplex Protease-Free workflow enables simultaneous RNA and protein visualization without enzymatic disruption, preserving antigen integrity and tissue morphology.

Result and discussion

Using this Multiomics assay we can study CD8 T-cell differentiation and activation. Co-detection of TNFA, TCF7, and IFNG transcripts with CD8 and PD1 proteins provides insights into specific T cell phenotypes and their activation/exhaustion status. Data reveal robust co-expression patterns of these targets, particularly in the tumor-cell neighboring stromal and immune cells in breast, cervical and stomach cancers, reflecting immune activation and exhaustion across distinct TME niches

Conclusion

This methodology lays a foundation for refining immunotherapeutic strategies targeting PD1+ TCF7+ CD8 T-cells, enhancing effector functions, and advancing precision oncology interventions.

EACR25-1447

Identifying immune cell phenotypes and their function by using high throughput spatial multiomics

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Introduction

Advances in our understanding of the tumor microenvironment (TME) will help inform the development of next generation immunotherapies and engineered immune-cell therapies. Spatially characterizing immune cells with cell marker protein and activation marker RNAs is crucial to determine therapeutic efficacy and potential therapeutic toxicity. To address this, we have developed a high throughput RNAscopeTM assay on the BOND RX to spatially visualize RNA and protein markers on the same slide

Material and method

We utilized the new RNAscopeTM Multiomic LS assay that can detect up to 6 RNA and protein targets on the same slide. The TSA-based amplification strategy offers signal boost for both RNA and protein targets. To optimize protein detection, the workflow is completely protease-free thereby preserving antigen integrity and tissue morphology. The assay can be customized to include any target RNA and proteins of interest. Here, we demonstrate the use of our pre-conjugated antibody panel which includes CD8, CD4, FoxP3 and PanCK to visualize tumor infiltrating lymphocytes (TILs). We also utilized unconjugated primary antibodies for CD68 and CD163 to detect tumor macrophages in multiple tumor tissues. Using the HALO[®] image analysis software from Indica Labs, we performed RNA and protein quantification as well as cell phenotyping and spatial analysis

Result and discussion

Infiltrating Cytotoxic T cell lymphocytes were detected using CD8, GZMB and IFNG co-expression. Regulatory T cells were detected using CD4, FOXP3 co-expression. PanCK was used as a tumor marker to delineate tumor from stroma. Similarly, tumor associated macrophages

were detected using CD163, CD68, IL10 and IL-1B expression. We were also able to identify distinct M1 and M2 macrophage populations in the tumor samples.

Conclusion

The protease-free RNAscope Multiomic LS assay was successfully able to interrogate different subpopulation of immune cells with high fidelity RNA and protein signal while maintaining tissue morphology. The higher plex capability provides detection of more markers simultaneously, in turn enabling immune phenotype profiling while maintaining the sensitivity and specificity. Finally, the assay affords a very high throughput on the BOND Rx allowing large-scale pre-clinical and patient sample analysis.

EACR25-1515

Genome-scale screenings identify determinants of sensitivity of cancer cells and tumors to NFYA inhibition

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Introduction

Oncogene addiction, the dependency of certain tumors on specific genes, now extends to transcriptional dependencies, highlighting transcription factors as potential therapeutic targets in cancer. The nuclear factor Y (NFY) is a heterotrimeric transcription factor composed of NFYA, NFYB and NFYC. NFY and its targets are upregulated in cancer, with NFYA, the regulatory subunit, particularly overexpressed in human tumors. However, NFYA therapeutic potential remains unexplored.

Material and method

To study vulnerability to NFYA inhibition, we analyzed genome-scale dependency maps from CRISPR and RNAi screens conducted in over 1,000 cell lines (DepMap). A combined scoring method (60 CRISPR:40 RNAi) integrating both approaches was applied. To identify determinants of vulnerability and resistance, gene correlation and enrichment analyses were performed. Finally, we derived a single score based on gene expression to estimate NFYA dependency across all cell lines and TCGA.

Result and discussion

The combined score revealed that ~30% of cancer cell lines are dependent on NFYA. Notably, NFYA inhibition is more selective than other NFY subunits, suggesting a better therapeutic window. Vulnerability to NFYA inhibition is not related to NFY subunits' mRNA, protein levels or copy number. To identify determinants of NFYA dependency, we focused on genes whose expression correlates with combined score. Resistance genes, whose higher expression is linked to lower dependency, are enriched in interferon response pathways. Vulnerability genes, whose higher expression is associated to higher dependency, are involved in estrogen response pathways. Indeed, among breast cancer subtypes, luminal cell lines are also the most NFYA-dependent. To infer NFYA dependency in non-screened cell lines and patient tissues, we computed a single score based on the expression of determinants. Fibroblasts are

among the least dependent, suggesting lower sensitivity of non-neoplastic cells to NFYA inhibition. In TCGA, normal tissues have higher score than matched tumors, further supporting lower sensitivity. This is promising for therapy, as it may help limit offside effects and toxicity in normal tissues. Consistent with the enrichment of resistance genes in interferon pathways, the single score positively correlated with immune infiltration, indicating that immune-cold cancers may benefit from NFYA inhibition.

Conclusion

Our findings establish NFYA as a selective cancer vulnerability, particularly in tumors with high estrogen response gene expression and low immune infiltration. By identifying resistance and vulnerability determinants, we provide a foundation for predictive biomarkers and patient stratification. More broadly, we illustrate how transcriptional dependencies can be identified from genome-scale screenings and translated to patient tumors.

EACR25-1519

Putative Novel Breast Cancer Genetic Drivers in Ghana: Insights from a Cohort Study

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Introduction

A growing number of young Ghanaian women are diagnosed with breast cancer, yet its genetic basis remains largely unexplored. The only existing study used a Eurocentric panel of 34 breast cancer susceptibility genes, identifying some novel variants but lacking the ability to uncover genes and gene variants unique to Ghanaians. Given the high genetic diversity in African populations and rising breast cancer cases, this study utilized whole exome sequencing as a more comprehensive approach to profile germline and somatic breast cancer genetic risk factors in Ghanaian women.

Material and method

Breast cancer patients scheduled for surgery were recruited from four health centers in Ghana after providing written informed consent. Saliva samples were collected from all patients; breast tissues were obtained only from neoadjuvant-naïve patients. Whole-exome sequencing was performed on 86 patient samples and the germline and somatic variants analyzed using established bioinformatics pipelines. Additionally, whole-exome data from 44 Ghanaians in a Ghanaian hearing impairment cohort served as controls.

Result and discussion

Analysis revealed 20 pathogenic germline variants across 20 genes, including novel variants in BRCA2, CHRNG, CEP83, MAN1B1, and CYP4F22, as well as a previously reported BRCA1 variant (rs80357708). Nine of these genes have been linked to breast cancer metastasis, proliferation, and immune evasion, with FUS, BRCA1, and BRCA2 being key components of the DNA damage repair network. While further high-powered and functional studies are needed for validation, existing data suggest these genes may influence breast cancer

prognosis. Additionally, 20 putative somatic drivers were identified, with interaction analysis revealing 12 co-occurring and 2 mutually exclusive somatic interactions. The most significant co-occurrence was between SCUBE2 and UGT2A1 in eight cases. SCUBE2 was identified as a novel breast tumor suppressor gene and a key predictor of taxane-based neoadjuvant chemo-resistance. With the emerging link between UGTs, cancer, and drug resistance, this co-occurrence may indicate potential drug resistance in the study cohort. Somatic mutations were predominantly single-base substitutions, driven by defective DNA double-strand break (DSB) repair via homologous recombination, spontaneous 5-methylcytosine deamination, and APOBEC cytidine deaminase activity. Among these, spontaneous 5mC deamination was the most enriched mutational signature, underscoring the need for further investigation into epigenetic predispositions to breast cancer in Ghanaian women.

Conclusion

Once validated, the identified germline drivers will expand the biomarker repertoire, enhancing breast cancer detection, prognosis, and genetic counseling in the Ghanaian population. The somatic drivers will provide further insights into sporadic tumorigenesis.

EACR25-1584

Decoding the Complexity of Prostate Cancer Evolution: Uncovering Heterogeneous Cell States by Integrative Approaches

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Introduction

Prostate cancer (PC) frequently acquires therapy resistance via lineage plasticity, enabling tumor cells to adapt to androgen deprivation (AD) by activating alternative transcriptional programs. While scRNA-Seq can capture diverse cellular states at high resolution, its use in clinical settings is constrained by cost, limited sample size, and lack of extended patient follow-up. By contrast, bulk RNA-Seq is widely available and accompanied by robust clinical data, yet lacks the cellular granularity provided by scRNA-Seq. To bridge these gaps, we propose a combined approach that integrates scRNA-Seq with customized deconvolution of large-scale bulk datasets to reveal subclonal architectures in both clinical samples and preclinical models.

Material and method

We integrated scRNA-Seq profiles from normal prostate, primary and castration-resistant (CR) tumors in two stages: first using scVI for unsupervised integration, then scANVI to incorporate cell-type annotations, preserving biological signals and enabling incremental updates to our reference with new data. Our atlas was also enriched with PDXs and in vitro cultures to track tumor evolution under AD and other pharmacologic pressures. We then applied a deconvolution method tailored to prostate

populations-specific signatures, allowing bulk RNA-Seq data to be broken down into pseudo-cells, that were mapped back to the single-cell reference.

Result and discussion

Our strategy captured a spectrum of tumor phenotypes, including AR-driven, neuroendocrine, WNT-dependent, or immune-modulated states, and additionally revealed novel subtypes, underscoring the deeper complexity of PC plasticity. Embedding bulk data provided the clinical context, such as prognosis and treatment response, to link transcriptional states with patient outcomes. We identified multiple coexisting clones within single tumors, mapping to distinct clusters and exposing pre-existing clonal heterogeneity in primary disease as well as clonal evolution under AD, thus enabling classification of primary and metastatic tumors by transcriptional profiling and specific genomic alterations. Notably, a subset of CR tumors with heightened immune infiltration emerged, pointing to a distinctive tumor micro-environment interplay and potential immunotherapeutic sensitivity. Moreover, applying literature-derived PC signatures onto PDXs uncovered distinct subclones within uniform model systems, clarifying their representativeness of specific clinical phenotypes and emphasizing the heterogeneity of late-stage disease.

Conclusion

By coupling single-cell and bulk transcriptome data, our framework delivers both the resolution to identify emergent subclones and the large-scale clinical perspective essential for translational insights. Our resource maps PC progression, aligns *in vitro* and *in vivo* models with patient subtypes, and ultimately can guide more precise therapeutic strategies.

EACR25-1669

Decoding atypical teratoid rhabdoid tumor subtype-specificity: single cell aspect of differentiation trajectories and potential maturation therapy

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Introduction

Atypical teratoid rhabdoid tumors (ATRTs) are among the most lethal pediatric central nervous system (CNS) tumors, which only harbor one single genetic driver mutation, the bi-allelic loss of the BRG1/BRM-associated factors (BAF) complex member SMARCB1. Based on DNA methylation profiles, three molecular subtypes have been further identified: ATRT-SHH, ATRT-MYC, ATRT-TYR. Each subtype has their own molecular and clinical features. Due to the very low occurrence rate (1–2 cases per million children per year), there are currently no subtype-specific treatments available, highlighting the necessity to better understand inter- and intra-subtype heterogeneity. Because paediatric cancers are often caused by block of developmental maturation, this knowledge will also be crucial for

development of maturation therapy (inducing cancer cells towards more mature, less aggressive state).

Material and method

We generated an unique single-nucleus transcriptome atlas of 19x ATRTs covering all three subtypes, validated by single-nucleus ATAC-seq and spatial transcriptomics, to study subtype-specific differentiation trajectories. Patient-derived-tumoroid models were used to validate potential maturation-including drugs.

Result and discussion

We discovered brain progenitor-like expression profiles within unique subtype-specific differentiation lineages. A shared cycling, progenitor-like cell population, interspersed throughout tumors, was observed across all ATRT samples. Additionally, top hits for maturation therapies, e.g., HDAC inhibitor Entinostat and PKC inhibitor Ro31-8220 were tested in our ATRT tumoroids, pointing that subtype-specific differentiation trajectories can be induced pharmacologically to push tumor cells towards a more mature and less-proliferative state.

Conclusion

Our study decodes ATRT subtype-specificity at single cell level and reveals that each ATRT subtype is enriched for distinct signaling trajectories mirroring normal fetal brain development, enabling the development of maturation therapies tailored towards ATRTs.

EACR25-1672

Inhibition of the heat shock response enhances modulated electrohyperthermia effects in triple negative breast cancer mouse model

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Introduction

Modulated electro-hyperthermia (mEHT) is a selective cancer treatment used in human oncology complementing other therapies. During mEHT, a focused electromagnetic field (EMF) is generated within the tumor inducing cell death by thermal and nonthermal effects.

Material and method

Here we investigated molecular changes elicited by mEHT using multiplex methods in an aggressive, therapy-resistant triple negative breast cancer (TNBC) model. 4T1/4T07 isografts inoculated orthotopically into female BALB/c mice were treated with mEHT three to five times.

Result and discussion

mEHT induced the upregulation of the stress-related Hsp70 and cleaved caspase-3 proteins, resulting in effective inhibition of tumor growth and proliferation. Several acute stress response proteins, including protease inhibitors, coagulation and heat shock factors, and complement family members, were among the most upregulated treatment-related genes/proteins as revealed

by next-generation sequencing (NGS), Nanostring and mass spectrometry (MS). pathway analysis demonstrated that several of these proteins belong to the response to stimulus pathway. Cell culture treatments confirmed that the source of these proteins was the tumor cells. The heat-shock factor inhibitor KRIBB11 reduced mEHT-induced complement factor 4 (C4) mRNA increase.

Conclusion

mEHT monotherapy induced tumor growth inhibition and a complex stress response. Inhibition of this stress response is likely to enhance the effectiveness of mEHT and other cancer treatments.

EACR25-1673

Advancing the genetic engineering toolbox by combining AsCas12a knock-in mice with ultra-compact screening

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Introduction

CRISPR-Cas9 technology has revolutionized genetic research, particularly in the discovery of novel tumour drivers and resistance factors through CRISPR screens. Cas12a (Cpf1) distinguishes itself from Cas9 by short crRNAs and a unique PAM site. Additionally, intrinsic RNase activity simplify multiplexed gene targeting via the processing of individual crRNAs from a pre-crRNA-encoding RNA.

Material and method

Here, we present a mouse model that constitutively expresses enhanced Acidaminococcus sp.Cas12a (enAsCas12a) linked to an mCherry fluorescent reporter. We generated compact, genome-wide Cas12a knockout libraries targeting each gene with four crRNAs encoded across one (Scherzo) or two (Menuetto) vectors, and performed multiple screens.

Result and discussion

We validated robust expression of Cas12a in our mouse model across multiple tissues, with a particular focus on hematopoietic organs, a key facet of our research. We demonstrate efficient single and multiplexed gene-editing in vitro, using primary and transformed cells from enAsCas12a mice. We further demonstrate successful in vivo gene-editing, using normal and cancer-prone enAsCas12a stem cells to reconstitute the hematopoietic system of wild-type mice. We demonstrated the utility of genome-wide Cas12a libraries across multiple screens: in vitro enrichment screening in lymphoma cells, in vitro drop-out screening in immortalised MDFs, and in vivo enrichment screening in the hematopoietic lineage of mice to identify lymphoma-driving events. The consistency and robustness of the data extracted from each of our screens underscore the high effectiveness and broad potential of these new tools. Finally, we demonstrate CRISPR multi-

plexing via simultaneous gene knockout (via Cas12a) and activation (via dCas-SAM) using primary T cells and mouse dermal fibroblasts (MDFs). This highlights the compatibility of our enAsCas12a mouse model with other CRISPR technologies.

Conclusion

Collectively, our enAsCas12a mouse model and accompanying crRNA expression libraries enhance genome engineering capabilities and complement current CRISPR technologies.

EACR25-1693

The computational strategy, popmax, decreases misclassification of germline variants

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Introduction

Classifying variants accurately is challenging for underrepresented populations. Here, we have utilized a computational strategy, popmax, to determine if the misclassification of germline variants can be decreased.

Material and method

Germline variants from Singapore and the United States were used to represent two ancestrally diverse populations. From Singapore, we had early-onset or familial colorectal cancer (CRC) cases ($n = 132$) and population-matched controls from the SG10K_Health cohort ($n = 9,740$). From the United States, 100 early-onset CRC patients and population-matched controls from gnomAD ($n = 134,187$) were used. The effect of using the popmax computational strategy was explored by comparing matched and mismatched (swapped) control cohorts, with and without popmax.

Result and discussion

In both CRC cohorts, population-matched classifications identified 62 pathogenic or likely pathogenic (P/LP) variants in 34 genes. The use of population-mismatched control cohorts resulted in an increase in the misclassification of non-P/LP variants as P/LP variants. However, these misclassifications were partially alleviated with the use of popmax.

Conclusion

Popmax decreased the number of misclassified variants in our cohorts, and can potentially be applied to studies of underrepresented populations with limited variant data of control cohorts.

EACR25-1694

Prevalence of Chromosomal Alterations in Acute Leukemias: A Descriptive Analysis in the Colombian Population

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Introduction

Acute leukemia encompasses a heterogeneous group of hematologic malignancies characterized by diverse chromosomal alterations that significantly influence prognosis, treatment response, and survival outcomes. Understanding the distribution and frequency of these alterations is crucial for refining diagnostic and therapeutic strategies. In Colombia, there is a limited understanding of the prevalence of these genetic abnormalities due to the scarcity of comprehensive studies. This descriptive study aims to identify and compare the chromosomal alterations present in adult and pediatric patients with acute leukemia in the Colombian population, providing valuable insights into the genetic landscape of these malignancies.

Material and method

Bone marrow samples were collected from 48 patients de novo diagnosed with acute leukemia, including 27 adults (≥ 18 years) and 21 children (≤ 17 years). Chromosomal alterations were detected using RT-PCR, assessing 28 different genetic alterations, including translocations and inversions associated with leukemia. Statistical analyses were performed using chi-square tests, considering a p-value < 0.05 as significant to determine differences between the groups.

Result and discussion

Acute myeloid leukemia (AML) was more frequent in adults (74.07%) compared to children (23.81%) ($p = 0.001541$), while acute lymphoblastic leukemia (ALL) predominated in children (76.19%). The Hemavision test was positive in 37.04% of adults and 19.05% of children ($p = 0.298243$). Among the chromosomal alterations detected, inv(16) (CBFB-MYH11) was present only in adults (14.81%) and absent in children ($p = 0.188206$). Similarly, t(15;17) (PML-RARA) was exclusive to adults (7.41%) ($p = 0.585053$). In contrast, t(12;21) (ETV6-RUNX1) was detected only in children (14.29%) ($p = 0.153473$), and t(4;11) (KMT2A-AFF1) was present exclusively in children (4.76%) ($p = 0.898686$). The alterations t(9;22) (BCR-ABL1), t(6;11) (KMT2A-AFDN), and t(8;21) (RUNX1-RUNX1T1) were rare and showed no clear differences between age groups. These findings highlight the distinct patterns of chromosomal alterations between adults and children, emphasizing the heterogeneity of acute leukemias.

Conclusion

This is the first study to describe the prevalence and distribution of chromosomal alterations in de novo acute leukemia patients in Colombia. The exclusive presence of

inv(16) and t(15;17) in adults and t(12;21) and t(4;11) in children highlights the importance of implementing age-specific diagnostic strategies. It is important to note that these data are preliminary, and we plan to expand the number of patients included in the study to increase the robustness of the findings.

EACR25-1713

Comprehensive analysis of somatic structural variations in Malignant pleural mesothelioma (MPM) using long-read sequencing

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Introduction

Malignant pleural mesotheliomas (MPMs) harbour extensive somatic genome structural variations. Molecular targeted therapy is lacking. Identifying somatic driver mutations in MPM is critical in understanding the development of mesothelioma and developing new therapy strategies. Long-read DNA sequencing using Oxford Nanopore Technology (ONT) can detect chromosomal rearrangements, shorter insertion/deletions, collectively known as structural variants (SVs) compared with short-read whole exome sequencing. ONT also directly profiles the methylome, allowing assessment of epigenetic silencing. In this proof-of-principle study, we aim to identify and validate somatic structural variants using long read sequencing in three MPMs using matched blood samples as a reference, and comparing with matched WGS short-read DNA sequencing. We also examine the matched transcriptomes data.

Material and method

Three MPM tumours with matched blood DNA samples were sequenced at high coverage using ONT PromethION. Structural variants were identified using minimap2 and Severus, then compared to variants identified by Illumina whole genome sequencing using BWA and Manta. The segmentation copy number alteration was detected by CNVkit. The gene expression was detected by using Salmon. Finally, the affected genes were examined their impact on survival time using TCGA data.

Result and discussion

The three MPM tumours were sequenced to between 38x and 42x coverage, with a median read length between 20 kb and 28 kb, and a maximum read length between 580 kb and 248 kb. The most common SV type was translocation (35.7%), followed by deletion (27.8%), insertion (21.1%), inversion (0.11%), and duplication (0.11%). We identified the double hits of MPM driver genes such as NF2, LATS2, BAP1 using both whole genome sequencing Illumina and whole genome sequencing

ONT. On the other hand, Severus identified between 61–156 novel SVs not reported by whole genome sequencing Illumina. These SVs affected multiple COSMIC cancer-associated genes including LRP1B, BIRC6, EXT1, NBEA, GPC5, LPP, ROBO2, JAZF1, CDH1, FLCN, SPECC1, and EPHA7. Additional, potential cancer genes were previously reported in other cancers such as GPC6 and PRKN. Furthermore, some of these SVs were involved in complex rearrangement on same haplotype. Gene expression levels of GPC6 and PRNK were associated with survival time.

Conclusion

Long read sequencing using Oxford nanopore technology identifies novel structural variants in MPM, missed by short-read sequencing, including potential complex events such as chromoplexy. For future studies, the ONT could be applied to larger cohorts of MPM patients to identify the double hit and complex rearrangements during MPM evolution, and to support new precision medicine strategies.

EACR25-1740

Targeting Aurora Kinase A in Breast Cancer Brain Metastasis

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Introduction

Breast cancer brain metastasis (BCBM) affects 10–38% of metastatic patients, particularly those with HER2-positive and triple-negative subtypes. BCBM is highly aggressive, with a poor prognosis and an average survival rate of less than two years. BCBM diverge from their primary breast tumours both genetically and clinically and decoding mechanisms allowing drug-tolerant metastatic tumour cells to persist therapy and proliferate in the brain remains a challenge.

Material and method

In this study, we performed DNA exome sequencing ($N = 38$), RNA sequencing ($N = 63$), and DNA methylation analysis ($N = 18$) of patient-matched primary and BCBM sample pairs to identify genomic and transcriptomic vulnerabilities. This approach in combination with a high-throughput drug screening in five BCBM cell lines (LY2-MET [ER+], T347 [ER+HER2+], SUM190-BR [HER2+], MDA-MB-231-BR [TNBC], and JIMT1-BR [HER2+]) revealed significant sensitivity to AURKA pathway inhibitors.

Result and discussion

Our analyses identified aberrant activation of AURKA in BCBM patient samples, with high AURKA mRNA levels correlating with poorer overall and post-metastasis survival. Both genetic and pharmacological inhibition of AURKA yielded significant anti-tumor effects across cell lines, patient-derived organoids, and brain organotypic cultures. *In vivo*, alisertib substantially reduced brain metastasis growth. Furthermore, transcriptomic and

phosphoproteomic analysis revealed that targeting AURKA modulated Notch signaling, which appears to enhance tumor proliferation. Elevated expression levels of AURKA and MAML1 were linked to worse post BCBM survival outcomes in patient samples.

Conclusion

Our findings establish AURKA as a critical vulnerability in BCBM. Targeting both its kinase-dependent and kinase-independent functions may offer new therapeutic strategies to improve patient outcomes.

EACR25-1768

Unraveling the role of LKB1 mutations in Non-Small Cell Lung Cancer brain metastasis formation

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Introduction

Non-small cell lung cancer (NSCLC) is a prevalent form of lung cancer that presents significant challenges due to its propensity to metastasize to distant organs, with brain metastases (BMs) being a frequent and severe complication. NSCLC cells may exploit specific molecular interactions and signaling pathways to cross the blood-brain barrier and form tumors in the brain's microenvironment, characterized by low oxygen, limited nutrients, and a unique immune landscape. Based on our analysis of a large genomic database, we aimed to investigate the impact of STK11 mutations in the formation of NSCLC BMs, focusing on the mTOR signaling pathway and its effects on cell survival under brain-mimicking conditions. STK11 encodes liver kinase B1 (LKB1), a highly conserved serine/threonine kinase that regulates essential cellular processes such as metabolism and growth.

Material and method

The study cohort included 58,101 patients with NSCLC who underwent comprehensive genomic profiling of at least 300 genes (Foundation Medicine, Inc. (FMI)). Samples from both primary and metastatic sites were analyzed to identify common genomic alterations in BMs. An in vitro model using A549 cells was employed to assess the effects of LKB1 overexpression on NSCLC cell viability. Cells were cultured under hypoxic conditions, glucose deprivation, and in astrocyte-conditioned medium (a-CM) to mimic the brain microenvironment. Additionally, in an ex vivo model, A549 cells were cultured on brain slices from mice. mTOR pathway activity was assessed through Western blot analysis. Viability assays were conducted to evaluate the impact of LKB1 expression on cell survival.

Result and discussion

Analysis of N = 2,815 BMs and N = 9,793 non-BMs in FMI's genomic database revealed a higher incidence of STK11 loss-of-function mutations in BMs (24%) compared to non-BMs (19.2%) ($P < 0.001$). LKB1 over-expression in A549 cells decreased cell viability under hypoxic and glucose-deprived conditions, highlighting its role in cellular stress responses. Similarly, LKB1 over-expression reduced cell viability in a-CM. Additionally, we observed a reduction in the proliferation of A549 cells overexpressing LKB1 when cultured on brain slices, further supporting its role in inhibiting cell growth within the brain microenvironment. Moreover, LKB1 over-expression reduced mTOR pathway activity in brain-like conditions, suggesting a potential mechanism for decreased cancer cell survival. Analysis of a-CM revealed elevated levels of hepatocyte growth factor (HGF), which also decreased the viability of NSCLC cells overexpressing LKB1 and downregulated mTOR pathway activity.

Conclusion

Our findings suggest that STK11 mutations contribute to NSCLC BMs by enhancing cell survival in the brain microenvironment through the mTOR pathway. This research highlights the potential of targeting mTOR signaling in patients with NSCLC BMs as a therapeutic strategy to improve treatment outcomes.

EACR25-1774

Genomic profiling of tumour and liquid biopsy melanoma samples with targeted next-generation sequencing

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Introduction

Genomic profiling of cancer with next-generation sequencing (NGS) is widely studied for cancer diagnosis, monitoring, and prognosis. Two main sources of tumour DNA for genomic analysis are: standard biopsy of primary or metastatic tumour and liquid biopsy-derived circulating tumour DNA (ctDNA). The first provides a greater amount of tumour DNA, while the other is low-invasive and accessible for longitudinal tumour DNA analysis. Here we present the results of targeted NGS performed on 126 melanoma samples, including 32 patient-matched tumour and liquid biopsies.

Material and method

Tumour DNA (standard biopsy, n = 45) and cfDNA (cell-free DNA, liquid biopsy, n=81) derived from 81 patients with stage IV or III melanoma were used. CfDNA was isolated with the QIAamp Circulating Nucleic Acid Kit (Qiagen). DNA libraries were prepared with a custom panel (30 genes) and the Ion AmpliSeq HD library kit. Sequencing and data analysis were performed using the Ion Torrent NGS platform, dedicated software, and the Alamut Visual Plus programme. The copy number variation analysis was performed using the ONCOCNV 7.0, CNVkit 0.9.10, and ExomeDepth 1.1.15 programmes. Statistical analysis evaluated the predictive power of the gene panel. Genetic profiles were analyzed using hierarchical clustering, multiple correspondence analysis (MCA), and univariate logistic regression in R (v4.4.1). Pairwise comparisons between patient subgroups were made using Fisher's exact test [1].

Result and discussion

The analysis detected approximately 180 variants in 62 patients (76.5%) that covered all genes in the panel with allele frequency (VAF) ranging from 0.1% to 86%. They were divided into pathogenic (mutations) and likely pathogenic variants of unknown significance (VUS-LP). The genes that were the most frequently mutated were BRAF, NRAS, NF1, ARID2, TP53, and the TERT promoter. The concordance analysis revealed a 64% overlap of all variants between liquid and a tumour biopsy and 70% concordance for pathogenic variants. Statistical analysis showed no genomic clustering for the immunotherapy response. For targeted therapy, some clustering patterns suggested potential genetic associations. For 7 patients, two or more LB samples were profiled before therapy and at the time of progression/start of a new therapy showing an increased, or decreased VAF of altered genes or the appearance of new variants.

Conclusion

Genomic profiling of tumour and liquid biopsy melanoma samples is a useful tool for the identification of mutated genes in melanoma and, in some cases, for the monitoring of genomic evolution during treatment. The usefulness of our 30-gene NGS panel in predicting response to therapies needs further investigation in larger patient cohorts.

This work was supported by the National Science Center, grant no 2021/43/B/NZ7/01812. The study was approved by the Ethics Committee (approval no. KB/430-81/22).

EACR25-1830

STAG2 mutations in the normal colon induce upregulation of oncogenic pathways in neighbouring wildtype cells

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Introduction

There has been recent interest in the role of somatic driver mutations in phenotypically normal organs, particularly in relation to oncogenesis. One such driver mutation is STAG2, a critical component of the cohesin complex involved in mitosis. Here, we sought to characterise the role of STAG2 mutants in the normal colon in relation to oncogenesis.

Material and method

We performed this study using patient-derived human colonic organoids, in which the STAG2 mutation was introduced using CRISPR-Cas9 gene editing. We performed single-cell RNA sequencing (scRNAseq) to determine transcriptomic changes in co-cultured wildtype and STAG2 mutant organoids.

Result and discussion

Co-cultured cells on scRNAseq clustered into four clusters. There was a higher proportion of STAG2 mutant cells in Cluster 3, while there was a higher proportion of wildtype cells in Clusters 0 and 2. Cluster 1 appeared to have equal proportions of STAG2 mutant and wildtype cells. Comparing between STAG2 mutant and wildtype cells, only GPS2, SPTSSA, H4C3, and HNRNPA0 had average log2FC >1 and were statistically significantly different. Analysis of STAG2 wildtype and mutant cells using Gene Set Enrichment Analysis (GSEA) for "Hallmark" gene sets (50 genes sets) revealed that only the "HALLMARK_TNFA_SIGNALING_VIA_NFKB" gene set was upregulated in wildtype compared to mutant at an FDR of <25%. Intriguingly, no gene sets were upregulated in STAG2 mutant relative to wildtype. Further analysis was conducted within clusters. In Cluster 0 and 2, we observed that the "HALLMARK_TNFA_SIGNALING_VIA_NFKB" which had been upregulated in wildtype cells was also upregulated in both clusters with FDR < 25% and nominal p-value < 5%. In Cluster 0, other gene sets which were upregulated included "HALLMARK_ANGIOGENESIS" and "HALLMARK_EPITHELIAL_MESENCHYMAL_TRANSITION". In Cluster 2, other gene sets which were upregulated included "HALLMARK_EPITHELIAL_MESENCHYMAL_TRANSITION", "HALLMARK_KRAS_SIGNALING_UP", and "HALLMARK_P53_PATHWAY". Notably, in Cluster 3, none of the Hallmark gene sets were observed to have been upregulated or downregulated at FDR < 25%.

Conclusion

These observations confirm that oncogenic pathways are upregulated in co-cultured wildtype cells while oncogenic pathways in STAG2 mutant cells themselves remain relatively quiescent, suggesting a novel cell-cell interactive modality of oncogenesis hitherto undescribed.

EACR25-1840

Genome-wide CRISPR screen identifies RAB14 as a new target for gastric cancer treatment

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Introduction

About 20% of gastric cancers (GC) exhibit HER2 overexpression. HER2-positive GC patients can be treated with HER2 inhibitors like trastuzumab. Due to HER2 expression heterogeneity in tumours, only ~47% of patients respond to this targeted treatment, and even those who do, eventually develop resistance to it. This project aims to explore the biology of HER2-GC and to uncover additional targets and modifiers of response to anti-HER2 therapies.

Material and method

We performed a pilot genome-wide CRISPR screen, in a HER2-positive GC cell line, to identify genes which lead to increase in sensitivity or resistance to HER2 small molecule inhibitor lapatinib. Validation was performed with target knock-out (KO) of the top hits in multiple GC cells lines using CRISPR/Cas9.

Result and discussion

KO of the top hit, RAB14, sensitized five different GC cell lines to multiple HER2 inhibitors. Unexpectedly, we found that loss of RAB14 was synergistic with HER2 inhibition, not only in HER2-positive, but also in HER2-negative cell lines. Rab14 was found overexpressed in GC. Our current data supports that RIC8A may have a similar effect to RAB14 in HER2-positive cells.

Conclusion

In summary, we identified two modifiers of response to HER2 inhibition, RAB14 (overexpressed in GC) and RIC8A, each synergizing with lapatinib to reduce cell viability. The notable synergistic effect of RAB14 depletion and lapatinib in HER2-negative cells, likely reflects lapatinib pleiotropy, providing an opportunity to treat HER2-negative GC, which accounts for 80% of GC cases and currently lacks targeted treatment strategies.

EACR25-1869

Dependency-Shock transcriptomic footprints as predictive tools in early anti-cancer drug discovery

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Introduction

The oncogenic shock model has been proposed to describe the interplay between pro-survival and pro-

apoptotic signals that follow an oncogene's inactivation and lead to cancer cell death. Building on this model, we call cancer dependency shock (DepSHOCK) the signal dynamics' interplay that follows the ablation of any gene essential for cancer survival, i.e., cancer dependency gene. Any DepSHOCK triggers the sudden inactivation of pro-survival pathways and activates death-inducing signals, with both events impacting down-streaming transcription factors, leaving a measurable gene expression footprint. Each DepSHOCK footprint can be deconvoluted to infer transcriptional markers of signals that emanate from a dependency gene and drive cancer cell survival through the constitutive activity of specific biological pathways. Our goal is to investigate this hypothesis across tissues, canonical oncogenic addictions, and novel candidate cancer therapeutic targets.

Material and method

We have established an experimental/computational platform for the generation and the analysis of transcriptional data generated upon drug and genetic perturbation of a selected panel of cancer cell lines and therapeutically relevant dependency genes. We generated a first post-perturbational dataset profiling 10 cancer cell lines with 9 drugs, such that each drug targets at least a cell line-specific cancer driver gene and for each drug there is at least one very sensitive cell lines (harbouring a driver mutation in the targeted gene). We are applying a variety of unsupervised machine learning methods to this data to identify genetic signatures that can be used to sense the presence of specific pro-survival signals, thus drug sensitivity and genetic dependencies in independent cancer samples.

Result and discussion

Our preliminary results show that the basal expression of specific dependency-shock signatures is predictive of drug response and genetic dependency in independent cancer samples comparably to pathway/gene-addiction signatures derived with much more complex supervised machine learning methods applied to much larger datasets of clinical and multiomic datasets.

Conclusion

Our main goal is to study the information content of the DepSHOCK footprints and their potential use as computational tools in early anti-cancer drug discovery through new statistical and machine-learning methods delivering mechanistically grounded therapeutic markers and hints for the design of combinatorial therapies.

EACR25-1879

Combined analysis of chromatin accessibility, promoter interactions and whole genome sequencing solved the missing heritability in gastric cancer

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Introduction

Missing heritability in hereditary diffuse gastric cancer (HDGC) ranges from 60 to 90%. HDGC-like families, despite fulfilling HDGC clinical criteria, lack actionable variants in adhesion molecules CDH1 and CTNNA1, and are not offered HDGC-targeted life-saving disease preventive measures. Thus, we hypothesized that novel inherited mechanisms within CDH1-regulatory elements (REs) contribute to HDGC predisposition.

Material and method

We called single-nucleotide/copy-number variants (SNV/CNV) from 19 HDGC probands whole-genome sequencing data, performed gene-ontology analysis, 4C-seq and ATAC-seq in healthy stomach epithelia, CRISPR-Cas9, RT-PCR and flow cytometry in cell lines, immunohistochemistry and microsatellite instability analysis in tumours and enhancer assays in mouse embryos.

Result and discussion

We show novel mechanisms affecting the CDH1-regulatory network, predisposing HDGC-like families to cancer in 16% of HDGC-like families. We found a family carrying a germline deletion of two hypomorphic tissue-specific REs within CDH3, and another with a germline RE deletion downstream of CDH1, both triggering CDH1 mRNA/protein downregulation. The same effect was triggered by a MLH1 germline deletion overlapping a stomach-specific RE in a family with gastric but no colorectal cancer. Tumour of CDH3 and MLH1 probands showed CDH1 reduced expression and presence of signet ring cells, a cell adhesion loss phenotype. Co-occurring deletions of stomach-associated REs and truncating variants, affecting mucin and immune-related genes were found in additional 9/19 (47%) families. In one patient, recurrent deletions were confirmed by long-read sequencing.

Conclusion

This pioneer study identifies monogenic-like heritability through REs deletions in the CDH1 locus or stomach-specific REs, and raises the hypothesis of polygenic heritability mechanisms involving co-occurring variants

favouring cancer predisposition via immune-deficient phenotypes in HDGC-like families.

EACR25-1892

Beyond BRAF V600E: Revealing the Hidden Clinical Relevance of Uncharacterized BRAF Variants

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Introduction

BRAF is a critical oncogene driving tumorigenesis in multiple cancer types, including melanoma, colorectal cancer, and non-small cell lung cancer. Advances in comprehensive genome profiling reveals a growing number of alterations whose clinical significance remains undefined due to the lack of functional testing. The clinical interpretation of these variants of unknown significance in BRAF pose significant challenges for personalized treatment, particularly non-V600 variants such as CRAF-dependent class-III BRAF variants. By focusing on the functional impact of these lesser-known BRAF alterations, this study aims to elucidate their role in MAPK pathway activation and assess their potential as actionable targets in cancer therapy.

Material and method

We collected BRAF variants detected in cancer patients analyzed at our center between 2021 and 2025. The cohort consists of 3520 and 972 samples for DNA and RNA, respectively. For functional testing, we selected a previously uncharacterized FNBP1::BRAF fusion and several uncharacterized non-V600 BRAF variants (E510K, G466A, E549Q, E695Q), which were introduced into plasmids via Golden Gate cloning or site-directed mutagenesis, respectively. HEK-293T cells were transfected with wild-type or mutant BRAF plasmids alone or co-transfected with CRAF. After 48 hours, proteins were isolated, and p-ERK levels analyzed by western blot. Cells transfected with FNBP1::BRAF were treated with MEK and RAF inhibitors for two hours to assess p-ERK inhibition.

Result and discussion

BRAF variants were processed to remove artefacts, low quality variants and intronic/synonymous variants,

yielding a total of 247 variants. Of those, 20% are of unknown significance, with 27% located in the kinase domain, 5% in the cysteine-rich domain, and 16% in the Ras-binding domain. Functionally, cells expressing FNBP1::BRAF, identified in a glioma patient, exhibited elevated ERK activation, with p-ERK levels similar to BRAF p.V600E-transfected cells. Treatment with RAF inhibitor Dabrafenib or MEK inhibitor Trametinib and their combination successfully reduced p-ERK levels. BRAF p.E501K and BRAF p.E549Q showed kinase impairment when mono-transfected, but CRAF presence triggered ERK hyperactivation, suggesting a class-III BRAF variant. In contrast, BRAF p.G466A led to elevated p-ERK signals, regardless of CRAF presence. BRAF p.E695Q did not cause pathway overactivation, regardless of CRAF-presence.

Conclusion

This study identifies FNBP1::BRAF as a previously uncharacterized potential driver of glioma. Trametinib and Dabrafenib effectively reversed its constitutive ERK phosphorylation. Additionally, BRAF p.E501K and BRAF p.E549Q are likely pathogenic class-III BRAF variant, as it exhibits CRAF dependent ERK hyperactivation. In contrast, BRAF p.G466A is a likely pathogenic BRAF variant that induces ERK hyperactivation independent of CRAF.

EACR25-1894

Intra-Tumour Heterogeneity in Cutaneous Squamous Cell Carcinoma Assessed by Spatial Transcriptomics

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Introduction

High-resolution study of the tumour microenvironment is made possible by spatial transcriptomics, which captures interactions between stromal, immune, and tumour cells. This work used a PolyA capture-based spatial transcriptomic technique to evaluate intra-tumour heterogeneity in three tumour regions and the normal adjacent skin from a patient with cutaneous squamous cell carcinoma (SCC). The tumour regions displayed diverse morphologies under a microscope, including big nests displaying squamous differentiation, medium-sized nests, and smaller nests within the desmoplastic stroma.

Material and method

Prior to RNA sequencing, four cryosectioned tissue samples were placed onto a Visium® slide and subjected to the Visium Spatial Gene Expression V1® protocol. Further downstream analyses, including normalization, clustering, and cell annotation were conducted using the Seurat package in R. Regulatory network inference was performed using SCENIC to identify key transcription factors and gene regulatory modules in each different tumour region. The intercellular communication networks within the tumour microenvironment were inferred using CellChat.

Result and discussion

In tumour regions, the Visium® protocol yielded 78,344 mean reads per spot and 2,026 median genes per spot. Two tumour regions exhibited diverse SCC cell subtypes, including typical epithelial SCC, basal-like SCC, and SCC with epithelial-mesenchymal characteristics. Myofibroblastic cancer-associated fibroblast (myCAF) demonstrated the highest transcription factor enrichment among stromal cells, which was primarily regulated by NR2F1. Significant interactions were observed between myCAF and SCC clusters, potentially sharing similar CXCL signalling pathways. The third tumour region, which displayed smaller nests in the desmoplastic stroma, predominantly exhibited basal-like SCC. Vascular cancer-associated fibroblast (vCAF) demonstrated the highest transcription factor enrichment, which was primarily regulated by KLF2 and NR2F2. Concurrently, vCAF, myCAF, and basal-like SCC exhibited the strongest interactions exclusively through GALECTIN, SELE, PTPR, Netrin and PDGF signalling pathways.

Conclusion

We showed that spatial transcriptomics analysis on frozen tissue samples is an effective method to study intra-tumour heterogeneity and potential molecular interactions within cutaneous SCC. The three tumour regions exhibited marked differences in tumour morphologies, which were associated with distinct cell composition, gene regulatory module, and cell interactions.

EACR25-1919

BUB1-Mediated G2/M Checkpoint Control and Mitotic Integrity: A Targetable Vulnerability in MPM

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Introduction

Malignant pleural mesothelioma (MPM) is a rare but highly aggressive malignancy with limited treatment options, a severe prognosis, and poor survival rates, necessitating the identification of novel therapeutic targets. BUB1, a mitotic checkpoint serine-threonine kinase, plays a pivotal role in the spindle assembly checkpoint (SAC) function and accurate chromosome segregation. Elevated BUB1 expression has been correlated with poor prognosis in multiple cancers. Here, we demonstrate the role of BUB1 as an emerging cancer-

essential gene and a potential druggable vulnerability in MPM.

Material and method

BUB1 was identified as a vulnerability for MPM cells by a whole-genome CRISPR screen and this role was validated by knockout and pharmacological inhibition (BAY-1816032). RNA sequencing was conducted to analyze transcriptomic profiles of BUB1-depleted MPM cells. Affected pathways and regulatory networks were defined by gene set enrichment analysis (GSEA). To explore BUB1's functional role in mitotic regulation, localization of key mitotic checkpoint proteins and BUB1 interaction partners MAD1, MAD2, and Shugoshin (SGO1) was assessed by immunofluorescence (IF) staining and the expression of BUB1 local network genes (CDC20, Cyclin A, Cyclin B, and p21) was measured by western blot. Additionally, time-lapse live-cell imaging was conducted to track mitotic progression and cytokinesis dynamics in real time.

Result and discussion

MPM cell survival and growth were significantly impaired by genetic depletion or pharmacological inhibition of BUB1, which also caused G2/M cell cycle arrest, cellular senescence, apoptosis, and a reduction in aggressive cancer cell traits. RNA-seq analysis of BUB1 knockout cells revealed widespread transcriptional changes associated with cell cycle regulation, G2/M checkpoint control, and mitotic reprogramming.

Mechanistically, BUB1 is essential for the proper localization of MAD1, MAD2, and SGO1, ensuring the integrity of the spindle assembly checkpoint (SAC). The loss of BUB1 impaired SAC integrity, leading to mitotic errors. These defects were accompanied by significant downregulation of CDC20, Cyclin A, and Cyclin B, alongside an upregulation of p21, as well as G2/M cell cycle arrest reinforcing the role of BUB1 in G2/M checkpoint control. Live-cell imaging further confirmed that BUB1 KO cells exhibited prolonged mitosis, frequent cytokinesis failure, and multinucleation, underscoring the essential function of BUB1 in maintaining mitotic fidelity.

Conclusion

Our findings establish BUB1 as a critical regulator of mitosis by modulating the G2/M checkpoint in MPM. The observed mitotic defects and altered transcriptional programs reinforce the role of BUB1 in MPM pathophysiology. These results highlight BUB1 as a key dependency in MPM and provide a strong rationale for targeting BUB1 in preclinical models of MPM therapy.

EACR25-1925

Illumina based ex situ whole transcriptome workflow providing large area capture substrate, up to 7.5 cm² active area, and a scalable end-to-end software solution

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Introduction

Spatial Omics, by preserving the architecture of the tissue, enables new insight into the biological processes

within their native micro-environments. A large capture area can allow for large tissues to be studied on a single substrate, without the need to break tissues up into multiple separate runs. However, the ability to study large tissues is challenging, as it requires a large capture area and the capability to process the correspondingly large amount of data. Illumina spatial technology is built for scalability and efficiency by leveraging Illumina's best-in-class software ecosystem, enabling an end-to-end spatial analysis workflow. Powered by DRAGEN™ and Partek™ Illumina Spatial Technology (IST) provides a scalable system for researchers to analyze large datasets, with user-friendly interfaces for image processing, secondary analysis, spatial visualization and tertiary analysis. Combining with tissue imaging, IST has automated cell-based binning through nuclei identification and cell border expansion, yielding improved cell typing and marker gene identification. In addition, IST gives the flexibility for users to export data for use with third-party spatial analysis tools.

Material and method

To demonstrate this large capture area and end-to-end analysis capability, we present kidney on a single IST spatial substrate and processed the data with IST. From the data, we get ~3 million cells being captured. We were able to process the entire dataset, including secondary and tertiary analysis, within 14 hours.

Result and discussion

Additionally, using 1 NovaSeqX 25B 200 cycle kit we detected a median of 1964 molecules and 991 genes per cell. Using 3 NovaSeqX 25B 200 kits, we detected a median of 3100 molecules and 1365 genes per cell. Further, Illumina Connected Multomics (ICM) provides accelerated discovery of biological insights via an intuitive interface and scalable tertiary analysis functionality.

Conclusion

This shows the ability of IST end-to-end solution to analyze large tissue samples and accelerate discoveries with scalable analysis software.

EACR25-1942

The landscape of copy number intra-tumour heterogeneity in breast cancer and its impact on the tumour ecosystem

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Introduction

The genomic landscapes of breast cancer are dominated by copy number aberrations (CNAs). High-throughput

bulk sequencing has revealed the extent of intra-tumour genetic heterogeneity (ITH) in breast cancer genomes and advanced our understanding of tumour progression and treatment response. However, the identification of sub-clonal CNAs from bulk data is challenging due to limitation in inferential statistics. Single nuclei-based copy number aberration (snCNA) profiling overcomes such limitations by providing direct quantification of ITH. The impact of CNA ITH on the tumour micro-environment has not been systematically investigated.

Material and method

We generated snCNA profiles through shallow whole genome sequencing for nearly 40,000 single nuclei isolated from primary breast cancers (METABRIC cohort, n = 142). After alignment and quality control, a per-nucleus absolute copy number profile was generated using an optimised computational pipeline. snCNA profiles were used as input for per-tumour clustering, phylogenetic analysis, ITH metrics computation and extraction of snCNA signatures. snCNA data were integrated with multi-omics data available for the same tumours: SNP6, targeted sequencing, mRNA and miRNA expression, methylation and imaging mass cytometry data using unsupervised and supervised statistical methods.

Result and discussion

snCNA profiles matched the bulk tissue CNA profile, particularly for clonal events, validating the snCNA profiling method. The CNA events driving each IntCluster subtype (e.g. ERBB2 amplification in IC5) were mostly clonal. The extent of ITH was highly variable, from tumours showing no evidence of heterogeneity to highly heterogeneous tumours by both ploidy and number of distinct CNA subpopulations. snCNA signatures capturing high ITH associated with presence of TP53 mutations, higher tumour grade and higher tumour ploidy. Phylogenetic analysis highlighted distinct patterns of tumour evolution. Significant associations with patients survival and multiple tumour micro-environment features were found. Further integration with multi-omic data is ongoing and results will be presented.

Conclusion

snCNA profiling in a well annotated cohort provides an unprecedented insight into the extent and patterns of copy number ITH in breast cancer.

EACR25-1945

A defined genomics approach for delineating the cellular states during pancreatic cancer initiation

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Introduction

Pancreatic ductal adenocarcinoma (PDAC) is one of the deadliest cancers. Patients seldom exhibit specific symptoms at the beginning, thereby usually diagnosed at advanced stage. The molecular mechanisms of PDAC initiation are still elusive, especially the cellular states that are conducive for the oncogenic drivers to hijack necessary for malignant transformation.

Material and method

To understand the cellular states critical for cancer initiation, we established a human PDAC model through novel cancer modelling approach that combines cell-fate reprogramming using defined transcription factors (TFs) and expression of PDAC-specific oncogenes for pancreatic cell transformation to delineate the early molecular events during PDAC carcinogenesis. The distinct cellular states are characterized by modern cutting-edge functional genomic methods such as RNA-seq, ATAC-seq and CUT&RUN in addition to in vivo functional analysis.

Result and discussion

We show that in collaboration with pancreatic ductal-specific TFs, mutant KRAS together with overexpression of MYC oncogene and loss of TP53 are sufficient and necessary for inducing cellular transformation.

Conclusion

Our data provide high-resolution profiling of the epigenome and transcriptome changes that contribute to cancer initiation. Importantly, analysis of the regulatory events by defined TFs elucidated critical signalling pathways that are activated for the oncogenic drivers to hijack during exocrine pancreatic cell fate determination, highlighting the strength of this dynamic cancer modelling approach for further dissection of the early events of pancreatic tumorigenesis.

EACR25-1985

Screening and functional characterization of small molecule compounds with selective cytotoxicity in Anaplastic Thyroid Carcinoma

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Introduction

Anaplastic thyroid carcinoma (ATC) is a highly aggressive malignancy with limited treatment options and poor prognosis. Identifying new therapeutic targets is crucial for improving patient outcomes. In this study, we

utilized microarray analysis to establish differential gene expression profiles in ATC cells, providing a molecular basis for drug discovery. This approach allowed us to identify dysregulated pathways and potential targets for therapeutic intervention.

Material and method

Microarray analysis compared gene expression profiles between ATC cell lines (C643 and CAL-62) and human primary thyroid epithelial cells (HPTE), identifying key dysregulated genes and pathways. Based on these findings, we screened the Small Molecule Immuno-Oncology Compound Library (Selleckchem library No. L4800) for potential therapeutic agents. Selected compounds were evaluated for cytotoxicity using cell viability assays, including the MTT assay, to determine their selective effects on ATC cells while preserving normal thyroid cell viability.

Result and discussion

Microarray analysis revealed significant alterations in gene expression patterns associated with cell proliferation, apoptosis, and immune modulation in ATC cells. We found 2164 overexpressed and 2425 down-regulated genes. The analysis has been done using Feature Extraction and Agilent GeneSpring GX program (Agilent Technologies). An unpaired t-test with Benjamini-Hochberg correction was applied, with a fold-change cutoff of 2.0 and a corrected p-value threshold of 0.05, utilizing Agilent single-color technology. Guided by these results, we identified candidate compounds with potential therapeutic efficacy. Among them, fludarabine phosphate exhibited the most potent cytotoxic effect, significantly reducing ATC cell viability time-dependently, while exerting minimal impact on normal thyroid cells. Further pathway analysis indicated that fludarabine's mechanism of action involves the inhibition of proliferative and survival signaling pathways in ATC cells. Cellular and molecular testing was performed at different time points establishing the best framework to use several compounds as potential therapeutics for ATC.

Conclusion

Our findings highlight the utility of microarray analysis on ATC cell lines in identifying potential candidates and therapeutic targets and guiding drug discovery efforts for ATC. Fludarabine phosphate emerged as a promising candidate for further preclinical evaluation. Future studies will focus on validating its molecular mechanisms and assessing its efficacy in vivo models to explore its potential as a targeted therapy for ATC.

EACR25-2024

Microhomology-Driven Genomic Alterations in Ovarian Cancer

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Introduction

Microhomology-mediated end joining (MMEJ) is an error-prone DNA repair mechanism that utilizes short homologous sequences to mediate double-strand break (DSB) repair. This process can lead to complex genomic rearrangements, including insertions, deletions, and

multinucleotide variants (MNVs). Understanding MMEJ activity is particularly relevant in tumors with homologous recombination deficiency (HRD), where alternative repair pathways may significantly contribute to genomic instability. Since ovarian cancer frequently exhibits HRD-related deficiencies, it serves as a key model for investigating MMEJ's role.

Material and method

We developed an algorithm to classify indels (insertions, deletions, and MNVs) as MMEJ-dependent. The analysis incorporated whole-genome sequencing (WGS) and whole-exome sequencing (WES) data, processed using bioinformatics tools including Strelka2, MuTect2, Pindel, and VarScan2. Statistical analyses focused on the frequency and characteristics of MMEJ-mediated indels, with a primary emphasis on ovarian cancer due to its well-established HRD deficiency. Additionally, a pan-cancer analysis across 33 cancer types was conducted to assess whether MMEJ is also involved in other malignancies.

Result and discussion

Our results demonstrated the influence of mutation detection algorithms and sequencing type (WES/WGS) on the statistics of microhomology-associated deletions. We examined the impact of the classification algorithm parameter (N) on the identification of MMEJ-dependent indels and determined that the optimal threshold is N = 4. This parameter represents the minimum number of matching nucleotides required between the mutation fragment and the adjacent sequence to classify it as MMEJ-dependent. We observed variations in the frequency of MMEJ-mediated deletions across different cancer types, with the highest proportions detected in testicular germ cell tumors, pancreatic adenocarcinoma, diffuse large B-cell lymphoma and ovarian cancer. Importantly, our analysis demonstrated that increased MMEJ dependency results not only from BRCA1/2 mutations but can also be driven by alterations in the RB1 gene. Furthermore, survival analysis revealed that a higher number of microhomology-mediated deletions is associated with improved patient survival, even in the absence of PARP inhibitor treatment.

Conclusion

We developed a novel method for detecting MMEJ-dependent indels and optimizing classification parameters, providing a robust approach to studying microhomology-mediated deletions. Our findings highlight the potential of these deletions as predictive markers for treatment response and patient prognosis. By elucidating the role of MMEJ in genomic instability, particularly in HRD-related cancers, this study paves the way for more precise diagnostic and therapeutic strategies in oncology.

EACR25-2038

Mapping Therapeutic Opportunities in LUSCThrough Targeted Compound Screening

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Introduction

Lung squamous carcinoma (LUSC), a subtype of non-small cell lung cancer (NSCLC), poses a significant therapeutic challenge due to the lack of reliable biomarkers and the high resistance to conventional therapies. Immuno-Oncology compounds that target key pathways such as angiogenesis, cell cycle regulation, and DNA damage and repair (DDR) offer promising therapeutic strategies. This study employs microarray analysis to establish a gene expression profile in lung squamous cell carcinoma (LUSC) cell lines, aiming to identify cytotoxic Immuno-Oncology compounds and evaluate their cellular and molecular effects.

Material and method

Gene expression profiling was conducted on two untreated LUSC cell lines (H1703 and SK-MES1) using Agilent's microarray technology. An integrated bioinformatics approach compared microarray data from these LUSC cell lines with publicly available TCGA datasets from LUSC patient samples to refine candidate compound selection. This analysis identified 233 commonly altered genes across all three datasets, emphasizing dysregulated cellular pathways. Based on these insights, 27 compounds from the commercially available Immuno-Oncology Compound Library (Catalog No. L4800, Selleck Chemicals) were screened for cytotoxic effects on the two cell lines. Active compounds were further assessed using the RealTime-Glo™ MT Cell Viability Assay to determine IC₅₀ values. Additional functional assays, including cell cycle, apoptosis, and colony formation assay were performed to characterize their cellular effects.

Result and discussion

Five compounds showed significant cytotoxic effects on LUSC cell lines, impacting key oncogenic pathways like angiogenesis, DDR, and cell cycle regulation. The cell cycle assay demonstrated phase-specific arrest, while apoptosis assays confirmed heightened apoptotic activity. The colony formation assay indicated reduced clonogenic potential, suggesting long-term inhibitory effects. The observed cellular responses correspond with the known mechanisms of action of these compounds, reinforcing their therapeutic potential in LUSC.

Conclusion

This study highlights the utility of molecular profiling in identifying dysregulated pathways in LUSC and guiding the selection of potential therapeutic compounds. The identified Immuno-Oncology compounds demonstrated promising cytotoxic effects, supporting their further evaluation as potential candidates for LUSC treatment. Future studies will focus on validating these findings in preclinical models to advance therapeutic development.

EACR25-2057

Whole-genome sequencing of 122 upper urinary tract urothelial carcinomas

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Introduction

Upper urinary tract urothelial carcinoma (UTUC) is a poor-prognosis cancer of the renal pelvis and ureters. Due to its rarity, previous large-scale genomic studies of urothelial carcinomas have focused on bladder cancer, which is the commonest malignancy of the urinary tract. Recently, our study identified five molecular subtypes of UTUC with different outcomes. To further understand the molecular pathogenesis of UTUC, we performed whole-genome sequencing (WGS) of UTUCs.

Material and method

We studied a total of 122 UTUCs which were surgically resected at the University of Tokyo hospital from 2000 to 2020. Matched germline control samples were also obtained from the normal renal cortex or peripheral blood samples. WGS was performed using NovaSeq at 120x and 30x depth for tumor and normal samples respectively. G-CAT, GRIDSS, and FACETS were used to identify single nucleotide variants/insertion-deletions, structural variants (SVs), and copy number variants. Amplicon-suite was used to identify extrachromosomal DNA (ecDNA).

Result and discussion

The median 11,000 mutations were detected per sample (range 2,700–780,000). Ten samples (8.1%) were hypermutated (>100,000 mutations), all of which had mutations in mismatch repair genes (MSH2, MSH6 and MLH1). dN/dS analysis identified 16 driver genes, of which the most frequently mutated driver gene was KMT2D in 54 samples (44.3%), followed by TP53 (33.6%), FGFR3 (32%), and ARID1A (23%). Recurrent non-coding mutations were also investigated, which identified known driver mutations in the promoter and 5' UTR regions of TERT. Comparing the frequency of mutations in the ureter and renal pelvis, TERT promoter and HRAS mutations were significantly more common in the ureter, while KMT2D and TP53 mutations were more frequently mutated in the renal pelvis. Mutational signature analysis using SigProfiler identified SBS1 and SBS5, which are clock-like signatures and increase with age, and SBS2/13 caused by APOBEC mutagenesis. Mutations caused by APOBEC were identified in 87 cases and hotspot mutations in the non-coding regions accumulated on target motifs of APOBEC. SV analysis identified driver SVs, such as SVs involving FGFR3 (N = 10), which included 7 FGFR3-TACC3 translocations. In addition, KLF5 duplications and AHR deletions were observed in 9 and 7 samples, respectively. L1 retro-

transpositions originating from TTC28 and PHACTR1 loci were also frequently detected as SVs. ecDNA was found in 58 samples (52%), including those containing oncogenes such as CCND1 and MDM2.

Conclusion

UTUCs were strongly affected by APOBEC mutagenesis, generating the hotspot mutations in non-coding regions. WGS identified non-coding driver mutations, driver SVs and ecDNAs, which have not been identified by the previous study. These data could contribute to the refined UTUC classification and further understanding of its pathogenesis.

EACR25-2106

Exposing G2/M Checkpoint Vulnerabilities: BUB1B and BUB3 as Essential Dependencies in Malignant Pleural Mesothelioma

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Introduction:

The G2/M checkpoint is a critical regulatory mechanism ensuring proper DNA replication and repair before mitosis preventing genomic instability and aneuploidy. Its dysfunction is implicated in various cancers, including Malignant Pleural Mesothelioma (MPM), a rare and aggressive cancer with poor prognosis. The BUB gene family, particularly BUB1 and its interaction partners BUB1B and BUB3, plays a key role in both the G2/M checkpoint and Spindle Assembly Checkpoint (SAC), ensuring accurate chromosome segregation. Whole-genome CRISPR/Cas9 screening in MPM cell lines identified BUB1B and BUB3 as essential genes, suggesting their involvement in MPM progression. This study aims to investigate the molecular effects of BUB1B and BUB3 in MPM, assessing their role in cell proliferation and survival to explore their therapeutic potential.

Material and method

Recently we conducted genome-wide CRISPR screening in H2052, H2452, and H28 MPM cell lines, identifying the BUB gene family (BUB1, BUB1B, and BUB3) as having a significant impact on MPM cell survival and proliferation. Following CRISPR/Cas9-mediated gene knockout, the dependency of MPM cells on BUB1B and BUB3 and their roles in aggressive phenotypes were assessed through functional assays such as BrdU incorporation, cell cycle, and 2D and 3D colony formation. Ongoing functional analyses aim to test the molecular and cellular implications of BUB1B and BUB3 in MPM.

Result and discussion

Genetic silencing of BUB1B and BUB3 individually profoundly impaired MPM cell proliferation and survival. Cell cycle analysis revealed significant accumulation in the G2 phase. Elevated expression of BUB1B or BUB3 was strongly associated with poor overall survival, as predicted based on their expression levels in publicly available datasets. Critically, DepMap analyses conducted in MPM cell lines revealed a

significant depletion of BUB1B and BUB3, supporting their impact on MPM survival. Interestingly, G2/M checkpoint molecules with high interaction scores in StringDB annotation have generally exhibited a depletion trend, highlighting that MPM cells are functionally dependent on proper G2/M modulation. Our ongoing studies aim to provide a deeper understanding of the functional and molecular effects of these genes on the G2/M checkpoint and their role in MPM pathobiology.

Conclusion

Findings highlight the critical role of BUB1B and BUB3 in the proliferation and survival of MPM cells, emphasizing their potential as both biomarkers and therapeutic targets. Silencing of these genes disrupted the cell cycle, particularly by inducing arrest in the G2 phase, pointing to their essential function in regulating the G2/M checkpoint. Further functional and molecular studies will be essential to fully understand the mechanisms by which BUB1B and BUB3 contribute to MPM pathogenesis. These investigations may offer valuable insights into novel therapeutic strategies targeting the G2/M checkpoint in MPM treatment.

EACR25-2186

Deciphering oncogenic gene regulatory mechanisms at nucleotide resolution using precision genome editing

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Introduction

Understanding oncogenic signaling and its regulation is critical for uncovering the mechanisms of tumorigenesis and tissue-specificity in cancer. However, the complexity of oncogenic transcriptional pathways and the limitations of currently used CRISPR-based technologies pose a significant challenge for studying signaling at the gene regulatory level. To overcome this, we have optimized and adapted a genome editing method to study the essentiality of oncogenic transcription factors by directly modifying their binding motifs at candidate regulatory loci of oncogenic driver genes.

Material and method

The genome editing method is based on homology-directed recombination (HDR) and it enables the editing of specific genomic regions precisely, simultaneously minimizing the confounding effects previously linked to other CRISPR-based approaches. To model oncogenic signaling in clear cell renal cell carcinoma (ccRCC), we used HIF2A-dependent cells with HIF-knockout cells as controls. Cells were transfected with ribonucleoprotein complexes and HDR templates, together with HDR enhancers or oligonucleotide modifications. The repair templates introduced either original or modified transcription factor binding motifs along with sequence tags, providing internal controls and increasing statistical power. The candidate regulatory regions were derived from CRISPR screens, and the essentiality of specific motifs was established by conducting *in vitro* and *in vivo* competition assays for edited alleles.

Result and discussion

By modifying repair templates and manipulating endogenous double-strand break repair pathways, we

optimized a precision CRISPR editing method, improving editing efficiencies. Using competition assays, we were able to demonstrate the effect of specific transcription factor binding motifs located in candidate regulatory regions on cell fitness in vitro and in vivo.

Conclusion

Using an optimized precision editing method, we were able to showcase the functional essentiality of specific transcription factor motifs in candidate regulatory regions of cancer-related genes downstream of the HIF2A pathway, thereby elucidating gene regulatory mechanisms in tissue-specific oncogenic pathways.

EACR25-2252

Common Genetic Dependencies of Sonic Hedgehog Medulloblastoma (SHH-MB) variants are Regulated by the Non-Malignant Microenvironment

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Introduction

Medulloblastoma, of which SHH-MB is a major subtype, is the commonest malignant childhood brain tumour. Analysis of the genetic landscape of these tumours has uncovered further subdivisions of SHH-MB, which has led to the view that their optimal treatment will require uniquely tailored therapies [1]. Superimposed on this classification scheme are key oncogenic mutations, for example affecting TP53 which have a profound effect on prognosis. We showed that SHH-MB cell states can mirror in vivo tumour cell states in a manner that is determined by the cerebellar microenvironment [2]. To determine whether there are shared cell-intrinsic determinants across SHH-MB variants, we sought to identify the key upstream regulators in the gene regulatory network and their direct targets (regulons) through computational approaches. Identifying these regulons could inform targeted therapies applicable across SHH-MB subtypes.

Material and method

The SHH-MB cell line, DAOY which harbours a TP53 mutation, was grown as monocultures or in co-culture with non-malignant human iPSC-derived cerebellar organoids, to simulate the microenvironment, for 25 days. Cells were processed for scRNA-seq then analysed using the three-step SCENIC pipeline [3]. First, candidate regulatory modules are inferred from gene co-expression patterns. Next, co-expression modules are pruned by eliminating indirect targets using transcription factor motif discovery, to define regulons for each transcription factor. Last, the activity of these regulons is measured in individual cells and used for clustering.

Result and discussion

There was a striking shift in the combinatorial pattern of regulons expressed by tumour cells in co-culture. Indeed, one of the top regulons, ELK1 in co-cultured tumour cells matched the second highest ranked regulon in a batch of four single cell sequenced TP53 wildtype ($n = 2$) and mutant ($n = 2$) patient tumours. Furthermore, another top regulon in DAOY cells in co-culture, CREB5 was a

homologue of the highest ranked factor, ATF4 (also called CREB2) in these patient tumours. Unexpectedly, high-risk TP53 mutant tumours shared identical top ranked regulons with TP53 wild-type tumours.

Conclusion

Our in silico analysis suggests common genetic dependencies across SHH-MB tumour subtypes which is regulated by the cerebellar microenvironment, and that could be exploited therapeutically.

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EACR25-2305

Prometastatic gene signatures of conventional and serrated colorectal carcinoma and their regulation by microenvironmental factors from primary and metastatic sites

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Introduction

Serrated adenocarcinoma (SAC) is a rare CRC subtype, which differs from conventional adenocarcinoma in morphology and expression of genes associated with extracellular matrix remodeling and epithelial-mesenchymal transition, presenting a worse prognosis. However, it is unknown at which stage this serrated phenotype is developed and how it is regulated by procarcinogenic factors at primary and metastatic sites.

Material and method

In this work, the prometastatic transcriptional pattern of conventional and serrated CRC was compared by studying expression levels and interrelations of a broad family of 72 CRC genes known to be regulated by *E. coli* endotoxins and soluble factors of tumor-activated hepatocytes and hepatic sinusoidal myofibroblasts, mimicking the colorectal cancer cell response to soluble factors from the HPMR promoting the hepatic metastasis disease.

Result and discussion

We identified a gene signature whose overexpression (>3 -fold change) was associated to serrated pathway carcinogenesis (DPEP1, PRDX4, SEM1, CLDN4, G6PD, PGK1, YWHAZ, PKM, HMGB1, NME1) and hepatic

metastasis development (CFTR, PRDX4, TIMP1, SEM1, CLDN4, SPP1, G6PD, PGK1, YWHAZ, PKM, HMGB1 and NME1), compared to their expression changes in conventional CRC versus normal mucosa. In addition, both unsupervised clustering and principal component analyses remarkably segregated normal and tumor tissue biopsies, primary tumors and metachronic (but not synchronous) metastases. Finally, a Pearson correlation matrix showed that around 50% prometastatic genes inversely correlated between serrated and conventional primary CRCs, and that this mainly occurred in serrated versus conventional CRCs.

Conclusion

These results demonstrate distinct prometastatic gene signatures for conventional and serrated colorectal carcinomas and suggest different transcriptional response patterns by effect of the same prometastatic micro-environmental factors at primary colonic mucosa and hepatic sites.

EACR25-2348

GEM-X Flex: Scalable and cost-effective whole transcriptome single cell analysis for cancer marker detection from FFPE tissue section slides

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Introduction

Formalin-fixed paraffin-embedded (FFPE) tissue blocks are the most widely used sample type in clinical research for preserving tissue morphology and supporting long-term storage. These samples can be particularly valuable for studying rare cancers or when sample procurement is challenging. The growing demand for comprehensive single cell gene profiling calls for methods that require less sample input, reduce costs and enable high-throughput analysis for large clinical studies.

Material and method

Large-cohort studies are often limited by financial constraints. To overcome this challenge, we performed a cost-effective, high-throughput experiment with eight different FFPE blocks (glioblastoma, colorectal, lung, breast, skin, endocervical cancers, normal kidney, and reactive lymph node), with two replicates per sample. Using the 16-barcode multiplexing approach of GEM-X Flex, we processed 320,000 cells pooled from all 16 samples in a single lane (20,000 cells per sample). The distinct barcodes allowed us to obtain high quality single cell sequencing data from each individual sample with $\sim 8\%$ undetected multiplets.

Result and discussion

We identified tumor markers specific to each cancer, including PROM1 (glioblastoma), BRCA1 (colorectal), KRT19 (lung), MUC1 (breast), GPC3 (breast), and MUC16 (endocervical). This method allows processing up to 2.56 million cells per chip across 8 lanes, reducing the cost per sample to just one cent per cell, making large-scale studies more cost-efficient. We also extended this approach to tissue sections already mounted on slides

and subjected to histological analysis, enabling the extraction of the most information from precious clinical samples. Using the GEM-X Flex assay, we generated whole transcriptome profiles from as few as 25,000 nuclei isolated from 5 μm FFPE tissue sections on standard superfrost slides. Gene expression profiles, cell type proportions, and assay sensitivity from these thin sections were comparable to those from 25 μm tissue scrolls from the same FFPE block, demonstrating that high-quality single cell RNA sequencing is achievable with minimal sample input. The ability to work with 5 μm tissue sections expands options for universal sample preparation across various assays. It also broadens sample procurement opportunities by using tissue sections instead of whole FFPE blocks, significantly lowering costs.

Conclusion

As a conclusion, the GEM-X Flex assay enables whole-transcriptome gene profiling at single cell resolution from 5 μm FFPE tissue sections. By combining H&E image analysis with RNA sequencing data from sequential sections of the same cancer block, pathologists can achieve more precise diagnostics. The assay's multiplexing capability significantly lowers costs and supports high-throughput studies, enabling large-scale tumor marker screening for early detection, progression monitoring, and therapeutic target development.

EACR25-2363

Long-Read Resequencing of Acute Myeloid Leukaemia Single-Cell RNA Sequencing Samples Improves Detection of Malignant Genomic Alterations

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Introduction

Acute myeloid leukaemia (AML) is an aggressive blood cancer, resulting in the accumulation of poorly differentiated white blood cells in the bone marrow. Normal and malignant haematopoiesis occur simultaneously in AML, requiring methods to separate these cell populations in bone marrow aspirate samples. Single cell transcriptomics (scRNA-seq) data have been used in recent years to infer the presence of genomic alterations (single-nucleotide variants, insertions/deletions, fusion genes) which allows cells with cancer-relevant alterations to be identified. One of the most popular scRNA-seq platforms, 10x Genomics®, typically uses Illumina® short-read sequencing, ultimately biasing transcript coverage towards the mRNA capture site. This limits detection of genomic alterations in regions distal to the capture site, complicating efforts to use these as markers of malignancy in cancers with a low mutational burden such as normal karyotype AML. Emerging single-cell technologies such as PacBio® Kinnex scRNA-seq utilises long-read sequencing to cover the whole transcript and is compatible with existing 10x cDNA

libraries. Here, we investigate the ability of Kinnex to identify the malignant cell population in primary, patient-derived AML samples previously sequenced with the 10x 3' workflow by identifying genomic alterations linked to malignant transformation.

Material and method

4 patient samples harbouring a range of different genomic alterations relevant to AML (e.g. TET2/DNMT3A point mutations, an NPM1 insertion, and a DEK::NUP214 fusion) were resequenced with the Kinnex scRNA-seq kit and processed with the single-cell Iso-Seq analysis pipeline. CTAT-Mutations and pbfusion were used for variant calling and fusion gene identification respectively. VarTrix was used to assign identified CTAT-Mutations variants to individual cells.

Result and discussion

Kinnex recovered 29% fewer cells than 10x across the 4 samples. For cells which were sequenced with both technologies, CTAT-Mutations identified approximately 63% more single-nucleotide cancer-relevant variants in the Kinnex data than the 10x. On average, the Kinnex data covered AML mutation hotspot sites in NRAS, TET2, FLT3, DNMT3A, and NPM1 in 1.84x more cells; e.g. in an NPM1 mutated sample, Kinnex data revealed ~3x more (1000 vs. 280) mutant NPM1 cells than 10x. The Kinnex data also identified the DEK::NUP214 fusion which was not detectable in the corresponding 10x data.

Conclusion

Despite the lower number of cells recovered, the Kinnex data identified more malignant cells with a higher confidence by improving coverage of AML mutation hotspots located distal to the 3' of relevant transcripts. Kinnex scRNA-seq data display a superior ability to detect multiple genomic alterations, highlighting the benefits of using long-read sequencing to characterise cancer samples in single-cell studies.

EACR25-2380

Extensive spatial heterogeneity of transcript variants in localised prostate cancer

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Introduction

Prostate cancer is the second most frequently diagnosed cancer in males worldwide. The majority of patients are diagnosed with multiple prostate-confined tumours with extensive spatial heterogeneity. While localised disease generally harbours few recurrent somatic point

mutations, gene fusions are a characteristic trait. These predominantly involve genes in the ETS family of transcription factors fused with androgen-regulated genes, causing overexpression of the ETS gene. TMPRSS2::ERG is the most prevalent, occurring in approximately 50% of cases. Our previous findings indicate that ERG overexpression is associated with a poor prognosis, but the topic is still debated. Moreover, the diversity in transcript variants has often been overlooked. Here, we aim to characterise the fusion transcript variants and explore intrapatient heterogeneity.

Material and method

We analysed a cohort of treatment-naïve, radical prostatectomy cases from Oslo University Hospital–Radiumhospitalet (2010–2012) using long-read RNA sequencing (Oxford Nanopore Technologies). The study included 110 fresh-frozen tissue samples (82 malignant, 28 benign) from 31 patients. Technical validation was performed using short-read RNA sequencing and real-time RT-PCR.

Result and discussion

In total, 92 samples from 28 patients passed the quality control of >200,000 reads, and were included in further analyses. The long-read sequencing data showed a high level of concordance with both short-read sequencing and real-time RT-PCR. TMPRSS2::ERG was the most common fusion transcript (found in at least one sample from 61% of patients), whereas TMPRSS2::ETV1 was observed in 4%. In fusion-positive samples, distinct ERG and ETV1 transcript variants were identified. These reflect the part of the ETS gene included in the fusion; however, the differences in transcript structures are not solely attributed to the truncated gene length. Intra- and interfocal heterogeneity in ERG transcript variant expression was found in 93% and 92% of patients, respectively. For ETV1, all patients exhibited both intra- and interfocal heterogeneity. Specific transcript variants, such as ETV1-207, appear to be associated with a more aggressive disease and poor prognosis. This finding, together with the spatial heterogeneity of transcript variants, may have implications for the prognostic utility of ETS genes. Additionally, non-ETS fusions were identified, such as AZGP1::GJC3 (50% of patients) and the novel ENSG00000284512::CMC2 (found in 14%).

Conclusion

A distinct expression pattern of ERG or ETV1 transcript variants exists in samples with TMPRSS2::ERG or TMPRSS2::ETV1 fusions. The extensive intrapatient heterogeneity in transcript variant expression should be considered during further development of ERG and ETV1 as prognostic biomarkers in localised prostate cancer. More research is warranted to explore the link between specific transcript variants and aggressive disease.

EACR25-2382

Spatial transcriptomic insights into the influence of cancer-associated fibroblast profiles in oral cancer spheroids

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Introduction

Oral squamous cell carcinoma (OSCC) presents high mortality and therapeutic resistance, influenced mainly by the complex tumor microenvironment, particularly cancer-associated fibroblasts (CAFs). CAFs actively remodel the extracellular matrix (ECM) and mediate signaling that may enhance tumor progression and invasion. Additionally, cell-in-cell (CIC) structures have been identified as markers of tumor aggressiveness in OSCC, yet the association between CAFs and these phenomena is still unclear. This study aims to clarify the interaction between CAFs, ECM remodeling, and tumor invasiveness in OSCC using advanced *in silico* and 3D spatial transcriptomic approaches.

Material and method

We conducted a combined *in silico* and experimental study. Initially, *in silico* analyses of publicly available transcriptomic data of OSCC were performed, focusing on genes related to ECM remodeling, epithelial-mesenchymal transition (EMT), invasion, and metabolic processes. Experimentally, OSCC cell lines representing metastatic (SCC04) and non-metastatic (H357) phenotypes, as well as dysplastic cell lines (DOK), were cultured alone or co-cultured in 3D spheroids with normal (NHOF), activated (MYO), or senescent (SEN) fibroblasts. Spatial transcriptomics analysis was performed after 6h, 24h, and 48h of co-culture. Data normalization utilized TPM (Transcripts Per Million), and differentially expressed genes (DEGs) were identified ($p < 0.05$). Functional enrichment analysis identified impacted biological pathways.

Result and discussion

In silico analysis showed significant upregulation of genes involved in ECM remodeling and EMT (e.g., MMP9, SNAI2) in metastatic compared to non-metastatic OSCC cells. Spatial transcriptomics of 3D spheroids confirmed that metastatic SCC04 spheroids exhibited statistically significant upregulation of genes involved in ECM degradation (MMP9), cell adhesion, and EMT (CAV1, SNAI2) compared to non-metastatic H357. Co-culture with normal or activated CAFs did not show significant immediate transcriptional alterations, though minor changes related to ECM organization were observed. Importantly, co-culture with senescent fibroblasts significantly influenced metabolic and inflammatory pathway activation in dysplastic cell spheroids, highlighting their early and critical role in tumor progression and CIC formation, suggesting a potential mechanistic link to enhanced invasiveness, though this requires further investigation.

Conclusion

Our results demonstrate that CAFs are significantly associated with transcriptomic alterations linked to ECM remodeling, EMT, and tumor invasion in OSCC spheroids. These insights underscore the importance of the CAF-tumor cell interplay in cancer progression, providing potential targets for future therapeutic interventions.

EACR25-2435

Transforming AI-Driven Drug Discovery with a 100 Million Single-Cell Dataset

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Introduction

Single-cell RNA sequencing (scRNA-seq) has become a powerful tool for studying complex biological systems, offering transcriptome-wide insights at single-cell resolution. AI-driven drug discovery requires large, high-quality datasets covering a broad range of experimental conditions to train and refine predictive models. Recent advancements in single-cell combinatorial barcoding have enabled profiling of transcriptomes from 10s to 100s of millions of cells in a single experiment. This presents an opportunity for combinatorial barcoding to provide over 100 million cells as training data and set a new standard for a more robust and comprehensive system for modeling drug perturbations in diverse cellular systems.

Material and method

A heterogeneous model system comprising over 50 cell lines was treated with ~300 cancer drugs at three concentrations for 24 hours. Cells were subsequently fixed to enable large-scale combinatorial barcoding in batches exceeding 10 million cells each. The Parse Biosciences GigaLab platform was employed for single-cell barcoding, which enables high-throughput while maintaining high transcript detection. Library preparation, including cDNA capture, PCR amplification, and clean-up, was automated in a 96-well format to ensure efficiency. The entire workflow, from treatment to sequencing-ready libraries, was completed within five weeks. Sequencing was performed on the UG 100™ (Ultima Genomics), and data were processed through the Parse Biosciences pipeline. Cell line identity was resolved using Demuxlet for downstream analysis.

Result and discussion

This study successfully generated transcriptomic data from over 100 million single cells across 60,000 experimental conditions, demonstrating the feasibility of scaling single-cell sequencing for large-scale AI applications. Automated workflows minimize batch effect while significantly accelerating processing time. The resulting dataset provides a powerful resource for AI-driven drug discovery, allowing researchers to model drug perturbations across diverse cellular backgrounds. These findings highlight the feasibility and impact of large-scale single-cell sequencing for AI applications in biomedical research.

Conclusion

By establishing a scalable and efficient single-cell sequencing workflow, this study sets a new benchmark for high-throughput transcriptomics in drug discovery. The ability to generate high-resolution data from 100 million cells provides unprecedented opportunities for modeling complex biological responses at scale. This approach will enable more accuracy and efficiency in future AI-driven studies in disease modeling, drug screening, and precision medicine, accelerating the development of novel therapeutics.

EACR25-2446**Optimizing low input and high-throughput cell fixation for single-cell RNA sequencing with combinatorial barcoding**

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Introduction

Single-cell analysis is a critical approach for extracting nuanced biological insights. Combinatorial barcoding is a highly efficient method for scRNA-seq at scale, offering both sensitivity in gene detection and flexibility in sample input. However, this approach becomes challenging when working with sample inputs of fewer than 100,000 cells. To address this limitation, we have developed a modified fixation and cell capture method optimized for low-input samples while maintaining compatibility with combinatorial barcoding. This advancement allows researchers to fix, store, and barcode cells efficiently without requiring costly or specialized instruments.

Material and method

The Low Input Fixation method enables the fixation and profiling of up to 384 samples in parallel, significantly reducing workflow duration while ensuring consistent handling. This minimizes both experimental variability and the burden of scaling. Fixed samples remain stable at -80°C, and capture efficiency remains consistently high for inputs as low as 10,000 cells. The workflow is designed to maximize sample recovery while providing accurate and comprehensive transcriptional profiling. To validate this approach, we applied it to a cancer drug screening panel, utilizing cell input amounts ranging from 10,000 to 20,000 cells per treatment.

Result and discussion

Robust cell capture minimizes cell loss during drug perturbations, leading to consistent gene detection. Whole transcriptome analysis using Evercode WTK identifies gene expression changes associated with each drug class and cell type. The results validate putative drug targets within the panel and demonstrate that panel-based perturbation allows for the scalable detection of off-target effects. The stability and efficiency of this method make it a powerful tool for studying transcriptional responses in low-input single-cell experiments.

Conclusion

This work demonstrates that combining low-input fixation with combinatorial barcoding enables high-throughput cancer drug and compound screening. By facilitating the efficient processing of low-input samples, this method expands the applicability of single-cell transcriptomics to a broader range of biological and clinical studies.

EACR25-2451**Towards a Quantitative Phenotypic Progression Model of Prostate Cancer**

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Introduction

Prostate cancer (PCa) is the most prevalent cancer among men, with significant global health implications. Among the three factors in the D'Amico classification system, Gleason Score (GS) remains the gold-standard for diagnosis but lack prognostic value. There is still need to develop better marker of Prostate cancer aggressiveness. We propose an Quantitative Phenotypic Progression Model of Prostate Cancer, leveraging tissue architecture (TissArch) features AND features measuring changing in the nuclei DNA organisation to provide a more reproducible and objective method that could complement the current PCa grading system.

Material and method

A total of 250 PCa patients were included in this study from the Prostate Cancer Centre. We identified 3 cohorts: cohort 1 composed of patients with GS 6 with no sign of progression and patients with GS 9 (n = 60); Cohort 2 composed of patients with GS6 under active surveillance that progressed (n = 42); Cohort 3 composed of 148 patients followed for PSA recurrence after brachytherapy. Cohort 2 and 3 are used for validation of the model. For training purpose, we randomly assigned 75% of GS6 patients (n = 31) form and 75% GS9 patients (n = 15) from the cohort 1, with the remaining samples used for testing (n = 14). Biopsies were stained by Feulgen, a DNA-stoichiometric dye. Scanned imaged were analysed by our in-house Hyperspectral Cell Sociology Imaging Pipeline (HCS), powered by Deep learning abased segmentation techniques. More than 100 Large scale DNA organisation (features were calculated for each segmented nucleus and about 50 TissArch features were extracted from Voronoi diagrams and other triangulations constructs.

Result and discussion

Using Linear Discriminant analysis, an overall accuracy of 97% on the training set and 90% on the test were achieved by Tissue Architecture analysis. Using Random Forest algorithms, LDO analysis reached a balanced accuracy of 85% for the training set and 86% for the test set. An LDO Score and a TissArch Score was then calculated for the remaining patients in the validation cohorts. We will analyze and discuss the potential of this parametric phenotypic canonical space to characterize the phenotypic progression "signature" of the patients and its correlation with the degree of aggressiveness of these lesions

Conclusion

This Quantitative Phenotypic Parametric model of Prostate Cancer progression could give additional insight into PCa biological dynamics and help in the clinical management of PCa patients.

EACR25-2452**Heterogenous fusion transcript landscape in multifocal primary prostate cancer**

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Introduction

Prostate cancer is the second most commonly diagnosed cancer in men globally, and is characterised by significant clinical and molecular heterogeneity. Most patients present with multiple primary tumour foci, each displaying distinct molecular profiles, with recurrent fusion genes playing a pivotal role in pathogenesis. These fusions typically involve ETS transcription factors such as ERG, ETV1, ETV4, and FLI1, with the TMPRSS2::ERG fusion observed in approximately 50% of cases. However, studies often rely on single focal sampling, thereby overlooking the considerable interfocal heterogeneity present within patients.

Material and method

In our study, multiple samples per patient were included to capture both interfocal and intrafocal heterogeneity. We analysed samples from 43 prostate cancer patients, including 2–3 malignant samples from 1–3 tumour foci and 1 benign sample per patient. High throughput RNA sequencing was performed on the 159 samples, followed by computational analysis using the fusion detection tools STAR-Fusion and Arriba to identify fusion transcripts. High-confidence fusions were prioritised based on detection by both tools, presence of multiple isoforms, breakpoints located at splice sites, and expression above 0.1 fusion fragments per million mapped fragments.

Result and discussion

We identified 3414 distinct fusion transcripts, with a median of 55 fusions per malignant sample. All known prostate cancer-related fusions displayed interfocal, or both intra- and interfocal expression variance, suggesting complex heterogeneity within and across tumour foci. TMPRSS2::ERG and SLC45A2::AMACR were the most frequently expressed fusions, present in 43% and 48% of samples, respectively, with approximately half of these samples expressing both fusions. The stringent filtering process yielded 16 high-confidence fusion candidates with evidence of pathogenic relevance; most of which were novel. Many involved genes with established cancer roles, such as IGF1R::CSPP1, MTUS1::OTOG, CTNND1::ETV1, and PDZRN3::CMTM8, and exhibited notable intrapatient heterogeneity. Most fusion transcripts were expressed at low frequencies, present in less than 5% of patients.

Conclusion

This study reveals a complex landscape of fusion transcript heterogeneity in multifocal primary prostate cancer, marked by substantial inter- and intrafocal variation. The discovery of novel fusion transcripts opens opportunities for future research into their functional roles in oncogenesis and offers promise for their potential as biomarkers and therapeutic targets.

EACR25-2468

Unraveling chemoresistance in breast cancer through circulating miRNAs in liquid biopsy

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Introduction

Neoadjuvant chemotherapy (AdjCh) is essential for breast cancer treatment, but resistance remains a major challenge. Circulating molecules, particularly extracellular vesicles (EVs) and microRNAs (miRNAs), are promising biomarkers. EV cargo exchange and circulating transcripts may drive resistance by altering gene expression and tumor behavior. Thus, this study explores the role of circulating RNAs as markers of chemoresistance.

Material and method

EVs were isolated from the culture media of breast cancer cell lines (MDA-MB-468, MDA-MB-231, and BT-549) with varying drug responses using PEG precipitation and differential ultracentrifugation (dUC). Small EVs (sEVs) were physically and biochemically characterized. Sensitive cells were exposed to 10,000 sEVs per cell from resistant cells. Transcriptomic analysis of EV cargo and recipient cells via microarrays was correlated with public TNBC datasets, identifying four key RNA transcripts linked to EV-mediated chemoresistance. Their functional roles were validated in vitro using siRNA and miRNA mimic transfections, assessing their impact on drug response and tumor aggressiveness. Circulating RNA was isolated from blood using Norgen kits, and miR-574-5p and miR-7977 expression was quantified via Qiagen digital PCR with TaqMan probes. miRNAnome was sequenced on an Illumina platform with Takara enrichment achieved 20 million reads of coverage.

Result and discussion

Given their stability and enrichment in circulation, miRNAs are promising biomarkers. We identified and functionally characterized miR-574-5p and miR-7977, secreted via small vesicles in chemotherapy-resistant cells. Their upregulation increased proliferation, viability, and motility, while transcriptomic analysis linked them to key cancer-related metabolic processes. Public datasets confirmed their enrichment in breast tissues resistant to neoadjuvant chemotherapy. As an initial approach, we assessed miR-574-5p and miR-7977 circulating expression in blood samples from 69 breast cancer patients treated with neoadjuvant chemotherapy using digital PCR. Their predictive performance for poor response, no response, or disease progression, quantified by the area under the curve (AUC) and optimized using

bootstrap analysis, reached 0.774. Furthermore, we evaluated the transcriptional landscape of circulating miRNAs in a liquid biopsy approach from 60 baseline samples of patients undergoing AdjCh, uncovering distinct expression patterns associated with treatment response.

Conclusion

This proof-of-concept study demonstrates how plasma miRNA profiles can reveal treatment resistance-associated miRNAs and provide biologically and clinically actionable insights, with potential implications for therapeutic decision-making.

EACR25-2487

Molecular Profiling of Brain Metastases from Distinct Primary Tumors Identifies Subtypes with Implications for Therapy and Biomarker Development

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Introduction

Brain metastases (BM) are the most prevalent tumors of the central nervous system (CNS), affecting up to 40% of cancer patients, occurring ten times more frequently than primary CNS tumors. Despite their prevalence, BM remains highly heterogeneous, shaped by both their primary tumor origin and the brain microenvironment. Evidence suggests that BM retain distinct gene expression patterns from their primary tumor, influencing tumor progression, therapeutic response, and biological pathways. Understanding transcriptomic changes associated with BM from distinct primary tumor sites is crucial for biomarker discovery and precision medicine.

Material and method

This study analyzed 43 BM samples from primary tumors from nine distinct primary sites. Patient samples were obtained from our local clinical hospital at Unesp. Total RNA sequencing was performed using the Illumina NovaSeq X platform, with gene expression quantified in transcripts per million (TPM) and normalized using DESeq2. Principal component analysis (PCA) and K-means clustering identified five molecular sample subgroups. Differential expression analysis was conducted using ANOVA ($p < 0.05$), followed by Tukey's post-hoc test. Biomarker selection focused on genes with the highest expression differences, prioritizing those with consistent expression patterns within subgroups for further validation.

Result and discussion

16,782 genes were assigned to the five molecular clusters based on transcriptomic profiling. Each cluster exhibited distinct pathway enrichment, influencing tumor behavior and treatment response. Clusters 1 and 4 were enriched in RNA processing, DNA repair, and protein degradation, suggesting stress responses and genomic instability. Clusters 2 and 5 were dominated by synaptic function and neurotransmitter regulation, highlighting potential interactions with the brain microenvironment. Cluster 3 was enriched in extracellular matrix (ECM) interactions, focal adhesion, and cancer pathways, emphasizing its role in tumor invasion and metastasis. Immune-related

pathways were underrepresented, likely reflecting immune evasion mechanisms in BM.

Conclusion

This study identifies distinct molecular subtypes in BM, each characterized by unique biological processes affecting tumor behavior and therapeutic response. Clusters enriched in RNA processing and DNA repair indicate genomic instability, while clusters with synaptic function suggest interactions with the brain microenvironment. ECM remodeling and adhesion pathways highlight mechanisms of tumor invasion and metastasis. The absence of immune pathway enrichment suggests immune evasion may play a critical role in BM progression, with implications for immunotherapy. These findings establish a foundation for biomarker discovery and targeted therapies, driving advancements in precision oncology to benefit patients with BM.

EACR25-249

The TP53:AHR genetic interaction regulates MAGE:TRIM28 activity to prevent cellular transformation

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Introduction

Within an organism, transformed cellular physiology is the concerted effect of intracellular alterations, interactions with other cells, hypoxia and nutrient-shortage tolerance, as well as the ability to grow in an anchorage-independent manner.

Material and method

To better mimic these physiological conditions, we developed a 3D soft-agar system with non-transformed human cells. We then analyzed global transcriptional changes of single and combinatorial gene knockout cell lines with bulk RNA-sequencing and publicly available TCGA LUAC patient data.

Result and discussion

We discovered a previously unknown genetic interaction between TP53 and the AHR/ARNT genes. Analysis of RNA-seq data originating from gene knockout cell lines revealed an increased expression of Type I Melanoma Antigen Gene (MAGE) family genes upon the synergistic loss of TP53 and AHR. Type I MAGE proteins have been shown to bind the E3-ligase TRIM28 which redirects its substrate specificity. On the same line, TCGA LUAC patient data show that expression of MAGEC2 correlates with shorter survival times, an effect which is even more pronounced when expression of ARNT and TP53 is low. While mechanistic studies are

ongoing, we also employed structural bioinformatics to model the TRIM28 dimer and its binding to MAGEC2.

Conclusion

Our findings point to a clinically relevant model in which the loss-of-function of TP53 and AHR/ARNT leads to the expression of type I MAGE genes, which causes an altered TRIM28 substrate specificity and the degradation of one or multiple anti-transformative factors.

EACR25-2496

Sex-Dependent Transcriptional Gene Expression in Urothelial Bladder Cancer

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Introduction

The incidence of bladder cancer is three times higher in men than in women. While the underlying causes of this disparity remain unclear, potential contributing factors such as smoking, hormonal effects, X chromosome inactivation, and anatomical differences cannot fully explain the significantly higher prevalence in males. More research is needed on the sex-specific biological mechanisms that cause this disparity. Aim: This study investigates sex-specific transcriptional differences in bladder cancer by analysing gene expression patterns. By identifying differentially expressed genes between male and female patients, I aim to uncover molecular mechanisms underlying sex-based disparities, particularly in the early stages of the bladder cancer.

Material and method

Bulk RNA sequencing data from the UROMOL2021 study were used for the analysis. This dataset includes NMIBC samples from 535 patients (121 females and 414 males) and 7 non-cancerous bladder tissues from the GTEx database. Differentially expressed genes (DEGs), gene set enrichment (GSE), progression-free survival (PFS), and tumour microenvironment (TME) analyses were performed to identify differences between male and female tumour tissues.

Result and discussion

DEG analysis revealed 94 genes with differential expression between male and female NMIBC patients, including 19 X-chromosome, 22 autosomal, and 53 Y-chromosome genes. In normal bladder tissues, only 16 genes exhibited differential expression between the sexes. Seven X-chromosome genes were significantly associated with PFS in male patients, compared to only two in female patients (log-rank test, $p < 0.05$). For autosomal genes, six were significantly associated with PFS in male patients not in female. In male patients, cancer-related pathways were significantly upregulated in tumour tissues, while no significant pathways were observed in female tumour tissues. However, in normal bladder tissues, up-regulation of cancer pathways was exhibited in male patients, while immune-related pathways were upregulated in females. Furthermore, gene ontology analysis of biological processes has

revealed that epigenetic regulation, X chromosome inactivation, and miRNA metabolic processes play a critical role in the sex-specific differences observed in NMIBC.

Conclusion

Comprehensive sex-specific data analysis of NMIBC patients indicates that most transcriptional differences are linked to X and Y chromosome genes. Notably, my findings highlight XIST expression and its regulatory mechanisms as key contributors to these differences, particularly in relation to PFS outcomes. The PFS analysis revealed that XIST expression affects males and females in opposite ways. These findings suggest that XIST and its associated mechanisms may provide a crucial explanation for sex-based differences in NMIBC.

EACR25-2553

Exploring Alternative Splicing in Breast Cancer: Direct RNA Nanopore Sequencing for Transcriptome Profiling

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Introduction

Breast cancer (BC) remains one of the most widespread diseases globally, profoundly affecting millions each year, particularly women. This study investigates the feasibility of utilizing direct RNA nanopore sequencing protocols from Oxford Nanopore Technologies (ONT) for transcriptome profiling in BC-related cell lines, including nontumoral MCF-10A cells, luminal A MCF-7 cells, and triple-negative MDA-MB-231 cells.

Material and method

The selected cell lines were cultured, RNA was extracted and sequenced, and the resulting data underwent processing through the bioinformatics pipeline FLAIR. This approach enabled the identification of alternative splicing (AS) events in various genes associated with tumorigenesis. Given the substantial volume of data obtained, the genes analyzed for AS events were chosen based on their roles in mRNA splicing and translation initiation pathways.

Result and discussion

Multiple AS events were identified in genes encoding RNA polymerase II subunits – POLR2G and POLR2H – as well as in the splicing factor gene SRSF2. Furthermore, EIF3A and EIF3E, which encode subunits of the eukaryotic initiation factor 3 (eIF3) complex essential for translation, exhibited novel isoforms and distinct expression patterns across the analyzed cell lines. The identification of novel isoforms in these genes suggests potential functional implications in BC development and progression. For example, intron retention in POLR2G and exon skipping in POLR2H may disrupt co-transcriptional splicing, thereby impairing RNA processing regulation. Additionally, nonsense-mediated decay (NMD)-sensitive isoforms detected in SRSF2 could influence its autoregulation, impacting cell survival. The discovery of a novel EIF3A isoform predominantly

expressed in MDA-MB-231 cells suggests potential alterations contributing to BC progression. Likewise, protein modeling of various EIF3E isoforms using AlphaFold revealed the possibility of distinct functional properties.

Conclusion

These results might be an essential step for further biologically significant discoveries in cancer transcriptomics, potentially advancing clinical management, diagnosis, and therapeutic strategies for BC patients.

EACR25-2557

Integrative analysis of epigenetic and transcriptional interrelations identifies histotype-specific biomarkers in early-stage ovarian carcinoma

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Introduction

Ovarian carcinoma (OC) is a deadly and heterogeneous disease comprising five major histotypes: clear cell carcinoma (CCC), endometrioid carcinoma (EC), low- and high-grade serous carcinoma (LGSC, HGSC), and mucinous carcinoma (MC). Despite this heterogeneity, OC is often treated as a homogenous disease, and reliable screening tests are lacking. Although progress has been made, there is a pressing need for biomarkers to refine patient stratification, guide treatment, and improve outcomes. Here, we elucidated the relationship between DNA methylation and gene expression patterns in OC to identify histotype-specific biomarkers.

Material and method

Differential DNA methylation and gene expression analyses were performed for 86 early-stage OC samples after histopathological reclassification stratified by histotype. The correlation between DNA methylation and gene expression was examined, and histotype-specific biomarkers were identified. Hierarchical clustering and predictive machine learning modeling were employed to assess the performance of the histotype-specific biomarkers using four external cohorts.

Result and discussion

OC histotypes exhibited distinct epigenetic, transcriptional, and functional profiles, with candidate histotype-specific biomarkers such as CTSE and VCAN effectively distinguishing CCC, HGSC, and MC on the transcriptional level. Gene expression for the candidate biomarkers was found to be reproducible across external cohorts, with histotype-specific differences remaining homogenous.

Conclusion

This study identified promising histotype-specific biomarkers for OC using integrative transcriptomic and epigenomic analysis. Furthermore, these findings indicate that additional stratification or potential reclassification of the EC histotype is warranted in future studies.

EACR25-2563

Molecular Characterisation of Trophoblast Tumours

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Introduction

Placental site trophoblastic tumors (PSTT) and epithelioid trophoblastic tumors (ETT) are rare malignancies arising from trophoblast cells post-pregnancy. Despite their aggressive cellular origins, these tumors exhibit slow progression and remain poorly characterized at the molecular level. This study employs a multi-omic approach to elucidate the molecular mechanisms underlying PSTT and ETT, with the aim of improving diagnostic and therapeutic strategies and informing broader cancer evolution models.

Material and method

Archival formalin-fixed paraffin-embedded (FFPE) samples of PSTT and ETT were analyzed using whole-genome sequencing (WGS), whole-exome sequencing (WES), RNA sequencing (RNA-seq), and shallow whole-genome sequencing (sWGS). A Nextflow-based bioinformatics pipeline processed sequencing data, identifying genomic alterations, copy number aberrations, and immune evasion mechanisms. HLA-typing was performed using WGS/WES data and validated via RNA-seq. Tumor mutational burden and immune selection pressure were assessed through variant analysis and immune dN/dS calculations.

Result and discussion

A significant correlation between the time since causative pregnancy and genomic instability was identified, with higher percent genome altered (PGA) in tumors arising more than four years post-pregnancy. HLA-typing revealed that immune evasion in PSTT/ETT mirrors their trophoblastic origin, with loss of HLA-A/B expression and retained HLA-C expression. Immune dN/dS analysis showed a lack of immunoediting, reinforcing the hypothesis that trophoblast tumors inherently evade immune detection rather than acquiring this ability.

Conclusion

This study provides novel insights into PSTT/ETT molecular evolution and immune evasion. The development of a time-dependent tumor progression model may enhance patient stratification and guide therapeutic interventions, with potential applications to other malignancies beyond PSTT and ETT.

EACR25-2564**Spatial Transcriptomics for characterising pathologic response to FOLFOX regimen in colorectal Liver Metastases**

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Introduction

Liver metastases are major cause of cancer deaths. Surgery is a potential cure, but recurrence is common, and only 20% are initially surgical candidates. Pre-operative FOLFOX chemotherapy is frequently used. As postoperative chemotherapy benefits are unclear, understanding preoperative response could improve treatment and relapse management. This study utilizes spatial transcriptomics to identify biomarkers predictive of FOLFOX response, enabling personalized adjuvant strategies based on hepatic growth patterns (HGP) of liver metastases.

Material and method

TMA of CRCLM, categorized by FOLFOX response and HGP, were created. Spatial transcriptomics (GeoMx) analyzed gene expression in tumor cells, CAFs, and immune cells. Data analysis used linear mixed models, GSEA, and cell deconvolution. Lasso regression and cross-validation were used to develop a predictive gene classifier.

Result and discussion

Analysis revealed distinct gene expression profiles between encapsulating and infiltrative hepatic gland patterns (HGP) within both tumor cells and cancer-associated fibroblasts (CAF). Gene candidates differentiating these HGPs are currently undergoing validation. In terms of pathological response, tumor cells from responders exhibited enrichment in inflammatory pathways, notably IL6-JAK/STAT3 signaling, while non-responders showed upregulation of DNA repair, chromatin remodeling, and proliferation-associated pathways. Notably, despite rigorous statistical analysis, no significant differential gene expression was detected in CAFs between responders and non-responders, regardless of HGP. However, non-responding areas, and non-encapsulating metastases, displayed a higher presence of contractile CAFs, despite ECM-producing CAFs being the most abundant subtype in both groups. Immune cell composition showed no major changes, except for increased neutrophils in non-responding areas and non-encapsulating metastases. Further immune cell characterization is ongoing, combining transcriptomic and proteomic data. Lasso regression identified four genes with high predictive value for treatment response, withheld due to patent concerns. Drug screening identified a transmembrane protein ligand (methanobenzazocine derivative) and a PROTAC.

Conclusion

Spatial transcriptomics revealed molecular differences in FOLFOX response and HGP biomarkers in CRCLM,

enabling biomarker-driven personalized post-hepatectomy treatment and relapse management. 4-plex staining validation is ongoing.

EACR25-2581**Clinical and Genetic Heterogeneity in Early-Onset Colorectal Cancer: Unclosing Distinct Molecular Entities**

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Introduction

The incidence of early-onset colorectal cancer (EO-CRC) has been increasing dramatically over the last few decades. The younger affected population tends to have more advanced stages, poor response to therapy and a worse prognosis among treated patients with metastatic CRC, thus emerging the need to investigate novel susceptibility genetic variants responsible for increased EO-CRC risk and to support therapeutic decisions.

Material and method

With the aim of identifying inherited variants underlying increased susceptibility for EO-CRC, 202 patients (≤ 50 years) who had previously undergone Next-Generation Sequencing-based germline genetic diagnosis using a custom multigene panel (Agilent) were analyzed, in a retrospective study, for germline genetic variants in selected cancer susceptibility genes and selected genes coding for components of relevant cancer mechanisms. The genetic variants of interest were selected based on their ClinVar classification, population frequency and predictive damaging in silico software.

Result and discussion

At the clinical level, women presented distal tumors ($p = 0.049$) and a family history (FH) of cancer ($p = 0.006$) more often than men. Patients under 30 mostly have no FH of cancer ($p = 0.014$). Moreover, metastatic disease was positively linked to a cancer FH in non-1st-degree relatives ($p = 0.013$) and inversely correlated with history of polyps ($p = 0.000$). Pathogenic/likely pathogenic variants (PVs) were identified in 20/182 (11%) patients, with 19/20 variants being identified in genes involved in different DNA repair mechanisms (ATM, MUTYH, FANCD2, ...). Furthermore, 105 putative damaging (PDVs) according the above mentioned criteria were identified in 84/182 (46%) patients. Among these PDVs, 57% are associated to DNA repair pathways and the remaining to other cellular mechanisms. Besides, 27/84 (32%) patients carried 2 or more PDVs and these presented less frequently FH of CRC ($p = 0.043$). Most DNA repair gene variants were related to homologous recombination DNA repair ($p = 0.013$) and PVs seemed to be associated with rectal cancer ($p = 0.043$) and

inversely related to a personal history of polys ($p = 0.041$). Lastly, since ATM showed the highest mutation rate in the entire cohort, with 4/20 (20%) of the patients with PVs presenting alterations in this gene ($p = 0.015$), we analyzed an additional validation cohort of 1652 patients with increased risk of CRC and identified 13 PVs and 23 PDVs in ATM gene, which appeared to be differentially distributed throughout the protein according to specific phenotypes.

Conclusion

EO-CRC population revealed heterogeneous features, defining specific clinical and genetic subgroups, with a subset associated to deficiency in distinct DNA repair mechanism, thus shedding light to the distinct etiologies of EO-CRC and the identification of specific traits that may be associated with greater tumor aggressiveness and to distinct responses to chemotherapy and PARP inhibitors.

EACR25-2592

Genomic Analysis of PARP Inhibitor Response in High-Grade Serous Ovarian Cancer: Insights from Whole Genome Sequencing

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Introduction

To evaluate the clinical utility of whole-genome sequencing (WGS)-based HRDetect in identifying homologous recombination deficiency (HRD) and its association with clinical outcomes in high-grade serous ovarian cancer (HGSOC) patients treated with PARP inhibitors (PARPi).

Material and method

This single-center study analyzed patients with stage III-IV high-grade serous ovarian or fallopian tube cancer treated at Asan Medical Center from 2017 to 2022.

Participants underwent surgery followed by adjuvant or neoadjuvant chemotherapy and maintenance therapy with olaparib or niraparib. Patients were stratified into four groups by BRCA status and progression-free survival (PFS). Retrospective data collection included demographics, treatments, and outcomes. Tumor and normal samples underwent WGS, and HRD was evaluated using HRDetect and Myriad genomic instability scores (GIS). Genomic features, including mutational signatures, structural variations, and copy number alterations, were analyzed.

Result and discussion

Among 37 patients, ScarHRD identified 30 as HRD-positive and 7 as HRD-negative, while HRDetect classified 17 as HRD-positive and 20 as HRD-negative. Discordant cases arose only when HRDetect labeled tumors as HRD-negative but ScarHRD classified them as HRD-positive. Tumors with key HRD-associated features, such as SBS3, ID6, RS3, and RS5, were consistently identified as HRD-positive by both methods. In contrast, tumors lacking SBS3 and displaying ambiguous characteristics, including age-related

mutational signatures or microsatellite instability, were classified as HRD-negative by HRDetect, even when ScarHRD deemed some of these HRD-positive.

Conclusion

This study suggests that WGS-based HRDetect can complement existing HRD assessment methods in HGSOC, offering a more comprehensive approach to identifying patients for targeted therapies.

EACR25-2603

Multimodal analysis of spatial transcriptomics data uncovers novel invasive lobular carcinoma subtypes

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Introduction

Invasive lobular carcinoma (ILC) is the second most common histological subtype of breast cancer. This study integrates morphological, spatial, and molecular data from spatial transcriptomics (ST) to analyze the ILC tumor microenvironment (TME), leading to a novel classification based on TME heterogeneity.

Material and method

ST (Visium 10X) was performed on 43 HR+/HER2- ILC frozen tumors and relative H&E slides were morphologically annotated. To improve resolution, ST spots were deconvoluted using external RNA sequencing data. Imaging and sequencing data were independently analyzed and then integrated using intNMF, leading to the identification of ILC4TME, a novel ILC classification. This classification was validated in external bulk RNA-seq using a surrogate gene signature-based classifier.

Result and discussion

Morphological and molecular analysis revealed distinct immune cell compartmentalization: adaptive immune cells (B and T cells) clustered near epithelial cells, while innate immune cells (macrophages and monocytes) associated with adipose tissue. In situ/normal glands correlated with better outcomes, while proliferative and metabolic-enriched tumor cells indicated worse outcomes. Multimodal integration of ST data identified four ILC subtypes (ILC4TME): proliferative (P, enriched in tumor cells and proliferation-related pathways), normal-stroma enriched (NSE, enriched in fibroblasts, carcinoma in situ, and EMT-related pathways), androgen receptor-enriched (ARE, enriched in AR expression, endothelial cells, and metabolic pathways), and metabolic-immune enriched (MIE, enriched in adipocytes, macrophages, endothelial cells, and metabolic pathways). Our classifier identified the same subtypes in METABRIC and SCAN-B ILC samples, where survival differences emerged: NSE and P subtypes were significantly associated with longer and shorter relapse-free intervals, respectively. Notably, the P subtype correlated with higher copy number aberrations in METABRIC and was linked to

larger tumors, higher grade, lymph node involvement, and high Ki67.

Conclusion

Multimodal integration of spatial transcriptomics (ST) data led to the identification of four distinct invasive lobular carcinoma (ILC) subtypes, each characterized by unique biological features and associated with different survival outcomes. This classification not only provides insights into the tumor microenvironment and disease progression but also highlights potential therapeutic targets. Importantly, these subtypes were also identified in more accessible bulk RNA sequencing data using surrogate gene signatures, facilitating their broader clinical applicability and potential for integration into routine diagnostic and prognostic assessments.

Carcinogenesis

EACR25-0022

Unravelling the role of AP-1 transcription factor in DNA damage signalling and response (DDR), platinum and PARP inhibitor resistance in ovarian cancers

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Introduction

Hypoxia is a characteristic of the microenvironment in high-grade serous ovarian cancer (HGSOC) and plays a role in resistance to platinum-based treatments and PARP inhibitors. It activates the activity of the AP-1 transcription factor, which promotes continuous cell proliferation, invasion, metastasis, and angiogenesis. However, the involvement of AP-1 in DNA damage signalling and repair, as well as its contribution to platinum and PARP resistance in HGSOC, remains uncertain.

Material and method

The activity of 48 different transcription factors (TFs) were assessed in PARP-sensitive and resistant high-grade serous ovarian cancer (HGSOC) cells (PEO1, PEO1R, and OVCAR4) using a luciferase-based reporter assay. The protein expression of AP-1 subunits, including c-JUN, JUND, JUNB, cFOS, and FOSL2, was analyzed under both normoxic and hypoxic (1% O₂) conditions. Immunohistochemical evaluations for JUNB, FOSL2, MRE11, CA-9, and CD-31 expression were conducted on tumor xenografts from PEO1 and PEO1R. CRISPR knockouts (KOs) of JUNB and FOSL2 were created and studied for their effect on DNA repair gene expression using DNA repair profiler PCR arrays, whole genome RNA sequencing, as well as proliferation, invasion, and sensitivity to cisplatin through clonogenic and 3D spheroid assays. Functional assays were performed to evaluate the accumulation of DNA double-strand breaks (DSBs), cell cycle progression, apoptosis, along with immunofluorescence, protein stability assays, and co-immunoprecipitation. Additionally, the clinical and

pathological significance of FOSL2, JUNB, and MRE11 expression was explored in a cohort of 331 patients with epithelial ovarian cancer.

Result and discussion

In platinum/PARP-resistant PEO1R cells, AP-1 transcription activity was significantly elevated compared to PEO1 cells, with JUNB and FOSL2 proteins being overexpressed under both normoxic and hypoxic (1% O₂) conditions. Tumor xenografts of PEO1 and PEO1R exhibited high JUNB and FOSL2 levels in hypoxic tumor regions. JUNB and FOSL2 knockout (KO) cells demonstrated reduced proliferation and increased sensitivity to cisplatin and the PARP inhibitor olaparib compared to controls, which was linked to increased double-strand breaks (DSBs), G2/M cell cycle arrest, and apoptosis. DNA repair profiling in KO cells showed downregulation of multiple DNA repair genes, including MRE11, a critical factor in the DNA damage response. JUNB and FOSL2 physically interacted with MRE11, enhancing its stability. RNA sequencing revealed enrichment of pathways related to platinum response, oxidative phosphorylation, and translation in KO cells relative to controls. Clinically, elevated expression of FOSL2, JUNB, and MRE11 correlated with significantly shorter progression-free survival (PFS) and poorer overall survival (OS).

Conclusion

Our findings suggest that JUNB and FOSL2 play a role at the intersection of hypoxia and the DNA damage response (DDR) in high-grade serous ovarian cancer (HGSOC). Beyond their predictive and prognostic value, JUNB and FOSL2 could serve as promising therapeutic targets, particularly in platinum/PARP-resistant HGSOC.

EACR25-0044

Targeting Replication Protein A (RPA) heterotrimeric complex to enhance platinum sensitivity and reverse PARP inhibitor resistance in ovarian cancers

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Introduction

Resistance to platinum-based chemotherapy poses a significant challenge in treating epithelial ovarian cancers, making the development of novel biomarkers and therapeutic targets essential. Replication Protein A (RPA), a heterotrimeric complex made up of RPA1, RPA2, and RPA3 subunits, functions as a single-stranded DNA (ssDNA)-binding protein that plays a crucial role in DNA replication, checkpoint regulation, and DNA repair.

Material and method

In this study, we assessed the clinicopathological significance of RPA1, RPA2, and RPA3 protein expression in a cohort of 331 ovarian tumors. We analyzed RPA1, RPA2, and RPA3 transcripts using publicly available datasets (n = 1287) and conducted detailed bioinformatics analyses in the ovarian TCGA cohort (n = 379). Preclinical experiments involved

depleting RPA1 or RPA2 in platinum-resistant ovarian cancer cells, followed by testing their sensitivity to cisplatin and PARP inhibitors (Olaparib, Talazoparib). Additionally, we evaluated the effects of HAMNO, a prototypical 70N-PPI inhibitor, in both PARPi-resistant and sensitive cells.

Result and discussion

Our findings revealed that high levels of RPA1 and RPA2 protein and transcripts were associated with platinum resistance and poor survival outcomes in human tumors. Furthermore, depleting RPA1 or RPA2 not only resensitized the cells to platinum therapy but also reversed resistance to talazoparib and olaparib. Treatment with HAMNO selectively enhanced the cytotoxicity of PARPi-sensitive compared to PARPi-resistant.

Conclusion

In conclusion, an RPA-directed precision oncology strategy holds promise as a potential approach for ovarian cancer treatment

EACR25-0170

Translational control in ovarian cancer: role of the glutaminyl-tRNA synthetase (QARS) in DNA damage response

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Introduction

High-grade serous ovarian cancer (HGSOC) is one of the deadliest gynecologic cancers. Treatment options have seen limited progress, despite the development of anti-angiogenic drugs and PARP inhibitors. While the latter are effective in homologous recombination-deficient (HRD) patients, resistance remains a challenge, highlighting the need for new therapeutic targets. We found that 50% of HGSOC patients exhibit MAP3K8 accumulation, activating the MEK/ERK pathway independently of KRAS/BRAF mutations. MAP3K8/MEK regulates the translation of specific mRNAs, with QARS (glutaminyl-tRNA synthetase) as the top target. QARS, like many ARSs (aminoacyl-tRNA synthetases), regulates substrates, not only via translation but also through aminoacylation, controlling various cellular processes. Here, we discovered a novel role for QARS in the DNA damage response (DDR).

Material and method

Polysome profiling investigated MAP3K8/MEK-mediated QARS translational regulation in ovarian cancer cell lines (OCCL), validated by immunohistochemistry with HGSOC tissue. Data from two patient cohorts highlighted QARS role in DDR. QARS function was examined in cell lines through overexpression of wild-type and catalytically inactive forms, analyzing DDR markers (DNA breaks via Comet assay, γH2AX, RAD51, and 53BP1 foci by immunofluorescence). Co-immunoprecipitation and *in vitro* glutaminylation assays were used to assess QARS involvement in DDR mechanisms.

Result and discussion

Inhibition of MAP3K8/MEK in OCCL decreased QARS protein levels without affecting mRNA expression, supported by human HGSOC tissue analysis showing a significant correlation between MAP3K8 and QARS protein levels. This indicates MAP3K8 regulates QARS translation without affecting transcription. QARS accumulation is associated with increased DDR; tumors sensitive to cisplatin-based chemotherapy exhibited elevated QARS expression. In line with patient observations, we provided evidence that QARS plays a role in DDR *in vitro*. Wild-type QARS overexpression reduces DNA damage and promotes double-strand break repair via homologous recombination, as shown by decreased γH2AX and increased RAD51 foci. In contrast, the catalytically inactive QARS mutant reversed these effects, highlighting the importance of its enzymatic activity in DDR. These results suggest QARS may accumulate in HRD patients sensitive to chemotherapy, to compensate and favour HR. This accumulation may enhance the DDR response in tumors, ultimately aiding their survival and chemotherapy response. We are currently investigating the molecular mechanisms by which QARS regulates DDR, identifying two potential QARS targets for translational regulation and/or glutaminylation.

Conclusion

MAP3K8 regulates QARS at the translational level in HGSOC, and QARS plays a critical role in promoting DNA repair. This study aims to identify new targetable pathways for HGSOC, a major clinical challenge.

EACR25-0259

Impact of EBV on Genetic Mutations in Diffuse Large B Cell Lymphoma: A Study Employing Conventional and High-Sensitivity Detection Methods in a Cohort from Argentina

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Introduction

Diffuse large B-cell lymphoma (DLBCL) is the most common subtype of non-Hodgkin lymphoma (NHL), representing approximately 30% to 40% of all newly diagnosed lymphomas. In 2017, the WHO confirmed a previous provisional entity, EBV+DLBCL, NOS. It has

been hypothesized that Epstein Barr virus (EBV) may act as an alternative or complementary mechanism to the genetic alterations involved in DLBCL development. Since traces of EBV infection were described by sensitive methods in cases considered negative by conventional techniques, the involvement of EBV infection in DLBCL pathogenesis is still under discussion. For that purpose, our aim was to analyze the genetic alterations, classify them according to their pathogenicity, and correlate these with the presence of the virus and its traces, detected using both conventional and high-sensitivity methods.

Material and method

A cohort of 35 patients with DLBCL was analyzed. EBV+DLBCL was defined by EBERs *in situ* hybridization (ISH), with 20% of EBERs⁺ tumor cells as cut-off. The presence of viral traces was explored by ViewRNA assay that detected LMP1 and EBNA2 transcripts. Genetic variants were evaluated using a custom-designed next-generation sequencing (NGS) panel (Qiagen), focusing on pathogenic and non-pathogenic variants and pathways enrichment analyses. Comparative analyses were conducted between three groups: EBV+ DLBCL, EBV-DLBCL with viral traces, and EBV- DLBCL without traces. Statistical analyses assessed the associations between genetic variants and the presence of EBV presence or its traces.

Result and discussion

NGS analysis identified pathogenic variants predominantly in ATM, TP53, PTEN, ARID1B, and KMT2A genes across all groups, suggesting shared mechanisms of DNA repair dysfunction and cell cycle regulation. No significant association was found between EBV presence or traces and the frequency of pathogenic variants when they were analyzed as a whole ($p > 0.05$, Fisher's test). However, EBV+ DLBCL exhibited unique alterations in BTK, HAX1, PAFAH1B1, and NAGLU genes, implicated in immune regulation, apoptosis, and mitochondrial dynamics, while 75% of EBV+ DLBCL displayed variations in C11orf65 gene. Notably, variants at the NOTCH signaling pathway were exclusively enriched in EBV+ DLBCL.

Conclusion

Our findings reveal no significant impact of EBV or its viral traces on the frequency of pathogenic genetic variants as a whole in DLBCL. This aligns with previous studies suggesting EBV contributes to lymphomagenesis through epigenetic or immunomodulatory mechanisms rather than specific genetic alterations. The high prevalence of mutations in ATM, TP53, and PTEN across all groups underscores the importance of genomic instability in DLBCL pathogenesis. In EBV+ DLBCL exclusive genetic alterations and NOTCH pathway enrichment indicate virus-specific mechanisms that may promote tumorigenesis. Further studies, are needed to validate these findings.

EACR25-0503

Late effect of radiotherapy: impairment of DNA double-strand break repair mechanisms in skin and lung fibroblast of previously irradiated sheep

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Introduction

Children who undergo radiotherapy (RT) for cancer at an early age face a significant risk of developing long-term complications. One of the most concerning late effects is the increased likelihood of developing radiation-induced cancer. This occurs because radiation exposure to normal tissues can lead to genetic mutations affecting DNA damage repair pathways, proto-oncogenes, and tumor suppressor genes. To simulate pediatric craniospinal irradiation, a sheep model was utilized, where five sheep received radiation treatment to the thecal sac, while four served as controls. However, surrounding tissues were also exposed. The goal of this study is to investigate how prior RT influences DNA double-strand break (DSB) repair kinetics. Three years post-treatment, lung and skin biopsies from areas exposed to both low and high doses of X-rays were collected and analyzed to compare the effects of radiation in previously treated versus untreated sheep.

Material and method

This study included a total of nine sheep, with five receiving radiation treatment and four serving as controls. The treated sheep underwent eight sessions of radiation therapy, receiving a total dose of 28 Gy targeted at the spinal thecal sac. The treatment areas were categorized into high-dose (HD, >17 Gy) and low-dose (LD, <2 Gy) regions. Three years post-treatment, lung and skin biopsies were collected from HD, LD regions, and untreated control sheep. Primary lung and skin fibroblast cells were isolated and cultured, yielding a total of 23 cell lines: nine control (five lung and four skin), six LD (Four lung and two skin), and eight HD (four lung and four skin) primary fibroblast cell lines. Cultured cells were irradiated with 2 Gy, and DSB repair kinetics were evaluated using anti- γ H2AX and anti-pATM immunofluorescence assays. The clonogenic and micronuclei assays were also performed.

Result and discussion

In lung cells, under baseline conditions, both LD ($p < 0.05$) and HD ($p < 0.01$) cells exhibited a significantly higher number of spontaneous γ H2AX foci compared to the control group. Ten minutes after a 2 Gy re-irradiation, only LD lung cells showed a significantly lower number of foci than the control. No significant differences were observed at these time point for skin cells. Interestingly, 24 hours post-irradiation, the number of residual γ H2AX foci remained higher in cells derived from previously irradiated sheep compared to those from non-irradiated sheep, for both lung and skin fibroblasts ($p < 0.01$). Finally, LD and HD cells displayed a significantly lower number of pATM foci 10 minutes after 2 Gy irradiation ($p < 0.01$) compared to the control cell line, in both skin and lung fibroblasts.

Conclusion

Our findings indicate that, in the long term, prior irradiation of normal tissues can lead to (1) increased genomic instability, (2) reduced efficiency of DNA double-strand break (DSB) signaling, and (3) impaired DSB repair in primary ovine lung and skin fibroblast cells.

EACR25-0802

Fibronectin leucine-rich transmembrane protein 3 is a potential driver of intrahepatic cholangiocarcinoma

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Introduction

Extensive research has identified oncogenic mutations, such as IDH mutations and FGFR fusions, as drivers of cholangiocarcinoma (CCA). However, these mutations are found in a limited subset of intrahepatic CCA (iCCA) cases, and resistance may develop. Thus, a comprehensive understanding of iCCA pathogenesis is crucial for developing effective therapies by elucidating mechanisms that drive tumor initiation and progression. Our study aims to identify molecular drivers of tumor progression using *in vitro* and *in vivo* models.

Material and method

We generated intrahepatic cholangiocyte organoids (ICOs) from mouse cholangiocytes carrying the KrasG12D mutation. These organoids were studied *in vitro* and *in vivo* after subcutaneous injection into immunodeficient mice. Human EGI-1 and Mz-Cha-1 CCA lines were used for molecular studies. Immunolabeling of human pathological samples was performed to investigate gene expression in iCCA. Mining of human transcriptome data further determined the expression of genes in human CCA.

Result and discussion

KrasG12D ICOs did not show morphological changes *in vitro* but induced subcutaneous tumors *in vivo* in immunodeficient mice, whereas wild-type ICOs did not. The resulting tumors displayed key features of human biliary lesions evolving into CCA, with desmoplastic stroma and cystic structures lined by cholangiocyte marker-expressing epithelial cells. RNA sequencing of KrasG12D and wild-type organoids identified candidate genes involved in tumorigenesis. Among these, fibronectin leucine-rich transmembrane protein 3 (FLRT3) emerged as a possible driver of iCCA progression. FLRT3 protein and mRNA expression was detected in a subset of human iCCA tumor cells. Functional inhibition of FLRT3 using shRNA significantly reduced organoid-derived tumor growth, as well as *in vitro* migration and invasion of human EGI-1 and MZ-Cha-1 cells.

Conclusion

In conclusion, we developed a versatile ICO-based model that closely mimics human iCCA and enables the identification of tumorigenic drivers. Our findings

suggest that FLRT3 stimulates iCCA progression. Further studies will clarify FLRT3-mediated tumor progression *in vivo* *in situ*, and explore how FLRT3 drives the progression of CCA.

EACR25-1000

The inter-relationship between arsenic exposure and oxidative stress biomarkers on the risk of cancer occurrence - A population-based cohort study

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Introduction

Arsenic exposure has been known as a risk factor for various cancers. However, the inter-relationship between arsenic exposure and cellular oxidative stress on cancer occurrence is still imperfectly understood. This study investigated the association between urinary arsenic metabolites and all cancer occurrence while examining the effects of oxidative stress biomarkers with a population-based cohort.

Material and method

This study conducted a 9-year follow-up on 6,402 residents aged over 40 years from the southwestern coast of Taiwan, and identified 305 all-cancer cases. The urinary arsenic metabolites were determined by inductively coupled plasma mass spectrometry, and oxidative stress biomarkers were assessed through urinary 8-hydroxy-2-deoxyguanosine (8-OHdG) and urinary malondialdehyde (MDA). Cox proportional hazards model was performed to calculate the multi-variate-adjusted hazard ratio (HR) of cancer occurrence.

Result and discussion

The analyses revealed a significant dose-response relationship between urinary arsenic and cancer occurrence. Participant's urinary arsenic in the highest tertile was at 1.45 folds (95% CI = 1.05-2.02) of developing cancers as opposed to ones in the lowest tertile. The results showed that the interactions of urinary arsenic and 8-OHdG or MDA were statistically significant. Participants with relatively higher urinary arsenic and higher urinary 8-OHdG were at the highest risk of developing cancers (HR = 1.44; 95% CI = 1.03-2.00) among four combination groups. Participants with relatively higher urinary arsenic and higher urinary 8-OHdG but lower MDA were at the highest risk of developing cancers (HR = 2.43; 95% CI = 1.37-4.31) than those with relatively lower levels of the three biomarkers.

Conclusion

Oxidative stress is considered an important pathway for cancer occurrence. However, the biomarkers of DNA damage and lipid damage moderate differently when considering the association between arsenic exposure and cancer occurrence. DNA damages and arsenic exposure play a synergistic role in the long-term arsenic carcinogenesis.

EACR25-1013**Secreted clusterin promotes the migration and invasion of triple-negative breast cancer cells***A. Ciringione¹, M. Marozzi², S. Belletti¹, F. Rizzi¹*¹*University of Parma, Department of Medicine and Surgery, Parma, Italy*²*University of Parma, Parma, Italy***Introduction**

Treatment of metastatic breast cancer (BC) remains a major problem. Thus, understanding the molecular processes that favor the acquisition of a motile phenotype, may help to find new “druggable” targets to block BC cells escape. Clusterin (CLU) is a secreted glycoprotein up regulated in BC that plays a role in cell adhesion, lipid metabolism, and membrane transporter recycling. We investigated the effects of CLU silencing on the migratory and invasive capacity of BC cells focusing our attention on the mechanisms that support extracellular matrix (ECM) remodeling and cytoskeleton fibers rearrangements.

Material and method

We abrogated CLU expression (siRNA transfection) in two BC cell lines at different stages of malignancy, MCF-7 and MDA-MB-231. We used transwell migration and invasion assays to study cell motility and molecular biology techniques, i.e., RT-PCR, Western Blot, immunocytochemistry, and Rho/GTP pull-down assay, to explore the mechanisms underlying the observed phenotypes.

Result and discussion

In the aggressive triple-negative breast cancer (TNBC) cell line, MDA-MB-231, CLU knock-down reduced both migration and invasion. This phenotype was accompanied by a dramatic change in the cell shape and an impressive reduction of F-actin-enriched (filopodia-like) cell membrane protrusions. Consistently, we observed a decreased activation of AKT and RhoA GTPase, both involved in the regulation of cytoskeletal dynamics and cell motility. Finally, we found a reduction of the activated form of NF- κ B and a coherent transcriptional down-regulation of MMP9. Conversely, CLU silencing had none of these effects on luminal-like MCF-7 cells. We also observed that CD44 and LRP1 receptors, which are involved in lamellipodia formation and cell motility, are more expressed in MDA-MB-231 than in MCF-7 cells.

Conclusion

Our work suggests that CLU contributes to the migratory phenotype of TNBC cells affecting the transduction of cell signalings that modulate the dynamics of the cytoskeleton and promote the ECM degradation through a mechanism that may involve the CD44/LRP1 axis. Of note, CLU is a known ligand of LRP1. Although our study needs to be extended *in vivo*, we are confident that it may have relevant translational implications, including the possibility of limiting the disease progression towards a metastatic stage in specific subpopulations of BC patients that may benefit from CLU antisense oligonucleotides (already available) in combination with chemotherapy.

EACR25-1051**Biological relevance of the long non-coding RNA NEAT1 in Squamous Cell Carcinoma progression***S. De Domenico¹, V. La Banca², A. Peschiaroli²,**S. Nicolai², S. D'amico²*¹*Tor Vergata, Experimental Medicine, Rome, Italy*²*CNR, IFT, Rome, Italy***Introduction**

Different Squamous Cell Carcinomas share genetic alterations, including Tp53 mutations and Tp63 modifications. Δ Np63, a truncated Tp63 isoform, is essential for stratified epithelial tissue integrity. We identified the lncRNA NEAT1 as a Δ Np63 target, showing its repression by Δ Np63 via HDAC recruitment is critical for epidermal differentiation. NEAT1 has also been recognized as a p53 target, important for transformation suppression, but its role in SCC pathophysiology is unclear. This study explores NEAT1 dysregulation and the influence of p53 mutations and Δ Np63 amplification on the NEAT1-dependent response to genotoxic stress in SCC and normal cell lines.

Material and method

We analyzed how Δ Np63 and p53 regulate NEAT1 expression under genotoxic stresses. We treated p53-positive and p53-negative/mutant SCC and normal cell lines with UVB radiation and Doxorubicin to induce DNA damage. NEAT1 isoform expression, p53 target gene activity, and apoptosis were analyzed using RT-PCR and Western blot. We repeated the same experiments while silencing Δ Np63 and p53 using siRNA. We assessed DNA damage after silencing NEAT1 before UVB exposure. Lastly, we evaluated if NEAT1 targeting could enhance HDACI cytotoxic effects, such as Givinostat, in human and murine SCC cell lines.

Result and discussion

NEAT1 was upregulated rapidly after genotoxic stresses, independently of apoptosis. Its induction correlated with cell cycle arrest genes, indicating its role in DNA repair and cell survival. Notably, NEAT1 upregulation was p53-dependent. Interestingly, silencing Δ Np63 further enhanced NEAT1 induction after genotoxic stress, indicating that p53 and Δ Np63 have opposing roles (p53 promotes, while Δ Np63 represses NEAT1 expression). This pattern was consistent in both normal and SCC cells. NEAT1 expression was downregulated in primary basal cell carcinoma (BCC), cutaneous SCC, and TCGA datasets, supporting its tumor-suppressive role.

Moreover, NEAT1 depletion increased DNA damage, indicating its importance in genomic stability. HDACI (Givinostat) treatment increased NEAT1 expression in a p53-independent manner. Our findings highlight a critical regulatory axis involving p53, Δ Np63, and NEAT1 in SCCs. p53 promotes NEAT1 expression in response to DNA damage, while Δ Np63 represses it. Disruption of this balance, with frequent TP53 mutations and Δ Np63 overexpression, may impair genomic stability, contributing to SCC tumorigenesis. NEAT1 functions as a tumor suppressor in SCCs by facilitating DNA repair. HDAC inhibition upregulates NEAT1 in a p53-independent manner, providing a potential therapeutic approach for SCCs.

Conclusion

This study establishes NEAT1 as a regulator of the DNA damage response in normal cell and in SCCs. Its down-regulation in SCCs may lead to genomic instability, thereby promoting tumorigenesis. Importantly, targeting NEAT1 enhances the cytotoxic effects of HDACIs, offering a promising therapeutic strategy.

EACR25-1075

The landscape of somatic mutations in Hepatitis B virus-infected livers and its role in the origins of liver carcinogenesis

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Introduction

More than 3.5% of the global population lives with chronic hepatitis B virus (HBV) infection. Over time, 15 to 40% of these individuals will develop cirrhosis, hepatocellular carcinoma (HCC), or liver failure, leading to ~800,000 deaths annually. In recent years, numerous initiatives have focused on studying cancer genomes and timing analyses have shown that many cancer-driving mutations arise years before diagnosis. As a result, research has increasingly shifted toward investigating genomic alterations in normal tissues. In the liver, the mutations that drive the early clonal expansions are still unknown. Additionally, although HBV insertions have been detected in HCC, the precise role in tumour initiation and progression is not yet understood. Here, we carried out a comprehensive analysis of somatic mutation in HBV-infected cirrhotic liver with unprecedented resolution, using whole-genome sequencing, cyto-histological techniques, and cytogenetics.

Material and method

We performed low-input DNA sequencing from >100 laser capture microdissections from different HBV-infected livers. We then performed computation analyses to call different types of somatic variation, to identify different clones, putative drivers of clonal expansion, identify mutational signatures, and reconstruct clonal evolution in regenerative nodules.

Result and discussion

We find that while microdissections from different nodules share very few somatic variants, distinct clones can be detected within individual nodules, indicating that clonal expansions are restricted to the nodular area. Notably, some clones carry variants affecting genes involved in cell proliferation and apoptosis. HBV-infected livers exhibit a patchy pattern, with nodules that either strongly express the HBV surface antigen (HBsAg) or appear negative. Interestingly, we identified cases where antigen-positive nodules accumulate distinct mutational signatures compared to antigen-negative nodules. This suggests that, the mutational processes active in HBV-positive nodules differ from those in HBV-negative nodules. Furthermore, while human hepatocytes are typically tetraploid, we observe that

positive nodules can be either tetraploid or octaploid, suggesting that viral infection may drive this transformation. The analysis of DNA insertions identified clonal HBV integrations and rearrangements, confirming that it represents an early event in cancer development. Finally, we found a positive association between clonal integrations and higher HBsAg levels, suggesting HBsAg expression from integrated HBV copies.

Conclusion

Clonal expansions in HBV-infected cirrhotic livers are restricted to regenerative nodules and often involve mutations in key oncogenic pathways. Infection promotes specific signatures in HBsAg-positive versus HBsAg-negative nodules, illuminating new biological insights into the mutational mechanisms of early liver carcinogenesis.

EACR25-1440

PD-L1 Expression by Macrophages and Myeloid-Derived Suppressor Cells in the Tumor Microenvironment of Epstein-Barr Virus-Associated Pediatric Hodgkin Lymphoma

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Introduction

In Argentina, EBV is associated with more than 70% of Hodgkin lymphoma (HL) cases in children under 10 years. PD-L1, a ligand expressed by HRS and micro-environment cells, plays a key role in promoting protumoral conditions immune escape. Our group described PD-L1 overexpression in the microenvironment of EBV+ HL in children, but the specific cell types involved remain unclear. PD-L1 expression by macrophages and Myeloid-derived suppressor cells (MDSCs) were described in adult HL at the tumor microenvironment, to contribute to immunosuppression. Our aim was to investigate whether MDSCs and macrophages express PD-L1 in pediatric EBV+ HL and compare its expression in EBV-infected tonsils.

Material and method

This study included 22 cHL and 36 tonsil formalin-fixed paraffin-embedded (FFPE) biopsies from pediatric patients. EBER in situ hybridization (ISH) was performed to assess EBV presence. Expression of CD33 (MDSC marker), CD68, and CD163 (macrophage markers), as well as PD-L1 expression, were analyzed by immunohistochemistry (IHC), and results were expressed as positive cells/mm².

Result and discussion

Thirteen cHL patients were EBV- and nine were EBV+. PD-L1+ cells were significantly higher in EBV+ patients ($p=0.0009$). No significant differences were found for CD33+, CD68+, or CD163+ cells, nor for CD33+/PD-

L1+, CD68+/PD-L1+, or CD163+/PD-L1+ between EBV- and EBV+ patients ($p > 0.05$). For tonsil analysis, 11 primary infected (PI), 10 carriers (C), 10 reactivated (R), and 4 non-infected (NI) patients were compared. CD33+/PD-L1+ expression was significantly higher in C compared to PI, R, and NI ($p = 0.0005$, $p = 0.0164$, $p = 0.0039$). No significant differences were found for CD68+ or CD68+/PD-L1+ between groups ($p > 0.05$). Similarly, no significant differences were observed for CD163+ or CD163+/PD-L1+ ($p > 0.05$, Mann Whitney test). Significant correlations were found between CD68+/PD-L1+ and PD-L1+ ($p < 0.0001$, $r = 0.5870$) and PD-L1+/CD163+ and PD-L1+ ($p < 0.0001$, $r = 0.9292$) in EBV+ tonsils. A significant difference in PD-L1 and PD-L1/CD163 expression was observed between primary infected and reactivated groups ($p = 0.0274$, $p = 0.0131$).

Conclusion

This study provides evidence that MDSCs and CD68+ and CD163+ macrophages may not be directly related to the overexpression of PD-L1 that characterizes the tumor microenvironment in HL. Remarkably, in children with persistent EBV infection, PD-L1 expression may be upregulated in MDSCs, suggesting a mechanism for immune response regulation.

EACR25-1547

Notch signaling during G2 Damage Checkpoint: a multifaced node to overcome drug resistance in T-cell acute lymphoblastic leukemia (T-ALL)

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Introduction

Drug resistance remains an important challenge in cancer treatment, and understanding the molecular mechanisms behind it is crucial. Among the many players, Notch signaling has emerged as a key regulator, not only in cancer progression but also in the development of resistance to conventional therapies. Its multifaceted role in both promoting and sustaining tumorigenesis makes it a critical target for oncologic intervention.

Material and method

In our previous work, we uncovered a crucial interaction between Notch1 and PLK1 kinase during the G2/M transition in squamous cell carcinoma. This interaction is vital for the DNA damage response, with PLK1 inhibition preserving Notch1 expression. To extend these findings, we explored T-cell acute lymphoblastic leukemia (T-ALL), an aggressive malignancy where Notch1 serves an oncogenic role. Using human leukemic T-cell lymphoblasts as an in vitro model, we investigated the dynamic regulation of Notch1 signaling in response to DNA damage.

Result and discussion

Our results suggest a complex network in cancer cells, where Notch1 signaling is both upregulated and downregulated during the DNA damage-induced G2 checkpoint. Notably, in T-ALL cells, we observed that Notch1 expression is sustained, allowing some cells to survive checkpoint-mediated arrest and evade apoptosis.

Remarkably, we found that while there is variability in the intrinsic ability of T-ALL cells to exhibit resistance to Notch1 inhibition under basal conditions, exposure to DNA damage triggers a fascinating adaptation at the G2 checkpoint, where Notch1 plays a crucial role in promoting resistance to chemotherapeutic agents, regardless of the cells' initial sensitivity to Notch1 inhibition.

Conclusion

These findings highlight a critical temporal window during which Notch1 signaling plays a pivotal role in the acquisition of therapy resistance, offering new opportunities to identify strategies to overcome Notch1 inhibitor resistance.

EACR25-1580

Alterations in laminin alpha2-chain impact melanoma progression

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Introduction

Extracellular matrix (ECM) remodeling has been described to directly impact the modulation of cancer hallmarks, particularly through the regulation of epithelial-mesenchymal transition (EMT), promoting metastatic spread. Laminins are ECM components, more specifically are part of the basement membrane, a specialized type of ECM that links cell membrane to ECM and promotes cell-ECM communication. Previous studies have shown that laminin remodeling alters cancer cell proliferation and migration, facilitating metastasis. However, whether changes in gene expression or mutations in laminins are per se key steps during cancer progression, remains largely unexplored.

Material and method

This study involved a comprehensive approach, including in silico analysis, the examination of patient samples, and in vitro experiments, to better understand the role of LAMA2 in melanoma progression.

Result and discussion

Considering that the ECM is an important component of the skin, we analyzed the frequency of genomic alterations in the five laminin alpha-chains(LAMA1 to 5) in melanoma samples, using cBioportal platform. Results showed that LAMA2 gene, encoding laminin alpha2-chain, crucial for the connection of the ECM to cell cytoskeleton, has the highest percentage of alterations in cutaneous melanoma. Also, within all types of cancer reported in cBioportal, cutaneous melanoma is the one where LAMA2 is most frequently altered. Moreover, our immunohistochemistry analysis of patient samples showed that laminin alpha2-chain levels were significantly lower in melanoma when compared to

melanocytic nevus. Similar results were obtained for collagen type IV, another basement membrane component, collectively suggesting a disruption of this structure in melanoma. To understand the impact of LAMA2-deficiency at cell level, we established LAMA2-knockout (KO) melanoma cells and explored proliferation, cell death, migration and DNA damage. Results showed that LAMA2-KO cells have reduced cell proliferation and migration and increased cell death and DNA damage. To better mimic the *in vivo* environment, melanoma WT and LAMA2-KO spheroids were also established to explore alterations in ECM proteins, EMT markers, proliferation and DNA damage. The absence of LAMA2 also prevented the detection of collagen IV, pointing towards the lack of basement membrane in these spheroids. To gain further insights into how laminin alpha2-chain impacts tumor progression, LAMA2 overexpressing cell lines were established and cellular processes were validated. Results show a significant recovery in DNA damage compared to LAMA2-KO cell lines, further confirming the importance of maintaining laminin alpha2-chain to prevent DNA damage.

Conclusion

This study will contribute to understand the role of the ECM in melanoma, particularly how LAMA2-deficiency may affect ECM structure resulting in cellular changes that may contribute for tumorigenesis.

EACR25-1614

Weaponizing the CRISPR/Cas9 technology

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Introduction

Most of current therapies against cancer can cause non-specific toxicity on healthy tissues because of their impact on important cellular mechanisms shared, to different extents, between cancer and healthy cells. For this reason, there is an urgent need for more specific and more effective therapies. To address this challenge, we developed a potentially therapeutic strategy to induce sequence-specific DNA double-strand breaks (DSBs) with the CRISPR/Cas9 technology. This approach can selectively target genomic sequences present only in cells to be eliminated. Possible applications include cancer cells harbouring genomic mutations or chromosomal rearrangements. Importantly, the effectiveness of this approach is independent of whether the aberrant genome sequences are expressed or not.

Material and method

As a proof of principle, an isogenic pair of cell lines with a healthy and a diseased counterpart was generated in two distinct cell systems. HeLa and RKO cells were infected with a lentiviral vector containing a promoterless Green Fluorescent Protein (GFP) construct to simulate genomic alterations present in cancer cells. The Cas9 endonuclease with the RNA guide targeting the integrated GFP sequences was retrovirally transduced in these cells. As a negative control, a Cas9 loaded with a

scramble guide that does not recognize any sequence in the human genome was used.

Result and discussion

DSBs generated by Cas9 within the GFP sequence reduce cell viability and increase mortality. This is associated with the activation of the DNA damage response (DDR), increased cell size, multinucleation, cGAS-positive micronuclei accumulation and the activation of an inflammatory response. However, since cells can survive to DNA damage insults thanks to DNA repair mechanisms, we investigated the combined effects of sequence-specific DSBs generation and DNA repair inhibition on cells viability. Impairment of the non-homologous end-joining (NHEJ) repair pathway – achieved by pharmacologically inhibiting the DNA-dependent protein kinase (DNA-PK) – further kill target cells when combined with Cas9-induced DSBs.

Conclusion

These results suggest the possibility to “weaponize” the CRISPR/Cas9 system for the elimination of cells with an aberrant genome.

EACR25-1647

The Role of YWHAG Silencing in Reducing Proliferation and Migration in Glioblastoma

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Introduction

Glioblastoma (GB) is the most undifferentiated and aggressive central nervous system tumor, and its pathogenic mechanisms remain poorly understood. In our previous study, YWHAG gene overexpression was postulated as biomarker of bad prognosis using human GB tissue samples. YWHAG encodes for the γ member of the 14-3-3 family of intracellular dimeric phosphoserine-binding proteins that regulate signal transduction, cell cycle, metabolic cascades and antagonize apoptotic cell death in response to a triggering stimuli. This work aims to elucidate the mechanisms through YWHAG impacts in GB prognosis, to assess its potentiality as a therapeutic target.

Material and method

YWHAG gene expression was silenced by siRNA in two IDH-wt GB cell lines, U251MG and LN229. YWHAG-silencing level was determined by western blot (WB) and RTqPCR. Cell proliferation and two-dimensional cell migration was measured by trypan blue exclusion and wound-healing assays respectively. Based on these findings, YWHAG expression was further silenced by a doxycycline-induced shRNA with an RFP reporter in U251MG. Transfection efficiency was evaluated by fluorescence using a LEICA DMI6000B microscope and LAS X software. YWHAG-silencing level was

determined by RTqPCR. All experiments were performed at different time-points. Results were obtained by comparing non-target cells versus siRNA or shRNA - transfected cells in at least three independent experiments. Data was analyzed using GraphPad Prism 8 software.

Result and discussion

In U251MG cells, the highest siRNA-YWHAG silencing rates were obtained at 72 h by RTqPCR (93.8%) and 96 h by WB (63.7%), and at 96 h by RTqPCR (84.3%) and WB (37%) in LN229. A statistically significant reduction of proliferation rate of U251MG siRNA-YWHAG was observed at 96 h after transfection (p -value = 0.0079). A trend to a reduction in cell migration was also observed at 96 h after transfection. Regarding LN229, despite the reduction of cell migration 120 h after transfection (p -value = 0.0001), no effect in proliferation was observed. Finally, U251MG cell line was selected for the establishment of the stable and inducible sh-YWHAG-silencing model. Suitable transfection rate by fluorescence signal was observed at 48 h after doxycycline treatment, with a promising 78% of gene silencing at RNA level in the sh-YWHAG cells.

Conclusion

YWHAG gene silencing causes a reduction in cell proliferation and migration in GB in-vitro models. To be deeply explored, a stable YWHAG inducible knocking-down model has been generated to confirm these results in preclinical advanced models, hence elucidating its potential role as biomarker and putative therapeutic target in GB.

EACR25-1773

Partial inactivation of VPS34 combined with oncogenic KRAS accelerates pancreatic cancer initiation and mimics early metastasis dissemination

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Introduction

Pancreatic ductal adenocarcinoma (PDAC) is a lethal disease with a poor prognosis. To improve the diagnosis and management of this disease, we need to understand the effectors responsible for the development of these tumours. The importance of the KRAS mutation (95% of cases), in cancer initiation is well known, but not sufficient to explain cancer development. Autophagy is a mechanism for degrading and recycling cellular components via lysosomes. Autophagy machinery proteins participate in pancreatic homeostasis and cancer progression. However, the role of VPS34, a PI3K that promotes autophagic flux, is unknown. We therefore investigated the role of VPS34 in pancreatic cancer development.

Material and method

To mimic low VPS34 protein activity found in patients with poorer prognosis, we developed a mouse model combining KRAS mutation with heterozygous inactivation of VPS34 (KCV34), using Cre recombinase

under the control of the Pdx1 promoter. KCV34 mice histological phenotype was quantified and compared to that of KRAS mutant mice (KC). We compared a panel of circulating markers present in the mice's plasma using Olink® technology. Cell lines obtained from a mouse in each of the KC and KCV34 groups were used to explain these histological analyses by studying their genome expression, autophagy flux and invasive capacities.

Result and discussion

We have observed a decrease in VPS34 levels in human precancerous lesion cells. An partial inactivation of VPS34 in murine model KCV34 showed a significantly decreased prognosis compared to KC mice. Histological analysis of HE staining revealed earlier and more aggressive lesions than in the KC cohort. We observed KCV34 tumours in mice of 4 to 6 months of age, some associated with metastases, whereas KC mice showed only few high-grade precancerous lesions at these ages. Analyses on derived cell lines showed a reduced autophagy flux in KCV34 compared with KC cells. However, migratory and invasive capacities did not appear to be increased. Acceleration of the tumorigenesis could be partly the results of a change in inflammatory context observed with VPS34 partial inactivation. Plasma of KCV34 mice showed decrease in IL23r level implicated in Th17 lymphocytes recruitment. Further analysis to understand the cellular mechanisms underlying this phenotype is required to fully understand the involvement of VPS34 in pancreatic carcinogenesis.

Conclusion

VPS34 partial inactivation induce a reduced autophagy flux that leads to an acceleration of pancreas carcinogenesis. This knowledge will help to decipher novel means of early diagnostic and treatment.

EACR25-1790

Development of an ex vivo model to determine early transcriptional changes in breast cancer

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Introduction

The early transcriptional events pre-disposing the mammary gland to transformation are poorly characterised. Mammographic density is an established risk factor for breast cancer and is associated with increased stiffness of the periductal stroma. However, the precise molecular mechanisms remain unknown. A major reason for this is the deficiency of physiologically relevant models of healthy tissue to study early transformation events.

Material and method

Intact mammary ducts were isolated from mice at 10-12 weeks of age and seeded ex vivo into 3D reconstituted basement membrane gels at different stiffnesses. Different methods were tested to induce oncogene activation and RNA sequencing was used to investigate mechanosensitive changes in signalling pathways downstream of oncogenic insults.

Result and discussion

Mammary ducts with luminal-basal polarity and an intact basement membrane can be isolated and co-cultured ex vivo with endothelial cell populations in a 3D mechanically tuneable system. Oncogenic Her2 over-expression can be induced in ducts using a doxycycline inducible promoter and protein and RNA can be extracted from these structures to examine immediate downstream signalling changes.

Conclusion

This model provides a novel approach to studying early events pre-disposing healthy tissue to tumourigenesis. By identifying transcriptional changes driving predisposition of the mammary duct to breast cancer, we can better characterise the events which are crucial for improvement of early detection and preventative therapies.

EACR25-1793

Effect of exposures to cancer risk factors on the clonal structure of normal kidney tubules

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Introduction

Cancer appears when mutated cells with a proliferative advantage expand, invading the surrounding tissue and forming a tumor. However, several studies have shown that potential cancer driver mutations are present in healthy tissues, implying that these mutations alone are not sufficient for tumor formation. Recent evidence also shows that many carcinogens are not mutagenic. These observations lead to a reconsideration of cancer as a multistage process, in which pre-existing mutated clones acquire a growth advantage upon the exposure to a promoter. Understanding the differences in the clonal architecture of normal tissues from donors with different lifelong exposures to cancer risk factors is key to understanding their potential role as tumor promoters and their relation to cancer risk. For clear cell renal cell carcinoma (ccRCC), with widely varying incidence worldwide, sex, hypertension and obesity are amongst the highest risk factors and none of them is known to be mutagenic.

Material and method

Duplex sequencing allows for the detection of somatic mutations present in very few cells in a tissue. We used this technology to sequence DNA from normal kidneys of 40 donors who suffered ccRCC, to a depth of 25,000x targeting 9 ccRCC driver genes. Furthermore, we sequenced DNA samples from tissue obtained via laser capture microdissection of normal kidney tubules – as the origin of the disease resides in the proximal tubules – from 90 different donors who suffered from ccRCC, targeting 192 genes to a depth of 2,000x. Samples were

obtained from the Mutographs biorepository, with rich clinical data for all donors. Cases from 8 different countries with varying incidence were selected considering relevant risk factors and using age and sex to balance groups. We developed two end-to-end pipelines that facilitated the mutation calling from duplex sequencing data, and then computed metrics to characterize the clonal structure of the normal kidney epithelial tissue.

Result and discussion

We detected a consistent increased presence of STAG2 mutant cells across all samples. Other genes such as CDKN1A or PTEN are positively selected but only in specific clinical groups, suggesting the presence of interindividual variability that we are currently investigating. The overall size of clones driven by mutations in these and other genes appear smaller than in other tissues. Interestingly, our analysis provides evidence that mutations in some genes may be under negative selection in normal kidney tubules.

Conclusion

This is the first characterization of the clonal landscape in normal kidney tubules. We identify some genes that when mutated provide a selective advantage in this epithelium. The degree of interindividual variability related to differences in certain risk factors could reveal how these factors tune the selection constraints favouring some mutated clones versus others. This would be the first step towards investigating how these affect cancer initiation.

EACR25-1796

A synergistic effect of RNF43 and BRAF mutations on colorectal carcinogenesis

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Introduction

Up to 30 percent of all colorectal cancer (CRC) cases develop from the serrated pathway. In this subtype of tumors, an association between mutations of the tumor suppressor RNF43 and mutations of the oncogene BRAF has been reported. In addition, recent studies uncovered RNF43 mutations as a predictive marker for better treatment response and survival outcomes in patients harboring the BRAF-V600E mutation and receiving anti-EGFR/BRAF therapy. However, the underlying molecular mechanisms explaining how RNF43 mutations may affect development and response of the mCRC BRAF-V600E patients have not yet been understood.

Material and method

We generated a novel mouse model by crossing Rnf43-ΔEx8 to Braf-V637E mice. These mice were monitored over time and sacrificed at specified time points with subsequent macroscopic examination, histopathological evaluation and immunohistochemistry staining.

Result and discussion

Rnf43-ΔEx8/Braf-V637E mice showed a more severe phenotype and increased tumor burden than single mutated Rnf43-ΔEx8 and Braf-V637E mice. The prominent tumorigenic phenotype of the double mutant mice was already macroscopically apparent after six weeks. Pathological evaluation of old mice confirmed a higher proportion of the Rnf43-ΔEx8/Braf-V637E mice presenting pathological alterations characteristic of serrated tumors as well as adenomas compared to the single mutated mice.

Conclusion

These results support a synergistic effect of RNF43 and BRAF mutations on CRC tumorigenesis.

EACR25-2011

VPS34-driven autophagy permits cell plasticity and cancer initiation

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Introduction

Autophagy, a self-digestive process, is a protective mechanism for tissue homeostasis. However, its importance during cell plasticity is not fully understood. Numerous human diseases are caused by epithelial cell plasticity, and animal models of pancreatic diseases are recognised as study models. Therefore, we developed a unique model in which VPS34, the highly conserved class III PI3K that promotes the autophagic flux, was inactivated in pancreatic exocrine epithelial acinar cells.

Material and method

Models that reconstitute early pancreatic cancer are recognised models to study cell plasticity. We hence generated a murine model of inducible VPS34 inactivation specifically in pancreatic acinar cells (ElasCreER/VPS34flx/flx), combined or not with KRAS mutation (KC/VPS34flx/flx). Phenotypic, histopathologic and single cell RNA sequencing analysis (scRNAseq) were realized. We used lentiviral vectors to overexpress VPS34 and demonstrate causative roles of VPS34.

Results were validated in Human samples.

Result and discussion

VPS34 inactivation (ElasCreER/VPS34flx/flx) induced a heterogeneous increase of lipids and markers of autophagy in the pancreas compared to control mice. The scRNAseq highlighted that VPS34 inhibition triggered the loss of an acinar subpopulation with a high mitochondrial activity for the benefit of 3 subpopulations expressing REG mRNAs, linked with pancreas regeneration. These cells had an altered expression of genes related to canonical autophagy and expressed embryonic markers. Those populations of cells were identified in Human pancreas associated with decreased

autophagy gene expression. VPS34-inactivated cells presented reduced protein levels of REG3A, due to an increased lysosomal activity. An overexpression of VPS34 was responsible for an increase of REG3A level and of AKT phosphorylation. A pharmacological activator of PI3K increased REG3A levels. Reversely, the PI3Kα pharmacological inhibition reduced the level of REG3A. The total inactivation of VPS34 in the pancreas protected from cell plasticity induced by inflammation and/or oncogenic KRAS alterations (KC/VPS34flx/flx).

Conclusion

VPS34 is a key protein for pancreatic cell plasticity, through positive control of autophagy, activation of class I PI3K and reduced selective degradation of REG3A by lysosome, which can be prevented solely with PI3Kα inactivation. Our unexpected findings transform the once unattainable goal of preventing environmentally-induced cancer initiation into a tangible possibility, paving the way for new protective strategies.

EACR25-2015

Use of the novel cell division assay (CDA) to evaluate T cell toxicity in response to lutetium-177-DOTATATE peptide receptor radionuclide therapy

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Introduction

The treatment with lutetium-177-DOTATATE (¹⁷⁷Lu-DOTATATE) improves survival in patients with metastatic neuroendocrine tumors but is limited by bone marrow toxicity. Individualized dose planning is challenging due to uncertainty in absorbed dose estimates and patient sensitivity to radiation. Here, we investigated the in vitro sensitivity of patient T cells to ¹⁷⁷Lu-DOTATATE as a tool in individualized dose optimization.

Material and method

Blood samples were collected from healthy individuals. Peripheral blood mononuclear cells were prepared and treated with different concentrations of ¹⁷⁷Lu-DOTATATE, corresponding to specific absorbed doses. Cells were also exposed to external beam radiation as a comparison. Cell survival and DNA damage was then analyzed using our in-house developed CDA and γH2AX assays.

Result and discussion

Our data indicate a dose dependent, but non-linear in vitro T cell sensitivity to ¹⁷⁷Lu-DOTATATE at treatment relevant doses. We detected an unexpected

hypersensitivity at very low (0.15–0.25) radiation doses. The ¹⁷⁷Lu-DOTATATE individual cell sensitivity correlated with EBR sensitivity. However, the kinetic of ¹⁷⁷Lu-DOTATATE double-strand break induction in T cells was different to EBR as indicated by γ H2AX staining.

Conclusion

The CDA could measure the in vitro sensitivity of T cells to ¹⁷⁷Lu-DOTATATE and may be a useful tool for evaluating individualized hematological toxicity.

EACR25-2133

Genomic Landscape of Skin and Internal Cancers in Xeroderma Pigmentosum Patients Reveals Signatures of Nucleotide Excision Repair Deficiency

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Introduction

Xeroderma Pigmentosum (XP) is a rare autosomal recessive disorder characterized by defects in Nucleotide Excision Repair (NER) or translesion synthesis. XP patients have an extremely high risk of skin cancer (1,000-fold increase) and an elevated incidence of internal tumors, including leukemia and gynecological and brain cancers (34-fold increase). However, the somatic mutagenesis mechanisms underlying this cancer predisposition remain incompletely understood from a genomic perspective.

Material and method

We assembled a unique cohort of skin tumors ($n = 39$) from five XP subgroups with the highest cancer susceptibility (XP-A, XP-C, XP-D, XP-E, XP-V) and internal cancers ($n = 23$) from XP-C patients, including leukemia, ovarian, pancreatic, thyroid tumors, and others. Whole-genome sequencing and detailed bioinformatic analyses were performed to characterize their mutational landscapes in comparison with sporadic cancers. In vitro (XP-C, XP-V) and in vivo (XP-C) mutation accumulation models were developed to investigate mechanisms mutagenesis in XP.

Result and discussion

An ultra-mutated tumor phenotype was observed in skin tumors with impaired GG-NER (XP-E: 350 mut/Mb, XP-C: 162 mut/Mb) and TLS (XP-V: 248 mut/Mb).

Mutational profiles of XP skin tumors with NER defects were dominated by C>T mutations, with each XP group exhibiting distinct trinucleotide context features. XP-A and XP-D tumors, with combined GG-NER and TC-NER defects, showed a uniform mutation distribution across chromatin compartments, indicating that NER modulates genome-wide mutation rate heterogeneity. XP-V skin tumors, characterized by TLS polymerase eta deficiency,

exhibited a unique mutational profile with a high frequency of TG>TT mutations (28%), absent in sporadic skin cancers. These mutations likely arise from error-prone bypass of rare photolesions in TpA and TpG contexts in the absence of polymerase eta. In internal tumors, XP-C cancers displayed a 21-fold increase in mutation rates compared to sporadic, tissue-matched counterparts. The mutational signature of XP-C internal tumors was consistent across different cancer types but markedly distinct from sporadic cancers. Mutations showed strong transcriptional bias, suggesting an association with unidentified endogenous bulky purine DNA lesions. To validate our findings, we sequenced hematopoietic stem cells from XPC knockout (KO) mice and observed a significant age-dependent increase in mutagenesis. The mutational signature in XPC KO mice resembled that of XP-C internal cancers, reinforcing our hypothesis.

Conclusion

The increased cancer susceptibility in XP patients is driven by an excess of unrepaired DNA lesions on the untranscribed strand, defective translesion synthesis, and impaired repair of endogenous oxidative DNA damage. These factors contribute to the elevated mutation burden and distinct mutational landscapes observed in both skin and internal tumors of XP patients.

EACR25-2276

Investigating the carcinogenic and epigenetic modulating effects of per- and poly-fluoroalkyl substances (PFAS) on breast cancer development

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Introduction

In the UK, there are 56,000 new cases of breast cancer annually. Genetics and lifestyle choices contribute to BC risk, however, there is a concern surrounding environmental toxins, particularly microplastics and per- and poly-fluoroalkyl substances (PFASs), known as “forever chemicals”. Microplastics and PFASs are heavily used in industrial practices and consumer products and are ubiquitous in the environment. PFASs have been detected in human blood and breast milk, while microplastics have been detected in lung and whole vein tissue, suggesting they may be able to cross the blood barrier into other tissues, such as the breast. Microplastics are known to adsorb and leach PFASs, thereby being a potential route of PFAS exposure in humans.

Material and method

Non-transformed breast epithelial cells (MCF-10A) were exposed to physiologically relevant and supra-physiological concentrations of perfluorooctanoic acid (PFOA) and perfluorooctanesulfonic acid (PFOS) for both acute (48 hours) and long-term exposure (30 cell passages). Analysis included investigating changes in cell transformation phenotypes such as cell proliferation, mammosphere formation, colony formation and cellular

metabolism. Further analysis through RNA sequencing, histone modifications and chromatin accessibility will be undertaken to further understand the mechanistic consequences of PFAS exposure to breast cells and whether they can induce breast cell transformation.

Result and discussion

Short-term exposure to 1 μM–100 μM PFOA and 0.0001 μM–1 μM PFOS increased non-cancerous breast cell growth, with a potential dose-response relationship effect on cell proliferation. At lower concentrations (10 μM PFOA and 0.0001 μM PFOS) minimal effects on cell number were observed however, when combined, there was a noticeable increase in cell number. In contrast, higher concentrations (100 μM PFOA and 1 μM PFOS) showed the highest increase in cell number individually but also induced cell death when combined. Long-term exposure of physiologically relevant concentrations (10 nM PFOA, 20 nM PFOS and a combination of both) resulted in an increase in cell number, with the highest increase being observed in the combination condition. Short- and long-term exposure to PFAS altered cellular metabolism causing a reduction in oxygen consumption suggesting mitochondrial damage or stress. Long-term exposure disrupted mammosphere formation, further indicating potential transformation of breast epithelial cells.

Conclusion

The results have shown that short- and long-term exposure to PFAS has affected the growth and transformation of breast epithelial cells, with long-term exposure of physiologically relevant concentrations mirroring more realistic human exposure conditions. Further analysis of epigenetic changes will aim to identify mechanisms causing phenotypic changes and therefore, potentially, how PFAS exposure may contribute to breast cancer development.

EACR25-2282

Obesity-Derived Circulating Extracellular Vesicles Promote Tumor-Stimulatory Functions of Stromal Fibroblasts in Breast Cancer

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Introduction

Obesity significantly impacts incidence, prognosis, and treatment response of breast cancer (BC). It induces systemic and local alterations that influence the tumor microenvironment, with a particular effect on stromal fibroblasts. This complex interaction involves adipokines, growth factors, and extracellular vesicles (EVs), which play a crucial role in cell-to-cell communication. Despite their emerging importance, the involvement of obesity-derived EVs in modulating the tumor-promoting properties of stromal fibroblasts remains unexplored.

Material and method

Circulating EVs were isolated from the serum of normal weight (NW) and overweight/obese (OW/Ob) healthy women and characterized in accordance to MISEV2023 guidelines. Immortalised human mammary control fibroblasts (CFs), their cancer-associated counterparts (exp-CAFs), and human CAFs, isolated from biopsies of primary BC, were used as experimental models for stromal fibroblasts. Human MCF-7 and T47D BC cells were employed as experimental BC models in co-culture experiments.

Result and discussion

Treatment with OW/Ob-EVs induced neoplastic traits of CFs in terms of cell motility, invasiveness, contractility along with an up-regulation of CAF-like markers compared to the NW-EV counterpart. Moreover, OW/Ob-EVs sustain the tumor-promoting functions of exp-CAFs and hCAFs. Co-culture assays revealed that conditioned medium (CM) from OW/Ob-EV-treated CFs, exp-CAFs or hCAFs enhanced anchorage-independent growth and migration of BC cells. Cytokine profiling indicated that OW/Ob-EV treatment triggered Fibroblast Growth Factor 19 (FGF19) secretion by CFs, a finding further validated by ELISA and qRT-PCR. Both pharmacological and genetic (lentiviral) inhibition of FGF19 signaling counteracted the pro-tumorigenic effects of OW/Ob-EVs on fibroblasts. We previously showed that serum-derived EVs exhibited BMI-dependent miRNA profiles, with let-7a being significantly downregulated in OW/Ob patients and inversely correlated with BMI. Notably, miRWalk analysis identified FGF19 as a let-7a target. A BMI-dependent decline in let-7a levels within EVs was also confirmed in our OW/Ob cohort. Treatment of CFs and hCAFs with OW/Ob-EVs led to a significant reduction in let-7a expression compared to NW-EVs, and functional assays confirmed the regulatory role of let-7a on FGF19 translation. Accordingly, let-7a inhibitor transfection – mimicking OW/Ob-EV cargo – enhanced FGF19 and CAF-like gene expression, as well as NF migration, contractility, and tumor/stroma crosstalk. Conversely, let-7a mimic transfection decreased FGF19 secretion, counteracting the aggressive phenotype of hCAFs.

Conclusion

Our findings offer new insights into the impact of obesity-derived EVs on tumor-stroma interactions and BC progression, emphasizing the let-7a/FGF19 axis as a promising therapeutic target for personalized treatment strategies.

EACR25-2384

Sex differences in selection of somatic mutations in normal human bladder

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Introduction

Bladder cancer has an unexplained three to four times higher incidence among males than females. Lawson et al. (Science, 2020) showed that, as in other normal tissues, the human bladder undergoes constant clonal evolution by the acquisition and selection of mutations. Although most clonal expansions do not result in cancer development, some of them constitute the first step towards malignant tumors. Factors known to increase the risk of bladder cancer, such as sex, could be playing a role shaping the clonal landscape of the normal bladder and promoting the evolution of protumorigenic clones. Nevertheless, it is not clear whether they also influence the clonal landscape of the normal urothelium. In this study, we characterize the clonal landscape of the normal urothelium of a group of individuals using ultradeep sequencing to unravel potential differences associated with sex and other bladder cancer risk factors.

Material and method

We obtained brushes of cells from ~2cm² of normal urothelium covering the upper part (dome) and lower part (trigone) of the bladder of 42 individuals at autopsy (73 samples in total). We performed bulk DNA error-correcting duplex-sequencing at a depth of ~7000X on a panel of 15 protein coding genes, known to be under positive selection in normal urothelium and/or relevant drivers of bladder cancer, and the TERT promoter. We used several complementary methods to detect signals of positive selection (e.g.: functional impact bias and dN/dS) in the identified mutations and conducted an association study to investigate the link between measures of clonal selection and common bladder cancer risk factors.

Result and discussion

We identified hundreds to thousands of mutations in each sample (~70000 mutations in total), many more than those identified in all sequenced bladder cancers in over a decade. We demonstrate that ultradeep sequencing of normal tissues provides a pathway for natural saturation mutagenesis in genes under positive selection. This allows the calculation of the magnitude and mode of selection at the gene level and at sub-genic structures, such as exons or domains. While the magnitude of positive selection in the urothelium at the dome and the trigone of the same individual is very similar, it is highly heterogeneous across individuals. We found that the magnitude of positive selection in the urothelium increases with age, and that there is significantly stronger positive selection of truncating mutations of RBM10, ARID1A, STAG2 and CDKN1A in males than in females.

Conclusion

The normal human bladder shows pervasive positive selection and differences in its clonal landscape between males and females, and with age. Ultradeep sequencing provides a path towards natural saturation mutagenesis.

EACR25-2391

Secretome profiling of TNBC cells under an inflammatory condition enhances tumorigenic properties in non-tumoral breast epithelial cells

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Introduction

Triple-negative breast cancer (TNBC) is the most aggressive subtype of breast cancer, characterized by poor prognosis and high recurrence rates. Inflammation is known to play a key role in tumorigenesis. Therefore, this study aimed to identify cytokines in the secretome of breast cancer cells exposed to an inflammatory environment and to evaluate their effects on the malignant transformation of non-tumoral breast epithelial cells.

Material and method

TNBC MDA-MB-231 cells were pretreated for 48 hours with an inflammatory cocktail (17 β -estradiol (10 nM), TNF α (10 ng/ml), and IL-6 (50 ng/ml), called ETI) and subsequently starved in media containing 0.5% FBS for additional 48 hours. Conditioned media (CM) were collected, and secreted proteins were precipitated by cold acetone. The analysis of secretome was performed by LC-MS/MS and validated using a Proteome Profiler Human Cytokine Array (R&D Systems). The effect of the CM on cell proliferation and mammosphere forming efficiency (MFE) of non-malignant MCF10A cells was evaluated. Additionally, the prognostic value of the identified cytokines in TNBC patients was assessed through in silico studies.

Result and discussion

Notably, exposure of MCF10A cells to CM of ETI-treated cells led to increased cell proliferation and enhanced MFE. Proteomic and cytokine array analyses revealed a significant upregulation of IL-6, ICAM1, and CXCL8 in the CM of ETI-exposed cells compared to CTRL CM. Overrepresentation analysis (ORA) indicated that the overexpressed proteins in ETI secretome were strongly associated with Signaling by interleukins and Extracellular matrix organization pathways. In silico analysis further demonstrated that TNBC patients with higher expression of the identified cytokine signature (IL-6, CXCL8, and ICAM1) had a worse prognosis in terms of relapse-free survival.

Conclusion

Our findings suggest that the secretome of TNBC cells under inflammatory conditions enhances tumorigenic properties and cell proliferation in non-tumoral cells. The identified cytokines may serve as key effector molecules in tumor progression, highlighting their potential as prognostic biomarkers and therapeutic targets in TNBC.

Drug Resistance

EACR25-0003

Acquired resistance mechanisms to KRAS G12C inhibitors in lung cancer

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Introduction

Approximately 20% of patients with lung adenocarcinoma (LuAD) have mutations in KRAS. KRAS alternates from an inactive GDP-bound to active GTP-bound state, activating downstream signaling pathways. This alternation is regulated by guanine nucleotide exchange factors (GEF) as SOS1 and GTPase activating proteins. About 85% of mutations are found at codon 12, and most of them consist of a change from glycine to cysteine (G12C). The first effective covalent G12C inhibitors (i) sotorasib and adagrasib bound to mutant cysteine, trapping KRAS in its inactive GDP-bound state. Sotorasib and adagrasib trials showed promising response rates but short duration of response (11 and 8 months, respectively). In this work we intend to study the possible mechanisms of acquired resistance to these inhibitors and possible strategies to overcome this resistance.

Material and method

We generated sotorasib-resistant (sr) KRAS G12C-mutated lung cancer (LC) cell lines (CLs) from two different commercial CLs (H358 and H2030) and we characterized them, genetically and molecularly, by exome (WES) and RNA sequencing as well as analysis of signaling pathways and KRAS activation status by western-blot and RAS assay test. We tested new drug combinations to revert off-target resistant mechanisms with a SHP2 inhibitor (TNO155) and a MEK inhibitor (trametinib), with and without sotorasib, and analyzed the efficacy by cell viability and colony formation assays. We validated these findings in other commercial LCCLs (H23, SW1573) and in a patient-derived CL from our hospital.

Result and discussion

We found the H2030 CL to be partially intrinsic resistant to sotorasib. Among the H358sr clones we identified KRAS G12C amplification in three clones, a secondary G12V mutation in one clone and AKT activation in two clones, as possible resistance mechanisms to sotorasib. We found that TNO155 couldn't overcome resistance but trametinib did. We also found that some sr clones are addicted to sotorasib. TNO155 and sotorasib showed synergy in the H2030 CL and in the patient derived CL.

Conclusion

On target KRAS mutations as mutant allele amplification and secondary KRAS nonG12C mutations are the most prevalent acquired resistance mechanisms. Upstream and downstream inhibitors as SHP2i and MEKi could be synergic in combination with sotorasib in partially resistant CLs.

EACR25-0051

Novel Topoisomerase I (Topo1) inhibitors with a non-Camptothecin core scaffold to overcome resistance to current Topo1-targeted drugs and ADCs

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Introduction

Camptothecin (CPT) derivatives as DNA topoisomerase I (TOPO-1) are widely used to treat various cancers. However, their clinical efficacy was significantly undermined by both inherent resistance and the rapid development of emerging resistance. Although resistance mechanisms are complicated, some key factors are identified such as TOPO-1 mutations and drug efflux. Unfortunately, all clinical stage CPT-derived TOPO-1 inhibitors shared the original CPT core structure, thus little chance to address resistance via structural diversity. In this study, we aimed to discover a novel class of non-CPT core TOPO-1 inhibitors that specifically address the key enzyme mutations thus overcoming resistance to current TOPO-1 drugs in the clinic.

Material and method

Using a Computer-Assisted Drug Design (CADD) approach, a non-CPT core scaffold was designed to specifically improve binding to the clinically relevant R364H mutant. Molecules from the novel scaffold performed very well in our computational model showing significantly improved hydrogen bonding and polar interactions with both wild type and R364H mutated TOPO-1.

Result and discussion

More importantly, compared to irinotecan these molecules demonstrated 50 to 1000-fold higher potency in patient-derived, irinotecan-resistant tumor organoid models, thus successfully addressed tumor resistance to current TOPO-1 inhibitors. In vivo antitumor activity was also validated in various PD animal models. A PCC molecule was identified and moved into IND-enabling development, which shows significant promise as next-generation therapies for cancers overcoming resistant to traditional TOPO-1 inhibitors. Recent breakthrough discoveries of ADCs attracted more attention to TOPO-1 inhibitors, however most if not all payloads targeting TOPO-1 still utilized the original CPT core (e.g. DXD, SN-38). Therefore, it is anticipated that ADC treatment will lead to new resistance, and our novel molecules with a non-CPT core could address future resistance via structure diversity.

Conclusion

A novel series of TOPO-1 inhibitors was discovered with non-CPT core that addresses two major resistance factors associated with current CPT-based therapies: impaired binding to TOPO-1 mutants and susceptibility to drug efflux. These compounds demonstrated potent *in vitro*, organoids and *in vivo* efficacy against various cancer models. A PCC molecule was identified and moved into IND-enabling development, which shows significant promise as next-generation therapies for cancers overcoming resistant to traditional TOPO-1 inhibitors.

EACR25-0163**Chemotherapeutic Response and Resistance in a 3D MDA-MB 231 Triple-Negative Breast Cancer Spheroid Model***B. Abrahams¹, C. Gouws², M. Sekhoacha³*¹*University of the Free State, Basic Medical Sciences, Bloemfontein, South Africa*²*North-West university, Centre of Excellence for Pharmaceutical Sciences, Potchefstroom, South Africa*³*University of the Free State, Pharmacology, Bloemfontein, South Africa***Introduction**

Triple Negative Breast Cancer (TNBC) is known as an aggressive and fatal subtype of breast cancer. Chemotherapy remains the only available systemic therapeutic treatment option. However, patients often develop resistance, creating a major obstacle for effective cancer therapy. 2D cell culture models are usually used for *in vitro* screening of novel compounds for potential anti-cancer effects; however, these models do not accurately represent the complexity of the tumor microenvironment. 3D models are more mimetic of *in vivo* physiological function and offers higher accuracy in predictive drug responses. Our study elucidates the development of a cisplatin-resistant TNBC 3D spheroid model to assess chemotherapeutic drug response.

Material and method

A single cell suspension of cisplatin-resistant MDA-MB 231 cells were used to construct 3D spheroids using the rotating clinostat-based CelVivo, ClinoStar® bioreactor system. A density of 8x10⁴ cells were seeded in reactors and sorted after 6-days of spheroid induction. Cisplatin-resistant 3D MDA-MB 231 spheroids were characterized over 28 days using the following viability parameters: planimetry, soluble protein content, intracellular adenosine triphosphate (ATP), extracellular adenylate kinase (AK) and glucose uptake. The metabolically stable experimental window of the spheroids was determined, followed by cisplatin and doxorubicin clinical dose treatment over 96-hours, to validate the spheroid model. Cisplatin and doxorubicin doses were calculated using the biomass of the spheroids in each bioreactor daily and the dose adjusted for treatment. Viability and growth were assessed daily over the 96h and compared to untreated spheroids.

Result and discussion

Characterization of the spheroids showed a viable model for 28 days. The metabolically stable period of the spheroids was determined between days 12-22, which indicated the optimal experimental window for the model. The spheroid model demonstrated variable responses to cisplatin and doxorubicin treatment. A clinical dose of cisplatin (4,435 x 10-6 µg/µg protein) had no significant effect on planimetry. However, an increase ($p < 0.05$) in protein (1.81 µg) at 48 h versus 0.39 µg at 24 h was noted. ATP and glucose levels increased between 72-96h indicating metabolic activity. Doxorubicin (1.064 x 10-5 µg/µg protein) however, elicited profound reduction in spheroid viability, marked by decreased planimetry relative to control ($p < 0.0001$). AK levels increased ($p < 0.05$) over the duration of treatment period (24-96 h). This coincided with a

decrease in ATP 24-48 h ($p < 0.05$) and glucose uptake by spheroids, relative to control.

Conclusion

The spheroid model maintained its induced cisplatin resistant phenotype against cisplatin with no significant effect on the viability of the model; however, it was sensitive to a doxorubicin clinical dose. Our model may provide potential benefit as a screening tool for novel drug therapeutics.

EACR25-0208**Three-dimensional (3D) Cancer****Microenvironment that Confers****Chemoresistance to DNA Damaging Drug***L. Zhang¹, C. Du¹, J. Shi¹*¹*Hong Kong Baptist University, Center for Quantitative Systems Biology and Department of Physics, Kowloon, Hong Kong, China***Introduction**

Cancer cell behaviors in tissues/organs critically depend on the three-dimensional (3D) architectures and micro-environments, and cannot be recapitulated by traditional 2D cell lines. The development of 3D cancer spheroid and tumor organoid models have enabled the study of 3D-specific cancer mechanisms using human-derived cells. One important research area where cancer cell behaviors differ significantly in 3D vs. 2D is anti-cancer drug response of solid tumors. Extensive effort has been made using 3D culture tumor models to unravel 3D-specific drug resistance mechanisms that can be targeted to reverse chemoresistance. However, our knowledge of 3D chemoresistance mechanism is still limited.

Material and method

Here we employed 3D cancer spheroid models to investigate 3D-specific mechanisms that underlie chemoresistance to etoposide, a commonly used DNA-damaging chemotherapeutic. By comparing the cellular responses of p53 wild-type human cancer cell lines grown in 2D monolayer versus 3D spheroids following etoposide treatment, we identified a new mechanism of 3D-specific chemoresistance to etoposide that is associated with a global change in kinase signalling, which possibly arises from the significantly higher cell-cell contact and membrane interaction in 3D.

Result and discussion

Specifically, by western blot, flow cytometry and single cell imaging analysis, we found the 3D drug resistance that we observed is unrelated to reduced drug intake by the 3D cancer spheroid cells. Instead, it is attributed to attenuated etoposide-induced p53 signaling in 3D.

Through ensemble kinase profiling of the 3D spheroid models, our data suggest that the spheroid cells exhibited altered activities of signalling kinases compared to 2D cells that led to reduced p53 upregulation. And inhibition of the activity of a number of signalling kinases by gene knockdown or small-molecule inhibitor can effectively enhance 3D cancer spheroid response to etoposide and reverse chemoresistance.

Conclusion

Our study not only provided novel insights into 3D-specific chemoresistance but also revealed new combinatorial targets that could be exploited to enhance

resistant solid tumor response to DNA damaging chemotherapeutic.

EACR25-0209

Overcoming Gemcitabine Resistance in MDA-MB-231 Cells: A Synergistic Approach Using ABT-737 and NVP-BEZ235 Dual Inhibition of PI3K/mTOR

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Introduction

Triple-negative breast cancer (TNBC) is an aggressive subtype that lacks progesterone (PR), estrogen (ER) and human epidermal growth factor receptor 2 (HER2). Gemcitabine (GEM) is a drug commonly used for chemotherapy, but GEM resistance often develops, leading to more severe outcomes and relapse. This highlights the need for new targeted therapies, especially in GEM-resistant TNBC cases. The PI3K/AKT/mTOR pathway and anti-apoptotic Bcl-2 family proteins play crucial roles in cancer cell survival. Targeting both pathways might be an effective therapeutic approach. In this study, we explored the combination of NVP-BEZ235, a dual PI3K/mTOR inhibitor, with ABT-737, a BH3-mimetic Bcl-2 inhibitor, as a strategy to sensitize triple-negative breast cancer (TNBC) cells to gemcitabine (GEM) and overcome resistance.

Material and method

MDA-MB-231 cells were treated with increasing doses of GEM for over 8 months to generate GEM-resistant cells MDA-MB-231GEMR. The resistance factor was calculated, and qPCR assessed the expression of resistant related gene. Parental MDA-MB-231 and MDA-MB-231GEMR cell lines were used to find the cytotoxicity and the IC₅₀ values by Resazurin assay. Cell migration and growth ability were analyzed with Scratch and Colony Formation Assays, respectively. Cell cycle analysis was conducted by flow cytometry, and gene expression of mTOR pathway and apoptotic related genes were analyzed by qPCR.

Result and discussion

MDA-MB-231GEMR cells were 5.06 times more resistant to GEM than parental MDA-MB-231 cells according to fold resistant calculation. qPCR showed significantly reduced expression of hENT1 (0.5 ± 0.1) in MDA-MB-231GEMR cells. The IC₅₀ values for ABT-737 and NVP-BEZ235 were $9.923\mu\text{M}$ and $0.345\mu\text{M}$, respectively. Treatment with the combination of NVP-BEZ235 and ABT-737 significantly inhibited cell growth and migration in MDA-MB-231GEMR cells, while cell cycle analysis revealed a notable SubG0 arrest, with a significant increase compared to the negative controls in resistant cells. Gene expression analysis revealed increased levels of Caspase-3 and decreased level of Bcl-2, and Mcl-1 expression compared to control, indicating the activation of apoptotic mechanisms. Consistent with expectations, the expression of PI3K and mTOR was reduced following treatment. Additionally, the increased expression of hENT1 suggests a potential reversal of resistance.

Conclusion

Our results suggest that the combination of ABT-737 and NVP-BEZ235 is a promising therapeutic strategy for overcoming Gemcitabine resistance in TNBC. This combinational approach enhances apoptosis and inhibits cell proliferation and migration by targeting both the PI3K/mTOR pathway and Bcl-2 family proteins. Further studies are needed to assess the potential of this strategy for GEM-resistant TNBC.

EACR25-0218

Targeted Delivery of NVPBEZ235 conjugated to gold nanoparticles for Overcoming Gemcitabine Resistance in Triple-Negative Breast Cancer

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Introduction

Triple-negative breast cancer (TNBC) is a highly aggressive subtype of breast cancer characterized by the lack of expression of HER2, estrogen and progesterone receptor (1). Due to limited targeted therapies and frequent development of chemoresistance, effective treatments remain a significant clinical challenge (2). Gemcitabine, a cytotoxic antimetabolite commonly used in chemotherapy, often develops resistance over time, decreasing the therapeutic efficacy (3). PI3K/AKT/mTOR pathway plays an important role in cell survival, growth, proliferation and motility. NVP-BEZ235, a dual PI3K and mTOR kinase inhibitor, decreases the proliferation of chemotherapy-resistant cancer cells (4). Phase II clinical trials of NVP-BEZ235 show anti-tumor efficacy in solid tumors however, side effects were also observed. Thus, employing targeted nanoparticle-based drug delivery systems may help overcome this obstacle. In this study, we aimed to overcome gemcitabine resistance in TNBC cell line (MDA-MB-231GEM) by delivering NVP-BEZ235 drug conjugated to targeted gold nanoparticle (t/BEZ@NP) developed in our laboratory.

Material and method

MDA-MB-231GEM was developed through incremental exposure to gemcitabine over a year. The resistance factor and the doubling time were analyzed to confirm the newly developed drug resistant cell line. Cytotoxic effect of t/BEZ@NP were evaluated via resazurin assay, while colony formation and wound healing assays were used to asses the growth and migration inhibition, respectively. Cell cycle and apoptotic assay were used to analyze the cell cycle arrest and apoptotic cell death induction.

Result and discussion

MDA-MB-231GEM cell line was successfully established with a fold resistance value of 116,04. t/BEZ@NP significantly reduced cell viability in MDA-MB-231GEM cells, with an IC₅₀ of 8.3 nM , compared to free NVP-BEZ235 (IC₅₀ = 10403 nM) at 48h. Colony formation assays demonstrated a reduction in both colony number and size following t/BEZ@NP treatment. Wound

healing assays showed impaired migration, with the lowest closure rate observed in the t/BEZ@NP group. Flow cytometry analysis indicated an increase in the SubG0 phase and a reduction in S phase progression, highlighting the antiproliferative and pro-apoptotic effects of t/BEZ@NP.

Conclusion

The combination of dual PI3K/mTOR inhibition with nanoparticle-based targeted therapy effectively overcame gemcitabine resistance in TNBC. t/BEZ@NP significantly enhanced cytotoxicity, suppressed proliferation, and impaired migration. This approach may offer a new avenue for improving treatment outcomes in patients with aggressive, drug-resistant breast cancer subtypes. *This project is funded by TUBITAK (122S737).*

EACR25-0229

Bacterial modulation of 5-FU chemoresistance in head and neck cancer

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Introduction

Head and neck cancer (HNC), the seventh most diagnosed cancer globally, primarily consists of squamous cell carcinomas (SCCs) that arise in the pharynx, larynx, and oral or nasal cavities. Chemotherapy is commonly used, but chemoresistance, a multifactorial process, often leads to poor outcomes. Changes in cancer cells affect their ability to absorb or metabolize drugs, and emerging evidence suggests that microbiome dysbiosis may influence cancer treatment efficacy and toxicity. In HNC, variations in chemotherapy responses have been linked to oral microbiome dysbiosis, indicating that tumor-associated bacteria may play a pivotal role in chemoresistance.

Material and method

Bacterial isolates were collected from the oral cavity and tumor samples of 39 patients. Complete bacterial genomes were sequenced using long-read (Nanopore) and short-read (Illumina) technologies. To evaluate 5-fluorouracil (5-FU) resistance, minimum inhibitory concentration (MIC) assays were performed using a high range of 5-FU (16–500 µg/ml). Mass-spectrometry was used to detect the presence of Pre-TA operon. Supernatants from 5-FU-resistant Streptococcus isolates were exposed to HNC cells to assess the potential for bacteria-induced 5-FU resistance in cancer cells.

Result and discussion

A total of 427 bacterial isolates were identified from the oral cavity and tumor samples. *Streptococcus* spp. was the most abundant genus, comprising 174 isolates. The MIC assay revealed that 36.2% of *Streptococcus* isolates could tolerate high doses of 5-FU (64 µg/ml). Resistance was not limited to a specific *Streptococcus* species, highlighting the diversity of resistance mechanisms across different isolates. Surprisingly, resistance was not attributed to the Pre-TA operon, which is commonly involved in 5-FU metabolism in other bacterial species.

This suggests that alternative, currently unknown genetic mechanisms may be contributing to the survival of these isolates under 5-FU stress. Eventually, exposure of SCC-1 and SCC-47 cells to the supernatants of 5-FU-resistant *Streptococcus* isolates demonstrated that certain bacterial strains could confer resistance to 5-FU in the HNC cells. This suggests that bacteria through secreted metabolites or other factors, may play a significant role in modulating cancer cell sensitivity to chemotherapy.

Conclusion

This study reveals the significant contribution of tumor-associated bacteria in the development of HNC chemotherapy resistance, particularly in relation to 5-FU. The findings suggest that bacteria may influence chemotherapy outcomes through direct interaction with cancer cells or by secreting factors that alter drug sensitivity. Identifying the molecular mechanisms and genetic components underlying these interactions will be crucial for developing novel therapeutic strategies to overcome chemoresistance and improve clinical outcomes for HNC patients.

EACR25-0247

Investigating AMBRA1's role as a prognostic biomarker of resistance for melanoma therapies

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Introduction

Targeted therapies (TT) and immunotherapies (IT) have revolutionized the treatment of metastatic melanoma, but low response rate and resistance remain a challenge. To overcome resistance, these therapies are usually combined or administered consecutively. Identifying biomarkers of resistance is crucial with significant implications for therapy selection. Recently, we found that the loss of Autophagy and Beclin 1 Regulator 1 (AMBRA1), a melanoma suppressor gene, activates the Focal Adhesion Kinase 1 (FAK1) pathway to drive TT resistance and can serve as a predictive biomarker for TT response.

Material and method

In this study, we used the Yale University Murine Melanoma 1.1 (Yumm1.1) cells to generate v-raf murine sarcoma viral oncogene homolog B1 inhibitors (BRAFi)-resistant cells. Ambra1 levels and Fak1 activation were assessed via western blot. BRAFi-resistant cells were extensively characterized through cellular assays and gene-expression profiling. Also, a syngeneic mouse model of melanoma was employed to evaluate the growth

of BRAFi-resistant Yumm1.1 tumors. The composition of the tumor immune microenvironment (TIME) in BRAFi-resistant Yumm1.1 tumors was assessed via flow cytometry.

Result and discussion

We identified that chronic exposure to BRAFi in murine Yumm1.1 melanoma cells resulted in a significant drop of Ambra1 expression compared to the sensitive line, similar to the drop observed upon acquisition of TT resistance in melanoma patients and human melanoma cells. Notably, Ambra1-Low-BRAFi-resistant Yumm1.1 cells exhibited high levels of phosphorylated FAK-Y397 (pFAK-Y397), when compared to the Ambra1-High-sensitive cell line. RNAseq analysis revealed a significant upregulation of cytokine receptor activity and interferon gamma signaling pathway, similar to the one observed in non-responder patients to TT and IT. In vivo, Ambra1-Low-BRAFi-resistant tumors exhibited accelerated growth compared to the Ambra1-High-sensitive tumors and were enriched in immunosuppressive immune populations. Additionally, we identified nuclear translocation of Fak1 and its downstream effector Yes-associated protein 1 (Yap) in both Ambra1-Low-BRAFi-resistant cells and tumors, potentially contributing to immune evasion via Programmed Cell Death Ligand 1 (PD-L1) upregulation.

Conclusion

Further studies are needed to investigate if AMBRA1-FAK1 axis is activated upon TT and IT resistance and if AMBRA1 can be used as prognostic marker of resistance for melanoma therapies.

EACR25-0257

Integrative Omics Insights into Metabolic Adaptations of TRAIL-Resistant

Glioblastoma

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Introduction

Glioblastoma (GBM) is the most common and aggressive primary brain tumor in adults, characterized by poor prognosis and high therapeutic resistance. This resistance, driven by tumor heterogeneity and the brain's complex anatomy, limits treatment success. Tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) selectively induces apoptosis in cancer cells, making it a promising therapy. However, GBM cells often exhibit innate or acquired resistance to TRAIL. Our previous studies generated TRAIL-resistant GBM models and identified the major apoptotic and intracellular alterations compared to their sensitive counterparts. Notably, emerging evidence suggests a strong link between metabolic reprogramming and therapy resistance in GBM. A better understanding of these resistance mechanisms could pave the way for improved therapeutic strategies.

Material and method

LC-MS-based untargeted metabolomics was performed to identify metabolite changes in TRAIL-resistant and sensitive A172 cells by analyzing the global metabolome. Gene expression profiling was conducted using RNA

sequencing, and these data were integrated with metabolomics data for a comprehensive analysis. Bioinformatics analysis was performed using R software, MetaboAnalyst, GSEA, and CytoScape pipelines to holistically evaluate the resistance mechanism.

Result and discussion

Integrating RNA sequencing and metabolomics provides a deeper understanding of TRAIL resistance in GBM by linking gene expression changes to metabolic alterations. Joint pathway and enrichment analyses showed significant changes in glycerophospholipid metabolism, glycolysis, oxidative phosphorylation (OXPHOS), and drug metabolism pathways in resistant cells. Integrated omics analysis demonstrated a positive correlation between OXPHOS-related metabolites and resistance, highlighting the potential role of mitochondrial metabolism in therapy resistance. These findings suggest that alterations in energy metabolism, membrane components, and drug metabolism contribute to TRAIL resistance.

Conclusion

Our findings highlight the role of metabolic adaptations in TRAIL resistance, revealing potential therapeutic targets for glioblastoma. Functional assays will further investigate the impact of key metabolic pathways on therapy resistance. Targeting these pathways may lead to novel strategies to overcome resistance and improve treatment outcomes.

EACR25-0278

In Silico Identification of Common Pre-existing Resistance Mechanisms to EGFR Inhibitors in Colorectal Cancer

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Introduction

Despite the genetic, transcriptomic, and epigenetic heterogeneity of cancers, resistance to therapy often arises through convergent mechanisms, suggesting that tumors rely on shared survival strategies regardless of drug selection or patient-specific factors. We hypothesized that common pre-existing phenotypic states across different tumors contribute to resistance against multiple drugs. To test this hypothesis, we developed an in silico strategy to associate transcriptomic programs with the mechanisms of action (MoA) of drugs. Specifically, we applied this approach to elucidate resistance mechanisms to epidermal growth factor receptor inhibitors (EGFRi) – a key treatment in colorectal cancer (CRC) whose efficacy is frequently limited by resistance.

Material and method

We analyzed transcriptomic profiles from 43 CRC cell lines from the Cancer Cell Line Encyclopedia (CCLE) and their drug response data for seven EGFRi from the Genomics of Drug Sensitivity in Cancer (GDSC) database. Moreover, we employed an external cohort (GSE162104) to validate our approach, as it involves cetuximab, an EGFRi absent in the GDSC. We applied Non-Negative Matrix Factorization (NMF) to decompose the transcriptomic profiles into 15 latent factors. A drug

enrichment score was then developed to identify latent variables associated with EGFRi sensitivity and resistance.

Result and discussion

The 15 latent factors successfully reconstructed the original matrix with an error of 4.84%. Using the drug enrichment score, we found that 3 out of the 15 latent factors were significantly associated with the EGFRi MoA: Factor 1 [ES = -0.755, p-value = 0.002], Factor 2 [ES = 0.756, p-value = 0.002], and Factor 12 [ES = 0.676, p-value = 0.020]. Functional analysis revealed that two of these factors were enriched in estrogen signaling pathways, while one was linked to immune response regulation. External validation confirmed that 22 out of the 230 differentially expressed genes associated with cetuximab resistance were also present among the top 500 genes contributing to factor construction. This suggests that certain resistance mechanisms are directly related to the drug's mechanism of action, independently of drug-specific factors.

Conclusion

Our findings indicate that, despite heterogeneity, different tumors can develop shared resistance mechanisms. Through the combined use of NMF and the drug enrichment score, our approach enables the identification of latent factors associated with EGFRi resistance, providing a framework to explore drug response mechanisms across different therapeutic contexts.

EACR25-0303

Variable responses to Akt kinase inhibition stem from non-genetic heterogeneity

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Introduction

Heterogeneity between cancer cells undermines the efficacy of therapies, with both genetic and non-genetic mechanisms generating diversity in cancer cell states. Delineating the mechanisms underpinning heterogeneity in cell state will reveal new strategies for improving the efficacy of cancer therapies. The PI3K/Akt/mTOR signalling pathway promotes cell and tissue growth, with several pharmacological inhibitors in clinical use. Although inhibition of either PI3K or Akt provides benefit in certain clinical contexts, durable responses are rare with reactivation of mTOR and S6 kinase frequently observed.

Material and method

We leverage complementary experimental approaches, including a modified Luria-Delbrück assay, to address

TNBC resistance to AKT inhibition in organoid models. We combine live imaging of AKT activity, glucose metabolism, and organoid growth with molecular approaches including DNA barcoding, RNA sequencing, and metabolomics.

Result and discussion

Linking heterogeneity in cell state to dynamic changes cell signalling, cell metabolism, and ultimately cell fate is challenging. I have found that targeting AKT isoforms leads to a heterogeneous growth inhibition in organoid models of triple negative breast cancer (TNBC). This identifies a subset of TNBC organoids that exhibit a signature positive for Krt14 and p63, similar to basal cells in the normal breast, that resist a bottleneck selection pressure imposed by Akt kinase inhibition. The resistant cell state is reversible and can be driven by ΔNp63α overexpression.

Conclusion

These analyses indicate that resistance to AKT inhibition in TNBC exploits a repertoire of cell states and switching mechanisms inherent to the biology of the breast.

EACR25-0351

Acquisition of resistance to targeted therapies in lung cancer: mechanistic basis and suppressive strategies

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Introduction

Targeted cancer therapies efficiently reduce tumor growth and progression. However, the development of resistance unavoidably occurs, representing a major cause of cancer-related deaths. Despite being the triggering event, we have demonstrated that the resistance mutation is present only in a fraction of relapsing tumor cells, evidencing that the resistance phenotype can be transferred among cancer cells. This challenges the prevailing view of resistance development based solely on cell division and selection of mutated cells.

Material and method

We used the human H1975 EGFRp.L858R/EGFRp.T790M Erlotinib-resistant and HCC827 EGFRp.delE746-A750 Erlotinib-sensitive lung cancer cell lines both in vitro and in a co-inoculation immunodeficient C57BL/6[-/-]; Il2rg[-/-] mouse model to study the transcriptomic, proteomic and epigenetic profile of therapy sensitive cells before and after development of resistance. This approach aimed to evaluate the impact of the stimulus from the resistant cell line on the acquisition of resistance in sensitive cells and investigate the stability of the acquired phenotypic resistance.

Result and discussion

We showed that supplementation with conditioned medium from therapy-resistant cells, as well as dual

inoculation of sensitive and resistant cells in our mouse model, leads to a faster and more stable resistance phenotype. In addition, the acquisition of resistance triggers a change in the transcriptional landscape, characterized by the enrichment of the endocytosis pathway, specifically through Caveolin-1 overexpression, which we further validated in human samples. Downstream pathway analysis has identified PI3K as a potential effector of resistance, whose inhibition can restore sensitivity to therapy as well as novel evidence regarding the role of AKT and ERK in cell survival and resistance to therapy. The methylation array analysis revealed that the therapy-resistant phenotype acquired appears to be accompanied by an epithelial-to-mesenchymal transition (EMT) through known mediators such as STK26.

Conclusion

These findings suggest that resistance can arise through alternative mechanisms, potentially influenced by interactions between cancer cells and underscore the complexity of resistance mechanisms, highlighting the potential of targeting intercellular communication to overcome resistance in lung cancer therapy.

EACR25-0379

BET inhibitor Birabresib synergizes with PARP inhibitor Talazoparib in urothelial cancer cell lines and patient-derived organoids

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Introduction

Patients with urothelial carcinoma (UC) often develop resistance to standard treatment. PARP inhibitors (PARPi) are an effective therapeutic option in cancers with BRCA1/2 mutations (BRCAne), which are rare in UC. We have previously shown that bromodomain and extra-terminal motif inhibitors (BETi) can induce a pharmaceutical BRCAne phenotype in UC cells (UCCs). In this study, we investigated the synergistic effects of a combination therapy with BETi Birabresib (Bira) and PARPi Talazoparib (Tala) in UCCs and their cisplatin-resistant sublines (LTTs) as well as patient-derived organoids (PDOs).

Material and method

T24 and J82 as well as their LTTs and benign HBLAK cells were used for dose response curve analyses after 72 hours of treatment. The Combenefit tool was used to analyse synergism of combination treatment. Reduced dosages were applied to assess effects on DNA damage repair, clonogenicity, cell cycle and apoptosis. Synergistic effects were validated on Cisplatin-sensitive and resistant PDOs.

Result and discussion

IC50 of UCCs for Bira and Tala were all in a low μM range. LTTs were more sensitive to Bira than their parental cell lines. While benign HBLAK cells and UCCs

had similar IC50 for Tala (IC50 T24: 0.2 μM; T24LTT & J82LTT: 0.4 μM; J82: 0.6 μM; HBLAK: 0.3 μM), J82 proved to be more resistant to Bira (IC50 J82: 20.0 μM; T24: 0.3 μM; HBLAK: 0.2 μM). Combined treatment of Bira and Tala resulted in a strong synergism in all UCCs, allowing a dose reduction for further analyses (0.5x IC50 Bira; 0.5x IC50 Tala). Combined treatment caused DNA damage, while impairing repair by homologous recombination. Cell cycle arrest in G2/M and apoptosis were induced, while long-term proliferation capacity was strongly reduced by the combination. Dose response analyses on Cisplatin-sensitive and resistant PDOs revealed similar IC50 values for Bira compared to UCCs (IC50 Azh6T: 1.4 μM; P106T: 0.9 μM), while showing resistance to PARPi (IC50 > 50 μM). However, when combining Bira and Tala on PDOs synergistic effects were again achieved with reduced dosages.

Conclusion

Combined treatment with Birabresib and Talazoparib had strong synergistic effects on all investigated models – UCCs, LTTs and PDOs - enabling a dose reduction. Synergistic response was also observed in models resistant to mono-treatment. Thus, we suggest combined treatment of Birabresib and Talazoparib as a new highly potent treatment option for all UC patients, irrespective of previous Cisplatin-based chemotherapy.

EACR25-0384

Overcoming paclitaxel resistance in ovarian cancer cells using DN200434, an inverse agonist of Estrogen-related receptor gamma (ERRγ)

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Introduction

Ovarian cancer remains a significant contributor to female mortality rates associated with gynecological malignancies. The development of resistance to paclitaxel (PTX), a frontline chemotherapeutic agent, poses a major hurdle in effectively managing this disease. Recent research has identified the nuclear receptor Estrogen-related receptor γ (ERRγ) as a key player in cancer progression, metastasis, and chemotherapy resistance. Notably, ERRγ is found to be upregulated in ovarian cancer, correlating with decreased patient survival rates. In this study, we investigated the efficacy of DN200434, an inverse agonist of ERRγ, in mitigating cancer cell proliferation and overcoming PTX resistance in SKOV-3.

Material and method

Parental SKOV-3 cells were subjected to incrementally increasing PTX concentrations over a period of 3 months to establish PTX-resistant SKOV-3 subline, SKOV-3-PTXR. The effect of DN200434 and PTX on SKOV3 and SKOV-3-PTXR cell proliferation was examined by CCK-8 assay. Apoptosis induced by DN200434 was evaluated in these cell lines using FACS-based Annexin-PI staining. The ability of DN200434 to reverse PTX resistance in SKOV-3-PTXR cells was investigated through CCK-8 assays. Cell cycle arrest and apoptosis resulting from combination treatment were analyzed using FACS-based PI and Annexin-PI staining,

respectively. An agarose-based 3-D spheroid model of SKOV-3-PTXR was employed to further validate DN200434's sensitization effect.

Result and discussion

SKOV-3-PTXR was successfully developed using a stepwise dose escalation approach with a resistance index of 50. The resistance remained stable after 6 months of cryopreservation. Further, both the cell lines exhibited dose-dependent growth inhibition and apoptosis induction when treated with DN200434. Moreover, combining DN200434 with PTX significantly enhanced cell cytotoxicity (50–55%) compared to treatments with DN200434 (20–25%) or PTX (10–12%) in SKOV-3-PTXR. Flow cytometry analysis revealed that DN200434 resensitized SKOV-3-PTXR towards paclitaxel via augmentation of PTX-associated metaphase arrest and apoptosis ability. Intriguingly, the phenomenon of resistance reversal is corroborated in 3-D agarose-based spheroid model.

Conclusion

The present study elucidates the potential of DN200434, a selective inverse agonist of ERR γ , as a potent therapeutic agent in ovarian cancer, demonstrating dose-dependent apoptosis induction. Moreover, the study establishes the role of the inverse agonist in overcoming paclitaxel (PTX) resistance, a significant challenge in ovarian cancer treatment. The observed resistance reversal by DN200434 is attributed to increased cell cycle arrest and apoptosis when combined with PTX. The sensitization effect of DN200434 in combination with PTX is corroborated in a spheroid model. These findings support the combination of PTX and DN200434 as a viable strategy for managing PTX-resistant ovarian cancer.

EACR25-0456

A newly discovered mTOR-STAT3 pathway regulates calcium release and calcium-mediated apoptosis at the ER in Triple Negative Breast Cancer

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Introduction

STAT3 is a pleiotropic transcription factor often constitutively activated in tumors including breast cancer. STAT3 canonical transcriptional activity is mediated by Y705 (Y-P) phosphorylation; in contrast, phosphorylation on S727 (S-P) orchestrates transcription-independent activities in distinct cellular compartments. Among them, we have recently described the ability of S-P STAT3 to localize to the ER and Mitochondrial Associated Membranes to control ER Ca²⁺ release and Ca²⁺-mediated apoptosis in STAT3-dependent Triple Negative Breast Cancer (TNBC) cells, by triggering the

proteasomal degradation of the Ca²⁺ channel IP3R3. Accordingly, STAT3 and IP3R3 protein levels are inversely correlated in the highly aggressive basal-like breast tumors, where STAT3 is often constitutively activated.

Material and method

To elucidate the mechanisms regulating STAT3 S-P at the ER, using Cytoscape we generated a network of human putative ER-STAT3 interactors based on the Search Tool for the Retrieval of Interacting Genes/Proteins (STRING) database, selected according to their ER gene ontology (GO) function. The only two serine kinases identified among the top ten putative ER-STAT3 interactors were mTOR and Akt1. The analysis of the TCGA TNBC RNAseq database showed that STAT3 mRNA expression levels positively correlated with those of mTOR but not Akt1.

Result and discussion

We confirmed STAT3-mTOR ER co-localization by immunofluorescence, and their interaction by co-immunoprecipitation from purified ER fractions and Proximity Ligation Assay (PLA) in human TNBC cells. Pan-mTOR inhibition by Torin-1 – but not mTOR Complex 1 inhibition via Rapamycin – could prevent both IP3R3 degradation and Ca²⁺-mediated apoptosis, mimicking the effects of STAT3 silencing. Accordingly, Torin-1 abolished STAT3 S-P within the ER, increasing intracellular Ca²⁺ concentration.

Conclusion

These data demonstrate for the first time that STAT3 Serine phosphorylation can be regulated in the specific cellular compartment where its functions are exerted, in this case occurring at the ER downstream of mTOR. In turn this specific regulation can lead to enhance apoptosis resistance via IP3R3 degradation. Preliminary data indicate that STAT3-mTOR might directly interact, since they can still co-immunoprecipitate in the absence of either mTORC1 or mTORC2 upon RAPTOR and RICTOR ablation, respectively. Accordingly, AlphaFold 3 modelling of an mTOR-STAT3-IP3R3 ternary complex indicates that STAT3 interacts with the Armadillo region of mTOR via its coiled-coil domain. The assessment of mTOR, STAT3, and IP3R3 protein levels in patient-derived tumor samples is in progress. Dissecting these molecular details, in addition to contributing to characterize the multiple non-canonical mechanisms of STAT3 action, may reveal novel therapeutic targets to disrupt apoptotic resistance in STAT3-dependent TNBC cells.

EACR25-0505

Activation of the Integrated Stress Response to overcome multidrug resistance in cancer therapy

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Introduction

Resistance to therapy is estimated to contribute to treatment failure in up to 90% of metastatic cancer patients and remains a fundamental challenge in cancer. In this regard, we recently found that several kinase inhibitors were able to kill multidrug-resistant cancer cells, through a mechanism independent of their proposed targets. Instead, these compounds killed cells showing multidrug resistance (MDR) by activating the so-called Integrated Stress Response (ISR) (Sanchez-Burgos et al., EMBO Mol Med, 2022). Other reports also converge on similar observations in that: (a) activation of the ISR can overcome MDR in cancer and (b) several drugs activate the ISR by an unknown mechanism.

Material and method

To systematically address the potential of drugs to activate the ISR, we conducted both chemical and CRISPR/Cas9 genome-wide genetic screens using a fluorescent reporter of CHOP, a pro-apoptotic factor that is expressed upon activation of the ISR.

Result and discussion

Chemical screens have allowed us to obtain a panoramic view of how drugs activate the ISR and to identify some surprising inducers of the response: benzimidazole derivatives. We subsequently tested these compounds in both *in vitro* and *in vivo* models of drug resistance, yielding very promising results. As mentioned above, in addition to chemical screens, we have also performed genome-wide CRISPR/Cas9 knockout screens to evaluate which targets, when depleted, lead to a more vigorous activation of the ISR. The idea here is to identify the best targets for which an inhibitor would be an efficacious ISR activator. The screen, also based on our CHOP reporter model, has led us to identify several promising hits, including some chaperones which we are currently investigating.

Conclusion

In summary, our work aims to provide a general overview of the genes and drugs that modify the ISR, and to use this information to develop novel strategies to overcome multidrug resistance in cancer therapy.

EACR25-0566

Evaluate the potential sensitization effect and underlying mechanism of tyrosine kinase inhibitor on cisplatin resistance oral squamous cell carcinoma

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Introduction

Oral squamous cell carcinoma (OSCC) is a common cancer, with cisplatin as the main chemotherapy drug. However, resistance often develops, linked to HIF-1 α activation. This pathway increases VEGF and BCL-2

levels, promoting angiogenesis and anti-apoptosis.

Targeting the HIF-1 α /VEGF/BCL-2 axis may improve treatment. Tyrosine kinase inhibitors (TKIs), such as lenvatinib, block key signaling pathways, including VEGF and BCL-2, to inhibit tumor growth and metastasis. However, their potential to enhance OSCC sensitivity to cisplatin remains unclear.

Material and method

In our study, MOC1 cells were cultured under hypoxic conditions (1% O₂) in a specialized medium containing growth factors and nutrients, successfully establishing cisplatin-resistant OSCC cell lines. The MTT assay was used to assess whether cisplatin and TKIs induced cytotoxicity in these resistant cells. Flow cytometry was then performed to determine whether the combination of TKIs and cisplatin enhanced both extrinsic and intrinsic apoptotic pathways. Additionally, transwell invasion and migration assays were conducted to evaluate whether TKIs combined with cisplatin increased the inhibitory effects on cisplatin-resistant OSCC cells. Finally, Western blotting was performed to investigate the molecular mechanisms underlying cisplatin resistance following TKI and cisplatin combination treatment.

Result and discussion

Our study established cisplatin-resistant OSCC cells under hypoxic conditions and found that cisplatin alone had limited cytotoxicity. Nevertheless, combining TKIs with cisplatin restored cisplatin sensitivity. Flow cytometry confirmed that TKIs enhanced cisplatin-mediated apoptotic pathways. Additionally, transwell assays showed significant inhibition of invasion and migration after combining TKIs with cisplatin. Finally, we showed that TKIs and cisplatin effectively suppressed angiogenesis and disrupted the anti-apoptotic mechanisms mediated by the HIF-1 α /VEGF/BCL-2 axis.

Conclusion

In conclusion, our results suggest that combined treatment with TKIs and cisplatin can enhance the response of OSCC cells under hypoxic conditions. Additionally, TKIs restored the sensitivity of OSCC to cisplatin through the HIF-1 α /VEGF/BCL-2 pathway.

EACR25-0574

Study of long non-coding RNAs in resistance to KRAS G12C inhibitors in lung cancers

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Introduction

Lung cancer is the leading cause of cancer-related mortality worldwide. KRAS mutations occur in 30% of lung adenocarcinomas, with half of these being of the KRAS G12C subtype. The development of covalent inhibitors targeting this variant, such as adagrasib, is a recent clinical breakthrough. However, despite promising phase I/II clinical trial results, only 40% of patients initially respond to these treatments and most of them will develop resistance. Current research on resistance mechanisms is limited and predominantly focuses on

genetic factors while non-genetic mechanisms, including those involving long non-coding RNAs (lncRNAs), remain largely unexplored. Our objective is to investigate the role of lncRNAs in resistance to KRAS G12C inhibitors in non-small cell lung cancer (NSCLC).

Material and method

Using the ADCA72 cell line derived from a KRAS G12C-mutated patient, we generated both adagrasib-sensitive and -resistant cells. Then, we conducted two complementary assays to identify candidates lncRNAs involved in resistance. Firstly, long-read nanopore sequencing was used to discriminate differentially expressed lncRNAs between adagrasib-sensitive and -resistant cells. Secondly, a genome wide lncRNA CRISPR activator screen was performed in ADCA72-sensitive cells to find which lncRNAs confer a survival advantage.

Result and discussion

Long-read sequencing analyses identified a specific subset of lncRNAs associated with resistance, including LINP1. Further experiments showed that LINP1 is mainly expressed in the nucleus of resistant cell suggesting that they could remodel chromatin and gene expression. LINP1 has been described to facilitate DNA damage repair via NHEJ pathway, therefore decreasing sensitivity to chemotherapy and radiotherapy. CRISPR screen analysis pinpointed previously uncharacterized lncRNAs, such as Linc01018, which we are studying in a functional way. Finally, cross-referencing of results revealed that some candidates were associated with resistance and survival advantage under adagrasib treatment using both nanopore technology and the CRISPR screen. Again, we are currently investigating their localization and functions.

Conclusion

Our study highlights the importance of lncRNAs in resistance mechanisms to KRAS G12C inhibitors in NSCLC. It suggests that non-genetic mechanisms involving lncRNAs contribute to the development of resistance. Further functional research into these lncRNAs could provide new therapeutic targets and strategies.

EACR25-0592

Targeting the Non-Homologous End-Joining Pathway Sensitizes MDM2-Amplified Liposarcoma to Doxorubicin-Induced Senescence in a p53-dependent Manner

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Introduction

Dedifferentiated liposarcoma (DDLPS) is a rare cancer defined by amplification of MDM2 and CDK4.

Conventional chemotherapy (doxorubicin) and targeted inhibition of MDM2 and CDK4 show sporadic responses, but most tumors display (intrinsic) resistance. We used

an unbiased approach to identify therapeutic strategies sensitizing to these current DDLPS therapies.

Material and method

Three parallel genome-wide CRISPR-Cas9 knockout screens were conducted in DDLPS cells to sensitize to palbociclib (CDK4 inhibitor), nutlin-3a (MDM2 inhibitor) or doxorubicin. Top screen hits were validated and characterized in both *in vitro* and *in vivo* models, while clinical data were leveraged to confirm molecular findings on a larger scale.

Result and discussion

We found pathways related to G1/S transition (CDK2, CKS1B, E2F3 and CCNE1) and Non-Homologous End-Joining (NHEJ; TDP2, PRKDC and XRCC4) to sensitize to palbociclib and doxorubicin respectively. Following validation of both pathways, we focused on mechanistic characterization of doxorubicin sensitization by genetic perturbation of TDP2 or pharmacological inhibition of PRKDC using pepotib. Synergy was achieved by prolonged administration of low-dose doxorubicin *in vitro* and *in vivo*, driven by increased cell cycle arrest and senescence. Senescent cells were triggered to undergo into apoptosis by subsequent senolytic therapy. Surprisingly, despite the amplification of MDM2, senescence was dependent on p53. Leveraging TCGA and DepMap data, we confirm potential p53 activity in DDLPS.

Conclusion

These findings provide a rationale for targeting the NHEJ pathway to enhance the efficacy of doxorubicin in an MDM2-amplified cancer, highlighting a potential therapeutic strategy that exploits p53-dependent cell cycle arrest and senescence for improved treatment outcomes. Furthermore, we provide, to our knowledge, the first evidence of maintained p53 activity in untreated MDM2 amplified DDLPS.

EACR25-0598

Regulation of FOXK2 by AKT in breast cancer cells: Serine 398 as a novel potential phosphorylation site

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Introduction

FOXK2 is a transcription factor which mediates drug sensitivity in breast cancer. Conversely, drug-resistant cells exhibit constitutively high FOXK2 protein levels

which seems to be inactive, suggesting that FOXK2 may be regulated by post-translational modifications. In silico analysis have found the AKT kinase as the main putative regulator of FOXK2. Therefore, the aim of our study is to evaluate whether FOXK2 is regulated by AKT-mediated phosphorylation and its impact on breast cancer chemoresistance.

Material and method

MCF-7 (chemosensitive) and MDA-MB-231 (chemoresistant) cells were used as models. FLAG-FOXK2 mutants were generated by site-directed mutagenesis using the overlap extension approach. Gene knockdown and overexpression were performed by transient transfections. MK-2206 was used for AKT pharmacological inhibition. Immunoprecipitation (IP) assays were performed using beads conjugated to specific antibodies. The NE-PER kit was used for subcellular fractionation. RNA levels were examined by real-time PCR and protein levels, by Western Blotting. The MTT and clonogenic assays were performed to assess cell viability and drug-induced cytotoxicity.

Result and discussion

AKT inhibition reduced exogenous and endogenous FOXK2 protein levels, with no such modulation in FOXK2 mRNA levels. Similarly to phosphorylated AKT, FOXK2 subcellular localization was predominantly nuclear in drug-resistant cells. Also, experiments with lambda-phosphatase and Phos-tag gels suggest that FOXK2 mobility profile is altered in AKT-modulated cells. Interestingly, FOXK2 IP eluates were probed using phospho-AKT substrate antibodies, further suggesting that FOXK2 might be a substrate for AKT phosphorylation. Notably, higher levels of phosphorylated FOXK2 were found in breast tumors samples from public databanks, including serine 398 (S398), which has also been identified as a putative phosphorylation site in FOXK2 in silico and in a large-scale proteomic analysis. These findings supported the construction of phospho-deficient (S398A) and phosphomimetic (S398E) FOXK2 mutants. Notably, the S398A mutant form of FOXK2 impaired cell growth, cell viability and clonogenic capacity compared with wild type and the S398E mutant version of FOXK2, even in the presence of chemotherapeutic drugs. Finally, preliminary IP assays using anti-FLAG antibodies suggest that the S398 residue in FOXK2 is phosphorylated by AKT.

Conclusion

In summary, AKT can regulate FOXK2 by phosphorylation in drug-resistant cells and S398 turns out to be a novel potential site in AKT-FOXK2 interaction interface. Understanding AKT-FOXK2 axis contribute to potentiate the effects of AKT inhibitors in breast cancer.

EACR25-0600

FOXK2 transcription factor in cancer: expression, function and potential as a biomarker

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Introduction

FOXK2 is a transcription factor that plays a dual role in cancer, acting either as a tumor suppressor or an oncogene, depending on the tissue type. In breast cancer, FOXK2 acts as a tumor suppressor, preventing carcinogenesis and favoring drug responses. Therefore, we aimed to evaluate FOXK2 gene expression as a potential biomarker in different tumor types and the role of FOXK2 at the interface between chemosensitivity and tumor progression in breast cancer.

Material and method

An in silico analysis compared FOXK2 gene expression between normal and tumor patient's samples in different tumor types by analyzing transcriptome-level data from TNMplotter platform. The impact of FOXK2 on patient's overall survival was evaluated by Kaplan-Meier curves from KMplotter. FOXK2 promoter methylation status was assessed by OncoDB; copy number variation and FOXK2 mutational status by XENA platform. The role of FOXK2 modulation in breast cancer was assessed by shRNA viral transductions in MCF-7 (chemosensitive) and MDA-MB-231 (chemoresistant) cell lines. The validation of these shFOXK2 cells was assessed by western blotting and real time RT-qPCR assays. Docetaxel and doxorubicin-induced cytotoxicity was evaluated by MTT and colony formation by clonogenic assays. To link FOXK2 gene expression and response to therapy on breast cancer patient's samples, we evaluated patient's pathological complete response to therapy and relapse-free survival at 5 years by ROCplotter.

Result and discussion

We have found FOXK2 overexpression in most tumor types, which was associated with high copy number variation, but not with FOXK2 gene mutations or promoter methylation status. This points to additional transcriptional or post-translational mechanisms governing FOXK2 regulation. From all tumors analyzed, low FOXK2 levels in patients with kidney renal clear cell carcinoma and liver hepatocellular carcinoma showed up a remarkable improvement on overall survival. Also, our in vitro results show that knocking-down FOXK2 renders breast cancer cells more resistant to chemotherapeutics. Additionally, grade II patients who received chemotherapy and relapsed before 5 years showed low FOXK2 expression. Similarly, reduced FOXK2 expression has also been found in non-responders patients, particularly luminal B, treated with the FEC chemotherapeutic combination. Therefore, FOXK2 low expression has been related to poor responses to treatment in breast cancer. Finally, ongoing in vitro experiments with shFOXK2 drug resistant cells are currently exploring the interface between breast cancer drug resistance and tumor progression.

Conclusion

Altogether, our findings suggest that analyzing FOXK2 gene expression might be a good predictor of response to therapy, diagnosis and prognosis in some tumor types.

EACR25-0601**Targeting DUSP6 to combat therapy resistance and brain metastasis in HER2-positive breast cancer**

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Introduction

Dual-specificity phosphatase 6 (DUSP6) is implicated in cancer progression and therapy resistance (2). In HER2-positive (HER2+) breast cancer, DUSP6 expression increases significantly during the transition from drug-tolerant persister (DTP) cells to expanding persister (DTEP) cells, a key stage in acquired resistance to HER2 inhibitors (HER2i). Elevated DUSP6 levels enhance apoptosis resistance, while its pharmacological inhibition restores HER2i sensitivity. Mechanistically, these effects are mediated by the impact of DUSP6 on the HER3-neuregulin axis, which is pivotal in breast-brain metastasis and therapy tolerance (1).

Material and method

Clonogenic assays assessed proliferation following treatment with HER2i (lapatinib) and DUSP6 inhibitor (BCI). Stable DUSP6 overexpression was achieved in the BT474 cell line via lentiviral transduction. Fluorescence-activated cell sorting was used to isolate highly EGFP-expressing cells, which were subsequently employed in ex vivo brain slice culture and live-cell imaging. In addition, intracranial xenograft models in mice were utilised to evaluate the impact of DUSP6 inhibition on tumour progression and therapeutic response.

Result and discussion

DUSP6 promotes therapeutic resistance in HER2+ breast cancer. Pharmacological inhibition of DUSP6 combined with HER2i resulted in a potent anti-proliferative effect in HER2i-resistant MDA-MB-453 cells, as demonstrated by clonogenic assays. Ex vivo brain slice culture further showed increased cell death in disseminated tumour cells upon combined DUSP6 and HER2 inhibition, underscoring the therapeutic relevance of this strategy in a physiologically representative environment. This model preserved brain architecture while enabling controlled experimental conditions. Conversely, DUSP6 over-expression in HER2i-sensitive BT474 cells conferred resistance to HER2i-induced apoptosis, as revealed in clonogenic assays. In vivo, intracranial xenograft models demonstrated that DUSP6-overexpressing cells induced tumour outgrowth, even when using non-brain-tropic cells, resulting in increased brain tumour burden, weight loss, and neurological symptoms in mice. These findings highlight the role of DUSP6 in facilitating brain metastasis and resistance to HER2-targeted therapies.

It is essential to investigate the molecular oncogenicity of DUSP6 and its contribution to brain colonisation.

Conclusion

DUSP6 is a candidate driver of HER2i resistance in brain metastasis. Dual inhibition of HER2 and DUSP6 exerts a significant synergy, suggesting a promising therapeutic approach. Subsequently, we aim to identify and characterise novel allosteric DUSP6 inhibitors. Developing and evaluating these inhibitors using preclinical models offers potential new therapeutic strategies for breast cancer and other subtypes.

EACR25-0637**Understanding and targeting mechanisms of resistance to the cytidine deaminase biodegrader and gemcitabine combination in cancer**

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Introduction

Despite the endeavours and achievements made in treating cancers during the past decades, resistance to classical chemotherapeutic agents and/or novel targeted drugs remains a major problem in cancer therapies. Indeed, drug resistance, either existing before treatment (intrinsic) or generated after therapy (acquired), is responsible for most relapses of cancer, which are one of the major causes of death of the disease. Deoxycytidine analogues (e.g. gemcitabine, cytarabine) belong to the antimetabolites class of chemotherapeutic agents, used to treat a variety of cancers. Cytidine deaminase (CDA) has been identified as a key regulator of resistance to these therapies by promoting their deamination and inactivation. In this context, the team developed a CDA biodegrader to deplete CDA in cells and tumours and sensitise them to deoxycytidine analogues. In vivo, while the combination CDA biodegrader and gemcitabine induced a first phase of tumours regression, it is followed by a second phase in which the tumours relapsed. These data suggest the appearance of mechanisms of resistance to this combination.

Material and method

We modelled this resistance in vitro by following the confluence of clonal populations of cancer cells expressing, or not, the biodegrader and treated, or not, with gemcitabine, by using real-time microscopy. Resistant cell lines (clones) were amplified and characterised (proliferation, IC50). Then, RNAseq was performed on one resistant clone to identify the molecular mechanisms that could be responsible for this resistance. We confirmed the RNAseq data at the transcriptional and protein levels. Finally, we will perform functional validation and targeting of these mechanisms by using siRNA and shRNA.

Result and discussion

We obtained three different resistant clones from our in vitro modelling experiments. Characterisation of these clones showed that the CDA biodegrader is still functional, suggesting that CDA-independent

mechanisms emerge to induce resistance. RNAseq data on one resistant clone show that a pathway is significantly and specifically altered in the resistant clone. We confirmed this at the transcriptional (RT-qPCR) and protein (Western blotting) levels. We also confirmed the expression of the driver of this pathway in all the resistant clones we obtained. Ongoing studies are focused on evaluating the impact of targeting and over-expressing this driver on the emergence of resistance.

Conclusion

This project contributes to the identification of resistance mechanisms to the CDA biodegrader/gemcitabine combination in cancer cells, in order to target them and improve the long-term efficacy of this innovative therapeutic strategy.

EACR25-0651

KRAS silencing impacts chromatin organization and transcriptional activity in colorectal cancer cells

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Introduction

The swift development of resistance to targeted inhibition of a critical oncogene to which cancer cells are addicted is a remarkable and complex phenomenon standing in the way of curing cancer patients. Colorectal cancer responses to the recently developed mutant-specific KRAS inhibitors fully typify this addiction/resistance paradox: although mutant KRAS is the most frequent oncogenic driver of colorectal cancer, in the clinic, no effective cell killing is achieved with its inhibition. Instead, tumor cells enter a cell cycle-arrested state, resuming growth shortly after treatment initiation. Our work focused on answering a critical question: how do mutant KRAS colorectal cancer cells so readily adapt to and tolerate KRAS inhibition?

Material and method

To answer this question, our analyses were contextualized within the framework of differing sensitivities to KRAS silencing observed in 3-dimensional spheroid cultures of mutant KRAS CRC cell lines. siRNA was used to silence KRAS expression. Cells were grown into spheroids and then analyzed by mass spectrometry. The expression of significantly altered proteins was validated by western blotting. Additionally, we investigated chromatin states using transmission electron microscopy, partial wave spectroscopic microscopy, and Hi-C. Transcriptional plasticity was evaluated through RNA sequencing.

Result and discussion

Our results showed that upon KRAS silencing, KRAS-silencing sensitive cell lines presented a significant reduction in the number of cells, accompanied by changes in the cell cycle and apoptosis, which supported their KRAS-dependency. Proteomics analysis revealed a

differential expression of several proteins associated with gene expression regulation, mRNA splicing and processing, and nucleosome assembly and repositioning in KRAS-silenced sensitive cells. Furthermore, these cells exhibited changes in the 3D physical organization of chromatin, namely alterations in eu/heterochromatin ratios, chromatin domain packing scaling, alterations in TAD number, and A/B compartment distribution. Additionally, longitudinal RNA sequencing following KRAS silencing revealed changes in the transcriptional performance in KRAS-inhibition sensitive cell lines.

Conclusion

Collectively, our findings highlight a potential mechanism involved in the rapid adaptation of mutant KRAS colorectal cancer cells to KRAS loss mediated by profound alterations in chromatin organization, emphasizing potential epigenetic vulnerabilities that could be exploited to enhance therapeutic efficacy.

EACR25-0659

Characterization of TRIB2 role in pancreatic cancer chemoresistance

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Introduction

Pancreatic cancer (PC) is classified as one of the most aggressive and deadliest cancers in the world (5-year survival rate of 10-25%; mortality/incidence ratio of 95%). However, the treatment options for these patients have not advanced significantly throughout the years and are frequently associated with the development of therapy resistance and patients' relapse. Recently, we discovered a novel mechanism of therapy resistance mediated by Tribbles homolog 2 (TRIB2). TRIB2 is a pseudo serine/threonine kinase that has shown to have a critical role in tumorigenesis and resistance to therapy. Higher TRIB2 levels seem to be associated with a worst prognosis in several solid tumors, including PC. However, the potential role of TRIB2 in pancreatic cancer chemoresistance remains unclear.

Material and method

To unveil the potential role of TRIB2 in PC, we generated gemcitabine-resistant cells. To achieve this, we treated the cells with consecutive cycles of 2',2'-difluoro-deoxycytidine (gemcitabine), increasing the dosage each time, over the course of 9 months. We further characterized these cells regarding their resistance, proliferation, cell death and wound-healing ability and analyzed TRIB2 mRNA and protein expression using RT-PCR and Western Blot techniques.

Result and discussion

During the generation of the resistant cells, we observed that gemcitabine induced morphological changes in the cells, that switch from an epithelial to a fibroblastic-like phenotype. However, this switch was reversible over time in the absence of the drug. Using the protocol described, we were able to generate resistant cells,

increasing their half maximal inhibitory concentration (IC₅₀). These cells seem to present lower cell death and higher cell viability when acutely treated with gemcitabine when compared with the parental cell line, but also an unexpected decreased migration ability (15-hour assay). Analysis of TRIB2, at the transcriptional and protein levels, revealed an increased TRIB2 expression in the resistant cell lines, when compared to the parental cells, which showed little to no expression of this protein. Higher levels of TRIB2 were detected in the emerging phenotypes, during the phenotypic switch, while the cells were being treated with gemcitabine.

Conclusion

Our results indicate a potential role of TRIB2 in the mechanism behind gemcitabine-resistance in PC, that could hypothetically involve the phenotypic switch during the generation of the resistant cells. With this project, we aim to investigate the role of TRIB2 in this mechanism and study its impact and clinical relevance in PC patient's standard of care.

EACR25-0711

Identifying Therapeutic Vulnerabilities in KRASG12D-Resistant Pancreatic Cancer Using High-Throughput Drug Screening

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Introduction

Pancreatic ductal adenocarcinoma (PDAC) is an aggressive malignancy with poor prognosis, driven predominantly by the KrasG12D mutation. Targeting KRASG12D with inhibitors such as MRTX1133 has shown promise, but resistance mechanisms including pathway rewiring limit efficacy. This study aims to identify therapeutic vulnerabilities of KrasG12D resistant PDAC models.

Material and method

A large and representative panel of molecularly characterized cell lines derived from murine PDAC (mPDAC) tumors were employed to identify resistant cell lines towards KrasG12D inhibition. Uniform manifold approximation and projection (UMAP) analysis from bulk RNA sequencing (RNA-seq) was used to assess transcriptomic diversity within the cohort. Drug response to MRTX1133 was conducted across 55 cell lines using a 3-fold 5-point concentration. Following seeding, cells were treated for 72 hours, and cell viability was measured using the CellTiterGlo assay. A systematic high-throughput combinational drug screen was conducted using two resistant mPDAC cell lines. The drug library consisted of DMSO controls and 95 compounds in preclinical and clinical investigation

targeting key oncogenic pathways. Drug interactions were assessed using ΔAUC-based synergy scoring, and top candidate synergistic drugs were further validated using colony formation assays. Bliss synergy score was used to assess the synergism between drug combination.

Result and discussion

Our mPDAC panel includes both KrasG12D-driven cell lines ($n = 51$) as well as Pi3k-driven cell lines ($n = 4$) to confirm the specificity of targeting KrasG12D. Furthermore, the panel encompasses key genetic alterations commonly found in PDAC, including Cdkn2a (p16), Tp53, Smad4, and Tgfb2 as well as a balanced representation of PDAC subtypes: epithelial, quasi-mesenchymal, and mesenchymal. Pi3k-driven cell lines showed resistance – thus underscoring the target specificity of MRTX1133 to KRASG12D – while KrasG12D-driven cell lines showed heterogeneous response. To investigate the resistance mechanism and identify the drugs synergizing with KrasG12D inhibition, we performed a systematic high-throughput combinational drug screen using two mesenchymal resistant mPDAC cell lines established in our lab. We found that Nintedanib, a multi-kinase inhibitor, was among the top synergistic hits. Clonogenic assays validated its synergistic effect in combination with MRTX1133 as bliss synergy scores above 10 in both cell lines.

Conclusion

Our study highlights the heterogeneity of KRASG12D-driven PDAC towards MRTX1133. Through high-throughput drug screening, we found that Nintedanib synergizes with MRTX1133 to overcome resistance in mesenchymal PDAC cell lines. These findings provide a potential combination strategy to improve therapeutic outcomes in KRASG12D-mutant PDAC.

EACR25-0713

Potential therapeutic implication of Epithelial Protein Lost in Neoplasm (EPLIN) in head and neck cancer and chemotherapy resistance

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Introduction

Epithelial Protein Lost in Neoplasm (EPLIN), also known as LIMA1, is an actin-binding protein implicated in cytoskeletal remodelling and tumour progression. While traditionally considered a tumour suppressor, emerging evidence suggests that EPLIN plays a potential oncogenic role in head and neck cancer (HNC). EPLIN has been linked to enhanced invasion and metastasis, but its role in drug resistance remains unclear in HNC. This study aims to investigate the relationship between EPLIN expression and drug response in HNC.

Material and method

EPLIN-overexpressing (Exp) and knockdown (KD) human head and neck cell models were established to evaluate the impact of EPLIN on drug sensitivity. Potential EPLIN-binding drugs were screened through molecular modelling and docking studies. Cytotoxicity assays were performed to assess cellular responses to chemotherapy (docetaxel, paclitaxel, neratinib, and cisplatin) and candidate drugs (digoxin). EPLIN expression levels in HNC tissues and their correlation with patients' drug response were evaluated using public datasets.

Result and discussion

Cell-based drug sensitivity evaluation indicated that high levels of EPLIN in HNC cells rendered resistance to cisplatin ($p = 0.01$) and, to a limited degree, to docetaxel ($p = 0.09$). The relationship between EPLIN and docetaxel response was further validated in HNC cell models. EPLIN knock down sensitised the cells to docetaxel with a decreased IC₅₀ at 1.04 μ M compared with control cells at 2.62 μ M. Conversely, EPLIN overexpression resulted in an increased IC₅₀ for docetaxel (1.91 μ M) compared to WT cells (0.7 μ M), indicating an elevated resistance to the drug. Digoxin, identified through molecular docking as a potential EPLIN-interacting drug, exhibited EPLIN expression-dependent drug resistance in HNC cells. Upon EPLIN knockdown, the IC₅₀ of digoxin in HNC cells was significantly reduced (98.48 μ M vs. 2.95 μ M). Conversely, EPLIN overexpression led to an increased IC₅₀ compared to WT cells (7.15 μ M vs 17.85 μ M), suggesting that EPLIN may contribute to digoxin resistance in HNC. These results highlight EPLIN's potential role in modulating digoxin sensitivity and its implications in therapeutic resistance. After EPLIN knockdown, HNC cells treated with 10 μ M digoxin exhibited further reductions in proliferation, adhesion, migration, and invasion, suggesting that EPLIN knockdown enhances the cytotoxic effects of digoxin and may contribute to increased drug sensitivity in HNC.

Conclusion

The present study is the first to report the connection between EPLIN expression and drug resistance in HNC, demonstrating that high EPLIN levels contribute to resistance against docetaxel, while EPLIN knockdown enhances drug sensitivity. Notably, digoxin exhibited significant EPLIN-dependent drug resistance, and this relationship provides a new potential therapeutic direction for the treatment of head and neck cancer.

EACR25-0739

Combination therapy with YAP/TEAD and RAS inhibitors overcomes phenotypic cell plasticity-driven resistance in NRAS-mutated melanoma

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Introduction

Melanoma is the most aggressive skin cancer, characterized by remarkable cancer cell plasticity, contributing to intra-tumoral heterogeneity and therapeutic resistance. NRAS-mutant melanoma remains a clinical problem, particularly in patients who do not respond to immunotherapies. As a second-line option, MEK inhibitors as single agents fail to provide a significant overall survival benefit. Therefore, there is an unmet need for new therapeutic strategies to improve the management of NRAS-mutant melanoma. Here we assessed in vitro and in vivo the response of NRAS-mutant melanoma cells to RMC-6236, a novel non-covalent inhibitor of both oncogenic and wild type RAS isoforms currently undergoing clinical investigation in various cancers.

Material and method

Loss-of-function approaches using RMC-6236, IAG933 (a YAP-TEAD interaction inhibitor), or siRNAs were employed to evaluate the impact of NRAS inhibition on phenotypic adaptation (RNA-seq, RT-qPCR, western blot analyses) as well as cell proliferation and survival (colony formation assay, flow cytometry) in human and murine NRAS-mutant cell line models. A murine melanoma model using MaNRAS cells grafted into syngeneic C57BL/6 mice was used to assess the effect of RMC-6236 on tumor growth and mouse survival.

Result and discussion

Our transcriptomic and proteomic analyses revealed that the anti-proliferative effect of RMC-6236 on NRAS-mutant melanoma cell lines is characterized by a phenotypic transition towards a less differentiated state, with increased expression of mesenchymal and extra-cellular matrix remodeling markers, along with the activation of a YAP-driven transcriptional signature and focal adhesion kinase (FAK) signaling. In vivo RMC-6236 slowed tumor growth and improved mouse survival. Melanoma cells treated with RMC-6236 in vivo exhibited reduced pigmentation and expressed mesenchymal and neural crest stem cell markers and YAP-target genes. The combination of RMC-6236 and IAG933 synergistically reduced proliferation, prevented phenotypic transition, and induced apoptosis in NRAS-mutant cells. These findings suggest that YAP-TEAD pathway inhibition by IAG933 targets the adaptive response induced by RMC-6236 and enhances treatment efficacy in vitro.

Conclusion

NRAS inhibition in melanoma cells induces a mesenchymal phenotypic transition linked to YAP pathway activation. YAP/TEAD inhibition can overcome resistance to NRAS inhibition by preventing adaptive phenotype switching and inducing tumor cell death. This work provides a scientific rationale for treating NRAS-mutant melanomas with a combination of RAS and YAP-TEAD inhibitors.

EACR25-0755

The interplay between mechanisms of resistance and therapeutic targets: identifying novel treatments for drug-resistant BRAFV600E pediatric high-grade glioma

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Introduction

The BRAFV600E mutation has been identified as a driver mutation in a subset of pediatric high-grade gliomas (pHGG). These tumors initially respond well to targeted BRAF and MEK inhibitors. Unfortunately, resistance inevitability develops, resulting in disease progression. There is limited understanding of the mechanisms underlying drug resistance in BRAFV600E pHGG, and no effective counter-therapies exist. This study aimed to identify drivers of resistance in BRAFV600E pHGG, enabling the identification of novel therapeutic targets.

Material and method

BRAF inhibitor-resistant, MEK inhibitor-resistant and BRAF+MEK inhibitor-resistant BRAFV600E pHGG cultures were derived through chronic exposure of a BRAFV600E patient-derived culture to vemurafenib (BRAFi), trametinib (MEKi) or a combination of both drugs, respectively. An omics-based approach (RNAseq/proteomics) was undertaken to identify mechanisms of resistance, allowing the subsequent identification of therapeutic targets.

Result and discussion

In vitro cytotoxicity assays confirmed the resistant nature of the BRAFV600E pHGG cultures. Similarly, dabrafenib (BRAFi)-treated animals orthotopically administered with the vemurafenib-resistant culture had significantly reduced survival compared to treated animals administered with the matched parental cells (65 days vs 79 days). All three resistant cell lines spontaneously transformed from a spheroid phenotype to adherent growth; proteomics pathway analysis correspondingly identified enrichment of extracellular matrix and cytoskeletal pathways. RNAseq identified >1500 differentially expressed genes in each resistant cell line, being enriched for neural development and plasma membrane/cell adhesion. Furthermore, the receptor tyrosine kinase (RTK) gene sets identified potent upregulation of several RTKs in vemurafenib-resistant cells, including NTRK2, SRC and EGFR, suggesting a key mediator of the acquired resistance and providing promising therapeutic targets. Repotrectinib (NTRK2/SRCi) and dacomitinib (EGFRi) were found to display potent synergistic activity when used in combination with vemurafenib (BRAFi) against both vemurafenib-resistant BRAFV600E pHGG cells and matched parental cells in vitro. EGFR/BRAF/MEK inhibition produced an even more potent triple combination therapy. Furthermore, scaffold proteins, integral to BRAF dimerisation, were also upregulated; the pan-RAF inhibitor, tovorafenib,

subsequently displayed potent synergy in combination with trametinib (MEKi).

Conclusion

This study has demonstrated that analyzing tumor mechanisms is a powerful tool for identifying effective therapies in treatment-refractory tumors. Using an omics-based approach in BRAFV600E pHGG, we have identified novel drivers of drug resistance, key therapeutic targets and several promising therapeutic options.

EACR25-0764

Targeting low fidelity polymerases to prevent acquired resistance mutations to EGFR-targeted therapy in lung cancer

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Introduction

Since the development of small-molecule inhibitors targeting mutated EGFR, lung cancer patients' outcomes have significantly improved. Nonetheless, tumors eventually develop resistance to these inhibitors, resulting in ineffective therapy and relapse. A major mechanism behind therapy resistance is the emergence of new mutations leading to amino acid substitutions in EGFR, such as T790M and C797S, which evade the inhibition. Although it is clear that EGFR-inhibitors cause an increased mutagenesis, the DNA damage mechanism behind the emergence of specific resistance mutations remain poorly understood. Aim: In this project, we study the role of low-fidelity polymerases (TLS) in the emergence of de novo resistance mutations in NSCLC.

Material and method

Frequency of resistance mutations was determined by digital droplet PCR (ddPCR); NSCLC cell lines were treated for 10 or 28 days with EGFR-TKI alone or combined with other pharmacological inhibitors. At appropriate time points, gDNA was harvested and subjected to ddPCR for T790M or C797S quantification using Taqman probes (BioRad). To determine cell viability, cells were incubated for 72h with the appropriate drug treatment, and cell viability was assessed by MTT assay. Gene expression levels were determined by RT-PCR. REV3L knock down was achieved by 2nd generation lentiviral transduction.

Result and discussion

We established an in vitro ddPCR assay to detect the mutations in a highly sensitive way, following their emergence over a 28-day time frame. As C797S was detected in several NSCLC cell lines prior to drug treatment, we are currently performing a fluctuation assay to distinguish colonies derived from pre-existing resistant clones, and de novo induced resistance mutations. Following TKI-exposure, REV3L, REV1 and other TLS polymerases were upregulated in PC9 cells. JH-RE06, a REV1-REV7 inhibitor that previously showed superiority in melanoma and colorectal cancer, did not affect the sensitivity of PC9 cells to erlotinib or osimertinib (MTT assay), however might prevent the emergence of resistance mutations. Similarly, REV3L and REV1 knockdown cell lines have been established,

and will be assessed for their mutability in response to EGFR-TKIs in vitro.

Conclusion

If our promising preliminary results of TLS inhibition preventing resistance mutations will be confirmed, *in vivo* PDX studies will be performed to further assess the efficiency of JH-RE06 to prevent relapses in NSCLC. This work will pinpoint new opportunities for drug development to overcome the current major drawback of anti-EGFR therapy and will overall aid in improving lung cancer patients' survival outcomes.

EACR25-0781

Targeting ErbB receptor family to intercept therapy adaptation in ALK-positive lung cancer

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Introduction

Anaplastic lymphoma kinase (ALK) translocations are found in about 3–5% of non-small cell lung cancer cases. Lorlatinib is a potent third-generation anti-ALK tyrosine kinase inhibitor (TKI) particularly effective in ALK-rearranged patients. Although lorlatinib is able to overcome acquired resistance to first- and second-generation ALK inhibitors, a fraction of patients treated in first line with this inhibitor rapidly relapse, suggesting early adaption to the third-generation TKI.

Material and method

ALK-rearranged H3122 cell line was interrogated by Human Phospho-RTK Array to define potential mechanisms of bypass signaling involved in adaptability to lorlatinib therapy. Western blot, Real-Time PCR, cell cycle and crystal violet analyses were performed to study and characterize the presence of drug tolerant cells upon lorlatinib treatment. H3122 cells were genetically engineered to express both Luciferase and EGFP (H3122-EGFP/Luc) for monitoring overtime the effects of single and combo regimens on drug tolerant cell survival and evolution.

Result and discussion

Lorlatinib treatment of H3122 cells resulted in growth arrest and apoptosis of the bulk population. However, a subpopulation, which we termed lorlatinib tolerant persisters (LTPs) rapidly emerged. LTPs were characterized by the upregulation, at the transcriptional and post-transcriptional level, of ErbB family members. The upfront combination treatment of the bulk population with the pan-ErbB inhibitor afatinib along with lorlatinib severely impacted on LTP viability. The long-term efficacy of single and combo regimens was then tested in hundreds of small pools of LTP-derived colonies that emerged upon lorlatinib treatment in 96-well plates. After

5 months of live cell tracking, the combination treatment completely eradicated 80% of LTP-derived colonies.

Conclusion

Overall, these data suggest that in a fraction of ALK-rearranged NSCLC tumors, drug tolerant cells could exploit ErbB signaling to overcome lorlatinib treatment. Targeting the ErbB family in combination with lorlatinib may represent a valuable therapeutic strategy to improve the clinical outcome of these patients by interfering with LTP survival and evolution.

EACR25-0797

The Role of NRG-1 in Resistance to Targeted Cancer Therapies in ERBB2-Amplified Breast Cancer

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Introduction

Receptor tyrosine kinases (RTK) and their ligands frequently play a crucial role in oncogene-addicted cancers. Consequently, targeting RTKs has become a cornerstone of cancer therapy, significantly improving survival rates. However, both intrinsic and acquired resistance to RTK inhibitors present significant challenges, necessitating further research to optimize therapeutic strategies. Among the various mechanisms of resistance, the role of RTK ligands remains critical, yet underexplored. This study aims to investigate the impact of neuregulin-1 (NRG1), an ERBB3 ligand, as a micro-environmental factor that confers resistance to ERBB inhibitors in ERBB2-amplified breast cancer.

Material and method

To validate and quantify NRG1-induced resistance, ERBB2-amplified breast cancer cell lines (BT-474 and SKBR3) were treated with ERBB2-targeting tyrosine kinase inhibitors (TKI) and analyzed with various cell-based assays, protein analysis techniques, and functional genomics approaches. These included cytotoxicity assays, Western blotting, high-throughput phosphoprotein arrays, and kinase- and phosphatome-wide siRNA screening.

Result and discussion

In ERBB2-amplified breast cancer cell lines, the phenomenon of NRG1-induced resistance was recapitulated with various ERBB2-targeting TKIs. Based on the findings, ERBB3 appears to be the key player in NRG1-induced resistance, despite being widely recognized as a pseudokinase that requires ERBB2 for activation. Interestingly, our data suggest that ERBB3 remains active in the presence of NRG1, even when the phosphorylation of other ERBB family members (EGFR, ERBB2, and ERBB4) is effectively inhibited by a pan-ERBB inhibitor such as neratinib. We hypothesize that NRG1-ERBB3-induced resistance may occur independently of ERBB2 activation, potentially involving previously uncharacterized kinase signaling pathways.

Conclusion

These findings highlight a critical mechanism of resistance that could inform the development of more effective therapeutic strategies.

EACR25-0897**Unraveling the Hidden Player: miR-188-5p at the Crossroads of DDR and PARP****Inhibitor Resistance in Ovarian Cancer**

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Introduction

MicroRNAs (miRNAs) play a critical role in chemo-resistance to poly(ADP-ribose) polymerase (PARP) inhibitors by modulating DNA damage response (DDR) pathways. PARP inhibitors (PARPi) induce synthetic lethality in homologous recombination (HR)-deficient tumors, particularly BRCA1/2-mutant ovarian cancers. However, resistance emerges through mechanisms such as HR restoration and activation of alternative DNA repair pathways. While the role of miR-188-5p in DDR and PARPi resistance remains unclear, previous studies suggest its involvement in tumor progression via the AKT/mTOR pathway. However, its specific role in high-grade serous ovarian cancer (HGSOC) is largely unexplored. This study aims to elucidate the regulatory network of miR-188-5p and its potential as a therapeutic target in PARPi-resistant HGSOC.

Material and method

To identify differentially expressed miRNAs in drug-resistant versus sensitive ovarian cancer models, we performed microarray and qRT-PCR analyses on in vitro and ex vivo cultured cells, as well as formalin-fixed paraffin-embedded (FFPE) patient samples [1]. Olaparib (Lynparza®)-resistant cell lines were transfected with miR-188-5p mimics using Lipofectamine®. Apoptosis and DDR mechanisms were assessed via Annexin-V/PI staining (flow cytometry), p-H2AX immunofluorescence staining, and expression analysis of DDR and apoptosis-related genes/proteins using Western blot and qPCR.

Result and discussion

miR-188-5p expression was significantly downregulated in olaparib-resistant OVCAR-3 cells, resistant FFPE HGSOC patient samples, and ex vivo patient-derived cultures. Combination therapy with olaparib and miR-188-5p mimics resensitized resistant cells in vitro and ex vivo, inducing apoptosis via caspase cascade activation, as evidenced by an increase in cleaved-caspase-3 and cleaved-PARP expression. Western blot analysis revealed decreased p-AKT levels in miR-188-5p mimic-transfected resistant cells, indicating suppression of survival pathways. This AKT inhibition led to Bid and Bim upregulation, promoting mitochondrial apoptosis. Additionally, p-AKT suppression triggered ATR phosphorylation, enhancing γ-H2AX-mediated DDR activation.

Conclusion

This study provides new insights into miRNA-mediated drug resistance mechanisms and their potential for enhancing PARP inhibitor efficacy in HGSOC. Our findings suggest that miR-188-5p re-expression restores DDR activation and apoptosis, overcoming chemo-resistance. These results highlight the therapeutic potential of miRNA-based strategies to improve patient

outcomes and combat chemotherapy resistance in clinical settings. The authors declare no financial conflicts of interest related to this study.

[1] Koç University Ethics Committee Approval: 2019.257.IRB2.079

EACR25-0923**Decoding drug resistance in cervical cancer cell lines: an AKT-independent mechanism**

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Introduction

Activation of the Fibroblast Growth Factor Receptor (FGFR) axis is important in cell proliferation, differentiation, migration and cell survival and its aberrant signalling is associated with cancer progression and drug resistance. The Aim of this study was to identify mechanisms of drug resistance in cervical cancer by generating three drug resistant (DR) human cervical cancer cell lines (HCCCL; Caski, HeLa and SiHa) to an FGFR inhibitor, PD173074 (PD).

Material and method

Transcriptomic analysis was performed to detect differentially expressed genes (DEGs) and protein-protein interactions between DR and parental HCCCLs. Findings were validated and signalling pathways further investigated via PCR, immunocytochemistry and western blot. The functional effect of altering these signalling pathways were assessed by cell proliferation, apoptosis and migration assay.

Result and discussion

Transcriptome analysis identified several DEGs in DR HCCCL. Protein-protein interaction modelling revealed that integrin alpha 2 interacts with the products of most DEGs shared by all three DR HCCCL. Phosphorylated FAK was localised in both cytoplasm and nucleus and increased in all three DR HCCCL compared to parental counterparts. In addition, in DR cells, integrin alpha 2 mainly localised to the cytoplasm, unlike the parental cells in which it was cell surface expressed. However, they exhibited higher levels of cytoplasmic expression compared to parental. Biochemical studies revealed no difference in AKT phosphorylation at either Ser(473) or Thr(308) sites in the presence or absence of PD after FGF2/4/7 stimulation in both parental and DR cells. However, Phospho-S6 was more abundant in DR cell lines compared to parental, independent of PD treatment. FAK phosphorylation and integrin alpha 2 protein levels were higher in all DR cells. FGF2 stimulation, in the presence of a FAK inhibitor, abolished FAK phosphorylation in parental cells, but it remained unchanged with or without inhibitor in all DR cells. S6 phosphorylation was elevated in DR cell lines compared to parental, with or without FAK inhibitor treatment. Interestingly, after FGF2 stimulation, phosphorylation of S6 was abolished in parental cells upon mTORC1 inhibition but remained unchanged in all DR cells.

Conclusion

Our findings suggest that activation of FAK and AKT independent mTOR pathways, as well as integrin alpha 2 protein expression, might play a key role in mediating drug resistance in HCCCLs.

EACR25-0975

Rituximab resistance shapes sensitivity to effector-cell mediated cytotoxicity in B-cell lymphoma

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Introduction

For over 30 years, rituximab (RTX) has played a pivotal role in the immunotherapy of CD20-positive B-cell lymphomas. Despite its efficacy, resistance develops in over one-third of patients, posing a significant clinical challenge. Although extensively studied, the mechanisms underlying RTX resistance remain unclear. Notably, our characterization of RTX-resistant cell lines revealed reduced sensitivity to effector-mediated cytotoxicity. This study aimed to identify molecular mechanisms responsible for this decreased sensitivity in response to effector cells such as NK and CAR-T cells.

Material and method

Three established B-cell lymphoma cell lines (SU-DHL4, Raji, RL) with acquired RTX resistance were used as in vitro models. Fluorochrome-conjugated antibodies were used to assess changes in the expression of activating and inhibitory ligands, adhesion molecules, and death receptors on the surface of these cells. Sequential stages of recognition and killing by primary NK and NK92 cells were analyzed using flow cytometry-based methods to evaluate degranulation, conjugate formation, and detachment. NK cell cytotoxicity was assessed with inhibitors targeting perforin (concanamycin A), granzyme B (3,4-dichloroisocoumarin), caspases (zVAD), and caspase 8 (z-IETD). RNA sequencing was employed to identify differentially expressed genes and signaling pathways.

Result and discussion

RTX-resistant cell lines exhibited heterogeneous surfaceome alterations:

- (i) key NK cell activating ligands (MICA/B, ULBPs, PVR, CD112, B7H6) were either unchanged or absent;
- (ii) among inhibitory ligands, only MHC class I was upregulated but did not affect NK cell sensitivity; and

(iii) adhesion molecules (CD54, CD58, CD59, CD102) and death receptors (TRAIL-R, Fas) varied across cell lines.

These alterations did not contribute to reduced sensitivity to effector cells. NK and NK92 cells retained their ability to degranulate, form conjugates, and detach from RTX-resistant cells at levels comparable to wild-type counterparts, indicating intact target-effector interactions. Studies with selective inhibitors demonstrated that NK cell mediated cytotoxicity primarily depends on degranulation and effective delivery of perforin and granzyme B. RNA sequencing further revealed differentially expressed apoptosis-related signaling pathways, with key resistance-associated proteins, including Bax, Bak, and Bcl-2 family members, involved in mitochondrial apoptosis.

Conclusion

Decreased sensitivity of RTX-resistant B-cell lymphoma cell lines to effector cells is driven by deregulation of apoptosis-related pathways, rather than defects in target effector cell interactions.

Funding: National Science Centre Poland: 2022/45/N/NZ6/01691, 2023/05/Y/NZ6/00174

EACR25-0984

Inhibition of the extracellular signal-regulated kinase 5 (ERK5) increases EGFR expression and its nuclear localization in HCC, generating new targeting opportunities

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Introduction

Hepatocellular Carcinoma (HCC) is the most common form of liver cancer and accounts for 90% of cases. Nonetheless, available molecular-targeted therapies are still unsatisfactory, so additional therapeutic targets must be identified. The extracellular signal-regulated kinase 5 (ERK5) is a member of the Mitogen-Activated Protein Kinases (MAPK) family, and is highly expressed in hepatocytes, and its gene has been reported to be amplified in HCC. Moreover, we recently reported that ERK5 regulates the development and growth of HCC, so that ERK5 targeting appears as a promising approach for the treatment of this type of cancer.

Material and method

The aim of this study was to investigate the interplay between ERK5 and EGFR using two hepatocellular carcinoma cell lines, Huh7 and HepG2. Gene silencing was performed with short hairpin RNA for ERK5. For the pharmacological treatments, ERK5 (JWG-071), EGFR (Gefitinib) α/β importin (Ivermectin) inhibitors effects were evaluated after 72h of treatment in term of cell viability using MTT assay. The mRNA expression was analyzed using quantitative RT-PCR. Protein expression levels and intracellular localization were detected by Western blot and immunofluorescence in confocal microscopy.

Result and discussion

We found that ERK5 knock down (KD) increases EGFR mRNA and protein levels, and downstream PI3K/AKT activation. Furthermore, the amount of EGFR protein was increased in cells treated with the ERK5 inhibitor, JWG-071, compared to control cells. The combination of JWG-071 and the EGFR inhibitor Gefitinib resulted to be more effective than single treatments in reducing cell viability and 3D spheroid growth in both cell lines. In addition, nuclear EGFR was higher in ERK5-KD cells compared to controls. Since EGFR shuttles to the nucleus via the α/β importin system, we performed combined treatment of JWG-071 and the α/β importin inhibitor, Ivermectin. This cotreatment showed a higher anti-proliferative effect than single drugs on HCC cell lines.

Conclusion

In conclusion, these results revealed new potential therapeutic strategies to explore for the treatment of HCC.

EACR25-0987

Crosstalk between oncogenic signaling pathways as driver of therapy resistance against small molecule inhibitors in Glioblastoma

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Introduction

Glioblastoma (GBM) is the most prevalent primary brain tumor in adults, with a median survival of 14 months despite multimodal treatment. Small molecule inhibitors (SMIs) targeting mutational drivers, such as the RAS/MEK/ERK-pathway, offer potential therapeutic avenues, yet clinical trials have not improved GBM survival outcomes. Using patient-derived GBM models (pdGBMs), we evaluated the cytotoxicity of SMI mono- and combi therapy and explored aberrant signaling pathways driving treatment resistance. Our analysis specifically focused on combinations with trametinib, which demonstrated the highest cytotoxic potential.

Material and method

Eight BBB-penetrable and clinically approved SMIs were used to target key GBM driver oncogenes. Cell viability was measured via CellTiter-Glo and the IC₅₀ for each inhibitor was determined for six molecularly distinct pdGBMs. Changes in phosphokinase activity after SMI monotherapy were assessed via WB and phosphokinase screening. Based upon these results, SMI combinations were made, targeting compensatory pathways driving treatment resistance. Bliss scores were established to test synergistic SMI combinations.

Result and discussion

SMIs targeting downstream effector kinases (Trametinib, Buparlisib, Abemaciclib; IC₅₀ 70nM-1μM) or

membrane-bound tyrosine kinase receptors (Afatinib, Capmatinib, Axitinib; IC₅₀ 1-15μM) reduced cell viability. However, residual cell proliferation was seen in all pdGBMs following single-agent SMI treatment, indicative for clonal expansion of treatment resistant tumor cells. Western blot analysis confirmed on-target drug activity. Subsequently, MEK-inhibition with trametinib induced compensatory signaling, including MEK/AKT-crosstalk, JNK/c-JUN upregulation and most notably MEKSer221 hyperphosphorylation. Combined trametinib and SP600125 (JNKi) mitigated JNK/c-Jun activation and synergistically reduced cell viability (Bliss >20), but failed to suppress MEKSer221 hyper-phosphorylation. In a small-scale SMI drug screen, trametinib combined with VEGFRi, axitinib significantly abrogated MEKSer221 hyperphosphorylation and led to a synergistic reduction in cell viability (Bliss >20). In contrast, co-treatment with Afatinib (EGFRi) or CHIR99021 (GSK3βi) did not exhibit synergistic effects (Bliss <10).

Conclusion

Trametinib reduces cell viability but triggers compensatory signaling, including MEKSer221 hyperphosphorylation. This study presents a mechanistically driven selection of tumor-tailored combination treatments to overcome resistance to SMI monotherapy in pdGBM models, highlighting VEGFR co-inhibition as a promising combinatorial strategy with trametinib.

EACR25-1015

Targeting Drug Resistance in Hepatocellular Carcinoma: Uncovering Metabolic Vulnerabilities for Therapeutic Re-Sensitization

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Introduction

Hepatocellular carcinoma (HCC) is the most common form of primary liver cancer, often arising from chronic liver inflammation due to hepatitis B virus, hepatitis C virus, alcohol-related liver disease (ALD), or metabolic-dysfunction-associated steatotic liver disease (MASLD). Current treatment options for advanced HCC include multi-kinase inhibitors (e.g., Lenvatinib, Sorafenib and Regorafenib) and immune checkpoint inhibitors, but these therapies are limited by drug resistance, which significantly reduces patient survival. Identifying novel vulnerabilities in resistant HCC cells is crucial to restoring treatment efficacy.

Material and method

To model drug-resistant HCC, three HCC cell lines (Hep3B, Huh7, Huh1) were adapted to a modular physiologic medium (MPM) recently established in our lab, providing a more physiologically relevant culture system. Dose-response experiments in the presence of key growth factors were performed to determine sensitivities to five kinase inhibitors: Lenvatinib, Sorafenib, Regorafenib, Roblitinib, and Erdafitinib, the latter two being fibroblast growth factor receptor inhibitors currently in clinical trials. To mimic tumour

conditions, HCC-oriented media enriched with growth factors were optimized to promote spheroid formation.

Result and discussion

Initial results demonstrated that MPM adaptation altered drug response patterns, underscoring the importance of physiologically relevant conditions. Next steps include the screening of a metabolism-oriented library to highlight metabolic vulnerabilities that could be leveraged to overcome resistance. The screen will identify promising candidate compounds capable of re-sensitizing resistant cells to kinase inhibitors. Further, advanced 3D models incorporating extracellular matrix, hypoxia, and other cell types (fibroblasts and endothelial cells) will be utilized to refine therapeutic windows and validate drug combinations in conditions that better reflect the tumour microenvironment.

Conclusion

This study aims to provide a framework for targeting metabolic vulnerabilities in HCC, offering potential new therapies to enhance treatment efficacy and to break or delay drug resistance. By integrating physiologically relevant culture systems and metabolic reprogramming strategies, future findings could contribute to the development of more effective interventions for overcoming therapeutic resistance in HCC.

EACR25-1057

Investigating estrogen receptor fusion proteins as a mechanism of resistance to endocrine therapies in breast cancer

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Introduction

Breast cancer is the most common cancer worldwide, with approximately 80% of cases classified as estrogen receptor-positive (ER+). In these cancers, estrogen activates ER, driving tumour growth through gene regulation. Endocrine therapies (ET) targeting ER are the standard-of-care treatment for ER+ breast cancer. However, over 20% of patients develop drug-resistant disease, highlighting the need for alternative therapeutic strategies. Chromosomal translocations involving ESR1, the gene encoding ER, have emerged as a resistance mechanism. These translocations generate ER fusion proteins that lack the ligand-binding domain, making them insensitive to ET, including selective ER down-regulators (SERDs), such as fulvestrant. Understanding the mechanisms of action of ER fusion proteins will be essential for developing appropriate therapeutic approaches.

Material and method

Towards this end, we have generated breast cancer cell lines expressing the ESR1-YAP1 fusion using CRISPR/Cas9 genome editing. RNA sequencing was performed on these models alongside wild-type ESR1 cells treated with fulvestrant or vehicle-control. Differential gene expression was analysed using DESeq2 and gene set enrichment analysis using Hallmark and breast cancer-specific gene sets identified enriched pathways. Heatmaps were generated using pheatmap with hierarchical

clustering and Z-score normalisation. Growth assays assessed cell proliferation in response to ET.

Result and discussion

ESR1-YAP1 fusion clones showed reduced expression of estrogen response gene sets, suggesting reduced estrogen-driven transcription, while showing positive enrichment for proliferation gene sets, indicating enhanced ligand-independent growth signalling. In fulvestrant-treated cells, the expression of estrogen-responsive genes was maintained in ESR1-YAP1 cells, whereas it was reduced in ESR1 wild-type cells. Consistently, the growth of ESR1-YAP1 cells was refractory to inhibition by ETs, including fulvestrant.

Conclusion

Our work underscores the potential role of ER fusions in mediating resistance to ET in breast cancer. Such models represent powerful isogenic models for investigating mechanisms by which ESR1 fusion genes drive breast cancer cell growth and offer the potential for identifying alternative treatment strategies following the emergence of ER fusion-directed resistance to ETs.

EACR25-1105

Increased Sensitivity to BCL-2 Inhibition in EGFR-TKI Resistant NSCLC Cell Lines

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Introduction

Among cancer entities lung cancer ranks first in incidence and mortality, with ~85% of cases classified as non-small cell lung cancer (NSCLC). In lung cancer, driver mutations are commonly found in receptor tyrosine kinases with Epidermal Growth Factor Receptor (EGFR) constituting the major driver of oncogenesis (29%, Chevallier et al. 2021). Mutant EGFR is targeted by multiple specific tyrosine kinase inhibitors (RTKis) for anti-cancer therapy. Therapy resistance against such RTKis and subsequent disease progression occurs frequently and remains a severe challenge. A promising approach to overcome resistance is the combination of RTKi with BH3-mimetics or proteasome inhibition (Muenchow et al. 2021, Tanimoto et al. 2021, Weller et al. 2022). The proposed mechanism is the induction of NOXA, which inhibits the anti-apoptotic Bcl-2 protein MCL-1. In this study, we assess the role of BCL-2 proteins in RTKi resistance in NSCLC cell lines and the effect of direct BCL-2 inhibition.

Material and method

NSCLC cell lines of differing EGFR mutation status (HCC4006 [ex19del] and H1975 [L858R/T790M]) were cultured in the presence of EGFR-TKI osimertinib (OSI) to generate EGFR-TKI resistant cell lines. Successful generation of resistance was verified via flow cytometric analysis of apoptotic cell death induction and via live-cell imaging-based proliferation assessment. Expression of Bcl-2 proteins was analyzed by RNAseq and Western Blot. Cells were incubated with OSI and/or Bcl-2 inhibitors and apoptotic cell death induction as well as cell viability were measured by flow cytometry and luminescence-based assays.

Result and discussion

RNAseq revealed an upregulation of BCL-2 in the RTKi resistant cell lines and derived clonal cell lines suggesting a resistance mechanism. We found resistant cell lines to be more sensitive to apoptosis induction via BCL-2 inhibition than parental cells, highlighting their dependency on anti-apoptotic proteins. Especially BCL-xL- and MCL-1-specific inhibitors demonstrated strong efficacy in combination with RTKi in NSCLC cells and RTKi-resistant NSCLC cells. Furthermore, combination of osimertinib with BCL-2i was sufficient to overcome RTKi resistance likely mediated by BCL-2 overexpression.

Conclusion

We find that RTKi resistant NSCLC cell lines show increased sensitivity towards inhibition of Bcl-2 proteins and that RTKi resistance of NSCLC cells is overcome through inhibition of these proteins by specific Bcl-2 inhibitors. These findings represent a general therapeutic approach that relies on the simultaneous blockade of driver mutation mediated EGFR activity and survival-promoting Bcl-2 proteins.

EACR25-1107

SPOCK1 is Linked to Mesenchymal-like Features and Drug Resistance in Melanoma Cells Treated with BRAF and MEK Inhibitors

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Introduction

In cutaneous melanoma, 52% of cases harbour mutations in the BRAF gene. Although targeted therapy, which involves the combination of BRAF and MEK inhibitors (BRAFi+MEKi), has revolutionized the treatment of BRAF-mutated melanoma, most patients experience disease progression within 8–12 months due to acquired drug resistance. Additionally, there are poor responders to immunotherapy-based second-line treatments. Understanding resistance mechanisms is thus of significant clinical interest. A previous study, using an *in vitro* resistance model revealed that BRAFi+MEKi resistance acquisition involves the enrichment of clones over-expressing genes characteristic of invasive and mesenchymal-like phenotypes, such as SPOCK1. Hence, our aim is to elucidate the role of SPOCK1 as biomarker and potential therapeutic vulnerability in resistance acquisition scenario.

Material and method

SPOCK1 RNA expression was analysed *in silico* in other melanoma BRAFi+MEKi resistant *in vitro* models (GSE134432 and GSE75299) and in melanoma tissue before treatment and at progression (GSE77940 and GSE61992). Additionally, an *in vitro* SPOCK1 knock-down was established using shRNA technique and functional studies of resistance reversion, proliferation,

migration and invasion were performed by using MTT, wound-healing and transwell assays.

Result and discussion

In silico analysis corroborated that SPOCK1 gene expression was increased in other melanoma cell lines resistant to targeted therapy. Furthermore, SPOCK1 expression was mainly higher in the resistant tumours compared to the basal ones. Functional *in vitro* analyses revealed that inhibiting the SPOCK1 gene did not reverse resistance to BRAF+MEK inhibitors, suggesting that SPOCK1 does not directly contribute to the molecular processes underlying resistance acquisition. However, SPOCK1 inhibition significantly reduced cell proliferation, migration, and invasion, suggesting its potential role in mesenchymal phenotype development and as a therapeutic vulnerability. Nevertheless, further studies in advanced preclinical models are guaranteed.

Conclusion

SPOCK1 is identified as a biomarker of acquired resistance to BRAFi+MEKi and it postulates as potential therapeutic vulnerability in resistance acquisition context.

EACR25-1127

Combinatorial assessment of DXd-based antibody drug conjugates with DNA damage response inhibitors in ovarian and endometrial cancers

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Introduction

Antibody-drug conjugates (ADCs) provide a novel treatment modality for target anti-cancer drug delivery. In this study, we investigated the *in vitro* efficacy of ADC-containing drug combinations in ovarian (OC) and endometrial (EC) cancers. As TROP2 and HER2 are commonly over-expressed in OC and EC, respectively, the TROP2 targeting datopotamab deruxtecan (Dato-DXd) and HER2-targeting trastuzumab deruxtecan (T-DXd) were chosen. As both ADCs use a potent topoisomerase I inhibitor payload (DXd), which induces irreparable DNA double-strand breaks, we aimed to uncover potential synergies between Dato-DXd and T-DXd and DNA damage response inhibitors (DDRIs), targeting ATM (AZD1390) and ATR (AZD6378), and chemotherapeutic agents in 2D cancer cell models. This study aimed to determine the potential of DXd-ADCs in combination with DDRIs and current first line chemotherapies.

Material and method

This study utilised a panel of six OC (PEO1, PEO4, IGROV-1, SKOV3, OVCAR4, COV318) and three EC lines (AN3CA, HEC1B, MFE280) cell lines. The anti-proliferative effects of Dato-DXd and T-DXd was assessed in their respective panels by 5-day acid phosphatase assay. Synergy with the DDRIs, AZD1390 and AZD6378, and chemotherapies, carboplatin and paclitaxel, were assessed by matrix proliferation assays

and analysed by Combenefit software. TROP2 and HER2 protein expression was examined by Western blotting.

Result and discussion

Dato-DXd showed single agent efficacy in the three highest TROP2 expressing OC cell lines (PEO1, PEO4, OVCAR4). Dato-DXd demonstrated synergy with carboplatin in these three cell lines and additivity in the lower TROP2 expressing cell lines. Paclitaxel did not enhance the anti-proliferative effect of Dato-DXd in the OC panel. However, synergy was observed across all cell lines with AZD1390 and in 4/6 cell lines with AZD6738. MFE280 showed the highest HER2 expression and sensitivity to T-DXd. Both AZD1390 and AZD6738 plus T-DXd were synergistic in all cell lines and outperformed carboplatin and paclitaxel.

Conclusion

Our findings indicate that combining Dato-DXd and T-DXd with specific DDR inhibitors potentially enhances anti-tumour activity in ovarian and endometrial cancer models, which offers potential pathways for clinical translation. These combinations, particularly those involving ATM and ATR inhibitors, support the need for further investigation in clinical settings.

EACR25-1205

Unraveling Therapeutic Resistance in Patients with Early-Stage HER2-Negative Breast Cancer in the I-SPY2 Clinical Trial

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Introduction

We previously established Responsive Predictive Subtypes (RPS) using pre-treatment gene expression (GE), reverse-phase protein array (RPPA), and clinical data from ~990 breast cancer patients of the I-SPY2 neoadjuvant platform trial (NCT01042379) that can guide treatment selection to maximize pathologic complete response (pCR) rates with subtype-matched therapies (Wolf et al, Cancer Cell 2022). Tumors of 29% of patients in the I-SPY2 cohort were characterized by low expression of immune genes and no DNA repair deficiency (DRD) (HER2-/Immune-/DRD-) and these patients experienced very low pCR rates with any modern therapy. The goal of our current analysis is to identify targetable biological processes that could improve treatment sensitivity in these cancers.

Material and method

Published transcriptomic and (phosho)protein I-SPY2-990 data were analyzed for enrichment of canonical

signaling pathways (Molecular Signatures Database gene sets, n = 2152) and epigenetic modulator genes (n = 94) to study two subsets of HER2-/Immune-/DRD- tumors: 1) hormone receptor-positive, HER2-negative (HR+ HER2-)/Immune-/DRD- (n = 191) vs. other HR+HER2-tumors (n = 185), and 2) triple-negative (TN)/Immune-/DRD- (n = 93) vs. other TN tumors (n = 270).

Comparisons were made using Wilcoxon rank sum tests, with p-values adjusted for multiple comparisons using the Benjamini-Hochberg method. All reported results have adjusted p-values <0.05.

Result and discussion

HER2-/Immune-/DRD- tumors had lower expression of a broad range of biological processes, measured by both GE and RPPA. HR+HER2-/Immune-/DRD- tumors, specifically, had low expression of proliferation signatures compared with other HR+HER2- tumors, whereas TN/Immune-/DRD- had similar proliferation levels compared with other TNs, reflecting subtype-specific differences. Interestingly, HER2-/Immune/DRD-tumors of both subtypes expressed higher levels of many epigenetic modulator genes, including HDACs and KATs, suggesting that epigenetic silencing may play a role in these non-responsive tumors. Moreover, both HER2-/Immune-/DRD- subtypes had higher enrichment of Wnt/beta-catenin, Hedgehog, TGF-β/Smad, and PPARγ signaling pathway gene sets compared with other tumors. Additionally, higher ER, pAR, and Cyclin D1 signaling (by GE and RPPA) were found in HR+HER2-/Immune-/DRD-, which previously have been associated with low proliferation and non-response.

Conclusion

HER2-/Immune-/DRD- resistant tumors are characterized by low expression of many cancer-relevant biological processes, including proliferation in HR+HER2/Immune-/DRD-, though HER2-/Immune-/DRD- cancers have higher expression of Wnt/beta-catenin, Hedgehog, TGF-β/Smad, PPARγ and AR pathways and epigenetic modulator genes known to participate in gene silencing. Our findings suggest potential novel targetable vulnerabilities to increase treatment sensitivity in these treatment-resistant tumors.

EACR25-1238

mTORC1 Fuels Resistance but Unlocks Metabolic Kill-Switch in PI3K-Mutant Breast Cancer

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Introduction

The alpha isoform-specific PI3K inhibitor Alpelisib improved the treatment outcomes for hormone receptor-positive, Her2-negative, and PIK3CA-mutated metastatic breast cancer patients. However, the emergence of resistance, often due to aberrant activation of the mTOR complex 1 (mTORC1) pathway, presents a significant clinical challenge. This study investigates targeting mTORC1-induced metabolic vulnerabilities in Alpelisib-resistant breast cancer.

Material and method

We compared therapy-naïve and Alpelisib-resistant PIK3CA-mutated breast cancer cells, T47D and MCF7, in response to the metabolic inhibitors dichloroacetate (DCA) and Metformin using competitive co-cultures, proliferation, cell viability, and apoptosis assays. The mTOR-dependence of metabolic drug responses was analyzed with mTOR kinase inhibitors. The role of autophagy in energetic homeostasis under metabolic perturbation was examined through untargeted metabolomics, autophagy flux analyses, fluorescence microscopy, western blot analyses, and CRISPR/Cas9-generated autophagy-knockout cells. The efficacy of metabolic compounds in eradicating PI3Ki-resistant and CRISPR/Cas9-engineered autophagy-deficient breast cancer cells was tested in vivo using an orthotopic xenograft mouse model. Immunohistochemistry was used to analyze a tissue microarray (TMA) from the breast cancer patient GAIN cohort to correlate overall survival with phosphorylation of the mTORC1 target 4E-BP1T37/46 and accumulation of the autophagy cargo protein p62/SQSTM1 as potential biomarkers for metabolic therapy response.

Result and discussion

Alpelisib-resistant PIK3CA-mutated breast cancer cells exhibited increased sensitivity to metabolic inhibitors due to mTORC1 activation, which promoted Alpelisib-resistance while inhibiting autophagy. In resistant and autophagy-knockout cells, metabolic inhibitors induced severe energy stress, leading to a critical depletion of aspartate, and ultimately apoptosis, whereas therapy-naïve breast cancer cells survived via autophagy both in vitro and in vivo. TMA analyses identified a correlation between 4E-BP1T37/46 phosphorylation and p62/SQSTM1 accumulation, with their combination serving as a biomarker for poor survival in hormone receptor-positive, Her2-negative breast cancer patients.

Conclusion

This study reveals that mTORC1 upregulation, a common cause of Alpelisib-resistance in breast cancer, creates vulnerabilities to metabolically active drugs by suppressing autophagy, and identifies the combination of 4E-BP1T37/46 and p62 as a biomarker for poor survival, indicating potential for identifying patients who may benefit from metabolic therapies in a novel precision medicine approach.

EACR25-1273

Pt1a and sorafenib combination treatment exerts synergistic anti-hepatocellular carcinoma effect through RNA m6A methylation modification

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Introduction

Sorafenib, a multi-target tyrosine kinase inhibitor for advanced hepatocellular carcinoma (HCC), provides only modest survival benefits due to acquired resistance. In our previous work, the novel platinum-based drug, [PtII (C^NN)(NHC₂Bu)]PF₆ (Pt1a), has demonstrated anti-cancer properties by targeting the epithelial-mesenchymal transition marker vimentin. As vimentin is a known mediator of sorafenib resistance, we investigated whether Pt1a can sensitize HCC cells to sorafenib.

Material and method

HCC cell lines and patient-derived organoids were used to assess the inhibition effect induced by the single or combination drug treatment of sorafenib and Pt1a in vitro, whereas the subcutaneous injection of HCC patient-derived xenograft and proto-oncogene (NRAS+ AKT)-driven HCC was employed as the in vivo mouse model. Thermal proteome profiling (TPP) was utilized to evaluate the interaction of the small molecule drugs with proteins. RNA sequencing (RNA-seq) was performed to explore the transcriptomic changes following different

drug treatments while glyoxal and nitrite-mediated deamination of unmethylated adenosines sequencing (GLORI-seq) was conducted to assess the RNA m6A levels at single base resolution. Pathway analysis of HCC datasets or in-house sequencing data was carried out by GO and GSEA analysis methods.

Result and discussion

In cultured HCC cells and patient-derived organoids, the combination of sorafenib and Pt1a synergistically reduced cell viability, attenuated self-renewal, and promoted cell apoptosis compared to single-drug treatment. In contrast, this synergistic effect was not observed in an immortalized human hepatocyte cell line. These findings were further validated in patient-derived xenograft and NRAS+AKT-driven HCC mouse models. Notably, in the NRAS+AKT-driven HCC mouse model, the combination of sorafenib and Pt1a demonstrated superior efficacy compared to anti-VEGF-A combined with anti-PD-L1 antibodies treatment, which is the current first-line treatment for HCC. Mechanistically, RNA-seq and TPP revealed enrichment of m6A-related pathways under combination treatment, with an elevated m6A level confirmed by m6A dot blotting. The m6A writer METTL14 was identified as a potential mediator of the synergistic effect through functional CRISPR-knockout experiments. Deregulated genes identified from RNA-seq, in common with genes containing m6A modification sites identified from GLORI-seq, were used to generate a combined signature, which predicted a better prognosis in HCC patients.

Conclusion

Our findings suggested Pt1a could synergize with sorafenib to inhibit HCC through METTL14-mediated m6A modification. This research provides valuable insights into the potential of Pt1a to overcome sorafenib resistance and opens avenues for novel treatment strategies for HCC.

EACR25-1275

SOAT1 Promotes Methotrexate Resistance in Choriocarcinoma

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Introduction

Choriocarcinoma (CC), a highly aggressive subtype of gestational trophoblastic neoplasia, is generally characterized by favorable responses to methotrexate (MTX)-based chemotherapy. Nevertheless, a subset of patients experience disease recurrence or MTX resistance after initial treatment. The molecular mechanisms of MTX resistance remain unclear, so further research to optimize clinical management is imperative. Our study identifies sterol O-acyltransferase 1 (SOAT1), a pivotal enzyme in cholesterol esterification, as a critical mediator of MTX resistance in CC.

Material and method

Transcriptomics combined with bioinformatics were used to screen differentially expressed genes between MTX-sensitive (JAR, JEG3) and MTX-resistant (JARR, JEG3R) CC cell lines. The expression profiles of SOAT1 were validated through qRT-PCR and Western blot in

these cell lines. Chemosensitivity was systematically evaluated using CCK-8 assay and cell apoptosis assay. To investigate the potential role of SOAT1 in vivo, we constructed mouse xenograft model by subcutaneously injecting JEG3R cells with or without stable SOAT1 knockdown.

Result and discussion

Our data revealed a significant upregulation of SOAT1 expression at both mRNA and protein levels in MTX-resistant CC cell lines relative to MTX-sensitive cell lines. SOAT1 knockdown markedly enhanced the chemosensitivity of MTX-resistant cells (JARR, JEG3R) to MTX, whereas SOAT1 overexpression conferred MTX resistance in parental CC cells (JAR, JEG3). In vivo experiments, SOAT1 knockdown significantly reduced the volume and weight of tumors, proving that SOAT1 knockdown could promote the efficacy of MTX in vivo. Mechanically, SOAT1 knockdown upregulated USP24 expression, a deubiquitinating enzyme, through a ubiquitin-dependent pathway.

Conclusion

This study provides novel insights into the role of SOAT1 in regulating MTX resistance, identifying its potential as a therapeutic target for reversing chemo-resistance in CC patients. These findings highlight the promise of targeting SOAT1 as a strategy to enhance chemotherapeutic efficacy in clinical practice.

EACR25-1297

Creating Repotrectinib Resistance in a Ba/F3 SLC34A2-ROS1 NSCLC cell line

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Introduction

1-2% of non-small cell lung cancer (NSCLC) is driven by constitutive activation of ROS1 due to a ROS1 rearrangement. ROS1+ NSCLC is treated with tyrosine kinase inhibitors (TKI) which inhibit the phosphorylation of ROS1 and as a consequence its downstream pathways. Treatment with TKIs is palliative and will eventually lead to resistance via accumulation of additional genomic aberrations in either the tyrosine kinase domain of ROS1 (on-target) or other genes leading to alternative survival routes (off-target). In this study we aim to generate a repotrectinib resistant cell line by long term treatment with TKIs to study treatment induced resistance mechanisms.

Material and method

Ba/F3 cells were transfected with a pCDNA3.4 plasmid containing the SLC34A2-ROS1 fusion gene. Sensitivity for repotrectinib was measured by Western blot for phosphorylated ROS1, MTS assays and calculation of the

IC₅₀ value. To generate resistant cells, we treated the Ba/F3 SLC34A2-ROS1 cells with an increasing dose of repotrectinib during the course of 4 months until growth at a 100-fold increase of the IC₅₀ value was observed. MTS assays were performed on the resistant cells cultured either one or two weeks with and without repotrectinib before conducting the MTS assay.

Result and discussion

IC₅₀ value of the parental Ba/F3 SCL34A2-ROS1 treated with repotrectinib was 0,15nM. Effective treatment was confirmed by western blot for phospho-ROS1 showing a strongly reduced phosphorylation between 0,1 and 1nM of repotrectinib. Resistance cell cultures were made using 5, 10 and a final concentration of 15nM repotrectinib. IC₅₀ values of the resistance levels cultured for one week without repotrectinib pressure were 3,56, 3,69 and 5,16nM for the 5, 10 and 15nM respectively. IC₅₀ values of cells continuing to grow with repotrectinib were higher with 9,32 and 11,07nM for the 10 and 15nM repotrectinib cultures. For the 5nM culture the IC₅₀ was as high as 47nM, this is possibly due to hormesis. These data show that removal of repotrectinib from the medium might make the cell lines more sensitive to repotrectinib again. Cells cultured with repotrectinib were smaller in size compared to the parental cells. When repotrectinib pressure was removed cells regained similar size as the parental cells but did however clump together which is not observed in the parental cell line.

Conclusion

We generated three Ba/F3 SLC34A2-ROS1 repotrectinib resistant cell lines. A short term repotrectinib withdrawal makes the resistant cells more sensitive to repotrectinib. Mechanisms underlying the observed resistance such as mutations in the tyrosine kinase domain or in genes affecting alternative signaling pathways are currently being examined.

EACR25-1298

Assessment of two new ROS1+ NSCLC patient-derived cell lines as in vitro models for TKI resistance studies

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Introduction

ROS1 fusion gene-positive (ROS1+) is found in 1-2% of the non-small cell lung cancer (NSCLC) patients. Initially these patients are treated effectively with tyrosine kinase inhibitors (TKIs), however patients

generally develop resistance to TKI treatment due to on- and off-target resistance mutations. In this study, we assessed TKI sensitivity of two new patient-derived ROS1+ cell lines as in vitro models for investigating the resistance mechanisms to ROS1 TKI treatment.

Material and method

Two cell lines were generated from pleural effusions obtained from CD74-ROS1+ NSCLC patients. The cell line from patient 1 was derived from cells obtained upon resistance to crizotinib (PC1 cells). The cell line from patient 2 was generated from cells obtained upon resistance to crizotinib followed by resistance to lorlatinib treatment (PC2 cells). The Archer lung fusion cancer and TSO500 panels were used to identify potential resistance mechanisms in these cell lines. To assess efficacy of TKIs in the patient derived cell lines, IC₅₀ values were compared to CD74-ROS1 Ba/F3 cells. Sensitivity to TKIs crizotinib, entrectinib, lorlatinib, repotrectinib and zidesamtinib was assessed using MTS assays.

Result and discussion

Presence of the CD74-ROS1 fusion gene was confirmed in all three cell lines. The IC₅₀ values of unmutated ROS1+ Ba/F3 cells for crizotinib, entrectinib, lorlatinib, repotrectinib and zidesamtinib were 31.4; 44.6; 14.3; 14.9 and 29.9 nM, respectively. PC1 cells had a TP53 p.M246I mutation and IC₅₀ values above 3000 nM for all five ROS1 inhibitors. Interestingly, these cells were resistant to lorlatinib, while the patient is – after being treated with lorlatinib+cis-platinum+pemetrexed, now currently successfully treated with lorlatinib mono-therapy. PC2 cells had a ROS1 p.G2032R mutation, along with mutations in PIK3CA, PIK3CG and ARID1A. These cells responded to repotrectinib (IC₅₀ 57 nM) and lorlatinib (IC₅₀ 556 nM), while response to zidesamtinib, crizotinib and entrectinib was poor with IC₅₀ values above 1500 nM. The p.G2032R mutation is a known resistance mutation for crizotinib and lorlatinib, but was shown to be responsive to repotrectinib and the ROS1 specific zidesamtinib. Thus, the results from the PC2 cell line indicate presence of other off-target resistance mechanism that lead to resistance to zidesamtinib.

Conclusion

In this study we showed that PC1 cells generated from a crizotinib-resistant patient were resistant to all ROS1 inhibitors tested. The G2032R mutated PC2 cells generated from a crizotinib and lorlatinib resistant patient were sensitive to repotrectinib, but not to zidesamtinib. This PC2 cell line can be used to assess off-target resistance mechanism to zidesamtinib.

EACR25-1307

Targeting Fascin as a Therapeutic Approach to Overcome Oxaliplatin Resistance in Colorectal Cancer

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Introduction

Both primary and secondary resistance to antineoplastic treatments remains a major challenge in colorectal cancer (CRC). This underscores the urgent need to develop tools that can predict and assess the response to antitumor therapies. Fascin, a protein involved in tumour invasion and metastasis, has recently been identified for its role in resistance to chemotherapy and immune checkpoint inhibitors, such as anti-PD1 in breast cancer. Given this, our group has extensive experience in characterizing fascin in CRC and discovering new drugs with anti-fascin activity. The main aim of this work is to evaluate the effectiveness of anti-fascin compounds in reversing oxaliplatin chemoresistance in in vitro CRC models.

Material and method

Eight colorectal cancer cell lines were used, four sensitive to oxaliplatin (HT29, LoVo, DLD1 and LS513) and four resistant derivatives of the former (HTOXAR, LoVOXAR, DLDOXAR and LSOXAR). These were cultured according to ATCC recommendations, under standard incubation conditions at 37°C with 5% CO₂. The cells were treated with oxaliplatin and fascin inhibitors (Imipramine and NP-G2-044), in monotherapy and in combination. Cell viability was assessed using the colorimetric XTT assay. Fascin levels in these lines were obtained from microarray data available in the GEO database, specifically from the GSE30011 study, and immunohistochemistry (IHC).

Result and discussion

In resistant cell lines, a higher IC₅₀ for oxaliplatin was confirmed comparatively to the sensitive lines, indicating a resistance to the drug. The anti-fascin compounds NP-G2-044 and imipramine, in monotherapy, reduced cell viability in all lines, with NP-G2-044 being the most effective. The combination of NP-G2-044 or imipramine with oxaliplatin significantly decreased viability in HTOXAR and DLDOXAR (up to 40% and 30%, respectively). In LoVOXAR, only the combination with NP-G2-044 was effective, reducing viability by 30%, suggesting a synergistic effect capable of reversing oxaliplatin resistance. However, in LSOXAR, the tested combinations did not improve the response to oxaliplatin. FSCN1 expression increased with oxaliplatin resistance in all the lines, except for LSOXAR, being notable in HTOXAR and DLDOXAR, which could explain the high efficacy of the combination treatments in the latter cell lines. IHC analysis further validated these results, confirming fascin's differential levels related to oxaliplatin resistance.

Conclusion

Our findings highlight the key role of fascin in oxaliplatin resistance in CRC. Although the anti-fascin compounds NP-G2-044 and imipramine in monotherapy could be viable therapeutic options, higher concentrations

would be required to achieve inhibitory effects comparable to oxaliplatin in resistant cells. However, the combination of fascin inhibitors with oxaliplatin exhibited a great ability to reverse drug resistance, showing promising therapeutic potential to overcome chemoresistance.

EACR25-1339

Abstract: Transcriptional Upregulation of ERG11, MDR1, and CDR1 Genes in Azole Resistance Among Candida Species from Head and Neck Cancer Patients

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Introduction

Head and neck cancer patients are highly susceptible to opportunistic infections, notably candidiasis, due to immunocompromised. *Candida* species, particularly *C. albicans* and *C. tropicalis*, are frequent colonisers in these patients, with azole antifungal resistance posing a significant clinical challenge. Resistance is often linked to the transcriptional up-regulation of efflux pump genes (MDR1, CDR1) and the ergosterol biosynthesis gene (ERG11).

Material and method

A prospective study was conducted at a tertiary care hospital in Northeastern India, enrolling 57 HNC patients from March 2023 to February 2025. Oropharyngeal swabs were collected and cultured on SDA. *Candida* spp. identification was performed using standard microbiological techniques. Antifungal susceptibility testing was conducted via the disk diffusion method against fluconazole, itraconazole, and voriconazole. Azole-resistant isolates underwent RNA extraction and qRT-PCR to assess transcriptional upregulation of ERG11, MDR1, and CDR1, using GAPDH as a housekeeping gene. Fold changes were calculated relative to a susceptible *C. albicans* reference strain. Data were analysed using SPSS v25 software.

Result and discussion

Of the 57 patients, 35 (62.1%) exhibited *Candida* spp. growth, with *C. albicans* (54.3%, n = 19) and *C. tropicalis* (34.3%, n = 12) predominating, alongside minor isolates of *C. glabrata* (11.4%, n = 4). Antifungal susceptibility testing revealed resistance in 14 isolates (40% of *Candida*-positive cases): fluconazole resistance in 12 isolates (85.7%), itraconazole in 9 (64.3%), and voriconazole in 5 (35.7%). Among resistant isolates, qRT-PCR showed significant transcriptional up-regulation: ERG11 was upregulated in 10 isolates (mean fold change: 3.8, range: 2.1–5.6), MDR1 in 8 (mean fold change: 4.2, range: 2.5–6.8), and CDR1 in 11 (mean fold change: 5.1, range: 3.0–7.9), with p < 0.01 for all genes compared to controls. *C. tropicalis* isolates exhibited higher CDR1 upregulation (mean: 6.3) than *C. albicans* (mean: 4.2), suggesting species-specific resistance.

patterns. These findings align with prior studies linking efflux pump overexpression and ERG11 alterations to azole resistance, though the high resistance rate in this cohort underscores the role of local factors, such as antifungal overuse in Northeastern India.

Conclusion

This study demonstrates that transcriptional upregulation of ERG11, MDR1, and CDR1 is a key driver of azole resistance in *Candida* spp. from HNC patients in Northeastern India, with *C. albicans* and *C. tropicalis* as dominant pathogens. The significant resistance rates and pronounced gene upregulation highlight an urgent need for tailored antifungal stewardship and alternative therapies in this region. These insights enhance our understanding of resistance mechanisms and advocate for routine molecular screening to guide treatment, ultimately improving outcomes in this vulnerable population.

EACR25-1344

Discovery of Molecular and Epigenetic Candidates Underlying Chemosensitivity of Colorectal Cancer

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Introduction

Oxaliplatin (OXA)-based chemotherapy is the therapeutic regimen for patients with advanced colorectal cancer (CRC). Although OXA treatment improves mortality and tumor recurrence, its long-term effectiveness is limited by acquired resistance. This study aims to identify novel genes contributing to chemo-resistance in CRC using parental (WT) and OXA-resistant (OXAREZ) CRC cell lines.

Material and method

The OXAREZ cell line was generated by introducing increasing concentrations of OXA to DLD-1 cell line. All the experiments were performed on WT cells compared to OXAREZ cell lines. Behavioral tests such as cytotoxicity, proliferation, colony formation, and wound healing abilities of the DLD-1 cell lines were evaluated under OXA exposure. Genome-wide DNA methylation profiling and total RNA sequencing were conducted to identify underlying molecular mechanisms of OXA-based chemoresistance. CRC stem cell markers, apoptosis, and cell cycle arrest profiles were measured using flow cytometry.

Result and discussion

The behavior of the resistant cells was remarkably altered compared to the WT cell line. The OXAREZ cells exhibited enhanced colony formation and proliferation upon OXA treatment, indicating an adaptive advantage that promotes survival under chemotherapeutic pressure. Additionally, the expression patterns of key stem cell

markers underwent notable changes following the development of OXA resistance. Notably, OXAREZ cells showed a complete loss of CD133 expression, a marker commonly associated with cancer stem-like properties, suggesting a shift in the cellular phenotype. Furthermore, DNA methylation analysis revealed hypermethylation differences in the promoter shelf regions of OXAREZ cells compared to WT cells, highlighting potential epigenetic modifications associated with chemoresistance. RNA sequencing identified several differentially expressed genes involved in crucial cellular processes, such as cell cycle regulation, apoptosis, and migration. Additional CRC cell lines are currently being generated to acquire OXA resistance. Comparative analyses between these resistant cell lines will provide deeper insights into common and cell line-specific mechanisms of chemoresistance.

Conclusion

This study explores the impact of OXA resistance on the CRC cell's behavior, revealing significant molecular and functional changes associated with acquired chemoresistance. Substantial modifications in DNA methylation patterns and gene expression profiles were identified, highlighting potential biomarkers and key pathways involved in resistance. The ongoing analyses aim to refine these findings and pinpoint actionable targets for therapeutic intervention.

The study was supported by the National Institute for Cancer Research (Programme EXCELES, ID Project No. LX22NPO5102), funded by the European Union–Next Generation EU, and Czech Science Foundation (Project No. GX21-04607X).

EACR25-1350

The mechanism of PPAR α -mediated cholesterol metabolism in regulating paclitaxel resistance in ovarian cancer cells

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Introduction

Ovarian cancer, the deadliest gynecological malignancy, has a poor prognosis, with over 75% of cases diagnosed at late stages and a global 5-year survival rate below 45% due to frequent chemoresistance. Dysregulated lipid metabolism is closely associated with chemoresistance. Peroxisome proliferator-activated receptor alpha (PPAR α), a key regulator of lipid metabolism, enhances drug sensitivity by promoting apoptosis, but its role in chemoresistance via lipid metabolic pathways remains unclear.

Material and method

Human serous ovarian cancer cell lines A2780 and SKOV3 were used as experimental models in this study. Paclitaxel (PTX)-resistant cell lines (A2780/TR and SKOV3/TR) were established via continuous low-dose PTX exposure. Resistance was assessed using CCK-8

assays, flow cytometry (apoptosis), and western blot (multidrug resistance protein 1 expression). Lipid droplets within cells were visualized using Nile Red staining. Untargeted lipidomics was performed on sensitive (A2780, SKOV3) and resistant (A2780/TR, SKOV3/TR) cells using ultra-performance liquid chromatography-mass spectrometry (UPLC-MS). Log₂(FC) values were calculated via Fold-Change, and significantly altered lipids were screened. Cholesterol (Chol) levels were measured using a Chol quantification kit, while key Chol regulatory factors were detected by western blot. PPAR α expression was modulated through knockdown, overexpression, and treatment with the pharmacological inhibitor GW6471 or agonist fenofibrate.

Result and discussion

Resistant cells exhibited fewer but larger lipid droplets ($P < 0.05$) and elevated CE levels ($\log_2(\text{FC}) > 9$). Total Chol and key metabolic proteins, including sterol regulatory element-binding protein 2 (SREBP2), 3-hydroxy-3-methylglutaryl-CoA Reductase (HMGCR), low-density lipoprotein receptor (LDLR), and acetyl-CoA acyltransferase 1 (ACAT1), were significantly upregulated in resistant cells ($P < 0.05$). Simvastatin treatment reduced resistance ($P < 0.01$), linking Chol metabolism to chemo-resistance. PPAR α was upregulated in resistant cells ($P < 0.05$), and its knockdown or inhibition increased resistance in sensitive cells ($P < 0.001$), while over-expression or activation reduced resistance in resistant cells ($P < 0.01$). Mechanistically, knockdown of PPAR α upregulated Chol levels ($P < 0.001$) and SREBP2, HMGCR, LDLR, ACAT1 expression ($P < 0.05$), while overexpression had the opposite effect ($P < 0.05$). PPAR α knockdown reversed simvastatin-induced resistance reduction ($P < 0.001$), indicating PPAR α regulates paclitaxel resistance via Chol metabolism. Future studies will validate these findings in clinical samples and murine models.

Conclusion

PPAR α regulates PTX resistance in ovarian cancer by modulating Chol metabolism. Targeting PPAR α and its downstream pathways may offer novel therapeutic strategies to overcome chemoresistance.

EACR25-1407

SRSF9-mediated alternative splicing impairs anti-tumor immunity and drives resistance to PD-L1/VEGF-A blockade in hepatocellular carcinoma

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Introduction

Immune resistance remains a significant barrier to the clinical efficacy of PD-L1/VEGF-A blockade in hepatocellular carcinoma (HCC). In this study, we identify

SRSF9, an RNA splicing factor, as a critical regulator of immune evasion in HCC and elucidate the mechanisms underlying treatment resistance.

Material and method

We conducted a CRISPR-based genome-wide knockout screen in an orthotopic HCC model receiving dual PD-L1/VEGF-A antibody treatment to uncover novel regulators of immune evasion. Key hits were validated using both the orthotopic HCC mouse model and lucOS-MYC/CTNNB1-driven spontaneous HCC model. We assessed the impact of SRSF9 knockout on T cell function, immune cell infiltration, and tumor growth, and evaluated whether the natural compound Apigenin could modulate SRSF9 expression and splicing activity to enhance the efficacy of PD-L1/VEGF-A blockade. Furthermore, to explore how SRSF9-mediated alternative splicing reshapes the tumor microenvironment (TME) and drives resistance to PD-L1/VEGF-A blockade, we then performed comprehensive analyses including bulk RNA sequencing, single-cell transcriptomics, and enhanced crosslinking and immunoprecipitation sequencing (eCLIP-seq).

Result and discussion

Our CRISPR screen identified SRSF9 as a key driver of immune resistance in HCC. Knockout of SRSF9 in tumor cells significantly enhanced CD8+ T/NK cell cytotoxic activity, suppressed M2 tumor-associated macrophage polarization, and reversed resistance to PD-L1/VEGF-A blockade. Transcriptomics and eCLIP-seq analyses revealed that SRSF9 directly binds and regulates the alternative splicing of critical immune signaling genes (e.g. CSDE1), thereby modulating both T cell function and TME composition. Importantly, treatment with Apigenin reduced SRSF9 expression and altered the splicing patterns of SRSF9 target genes, leading to improved T cell activation and significant anti-tumor responses, with synergistic effects when combined with PD-L1/VEGF-A blockade.

Conclusion

Our findings demonstrate that SRSF9 plays a crucial role in promoting immune resistance in HCC. Targeting SRSF9 with Apigenin represents a promising strategy to overcome immune evasion and boost the efficacy of immune checkpoint inhibitor-based therapies in patients with advanced or refractory HCC.

EACR25-1413

Revealing Distinct Oxaliplatin Resistance Mechanisms in HCT116 Cells Through Genomic and Transcriptomic Analysis Under Continuous and Intermittent Treatment Regimens

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Introduction

Chemotherapy resistance remains a major challenge that reduces treatment effectiveness and accelerates disease progression in colorectal cancer. Identifying the underlying molecular interactions driving resistance development; therefore, is essential for improving novel therapeutic strategies. Additionally, development of drug

resistance under different treatment regimens may result in distinct molecular changes, which may eventually influence the outcomes of the treatments. We aimed to investigate both genomic and transcriptomic alterations associated with drug resistance in CRC cells through the implementation of two different treatment regimens. By integrating whole-exome sequencing (WES) and RNA sequencing (RNA-seq) data, we sought to clarify molecular mechanisms contributing to resistance development under different treatment conditions.

Material and method

To generate resistant cell populations, HCT116 cell lines were treated with oxaliplatin under two different treatment regimens. Cells were subjected to either constant drug exposure or periodic withdrawal and re-exposure. Once resistance was confirmed via cell viability assay, WES and RNA-seq were performed to reveal both genomic and transcriptomic alterations specific to each regimen to identify differentially expressed genes, enriched pathways, and potential resistance mechanisms.

Result and discussion

WES analysis revealed differences between mutational landscapes, whereas RNA-seq analysis further emphasized the differential expression patterns between intermittent and continuous treatment regimes. These findings suggest that drug exposure patterns have a significant impact on the adaptive resistance mechanisms in HCT116 cell lines, highlighting the potential of treatment optimization to prevent chemotherapy resistance.

Conclusion

In summary, we demonstrated that oxaliplatin resistance in HCT116 cell lines is driven by both genomic and transcriptomic alterations and treatment specific adaptations in response to drug exposure regimens. These findings indicate the importance of implementing optimized treatment strategies in colorectal cancer treatment to enhance treatment efficacy.

EACR25-1424

AP-1 transcription factor is a targetable mediator of osimertinib resistance in EGFR-mutant non-small cell lung cancer

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Introduction

Non-small cell lung cancer (NSCLC), which constitutes 85% of all lung cancer cases, is often driven by oncogenic mutations within the kinase domain of the epidermal growth factor receptor (EGFR). Osimertinib, a third-generation EGFR tyrosine kinase inhibitor (EGFR-TKI), has revolutionized the treatment of EGFR-mutant non-small cell lung cancer (NSCLC). However, acquired resistance remains a major challenge, with non-genetic mechanisms increasingly recognized as key drivers. Here, we employ a multi-omics approach to characterize genome-wide chromatin accessibility and transcriptional landscapes between osimertinib-sensitive and -resistant

EGFR-mutant NSCLC cells. Our findings reveal a strong correlation between epigenetic remodeling and transcriptomic alterations that define the resistant state.

Material and method

Osimertinib-resistant subclones of HCC827, PC9, and H1975 cells, lacking secondary EGFR mutations, were generated using a dose-escalation approach. CRISPR/Cas9-based functional genomics screen targeting epigenetic regulators and transcription factors identifies critical resistance-associated regulatory networks, prominently featuring the NuRD and PRC2 complexes. Notably, among the top hits, FOSL1 and JUN – core components of the AP-1 transcription factor – emerge as central mediators of resistance. Mechanistically, we demonstrate that cis-regulatory elements with altered chromatin accessibility in resistant cells are enriched for AP-1 binding motifs, allowing AP-1 to drive a gene expression program that sustains the druggable MEK/ERK signaling axis, thereby enhancing cell viability and fitness.

Result and discussion

Crucially, genetic depletion or pharmacological inhibition of AP-1 restores osimertinib sensitivity and reverses resistance-associated phenotypes, including epithelial-to-mesenchymal transition, upon anti-EGFR rechallenge. This effect is mediated through suppression of AKT and ERK signaling, underscoring AP-1 as a key vulnerability in the resistant state.

Conclusion

These findings provide novel insights into the epigenetic and transcriptional regulation of osimertinib resistance in EGFR-mutant NSCLC. Targeting AP-1 represents a promising therapeutic strategy to overcome osimertinib resistance and enhance the efficacy of EGFR-TKIs in NSCLC.

EACR25-1446

Overactivation of oncogenic signaling steers cancer cells toward less malignant phenotypes

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Introduction

Over the past few decades, extensive research has been conducted on cancer and a myriad of compounds have been developed. Despite significant progress for some cancers, the overall efficiency of these drugs remains limited by the emergence of resistance. Cancer cells often acquire resistance to targeted therapies by gaining further oncogenic alterations, reactivating feedback loops, and becoming more aggressive and refractory to subsequent treatments. We recently proposed an unconventional approach for cancer therapy involving the activation of oncogenic signaling along with the inhibition of stress responses. The underlying rationale is that in cancer cells, the aberrant oncogenic signaling is accompanied by cellular stress which increases the necessity for stress responses to maintain cell viability. Therefore, the overactivation of oncogenic signaling can overload cancer cells with cellular stress and be highly toxic to

them, especially when combined with stress-response inhibitors. We have recently shown that the inhibition of protein phosphatase 2A (PP2A) led to hyperactivation multiple oncogenic signaling pathways in colon cancer models and was associated with engagement of multiple stress response pathways. Compound and CRISPR screens revealed that the inhibition of the mitosis gatekeeper kinase WEE1 as the major vulnerability of such hyperactivated cells. The combination of PP2A and WEE1 inhibitors proved very efficient in killing different cancer cell in vitro and in vivo. As for any other treatment, resistance to this approach can also emerge. Given the fundamentally different mechanism of toxicity, we aimed to explore how cancer cells acquire resistance to overactivation therapy.

Material and method

The experiments were performed on colorectal cancer cell lines. We performed viability, invasion and seahorse assays, genome-wide screens, RNA sequencing and animal experiments.

Result and discussion

Our study showed that the resistance to the combination of PP2A and WEE1 inhibitors led to transcriptional suppression of oncogenic signaling and cellular stresses. Moreover, the resistant cells switched from glycolysis to oxidative phosphorylation for their metabolic needs. In line with the in vitro data, the acquired resistance restrained the ability of cancer cells to form tumors in vivo. Altogether, our data suggest that the acquired resistance, developed to evade the stressful state caused by the activation therapy, is related with reduced tumorigenicity.

Conclusion

These data suggest that the combination of overactivation of oncogenic signaling together with suppression of stress responses can force cancer cells to give up their malignant traits. Our study proposes that this unconventional approach targets the pathological behavior of cancer cells and can be explored therapeutically as it may provide unforeseen ways to suppress oncogenic signaling.

EACR25-1461

An unbiased in vitro screen for uncovering HER2 drug resistance mutations

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Introduction

The HER2 receptor tyrosine kinase (RTK), also known as ERBB2, has been extensively studied for developing tyrosine kinase inhibitor (TKI) drugs. HER2-activating point mutations and small indels have been found in various human cancers. These mutations can drive malignancies similar to HER2 amplification and are responsive to HER2-targeted TKIs. However, recent studies have revealed disease progression in cancer patients treated with TKIs due to the development of additional on-target mutations. Moreover, different TKIs might retain activity in the presence of on-target resistance mutations for another specific TKI. Therefore, it is crucial to identify the resistance mutation spectrum

of different HER2 TKIs. This study addresses the development of on-target HER2 resistance mutations using the in vitro screen for activating mutations (iSCREAM), a functional genetics screen previously developed in our laboratory.

Material and method

To perform a modified version of iSCREAM, a retroviral library of randomly mutated HER2 containing one of the well-known HER2 activating mutations (S310F and V777L) is utilized to generate stable Ba/F3 lines ectopically expressing the HER2 mutant library. Ba/F3 cells, which rely on exogenous Interleukin-3 (IL-3) for survival, are induced to gain IL-3 independence through the expression of HER2 oncogenes. The cells are grown under IL-3-depleted conditions to establish a population independent of IL-3. Under HER2 TKI treatment, clones containing on-target resistance mutations are selectively enriched. Sequencing of the HER2 retroviral insert from the surviving Ba/F3 cell pool is performed to identify these mutation hits. The identified mutation hits will be further validated through in vitro cell models and in silico analysis.

Result and discussion

In IL-3-independent Ba/F3 cells with HER2 mutations, different proliferation rates and HER2 TKI sensitivities confirmed the efficacy of the activating mutation panel for iSCREAM. Cells addicted to HER2S310F and HER2V777L showed higher growth rates and TKI sensitivity. Additionally, the proliferation of HER2S310F and HER2V777L Ba/F3 cells, with and without random mutations under TKI treatment, indicates that random mutational variations promote TKI resistance. The ability of surviving cells to regrow under HER2 TKI pressure largely depends on the mutations present within the library.

Conclusion

Oncogene-targeted therapy using TKIs may lead to resistance due to on-target mutations that kill the activity of the drugs. On the other hand, some other targeted TKIs may retain functionality in the presence of on-target resistance mutations. Therefore, this study will identify and validate the HER2 on-target resistance mutation spectrum of different HER2 TKIs. This can provide invaluable information in choosing suitable HER2 TKI therapy for tumors harboring HER2 resistance mutations to other TKIs.

EACR25-1467

Quantitative proteomics of drug-metabolizing enzymes and transporters in patients with appendix tumours

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Introduction

Cancer may affect systems parameters, including the abundance of drug-metabolizing enzymes (DMEs) and transporters, leading to high variability in pharmacokinetics. However, such data are scarce, especially for rare cancer types. Appendix tumours are rare entities that usually metastasize to the peritoneum, for which there is no targeted treatment. This study aimed to quantify, for the first time, DMEs and transporters in the appendix, peritoneum and exosomes from plasma from patients with appendix tumours (AT) and healthy subjects.

Material and method

Tissues were homogenized, and epithelial cells were extracted from appendix (5 healthy, 16 non-tumor peri-carcinomatous, 15 tumor) and peritoneum (44 non-tumor peri-carcinomatous, 63 tumor) tissues. Exosomes from plasma (6 healthy subjects, 19 patients with appendix tumors) were prepared (liquid biopsy). Filter-aided sample preparation was used for protein digestion, and global liquid chromatography–mass spectrometry for quantification of proteins.

Result and discussion

Among cytochrome P450 (CYP) enzymes, CYP3A4 showed the most significant change in cancer patients; 400-fold decrease in tumor compared with non-tumor appendix, and 4-fold decrease in tumor compared with non-tumor peritoneum. On the contrary, CYP1B1 increased (2-fold) in tumour appendix and peritoneum. Uridine 5'-diphospho-glucuronosyltransferases (UGTs) also showed differential expression. For example, UGT2B17 was 70-fold lower in tumor relative to non-tumour appendix, and 16-fold lower in tumour relative to non-tumour peritoneum. However, UGT2B4 and UGT2B15 were upregulated in tumor appendix. Non-CYP and non-UGT DMEs (carboxylesterases, flavin-containing monooxygenases, microsomal glutathione S-transferases, sulfotransferases, aldehyde oxidase, aldehyde dehydrogenases, alcohol dehydrogenases etc.) were also quantified. They were generally decreased in tumor compared with non-tumor peritoneum.

Interestingly, most of them were upregulated in tumor and non-tumor appendix compared with healthy controls. ATP-binding cassette (ABC) transporters showed distinct expression profiles in cancer too; for example, BCRP was lower in tumor compared with non-tumor (9-fold) and healthy (3-fold) appendix, while P-gp was not detected in tumor. However, BCRP and P-gp showed similar levels in tumor and non-tumor peritoneum. In exosomes, differential expression was also observed; for example, ADH1B, CES1/2, EPHX1, MGST1, UGT1A1, P-gp and MRP3 were exclusively detected in AT patients, whereas NAT1 was only detected in healthy samples.

Conclusion

Overall, the abundance of DMEs and transporters is altered in patients with AT. This may affect the clearance and disposition of drugs metabolized by these DMEs or transported via these transporters. Therefore, our findings could facilitate informed clinical decisions related to drug

exposure, resistance and response to treatment for appendix tumors.

EACR25-1477

Establishment of chemoresistant patient-derived tumor organoids (PDTO) to identify relevant biomarkers for second-line treatments in ovarian cancer

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Introduction

Ovarian cancer has a poor clinical prognosis due to innate or acquired chemoresistance following carboplatin/paclitaxel treatment. Although PARP inhibitors have revolutionised patient management, they are only offered to patients with Homologous Recombination Deficiency (HRD) who have responded to carboplatin treatment. As carboplatin and PARP inhibitors are genotoxic agents that exploit the HR DNA repair pathway, cross-resistance between these two therapeutic classes is emerging, suggesting at least partial overlap in resistance mechanisms. Our understanding of the mechanisms involved in the acquisition of chemo-resistance remains very limited and no relevant tools are currently available to guide treatment in patients who can no longer benefit from these therapies. Definition of the molecular alterations and vulnerabilities to be targeted during chemoresistance is though crucial for ovarian cancer management. As access to biological samples from patients resistant to carboplatin and PARP inhibitors is very complex, we have focused on Patient-Derived Tumor Organoids (PDTOs) models which are world-renowned for reflecting molecular characteristics of the tumor and patient’s clinical response. In this context, we have established chemoresistant PDTOs from sensitive PDTOs that have undergone repeated treatments with carboplatin. This model would accurately enable us to follow the mechanisms involved in acquired chemoresistance.

Material and method

Three chemosensitive ovarian PDTOs lines were treated

with 10 μ M carboplatin (corresponding to their IC50) during 1 week for 6 cycles and CellTiterGlo assay was performed to estimate PDTOs viability and IC50 at the end of the 6th cycle. After each cycle, PDTOs were harvested to realize transcriptomic and proteomic analyses, to perform immunochemistry assay and to assess HRD profile.

Result and discussion

Six cycles of carboplatin were sufficient to induce resistance in PDTOs to carboplatin and 2 PARP inhibitors (Olaparib and Niraparib), assessed by the increase of IC50 between the 1st and the 6th cycles. The preliminary results indicate that chemoresistance acquisition is accompanied by an increase expression of the anti-apoptotic protein Bcl-xL and a decrease expression of the pro-apoptotic proteins Puma and Noxa resulting in an imbalanced ratio that prevent apoptosis. In addition, activating phosphorylation of NF κ B (P-p65Ser536), a transcription factor known to be involved in carboplatin resistance in ovarian cancer, was also increased in resistant lines, supporting the reliability of our model.

Conclusion

For the first time, our study highlights the possibility to establish an acquired resistance to carboplatin and PARP inhibitors in ovarian PDTO model. Future characterization of molecular pathways activation and biomarkers expression kinetics will represent an important scientific breakthrough and will offer new hope for second-line treatment of ovarian cancer.

EACR25-1480

CIC: The Master Regulator of Resistance to MAPK Pathway Inhibition

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Introduction

Drug resistance continues to be the main obstacle to achieving cures in patients with cancer. We recently developed Multiple Low Dose (MLD) therapy, having shown that partial inhibition of the EGFR-RAF-MEK-ERK kinases using a low dose (IC20) of small molecule inhibitors targeting each of these kinases stands out from conventional High Dose (HD) single-agent strategies because of its ability to prevent resistance. However, the underlying mechanism(s) that result in the effectiveness of the MLD strategy are not fully understood.

Material and method

To identify modulators of response to MLD therapy, we generated lung adenocarcinoma PC9 cells resistant to EGFR, RAF, MEK and ERK inhibitors, respectively, and performed a genome-wide loss-of-function CRISPR-Cas9 screen on the parental line.

Result and discussion

We found that PC9 cells were able to acquire spontaneous resistance to treatment with high doses of EGFR, RAF, MEK, ERK or RAF+MEK inhibitors. This was achieved via different mechanisms, all of which ultimately lead to MAPK pathway reactivation.

Interestingly, all these drug-resistant cells are still responsive to MLD therapy. However, knock-out of Capicua transcriptional repressor (CIC) renders PC9 cells resistant to all treatment strategies, independent of MAPK pathway reactivation. Furthermore, we also find CIC LOF mutations in tumors from patients resistant to MAPK pathway inhibitors.

Conclusion

These observations suggest that CIC is a Master regulator of resistance to MAPK pathway inhibition, paving the road for future clinical studies to assess CIC's expression as a predictive biomarker of response to MAPK pathway inhibitors.

EACR25-1569

Lysosomal Ca²⁺ release via TRPML3 enhances drug sensitivity of gefitinib-resistance NSCLC cells

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Introduction

Lysosomes have recently gained prominence as pivotal signaling hubs implicated in drug resistance within cancer cells. However, the precise role of Transient receptor potential mucolipin 3 (TRPML3), an endolysosomal Ca²⁺-permeable channel known to regulate lysosomal trafficking during endocytosis and autophagy, remains enigmatic in the context of cancer progression. This study aimed to elucidate the involvement of TRPML3 in modulating exosomal release triggered by lysosomal exocytosis during the development of gefitinib resistance in non-small cell lung cancer (NSCLC).

Material and method

Exosomal counts and cell-cycle analysis were conducted by flow cytometry and western blot analysis. Ratiometric assays and enzymatic activity assessments were performed to investigate Ca²⁺ signaling, lysosomal pH, and lysosomal exocytosis

Result and discussion

Our finding revealed that gefitinib-resistant NSCLC cells, HCC827/GR, exhibited significantly higher basal exosomal release and lysosomal exocytosis compared to gefitinib-sensitive NSCLC cells, HCC827. This difference was associated with an increased expression of TRPML3 in HCC827/GR cells. Furthermore, we observed a close correlation between the elevated exosomal release and lysosomal exocytosis and the upregulation of TRPML3 expression. Notably, the triggering of lysosomal Ca²⁺ release through TRPML3 was facilitated by gefitinib-induced elevation of lysosomal pH. Our investigation demonstrated that deficiency of TRPML3 resulted in gefitinib-induced cell death, as indicated by the accumulation of Sub-G0 population, hindered cell proliferation, and facilitated poly (ADP-ribose) polymerase cleavage.

Conclusion

In summary, our data demonstrate the emerging role of TRPML3 as a molecular factor in anti-cancer drug resistance. By effectively sensing lysosomal pH acidification, TRPML3 orchestrates lysosomal Ca²⁺

release, subsequently influencing lysosomal trafficking, exocytosis, and exosomal release. This study contributes to comprehending the defense mechanisms that developed by acquired drug resistance to tyrosine kinase inhibitor gefitinib in NSCLC cells.

EACR25-1572

Unlocking New Therapies: Targeting GSDMB Overexpression in HER2-Positive Tumors

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Introduction

Gasdermin B (GSDMB) protein has a dual function in cancer, rendering it as a potential therapeutic target. On the one hand, this protein has the intrinsic capacity to induce pyroptosis, a mechanism that may be capable of eradicating certain tumor cells. On the other hand, data from our lab revealed that GSDMB is overexpressed in a subset of HER2+ breast (BC) and gastro-esophageal tumors, where it correlates with unfavorable clinical outcomes, facilitating multiple pro-tumor activities, including invasion, progression, metastasis, and resistance to anti-HER2 therapies. In view of the above, the objective of this study is to identify and characterize novel GSDMB-targeted anti-tumor compounds.

Material and method

Using different HER2/GSDMB+ BC models, we tested the effects of compound A selected through high-throughput drug screening, using different complementary in vitro approaches such as cell viability (Alamar Blue) assay or lactate dehydrogenase (LDH) enzyme release assays and others. Additionally, protein expression analyzed by Western blot and in silico docking studies were performed. Furthermore, these findings were tested and validated in preclinical animal models.

Result and discussion

Following a large high-throughput screening, one orphan FDA-approved compound (compound A) was identified as a potential hit, and subsequently validated, exhibiting a positive GSDMB-dependent cytotoxic effect reducing the viability of GSDMB-overexpressing tumor cells.

Preliminary in silico studies indicated a potential direct binding of compound A with GSDMB, suggesting the possibility of a thermodynamically stable complex and reinforcing the concept of drug-target specificity. Consequently, we tested the potential induction of pyroptosis following compound A treatment.

Interestingly, this compound not demonstrably influence pyroptotic cell death (as measured by LDH release), suggesting that it may eradicate GSDMB+ cells through alternative cell death mechanisms. While no evidence of apoptosis has been observed, but a slightly induce senescence was observed. Finally, the therapeutic capacity of this compound was evaluated in preclinical in vivo mouse models using mice bearing orthotopically injected GSDMB-expressing and silencing HER2+ BC cells. These results demonstrated a reduction in tumor growth and tumor weight ex vivo, as well as a higher percentage of tumor necrosis ex vivo in those tumors expressing GSDMB versus those silenced, indicating a promising therapeutic efficacy of this compound in vivo.

Conclusion

The potential utility of compound A as a novel drug targeting GSDMB has been identified and validated at least in HER2 BC. This drug could preferentially reduce the viability of GSDMB-overexpressing BC cells, and it may serve as a starting point for the development of a novel and effective therapeutic approach for HER2/GSDMB+ BC.

EACR25-1592

FGFR signaling as a key mediator of resistance to alpelisib/fulvestrant combination in PIK3CA-mutant luminal A breast cancer

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Introduction

Luminal A breast cancer is characterized by HR positivity and HER2 negativity. Mutations in the PI3K pathway are common in these tumors. While PI3K inhibitors, such as alpelisib, are FDA-approved for treating PIK3CA-mutant breast cancers, the inhibition of the PI3K pathway can lead to the activation of compensatory signaling pathways, resulting in drug resistance. The objective of this study is to identify possible resistance mechanisms associated with the FDA-approved combined use of alpelisib and fulvestrant in the treatment of PIK3CA mutant breast cancer. Transcriptomic analysis reveals the activation of FGFR signaling as a key resistance mechanism. We further demonstrate the synergistic growth inhibition activity of pemigatinib, an

FDA-approved pan-FGFR inhibitor, when combined with alpelisib in cell lines and 3D tumor spheroids resistant to alpelisib/fulvestrant treatment.

Material and method

MCF7 and T47D cell lines with acquired resistance to the alpelisib/fulvestrant combination were generated and subjected to comparative transcriptome analysis to identify potential resistance networks. Immunoblotting was used to validate key components of the resistance-associated signaling pathways. To elucidate the functional impact of FGFR-dependent resistance mechanisms, resistant cells, and 3D tumor spheroid models were treated with the combination of alpelisib/pemigatinib. Additionally, FGFR2 was overexpressed in fulvestrant-resistant cells to assess its role in acquired alpelisib resistance. Ongoing *in vivo* experiments are being conducted to evaluate the efficacy of this combination treatment in preclinical tumor models.

Result and discussion

Transcriptome analysis implicated FGFR and downstream MAPK signaling pathways as key factors contributing to resistance. Immunoblotting confirmed the activation of these pathways in resistant cells. Treatment with the combination of alpelisib/pemigatinib demonstrated synergistic growth inhibition in 2D cells and 3D tumor spheroids. Notably, combinatorial treatment in both models led to a pronounced reduction in S6 phosphorylation and resulted in lasting tumor regression in 3D spheroids, surpassing the effects of individual treatments. Furthermore, overexpression of FGFR2 in fulvestrant-resistant cells significantly increased alpelisib resistance, suggesting that FGFR signaling may have a critical role in the acquired alpelisib/fulvestrant resistance. The combinatorial effects of alpelisib and pemigatinib on *in vivo* tumor growth are being evaluated.

Conclusion

Our study identifies FGFR signaling as a key mediator of resistance to alpelisib and fulvestrant in PIK3CA-mutant luminal A breast cancer cells. These findings suggest that targeting the FGFR pathway in combination with PI3K inhibition may represent a potential strategy to overcome drug resistance in advanced-stage luminal A breast cancer.

EACR25-1612

Targeting Ferroptosis to Enhance the Efficacy of BRAF/MEK Inhibitors in Overcoming Resistance in Melanoma

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Introduction

Melanoma is one of the most aggressive cancers, often displaying resistance to targeted therapies, particularly those involving BRAF/MEK inhibitors. While these therapies offer significant benefits in certain melanoma subtypes, acquired resistance remains a critical challenge. Ferroptosis, an iron-dependent form of cell death, has

recently emerged as a promising strategy to counteract this resistance. This study aims to explore the role of ferroptosis in melanoma and evaluate its potential as a therapeutic strategy in combination with BRAF/MEK inhibitors.

Material and method

Human melanoma cell lines (A375, SK-MEL-28) with BRAF mutations were used to model melanoma resistance. Ferroptosis induction was assessed using specific inhibitors and activators of this pathway, such as RSL3 and erastin. Cell viability assays, gene expression analysis, and western blotting were performed to measure the effects of ferroptosis induction alone and in combination with BRAF/MEK inhibitors (e.g., vemurafenib and trametinib). Additionally, the response of melanoma cells to this combination treatment was evaluated through oxidative stress and cell death markers.

Result and discussion

Our results show that induction of ferroptosis in melanoma cells significantly reduced cell viability and enhanced cell death, particularly when combined with BRAF/MEK inhibitors. Ferroptosis induction elevated oxidative stress levels, which appeared to sensitize melanoma cells to the effects of BRAF/MEK inhibitors. The combination treatment resulted in a synergistic therapeutic response, overcoming the resistance commonly seen with BRAF/MEK monotherapy. Gene expression analysis revealed upregulation of pro-ferroptotic and stress-response genes, supporting the potential of ferroptosis in combination therapy.

Conclusion

The induction of ferroptosis presents a promising strategy for enhancing the efficacy of BRAF/MEK inhibitors in the treatment of melanoma. Our findings suggest that ferroptosis could overcome treatment resistance and improve therapeutic outcomes in patients with advanced melanoma. Further preclinical and clinical studies are warranted to validate these results and optimize combination strategies involving ferroptosis and targeted inhibitors.

EACR25-1617

SOS1 inhibition enhances the efficacy of and delays resistance to oncogene-targeted therapies in lung adenocarcinoma

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Introduction

Lung cancer is the leading cause of cancer-related death worldwide; adenocarcinoma is the most common subtype of lung cancer. Oncogenic driver mutations in the RTK/RAS pathway occur in 75–90% of lung adenocarcinoma (LUAD). Receptor Tyrosine Kinase (RTK) and RAS pathway-targeted therapies have substantially improved outcomes in LUAD. However, in most cases both intrinsic and acquired resistance to these agents limits

their long-term effectiveness. Resistance to osimertinib in EGFR-mutated tumors, KRASG12C inhibitors in KRASG12C-mutated tumors, or the MEK inhibitor trametinib in KRAS-mutated and NF1-LOF tumors most often develops via activation of multiple RTKs, often within the same tumor. These data suggest that combating therapeutic resistance via individual RTK inhibitors will be ineffective. In contrast, we hypothesize that broad inhibition of RTK signaling via a common downstream signaling node has the potential to enhance the efficacy of and delay resistance to targeted therapies in a majority of LUADs.

Material and method

To test this hypothesis, we built an experimental framework that models the evolution of cancer cells to therapeutic pressure *in situ*. Using this framework, we assess for therapeutic combinations that (i) enhance therapeutic efficacy to overcome intrinsic/adaptive resistance, (ii) limit the survival of drug-tolerant persister (DTP) cells capable of driving acquired resistance, and (iii) delay the onset of and/or block the development of resistant cultures.

Result and discussion

Using this framework we show that proximal RTK signaling intermediates SHP2, SOS1, and SOS2 are therapeutic targets whose inhibition both enhance the efficacy of and delay resistance to RTK/RAS pathway-targeted therapies by targeting DTPs in EGFR-mutated, KRASG12-mutated, and NF1-LOF LUAD cells. We further show that the effectiveness of co-targeting proximal RTK signaling showed genotype-specificity; SOS1 inhibitors did not synergize with or prevent resistance to MEK inhibitors in KRASQ61-mutated cells or cells harboring PIK3CA co-mutations. Finally, we show that SOS1i can re-sensitize G12Ci and EGFRi DTPs to oncogene-targeted therapies, and combined EGFRi+SOS1i treatment inhibited EGFR-mutated tumor growth *in vivo* to a greater extent than EGFRi alone.

Conclusion

Our data present a framework for rapidly evaluating and selecting optimal combinations therapies prior to moving into extensive longitudinal pre-clinical animal studies. Our data further show that targeting proximal RTK signaling in combination therapy using a SOS1 inhibitor is a potential strategy to prolong the effectiveness of oncogene-targeted therapies for a majority of LUAD patients.

EACR25-1622

Investigation of the expression of drug resistance genes after Sorafenib administration

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Introduction

Hepatocellular carcinoma (HCC) is the most common primary liver cancer, accounting for 85–90% of cases and is a leading cause of cancer-related mortality worldwide. HCC represents a classic paradigm of inflammation-

related cancer, with various risk factors contributing to chronic liver inflammation and subsequent tumor development. It is characterized by high intrinsic drug resistance, leading to limited chemotherapeutic efficacy and relapse after treatment. Sorafenib is an FDA-approved first-line drug for the treatment of advanced HCC, which can extend overall survival in patients. As a multikinase inhibitor, sorafenib inhibits tumor cell proliferation and angiogenesis while inducing cancer cell apoptosis. However, its efficacy is limited due to primary and acquired resistance, poor solubility, fast metabolism, and low bioavailability. The ongoing research aims to overcome sorafenib resistance and improve its efficacy in HCC treatment. Understanding these mechanisms is crucial for developing novel therapeutic strategies to overcome drug resistance and improve patient outcomes in HCC treatment. In this study, we will examine the expression of genes involved in multidrug resistance after sorafenib resistance in hepatocellular cell model Hep3B cells.

Material and method

Hep3B cells were cultured in high glucose DMEM containing 10% FCS and treated with predetermined sorafenib. After sorafenib resistance is established, resistance formation will be checked by the MTT assay, colony formation, and scratch test. Hep3B-SR and Hep3B cells were trypsinized, and the cell pellets were collected. Total RNA was isolated using a cell pellet with an RNA extraction kit. Total RNA was reverse transcribed into cDNAs using a cDNA synthesis kit. cDNA was used as a template for real-time analysis. The human beta microglobulin gene was used as an internal control. MDR1, MRP1, MRP2, MRP3, and BCRP in multidrug resistance-associated gene expressions were determined using specific primers at the mRNA level.

Result and discussion

Sorafenib resistance in Hep3B-SR cells was demonstrated by MTT assay, scratch test, and colony formation. Gene expression analysis also showed that MDR1, MRP1, MRP2, MRP3, and BCRP genes, which are related to drug resistance, were differentially regulated in their expression.

Conclusion

The observed sorafenib resistance in Hep3B-SR cells is likely the result of a multifaceted process involving the upregulation of various drug efflux transporters. This understanding could potentially lead to the development of targeted therapies to overcome sorafenib resistance in HCC, possibly by inhibiting specific transporters or their regulatory pathways.

EACR25-1644

Targeting CCL19/21-CCR7 axis to overcome resistance to ALK inhibitors in ALK+ lymphoma

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Introduction

Anaplastic Large Cell Lymphoma (ALCL) is a T-cell lymphoma driven by the constitutive activation of the Anaplastic Lymphoma Kinase (ALK) due to a chromosomal translocation of the gene, resulting in the formation of the oncogenic chimeric protein NPM-ALK. Many patients with ALK+ ALCL tumors that undergo remission for years quickly relapse upon TKI cessation due to a re-activation of drug-tolerant persister cells (DTPC). We have demonstrated a role for the tumor microenvironment (TME) as a key element in supporting the survival of DTPCs. We identified a role of the CCL19/21-CCR7 survival pathway in DTPCs resistant to ALK TKI. In this work, we investigate whether blocking CCR7 with a newly developed IgG human antibody (CAP-100), combined with ALK TKI crizotinib can prevent relapses by eradicating DTPCs.

Material and method

CCR7 expression and CAP-100 binding were tested by flow cytometry with a commercial CCR7 antibody and CAP-100 in ALK+ ALCL cell lines and PDX-derived cell line. Patient-Derived Xenograft (PDXs) mouse models were made by subcutaneously injecting ALK+ ALCL cells in NSG mice (PDX-DN and PDX-MTK). For in vivo experiments mice were split into four cohorts of treatment: vehicle, CAP-100, crizotinib, and a combination of both drugs. Crizotinib was given orally at 50 mg/kg/day in PDX-DN and at 100 mg/kg/day in PDX-MTK; CAP-100 was given by peritoneal injection every 3 days at a dosage of 10mg/kg in both PDX models. Tumor growth was tracked with a caliper every three days. Mice were euthanized at a human endpoint. Tumors were resected and snap-frozen or formalin-fixed for further analysis. Immunohistochemistry (IHC) analysis was performed to assess ALK positivity on tumor tissues and organs.

Result and discussion

CCR7 expression was assessed in ALK+ALCL cell lines and both PDX models, showing positivity in all lines and PDXs with some heterogeneity. In therapeutic experiments, after two weeks of treatment, mice treated with crizotinib alone or with the combo showed decreased tumor mass in both PDX models. In PDX-DN model, after treatment suspension mice that received crizotinib alone all relapsed. In contrast, mice treated with the combo crizotinib+CAP100 had significantly extended survival, and only one out of eight mice had a late relapse. In the PDX-MTK model, mice treated with crizotinib alone developed brain dissemination; mice treated with the crizotinib+CAP100 showed significantly less brain involvement, also confirmed by immunohistochemistry for ALK

Conclusion

CAP-100 boosts crizotinib activity in ALK+ ALCL PDX models by aiding the killing of DTPC in a subset of mice. The addition of CAP-100 to crizotinib markedly reduced the dissemination of lymphoma cells to the brain confirming our previous data that showed that genetic knock-out of CCR7 impaired lymphoma dissemination to the brain. Overall, these data show that the addition of CAP-100 to an ALK TKI could improve the cure rate and prevent lymphoma spread to critical sites like the brain.

EACR25-1655

Intrinsic Mechanisms of Resistance to CDK4/6 Inhibitors in Luminal Metastatic Breast Cancer

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Introduction

Hormone receptor-positive (HR+) and HER2-negative breast cancer accounts for approximately 70% of cases. In early stages, standard treatment includes surgery and endocrine therapy (ET), reducing the risk of late recurrence. However, over 30% of patients develop advanced disease, for which CDK4/6 inhibitors (CDKi) represent the standard treatment. Approximately 20% of patients show intrinsic resistance to combined therapy, and no reliable tools exist to predict treatment response. Therefore, managing patients with resistance to CDKi presents a significant clinical challenge. Preliminary studies from our group have shown increased STAT3 expression in circulating tumour cells from patients treated with CDKi who did not respond to first-line treatment (recurrence within 6 months by RECIST criteria). Therefore, we aim to demonstrate whether STAT3 is associated with the mechanisms underlying intrinsic resistance to CDKi in HR+/HER2- metastatic breast cancer.

Material and method

In vitro assays were performed using luminal breast cancer cell models. STAT3 was activated with IL-6 and inhibited using C188-9, a STAT3 inhibitor. STAT3 modulation was confirmed by Western blot. MTT assays were performed to study cell viability and resistance to CDKi (Palbociclib). Transwell migration assays were conducted to assess functionality. Transcriptomic analysis by RNA sequencing was performed externally. Statistical significance was determined by the Mann-Whitney U test ($p < 0.05$) or Wald binomial test ($|fc| \geq 2$ and raw p -value < 0.05).

Result and discussion

IL-6-induced cell models showed enhanced STAT3 activation, as indicated by the STAT3/p-STAT3 ratio by Western blot. In contrast, inhibited cell models showed reduced phosphorylation levels in STAT3, confirming the inhibitory effect of C188-9. Thus, they are suitable models for studying the link between STAT3 activation and resistance to CDKi. The activation of STAT3 (via IL-6) induces resistance to CDKi, and its inhibition in induced cells increased the sensitivity to CDKi ($p = 0.028$), suggesting that STAT3 inhibition may reverse resistance to CDKi. Moreover, STAT3 activation

associates with increased cell migration and enhanced viability. Differential gene expression analysis between cell lines with activated/inhibited STAT3 identified a set of genes that by Gene Ontology analysis revealed an enrichment in key pathways related to tumour progression and therapy resistance (PPI enrichment p-value: 0.00148) that need further validation.

Conclusion

Preliminary data suggest that STAT3 activation induces resistance to CDK inhibitors in HR+/HER2-cells, while its inhibition sensitises these cells to treatment. These findings highlight the potential of STAT3 as a response biomarker and a possible therapeutic target to counteract resistance to CDK inhibitors in this breast cancer subtype.

EACR25-1664

Genomic Instability and Epigenomic Dysregulation as Hallmarks of mCRC Therapy Resistance – Findings from Broad ctDNA Molecular Profiling (METACC)

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Introduction

Despite its significance, the mechanisms behind therapy resistance in metastatic colorectal cancer (mCRC) remain poorly understood. Circulating tumor DNA (ctDNA) offers a unique opportunity to explore them, as it thoroughly captures intratumor heterogeneity and facilitates longitudinal monitoring. However, most studies have focused on known mutations, mainly related to anti-EGFR resistance. The METACC study aims to go beyond mutation-centric approaches to uncover novel mCRC therapy resistance mechanisms.

Material and method

We analyzed a retrospective cohort of 30 microsatellite-stable mCRC patients, with plasma samples collected at diagnosis and at progression to first-line therapy (chemotherapy ± anti-VEGF or anti-EGFR). Whole-exome sequencing (WES) was performed on both timepoints and on matched WBCs (to exclude non-tumoral variants). Unique molecular identifiers and high-sensitivity variant calling enabled robust detection of low-frequency variants. McNemar's test identified mutations enriched at progression, and multivariable Cox regression assessed their impact on survival. Methylation changes were analyzed using Infinium MethylationEPIC v2.0 BeadChips (~930k CpGs) with a plasma-adapted

workflow. SeSAMe processing and subsequent DMRCate analysis identified differentially methylated regions (DMRs), prioritizing those overlapping with regulatory regions.

Result and discussion

Plasma WES identified ARID1A mutations as the main acquired event (progression vs. diagnosis: 14/30 vs. 5/30; p = 0.022), shortening time to progression (HR = 3.2, p = 0.036). Variant allele frequency (VAF) gains in ARID1A correlated with increases in the tumor mutational burden (TMB) ($\rho = 0.37$; p = 0.037), in line with the gene's role in maintaining genomic integrity. Progression also concurred with VAF increases in genes associated with therapy resistance via 1) epigenetic dysregulation (e.g.: NF1, KMT2D), and 2) DNA mismatch repair (MSH2, MLH1). The latter strongly correlated with TMB ($\rho = 0.72$; p < 0.001), further implicating genomic instability. Differential methylation analysis identified 16,026 DMRs (198 with $|\Delta\beta| > 0.2$), including the hypermethylation of an ARID1A enhancer ($\Delta\beta = 0.29$), suggesting a dual mechanism of ARID1A inactivation. Enrichment analysis linked these epigenetic alterations to three major pathway groups: 1) tumor metabolism and microenvironment, 2) anti-EGFR resistance, and 3) longevity/mTOR signaling pathways. Notably, hypermethylation of the mTOR inhibitor DEPTOR ($\Delta\beta = 0.35$) and hypomethylation of SOX2-OT ($\Delta\beta = -0.33$) suggest a role in enhancing tumor plasticity and stemness.

Conclusion

Comprehensive ctDNA profiling reveals tumor plasticity as a key mechanism of resistance to therapy in mCRC, driven by both genomic instability and epigenomic dysregulation. Expanding the METACC cohort will help explore actionable biomarkers like ARID1A loss, which may inform immunotherapy strategies.

EACR25-1689

Overcoming Antibody-Drug Conjugate Resistance in HER2-Positive Breast Cancer: Insights into Molecular Mechanisms and Targeted Therapeutic Approaches

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Introduction

HER2-positive breast cancer accounts for 15–20% of all breast cancer cases. Second-generation antibody-drug conjugates (ADC) trastuzumab deruxtecan (T-DXd; Enhertu®) and trastuzumab duocarmazine (SYD985) have shown effectiveness in HER2-positive and HER2-low metastatic breast cancer. However, not all patients respond due to intrinsic or acquired resistance, as observed with other HER2-targeting therapies. Efforts to understand the mechanisms involved in resistance are essential for identifying strategies to overcome it.

Material and method

Cas9 expressing HER2-positive HCC1954 and MDA-MB-361 cells were transduced with the MinLibCas9 single guide RNA (sgRNA) library and exposed to T-

DXd and a SYD985 biosimilar as well as their respective cytotoxic payloads (DXd, a topoisomerase 1 inhibitor and DUBA, a DNA alkylator) for 30–45 days. High-throughput sequencing identified gene knockouts enriched or depleted in response to treatment. BT-474, HCC1954, and MDA-MB-361 cells were continuously cultured in T-DXd for 30–45 days to establish resistant models. RNA was extracted and subjected to RNA sequencing (RNASeq) to identify transcriptional changes associated with resistance. Antiproliferative activity of treatments was evaluated by thymidine incorporation assay.

Result and discussion

Whole-genome CRISPR/Cas9 screens revealed a high correlation of gene hits between the ADCs and their payload, particularly for T-DXd and DXd. Gene hits showed limited overlap between T-DXd and the SYD985 biosimilar, suggesting distinct resistance mechanisms for different ADCs. The top 500 candidate genes were identified for further validation using a focused sgRNA library. T-DXd resistance emerged after continuous culture in all three cell lines. HER2 expression was retained in BT-474 and MDA-MB-361 but decreased in HCC1954 cells. BT-474 and MDA-MB-361 resistant cells retained sensitivity to the ADC trastuzumab emtansine and the tyrosine kinase inhibitors neratinib and lapatinib. All resistant models also exhibited cross-resistance to the DXd payload. RNASeq analysis revealed upregulation of interferon-alpha and interferon-gamma signalling pathways across all resistant cell lines, while RET emerged as the most significantly down-regulated gene. Combination therapy with T-DXd and the RET inhibitor selretinib demonstrated synergy in all three treatment-naïve cell lines.

Conclusion

Our study provides insight into the molecular basis of ADC resistance, identifying potential targets to overcome resistance and enhance ADC efficacy in HER2-positive breast cancer. The identification of RET downregulation in resistant cells and the observed synergy between T-DXd and selretinib highlight a potential strategy for limiting T-DXd resistance.

EACR25-1698

Exploring potential strategies overcoming the PARPi resistance in the Olaparib induced MDA-MB-436 resistant model

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Introduction

Poly (ADP-ribose) polymerase inhibitors (PARPi) have emerged as a promising therapeutic approach in the treatment of various cancers, particularly those with deficiencies in DNA repair mechanisms. However, the clinical efficacy of PARPi is often limited by the development of resistance, which poses a significant challenge to their long-term use. Elucidating the molecular mechanisms underlying PARPi resistance is crucial for developing novel therapeutic agents and

strategies to overcome this resistance and optimize patient treatment regimens.

Material and method

Olaparib-resistant MDA-MB-436 breast cancer cell line was generated through prolonged in vitro exposure to escalating doses of Olaparib. In vitro and in vivo efficacy studies were conducted to validate the resistance to Olaparib.

Result and discussion

The MDA-MB-436-R-Olaparib model exhibited robust drug tolerance both in vitro and in vivo, with cross-resistance to other PARP inhibitors. Notably, cell viability assays revealed that the MDA-MB-436-R-Olaparib cells had reduced sensitivity to cisplatin compared to parental MDA-MB-436 cells.

Conclusion

In summary, this Olaparib-resistant model facilitates a systematic investigation of PARPi resistance mechanisms and discovery of combinatorial therapeutic strategies.

EACR25-1707

Chlorpromazine nanoparticles enhance anti-cancer effect of Doxorubicin nanoparticles preventing their extrusion

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Introduction

Anti-cancer agents like Doxorubicin (DOX) encapsulated in biodegradable PLGA nanoparticles (NPs) can be used to overcome challenges like off-target effects, drug resistance and dose limiting toxicity. Previous findings in our lab show that NPs are also actively exocytosed out of the cell and modulation of intracellular trafficking of NPs through the subcellular membranous structures, using small molecules like Chlorpromazine (CPZ), may play a key role in enhancing NPs retention within the cells and allow greater drug unloading in a single pass. We have tested this effect of CPZ to enhance anti-cancer effect of Doxorubicin (DOX) incorporated NPs.

Material and method

DOX and CPZ drug incorporated PLGA NPs were synthesized by single emulsion solvent evaporation method and characterized. Drug encapsulation efficiency was calculated. The IC₅₀ of DOX and CPZ was calculated in three cell lines: LN229, HeLa and U87 MG for 48hr treatment. Percentage viability of cells following 48hr treatment with various combinations of DOX and CPZ NPs were checked in the three cancer cell lines by MTT assay with appropriate controls.

Result and discussion

The average size of nanoparticles was less than 230nm and average zeta potential was less than -7mV. MTT assay 48 hrs following treatment with various combinations of DOX and CPZ NPs demonstrated:

- i) PLGA NPs itself were not toxic.
- ii) Significant cytotoxicity was observed by both DOX in solution and DOX incorporated NPs when given at doses below IC₅₀.
- iii) CPZ has some cytotoxic effects of its own when given in solution whereas no significant cytotoxicity was observed when incorporated within NPs.
- iv) Cytotoxicity due to DOX NPs was significantly enhanced in the presence of CPZ in solution as well as

CPZ-NPs but co-treatment with CPZ NPs showed better cytotoxicity as compared to CPZ in solution.

Conclusion

Our results demonstrate that co-treatment of DOX-NPs along with CPZ in solution or as CPZ NPs significantly enhance the cytotoxic efficiency of the anticancer drug, thereby reducing the dose of anticancer drug required. Co-treatment of DOX-NPs with CPZ-NPs showed better cytotoxicity as compared to CPZ in solution.

EACR25-1709

Involve ment of Androgen Receptor/miR-181a-5p/c-Met Axis in the Progression of Prostate Cancer

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Introduction

Prostate cancer (PCa) is one of the most common cancers worldwide, and its incidence is still increasing. Androgen deprivation therapy (ADT) is an essential treatment method in the management of PCa. However, most patients will eventually develop resistance to ADT and will have a poor prognosis. c-Met is a receptor kinase causing ADT resistance. Research showed an inverse correlation between androgen receptor (AR) and c-Met in the primary PCa tumors. Recently, miRNA-related therapeutic approaches have gained attention due to their potential in reversing the pathology of diseases. In this study, we investigated the role of miRNAs in regulating AR and c-Met signaling in the progression of PCa.

Material and method

Large patient cohorts were analyzed by bioinformatic tools to determine the expression pattern of c-Met, AR, and miRNAs in PCa. TransmiR were used to find miRNAs transcriptionally regulated by AR. Precursors and mature-miRNA, AR, and c-Met expressions were studied in PCa cells by qPCR. The activation and expression level of c-Met and AR were analyzed by Western Blot (WB). Cells were transfected with miRNA oligos (mimic or inhibitor) and their controls to investigate the effect of miRNA on c-Met and/or AR signaling and biological behaviors of cells.

Result and discussion

In silico analysis of AR, miR-181a-5p and c-Met expression confirmed that AR and miR-181a-5p levels are higher in the PCa primary tumors than in normal prostate tissues, whereas c-Met expression levels were lower in the same cohort. Bioinformatic analysis revealed that AR might regulate miR-181a-5p expression because hsa-mir-181a-2 and hsa-mir-181a-1 genes, which form mature miR-181a-5p, have “androgen response element” in their promotor regions. Moreover, AR negative cell line (PC3) expressed high c-Met and miR-181a-5p,

whereas cells (LNCaP) with high AR levels have low c-Met expression. Transfections with miRNA-oligos showed that miR-181a-5p regulates c-Met signaling and the biological behaviors of PCa cells, including the proliferation migration and clonogenicity. Overall, it seems that in prostate cancer progression, AR regulates miR-181a-5p expression to suppress c-Met expression; however, when signaling is inhibited by AR-targeted therapies, a decrease in miR-181a-5p expression is observed.

Conclusion

These findings display a novel molecular mechanism of AR/miR-181a-5p/c-Met axis in the progression of PCa. Strategies to increase miR-181a-5p levels might be an alternative approach for the inhibition of c-Met to prevent resistance to ADT.

This project is funded by TUBITAK (project #223S749).

EACR25-1715

miR-212-3p Down-Regulates the MAPK/ERK Pathway to Mitigate Oxaliplatin Resistance in Colorectal Cancer

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Introduction

Colorectal cancer (CRC) is one of the three most lethal cancers in the world. Chemotherapy, such as FOLFOX, is an oxaliplatin-based regimen used as an adjuvant treatment. However, prolonged chemotherapy often leads to drug resistance, reducing efficacy and increasing recurrence rates. It was reported that the ERK signaling pathway is involved in regulating oxaliplatin resistance. MicroRNAs (miRNAs) are critical regulators of chemotherapy resistance and drug efflux. We found that miR-212-3p is related to the MAPK/ERK pathway. This study investigates the role of miR-212-3p in overcoming drug resistance and highlights its potential as a therapeutic target to enhance treatment outcomes in CRC.

Material and method

Human CRC cell lines (HCT116, LoVo, SW480) and their corresponding oxaliplatin-resistant cells (oxaR#9, LoVoR, SW480R) were used to investigate the potential mechanisms of oxaliplatin resistance. After the transfection of miRNA mimics or siRNA, the expression level of miR-212-3p and FGF7 was analyzed by qPCR, and the ERK pathway-related proteins were detected by Western blot analysis. The CCK-8 assay and colony formation were used to evaluate the cell viability of oxaliplatin-resistant CRC.

Result and discussion

Previous reports and our results demonstrated that the ERK signaling pathway is highly activated in oxaliplatin-resistant CRC cells. A synergistic effect was observed when the ERK inhibitor trametinib was combined with oxaliplatin, resulting in enhanced anti-tumor effect of oxaliplatin-resistant CRC cells. We identified that the miR-212-3p had significantly reduced expression in oxaliplatin resistant CRC cells and associated with poor prognosis. Overexpression of miR-212-3p in drug-resistant CRC cells reduced oxaliplatin resistance,

induced apoptosis, and inhibited the ERK pathway. Additionally, FGF7 was confirmed as an miR-212-3p downstream gene. The high expression level of FGF7 was associated with poor prognosis, and its expression is significantly higher in resistant cells than in parental cells.

Conclusion

In this study, we showed that the overexpression of miR-212-3p significantly reduced drug resistance in CRC cells and inhibited ERK signaling. MiR-212-3p can directly regulate FGF7, and its overexpression is associated with poor prognosis. The potential of miR-212-3p as a biomarker to identify oxaliplatin-resistant patients who may respond to trametinib provides new options for personalized treatment approaches.

EACR25-1777

Functional and molecular changes implicated in doxorubicin resistance in breast cancer

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Introduction

Breast cancer (BC) is a prevalent oncological disease among women worldwide. Although therapeutic options for BC have improved significantly in recent years, the emergence of therapeutic resistance substantially decreases treatment efficacy. Doxorubicin (DOX), a chemotherapeutic agent classified as a topoisomerase II inhibitor, is commonly used in the treatment of triple-negative BC and is also employed in advanced stages of hormone receptor-positive or HER2-positive BC. This work describes the derivation of a cell line model to explore the mechanisms contributing to acquired DOX resistance in BC.

Material and method

DOX-resistant cancer cells were derived from three BC cell lines (MDA-MB-231, JIMT-1, and T-47D) through continuous exposure to increasing DOX concentrations. A luminescent cell viability assay was used to assess the sensitivity of BC cells to chemotherapy. Functional assays included cell proliferation and migration analysis using the IncuCyte ZOOM system, *in vivo* tumorigenicity tests in SCID/bg immunodeficient mice, and β -galactosidase staining. Molecular and genomic changes in the resistant cancer cells were assessed using the RT2

Profiler PCR array, qRT-PCR, western blotting, array-based comparative genomic hybridization, and whole exome sequencing.

Result and discussion

The derived BC cells exhibited a 5 to 8-fold increase in IC₅₀ values upon the treatment with DOX, confirming their resistant phenotype. Cell proliferation and tumor growth were decreased in DOX-resistant cancer cells, but no statistically significant changes in cell migration were observed. Additionally, MDA-MB-231 and T-47D DOX-resistant cells displayed reduced β -galactosidase activity, a marker of cellular senescence. Molecular analysis revealed the deregulation of genes involved in drug resistance mechanisms, including those encoding ABC-transporters, genes involved in apoptotic signaling, drug metabolism, and genes and proteins implicated in DNA methylation processes. These changes partially corresponded with the observed copy number alterations in drug-resistant cells. Finally, single-nucleotide variants were prevalent mutations in the resistant cancer cells, affecting multiple genes, including the target enzyme of DOX.

Conclusion

The acquisition of DOX resistance in BC is a complex process involving diverse changes at genomic, molecular, and functional levels. These changes include well-described multi-drug resistance mechanisms, alterations specific to DOX resistance, and newly observed changes in drug-resistant BC cells that warrant further investigation.

This work was supported by the EraCoSysmed project RESCUER and VEGA 2/0067/22. We acknowledge the Genomics Core Facility and Bioinformatics Core Facility of CEITEC Masaryk University of A4L_ACTIONS, supported by the European Union's Horizon 2020 under grant agreement No. 964997.

EACR25-1781

Sensitization of ovarian cancers to PARP inhibitors by induction of an HRD phenotype: towards a medicine of precision based on UBE2N inhibition

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Introduction

Ovarian cancers (OC) represent the leading cause of death from gynecological cancers worldwide, advanced stages displaying a survival rate below 40%. First-line treatment associates surgery and chemotherapy based on carboplatin and PARP inhibitors (PARPi). However, PARPi effectiveness relies on deficiencies in Homol-

ogous Recombination DNA repair (HRD status), present in 50% of OC patients. Double stranded DNA breaks can be repaired by Non-Homologous End Joining (NHEJ) or Homologous Recombination (HR) pathways. While HR is considered faithful, NHEJ is not and leads to cell death. Hence, defects in HR (HRD) sensitize to DNA damaging drugs. Our project is to develop a precision medicine approach to induce an HRD phenotype via UBE2N inhibition, an essential mediator of HR, to sensitize ovarian tumours to DNA damaging drugs (carboplatin and PARPi).

Material and method

We used an ovarian cancer cell line and PDTO (Patient-Derived Tumor Organoids) models generated from tumor of patients grown in 3D in extracellular matrix. To inhibit UBE2N, we use an inhibitor described by the literature, NSC697923 and the lipotransfection of Cas9-RNP. HR DNA repair following UBE2N inhibition (UBE2Ni) will be assessed using multiple approaches: the comet assay (DNA damage), the RECAP test (activation of HR DNA repair mechanisms), the DR-GFP reporter system (effective HR DNA repair), and micronuclei quantification, a marker of genomic instability induced by the HRD phenotype. In parallel, we will investigate a potential correlation between the increase in DNA damage and/or the decrease in DNA repair by HR induced by UBE2N inhibition and its ability to sensitize cells to the action of DNA-damaging therapies (carboplatin, PARPi). Sensitization will be assessed by monitoring cell morphology using the IncuCyte S3 and CellDiscoverer 7 real-time imaging systems, as well as through viability assays and colony-forming assays.

Result and discussion

UBE2Ni by the NSC697923 or by knockout increases micronuclei and DNA damage and decrease HR DNA repair. In addition, UBE2Ni sensitizes an ovarian cancer cell line and a PDTO HRP model to PARPi whereas it does not sensitize an already HRD model.

Conclusion

These encouraging preliminary results will be validated in more PDTO models, and the effects on DNA repair more finely characterized. They underscore the interest of UBE2Ni to sensitize OC to PARPi and justify the establishment of a drug design program with the Normandy Center for Drug Studies and Research (CERMN) to identify UBE2Ni for clinical using. Our strategy could be applied to other cancer locations where DNA-damaging therapies are currently used such as pancreatic cancer (oxaliplatin-based chemotherapy and PARPi), breast cancer (carboplatin, PARPi), and head and neck cancers (radiotherapy and cisplatin), thereby increasing its relevance in oncology.

EACR25-1827

A not expected effect of longterm temozolamide treatment on resistance of glioblastoma cells – an in vitro study

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Introduction:

Glioblastoma multiforme (GBM) is the most common malignant brain tumor with a very poor prognosis. The standard treatment for GBM is surgical resection followed by chemoradiotherapy with temozolamide (TMZ), an alkylating agent which methylates the DNA at various sites. Methylation of the guanine at the O6 position leads to a falsely inserted base nucleotide during transcription, which subsequently activates miss match repair (MMR), resulting in numerous single strand breaks and finally in cell death. Although TMZ extends the survival rate, tumors rapidly develop resistance, which is mainly based on the O6-methylguanine-DNA-methyltransferase (MGMT) expression as well as base excision repair (BER) and MMR. Clinical studies show that low expression of MGMT in glioma patients resulted in a better therapeutic outcome. To increase TMZ efficiency in GBM, a combined treatment with highly specific MGMT inhibitors like Lomeguatrib (LG) is used.

Material and method

We studied both a commercially available TMZ resistant (T98G) and non-resistant (U373) glioblastoma cell line as well as the resistant cell line U373R which was generated by us via long term TMZ treatment of U373. Normal human dermal fibroblasts (NHDF) were used as control for normal (healthy) cells. All cells were treated with increasing concentrations of TMZ from 10 µM to 1000 µM for 48 to 96h and/or 50 µM LG, respectively. Cell viability was determined by MTT and SRB Assay. MGMT occurrence was examined via western blot and qPCR. Incubation with 10 µM 5-Aza-2'-desoxycytidine (Decitabin) for 5 days was used to determine the effect of the that methylation inhibitor on MGMT expression.

Result and discussion

U373 cells show a significant decrease in cell viability after TMZ treatment compared to U373-R and T98G. For T98G, this result can be explained by a generally high expression of MGMT. Surprisingly, the U373R cells showed resistance from TMZ treatment without showing any MGMT expression. The use of the DNA-hypo-methylating agent Decitabin indicates an involvement of MGMT promotor hypermethylation in the observed downregulation of MGMT in U373R. To find out whether the MMR is also involved in the observed TMZ resistance, qPCR was performed to check for basal expression of MMR subunits, and indeed, a lowered expression of the 2 subunits MSH2 and MSH6, being major players in MMR, was observed in U373R compared to U373 and T98G.

Conclusion

The observed data verify that there are different resistance mechanisms in GBM cells to overcome TMZ toxicity. Although it is known that other resistance mechanisms apart from MGMT overexpression exist in GBM, it was shown for the first time that long term treatment with TMZ results in a loss of MGMT instead of an increase in expression. Those mechanisms must be evaluated to get a better understanding of the origin of the resistance, and these data may support the development of new treatment strategies.

EACR25-1885**Intercellular Communication via Therapy-Induced Secretome Drives****Chemoresistance in Ovarian Cancer Cells**

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Introduction

A key difference between cancer and healthy tissues is the significant plasticity of cancer cells in response to stress. Recent studies have demonstrated that this adaptation is mediated not only by intracellular signaling pathways but also by enhanced intercellular communication. Using ovarian cancer cell lines, we showed that the secretome of apoptotic cancer cells differs significantly from that of non-apoptotic cells and can induce phenotypic changes in recipient tumor cells, leading to the development of chemoresistance.

However, the molecular mechanisms underlying this process remain largely unexplored.

Material and method

We investigated the therapy-induced secretion (TIS) of apoptotic cancer cells and its impact on chemoresistance using ovarian cancer cell lines SKOV3, OVCAR3, and MESOV. Non-cancerous cell lines (HaCaT, FT282, and fibroblasts) were used as controls. Proteomic analysis was performed using a Q Exactive HF mass spectrometer. Recipient cells incubated with therapy-induced or control secretomes were analyzed using the Illumina HiSeq 2500 sequencing system. Drug sensitivity was assessed using the MTT assay. Functional assays, including immunocytochemistry, fluorescence microscopy, and flow cytometry, were conducted to evaluate DNA repair activity and cell cycle progression. Calcium dynamics were measured using transient transfection with a calcium sensor combined with live fluorescence imaging on the Celena X system.

Result and discussion

TIS specifically induced chemoresistance in cancer cells but not in normal cells. Proteomic analysis revealed that chemotherapy-induced secretion is a dynamic process, different sets of proteins secreted at different time points. These proteins were involved in metabolism, DNA repair, translation, pre-mRNA splicing, and cell cycle regulation, including factors which were not previously recognized as secreted molecules. Functional studies confirmed that incubation with TIS led to the upregulation of molecular pathways associated with DNA repair and cell cycle progression in recipient cells. Furthermore, incubation with TIS led to the acquisition of resistance to DNA-damaging agents by prolonging the S-phase of the cell cycle and activating the DNA repair system.

Conclusion

Our findings demonstrate that chemotherapy triggers a dynamic secretion of signaling molecules that modulate the cell cycle and enhance DNA repair in recipient cancer cells, thereby promoting chemoresistance. These findings suggest a complex regulatory mechanism underlying therapy-induced intercellular communication, which may

be an important factor in tumor adaptation to therapy.

The work was supported by the grant of the RSF №25-15-00520.

EACR25-1893**On-target mutations confer resistance to WRN helicase inhibitors in Microsatellite Unstable Cancer Cells**

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Introduction

Werner helicase inhibitors (WRNi) are in clinical development for treating microsatellite instability (MSI) tumors, which depend on WRN due to defective mismatch repair. However, acquired resistance may limit their efficacy. Here, we systematically investigate resistance mechanisms to WRNi in cancer cells, focusing on on-target WRN mutations, cross-resistance among clinical-grade inhibitors, and potential therapeutic strategies to overcome resistance.

Material and method

We performed genome-wide CRISPR screens, pharmacogenomic profiling, and time-to-progression (TTP) assays to identify WRNi resistance mechanisms. Semi-saturation mutagenesis of WRN and long-term drug exposure studies were used to track resistance emergence. Structural modeling predicted the impact of mutations on drug binding, while viability assays and DNA damage markers validated cross-resistance. Patient-derived organoids (PDOs) from MSI colorectal cancer were used to assess clinical relevance.

Result and discussion

CRISPR screens confirmed WRN's essential role in MSI cells, with no genetic bypass mechanisms identified. Pharmacogenomic profiling of WRNi revealed modulators of sensitivity, including SMARCAL1, a chromatin remodeler linked to WRN-MSI synthetic lethality. TTP assays and mutagenesis identified recurrent on-target WRN mutations driving resistance, including G729D, which disrupts WRNi binding, causing broad cross-resistance, and I852F, which selectively confers resistance to HRO761 while preserving sensitivity to VVD-133214. A non-canonical splice site mutation led to exon skipping but retained WRN function, allowing continued MSI dependency. These findings were validated in PDOs, where resistance to HRO761 emerged through the same exon-splicing mutation. Notably, WRNi-resistant cells remained sensitive to ATR inhibitors and irinotecan, supporting a WRN-specific resistance mechanism.

Conclusion

Our study identifies on-target WRN mutations as key drivers of three clinical grade WRNi resistance and highlights strategies to overcome it. By characterizing cross-resistance across clinical-grade WRNi, we propose switching inhibitors with different mechanisms of action to restore sensitivity. These findings provide a framework for biomarker-driven patient stratification, resistance

monitoring through ctDNA, and combinatorial approaches to improve WRNi efficacy in MSI cancers.

EACR25-1980

BMI1 as a therapeutic target to overcome chemoresistance in high-grade serous ovarian cancer

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Introduction

High-grade serous ovarian cancer (HGSC) is the most common and lethal form of ovarian cancer, characterized by high relapse rates and resistance to platinum-based chemotherapy. Despite advancements, chemoresistance remains a major challenge. Our previous research has contributed to understanding tumor heterogeneity and resistance mechanisms in HGSC by identifying key molecular drivers. A frequent alteration is homologous recombination deficiency, which leads to genomic instability while paradoxically sensitizing tumors to platinum-based treatment. BMI1 (B-cell-specific Moloney murine leukemia virus integration region 1), a polycomb complex protein, regulates chromatin remodeling, stemness, epithelial-mesenchymal transition, and homologous recombination-mediated DNA damage repair. High BMI1 expression has been associated with poor prognosis and chemoresistance in various cancers. Importantly, our unpublished work shows that high BMI1 expression in HGSC is an independent predictor of reduced response to platinum-based chemotherapy and overall survival, making it a potential candidate for therapeutic interventions.

Material and method

To investigate BMI1's biological role in HGSC, we generated CRISPR/Cas9-edited HGSC cell lines (e.g., CAOV3, Kuramochi, A2780) with BMI1 overexpression (BMI1-OE) and knockout (BMI1-KO) and validated them by Western blotting. We will use the cell lines to study BMI1's impact on tumor progression in vitro and in vivo. RNA sequencing and multiple (2D and 3D) functional in vitro assays (e.g., cell proliferation assay, 2D migration assay, 3D invasion assay) are used to further characterize BMI1-related pathways.

Result and discussion

We have successfully generated and validated BMI1-OE and BMI1-KO HGSC cell lines (e.g., CAOV3, Kuramochi, A2780). Preliminary functional data suggest that BMI1 overexpression promotes tumor aggressiveness, while knockdown reduces invasive properties. Our recent unpublished work showed that BMI1 expression is elevated in metastatic HGSC lesions. High BMI1 expression correlates with poor prognosis, as indicated by a shorter platinum-free interval and overall survival. Artificial intelligence (AI)-assisted RNA-ISH quantification of BMI1 further supports this correlation. An important avenue of our research is to explore how homologous recombination functionality is altered in the

BMI1-modified cell lines. Additionally, we will assess the therapeutic potential of small-molecule BMI1 inhibitors in combination with platinum-based chemotherapy in vitro and in vivo.

Conclusion

Our study highlights BMI1 as a promising prognostic marker and therapeutic target in HGSC. By integrating patient sample analysis with functional studies, we aim to provide insights into BMI1's role in chemoresistance. This research aims to develop BMI1-targeted therapies to overcome platinum resistance in HGSC.

EACR25-1999

The Role of AP-1 Target Transcript Vav3.1 in the Regulation of Osimertinib Resistance in Lung Adenocarcinoma

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Introduction

Lung adenocarcinoma (LUAD) is one of the deadliest cancers, with EGFR mutations – particularly EGFR-TKI sensitizing mutations – being common. Currently, osimertinib is the most used and effective FDA-approved EGFR-TKI; however, resistance is inevitable. Focusing on unknown epigenetic and transcription factors contributing to osimertinib resistance, we recently employed a CRISPR knockout screening that identified the AP1 transcription factor (FOSL1 and JUN) as a key modulator of resistance. Silencing FOSL1 or JUN restored drug sensitivity, revealing a synthetic lethal interaction with the EGFR pathway. Integrative multi-omics analyses (RNA-Seq, ATAC-Seq, CRISPR knockout screening) highlighted VAV3, a key molecular-switch in Rho-GTPase family, and specifically its major transcript VAV3.1 as a promising resistance-associated gene. Ongoing studies aim to clarify the role of VAV3.1 in the "transcriptional regulation – resistance" axis.

Material and method

To investigate the role of VAV3.1, multiple bio-informatic analyses and in vitro experiments were conducted. qRT-PCR analysis confirmed the dominance of VAV3.1 over VAV3 in HCC827-OsiR cells, also validated by RNA-Seq. VAV3.1 was then knocked out using CRISPR-Cas9 methodology. Possible synthetic lethal interactions between VAV3.1 knock-out and osimertinib were tested through 2D colony formation, competitive cell proliferation, and BrdU assays. Western blotting was performed to explore the effects of VAV3.1 on survival pathways. Vav3.1 was then overexpressed in parental HCC827 cells to assess the changes in proliferation and survival capabilities.

Result and discussion

Our findings validated that VAV3.1, containing a unique 110-nucleotide sequence that suggests a distinct promoter regulation, was the dominant transcript of VAV3 in our cells. Using integrative multi-omics studies, VAV3.1 was identified to have significant changes in expression and chromatin accessibility, suggesting a role in resistance. However, in vitro assays showed a minor effect of VAV3.1 silencing in osimertinib sensitization.

Conclusion

Here we provide the first preliminary findings of a unique VAV3 transcript, VAV3.1, as a downstream target of the AP1 family in osimertinib resistance. Our findings highlight the complexity of VAV3.1's involvement in drug resistance that could stem from the polyclonal nature and heterogeneity of our cells. Ongoing experimental strategies aim to further investigate the precise role of VAV3.1 in resistance mechanisms for a more comprehensive understanding of its function.

EACR25-2027

Unravelling PARP inhibitor resistance mechanisms in ovarian cancer

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Introduction

Ovarian cancer is the most lethal gynecological tumor. PARP inhibitors (PARPi) have revolutionized the high grade serous ovarian cancer (HGSOC) management. However, most of HGSOC patients will suffer disease progression, highlighting the importance of studying PARPi resistance mechanisms.

Material and method

39 archival tissue samples from diagnosis or debulking surgery were obtained of patients with platinum-sensitive relapsed HGSOC treated with PARPi as maintenance prior to 2020 in 3 Spanish institutions. Sixteen paired archival samples at progression were also retrieved. The expression of 180 genes related to DNA damage and repair was analyzed, using Nanostring technology. To identify intrinsic resistance biomarkers (BKs), PARPi progression-free survival (PFS) was analysed according to high or low gene expression (cutoff: median) in baseline samples. Differentially expressed genes (DEG) analysis between baseline and progression samples ($|FC|=1.5$; $p\text{-value} \leq 0.05$) was performed to identify acquired resistance BKs. Moreover, OVCAR3 and OVCAR4 cell lines were long term exposed with increasing doses of niraparib, generating two in vitro PARPi-resistant models.

Result and discussion

In our set of baseline samples, high expression of XRCC2 and POLE2 correlates with significantly better PFS-PARPi in patients, whereas high expression of ERCC1 is associated with significantly shorter PFS-PARPi. DEG analysis revealed that CDKN1A, CDKN1B, CDKN2C and WEE1, which are strong negative regulators of CDK-Cyclin complexes, were robustly downregulated at progression. In vitro studies showed that niraparib treatment triggered a CDK4 expression reduction, a G2/M phase arrest and increased homologous recombination activity (RAD51 increase) in PARPi-sensitive cells. In contrast, CDK4 was overexpressed in niraparib-resistant cells, enabling the cell cycle progression despite niraparib.

Conclusion

XRCC2, POLE2 and ERCC1 genes emerge as potential BKs of intrinsic resistance and that cell cycle overactivation could be an acquired resistance mechanism to PARPi in HGSOC.

EACR25-2033

Identifying miR-7-5p/KEAP1/NRF2 and miR-122-5p/ADAM10/IL-6 signaling axes in Castration and Enzalutamide-resistant prostate cancer cells

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Introduction

Oxidative stress and inflammatory cytokines play crucial roles in developing resistance to the standard treatment approaches for castration-resistant prostate cancer (CRPC) and Enzalutamide-resistant prostate cancer (ERPC). Imbalanced oxidative stress with increased reactive oxygen species (ROS) and inflammatory cytokines, especially interleukin-6 (IL-6) sustains the AR-independent survival pathways, promoting the progression of CRPC and ERPC. The identified regulators of oxidative stress and tumor progression, KEAP1 and ADAM10, have been demonstrated to be the targets of miR-7-5p and miR-122-5p, respectively. In this study, we aimed to evaluate the involvement of miR-7-5p and miR-122-5p in CRPC and ERPC in vitro models regarding their association with total antioxidant status (TAS), total oxidant status (TOS), and IL-6 levels.

Material and method

The CRPC cell line model was generated by long-term androgen deprivation of LNCaP cells, while ERPC was modeled by the treatment of CRPC cells with increased doses of Enzalutamide for 3 months. For gene expression analyses, total RNA was isolated and the expression levels of KEAP1, NRF2, ADAM10, miR-7-5p, and miR-122-5p were assessed using qRT-PCR. The released levels of IL-6 were determined by the ELISA method, and the colorimetric TAS/TOS assay was used to determine the oxidative stress status of the cells.

Result and discussion

The expression level of miR-7-5p was shown to be significantly increased in the ERPC, compared to CRPC cells. In association with this, KEAP1 was significantly decreased in the ERPC cells, highlighting the severity of oxidative stress. NRF2 expression level was determined

to remain unchanged in ERPC cells, compared to CRPC. Both CRPC and ERPC cell lines showed highly increased oxidative stress. However, alongside NRF2 status, no significant difference was evaluated between CRPC and ERPC cells in the TOS levels, while TAS levels were found to be slightly decreased in ERPC cells. miR-122-5p expression showed a significant upregulation in ERPC, compared to CRPC cells, while its target gene, ADAM10, was shown to be significantly downregulated in ERPC cells. ADAM10 has been identified to negatively regulate IL-6, which we found to be increased in ERPC cells, though not statistically significant.

Conclusion

We showed that miR-7-5p and miR-122-5p are differentially expressed in ERPC when compared to CRPC. This may potentially impair oxidative stress balance by suppressing KEAP1, and sustain survival pathways by suppressing ADAM10, which favors IL-6 release. This study highlights the interplay among miR-7-5p, miR-122-5p, oxidative stress, and cytokine signaling, providing novel insights into the drug resistance mechanisms and potential therapeutic strategies for advanced prostate cancer.

EACR25-2034

Identification of microRNAs and their target genes associated with acquired resistance of primary melanoma cells to targeted therapy

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Introduction

Melanoma is aggressive skin tumor prone to metastasis with frequent mutations in the components of MAPK signaling pathway. Due to fast response, BRAF-mutated melanoma was initially treated with monotherapy targeting BRAF kinase (BRAFi), while lately therapy includes combination of inhibitors that target both BRAF and MEK kinases (BRAFi/MEKi). Although initially response to targeted therapy is promising, disease relapses due to acquired therapy resistance. It has been shown that acquisition of tumor cell resistance to treatment can be associated with altered expression levels of microRNAs (miRNAs). Nevertheless, the association of specific miRNA expression profile and acquired melanoma resistance to BRAFi or BRAFi/MEKi therapy has not been thoroughly investigated.

Material and method

Comprehensive analysis of miRNA expression using NGS technology was performed on primary melanoma cells that differ in sensitivity to targeted therapy. Differentially expressed miRNAs were determined via sRNAde toolbox between treatment-sensitive primary melanoma cells and melanoma cells with acquired resistance to BRAFi or BRAFi/MEKi targeted therapy. After identifying miRNAs and validating their altered expression using RT-qPCR, we checked which predicted target genes are regulated via selected miRNA using several tools such as TargetScan, mirDIP, miRDB and miRTarBase. After selection of target mRNA, their

differential expression between primary cell lines was confirmed by RT-qPCR.

Result and discussion

We identified 700 miRNAs by miRNA-seq, from which 58 were shown to be differentially expressed. We have chosen to validate the miRNAs whose expression between sensitive and resistant cells differs at least 5 times. Interestingly, we observed reduced expression of hsa-miR-96-5p in BRAFi and BRAFi/MEKi resistant cells compared to treatment-sensitive cells. Next, we checked which predicted target genes are regulated via differentially expressed miRNAs and identified FOXO3 as hsa-miRNA-96-5p regulated gene. We validated differential expression of FOXO3 and observed its increased expression in targeted therapy resistant cells compared to treatment-sensitive cells.

Conclusion

Our results show that primary melanoma cells resistant to BRAFi or BRAFi/MEKi have higher expression of FOXO3 gene, so our further research will be focused on unraveling molecular mechanisms of FOXO3-dependent acquisition of resistance to BRAFi or BRAFi/MEKi in melanoma cells.

EACR25-2042

Impact of Mechanical Stress and Confinement on the Alteration of Protein Expression and Emergence of Time-Dependent Resistance to Cisplatin

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Introduction

The tumor microenvironment is a key driver of disease progression, with mechanical cues increasingly recognised as critical regulators of cancer development, alongside genetic and metabolic factors. This study explores how mechanical stress and confinement influence the expression of oncogenic features, by mimicking the forces acting on cancer cells in the early stages of neoplastic diseases.

Material and method

Using the MDA-MB-231 cancer cell line as a triple negative breast tumour model, the cells were cultured in a polymer-based semi-degradable hydrogel scaffold. The in vitro tumour model, named Tumour-Like Microcapsules, is composed of an alginate and gelatin blend with dimensions ranging from 400 to 600 µm and was designed to mimic the elasticity of early-stage breast tumours. The cells were immobilised and grown in the 3D tumour model, without cisplatin in the culture medium, using cells cultured as suspended spheroids and cells grown in cell culture flasks as 3D and 2D control, respectively. The cells were then isolated from the 3D milieus at different time points after seeding/

immobilisation (i.e., day 1, 2, 4, 7, 10 and 14). The cell resistance to anticancer drug cisplatin was assessed after reseeding the cells in 2D to avoid mass transfer restrictions. To gain deeper insight into the molecular mechanisms underlying this resistance, we conducted a temporal proteomic analysis to compare the protein expression profiles across the different culture conditions and examine how these evolve. Alterations in cell proliferation and increased cell heterogeneity upon confinement were also investigated.

Result and discussion

Cells cultured as spheroids and in the hydrogel scaffold exhibit a time-dependent increase in resistance to the anticancer drug cisplatin, which correlated with mechanical preconditioning. Our findings indicate that resistance emerges within two days post-seeding/immobilisation in both 3D models, though the temporal patterns differ. Notably, cells cultured in the micro-capsules display a distinct decrease in cisplatin sensitivity, aligning with the 3D mechanical preconditioning. The half maximal inhibitory concentration (IC₅₀) comparison shows more than a 2-fold increase between the IC₅₀ of 2D cultured cells and the cells cultured in the microcapsules for 14 days, 8.1 µM and 19.5 µM, respectively. Preliminary transcriptomic and proteomic results show that cells cultured in the different milieus express significantly distinct protein signatures related to proliferation, senescence and xenobiotic metabolism, which suggest the contribution of mechanical stress and confinement to the increase of primary chemoresistance.

Conclusion

Overall, this study highlights the role of mechanical forces in shaping cancer cell behaviour and resistance mechanisms. Understanding these influences could provide a better treatment strategy tailored to different stages of tumour progression.

EACR25-2062

Uncovering Transcriptional Signatures of Differential Drug Response in Archetypical Malignant States in Metastatic Breast Cancer (MBC)

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Introduction

Cancer treatment has significantly evolved, yet metastatic breast cancer (MBC) remains challenging due to limited durable responses. Genomic data have dominated research, but transcriptional profiling offers critical insights into distinct cellular states ("Archetypes") underlying drug resistance.

Material and method

Our analysis utilized harmonized genomic, transcriptomic, and clinical data from 775 MBC samples across multiple cohorts (DFCI, Broad Institute, POG570, MET500, META-PRISM, AURORA). We employed

computational clustering to define transcriptional archetypes and correlated these with clinical outcomes.

Result and discussion

Our earlier results revealed central Archetypes in Estrogen-Receptor (ER+) MBC that represent two strategies of ER therapy resistance based on evolutionarily acquired alteration in post-treatment biopsies – (1) ER reactivation and (2) Growth Factor Receptors (GFR) bypass. In this research, we characterized each of these dominant modes of resistance, including the distinct patterns of gene expression, their metastatic potential, and profiles of drug-response in clinically annotated MBC (Fig 1B). Our study suggests that these transcriptional archetypes have clear implications for the choice of next therapies (Fig 1C, Fig 1D). To explore the global transcriptional archetypes in MBC, our ongoing efforts, we leverage the harmonized MBC data (Fig 1A) to infer the transcriptional profiles that correspond to MBC driver genes and cluster these profiles into the dominant MBC transcriptional archetypes. Finally, we use a similar approach to study primary breast cancer from TCGA14, 15 ($n = 957$) and METABRIC16 ($n = 1980$) to highlight commonalities and differences between primary (treatment-naïve) and metastatic (drug-resistant) breast cancer archetypes.

Conclusion

This study highlights transcriptional archetypes as pivotal in understanding and overcoming drug resistance in MBC, offering clinically actionable insights for personalized therapy in precision oncology.

EACR25-2063

Targeting ferroptosis resistance by inhibiting SCD1 in TNBC enhances tumor response to immunotherapy

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Introduction

Breast cancer (BC) is generally considered a "cold" tumor and exhibits heterogeneous phenotypes, particularly Triple-Negative Breast Cancer (TNBC), which is resistant to immune checkpoint inhibitors. To overcome this resistance, we are investigating the impact of reversing ferroptosis resistance on immunotherapy

Material and method

The assessment of human breast cancer cell lines treated with ferroptosis inducers (FINs) was performed using MTT and cell death assays with PI staining. Cells were treated with vehicle or escalating doses of RSL3, a GPX4 inhibitor, as well as FABP4 (FABP4) and SCD1 (SCD1i) inhibitors. Proteomic analysis was conducted on both conditioned medium and total cell lysates of MDA-MB231 cells. ELISA was used to quantify IL-8 and MIF levels in the medium of treated cells. Immune activation assays were performed using splenocytes incubated with conditioned medium from FIN-treated cells, followed by immune profiling via FACS analysis. In vivo response to PD-1 inhibitor, SCD1i, and their combination was evaluated using the mouse TNBC (E0771) model.

Result and discussion

In this study, we first assessed the resistance and sensitivity of several human breast cancer (BC) cell lines

to ferroptosis inducers (FINs) in vitro. These experiments revealed acute resistance to RSL3 or SCD1i in certain BC cell lines. The most sensitive cell line, MDA-MB-231, was further analyzed by proteomics after treatment with RSL3 and SCD1i. The data revealed a similar protein expression pattern, characterized by the release of proteins involved in inflammatory pathways, including IL-8 and MIF. A similar but less pronounced response was observed in cells treated with FABP4i at 6 hours, but not at 24 hours. Notably, a panel of inflammatory proteins was found to be upregulated by 26- to 46-fold following SCD1i treatment, and by 3- to 5-fold after RSL3 treatment, suggesting that SCD1 inhibition led to the release of inflammatory factors. The increased secretion of MIF and IL-8 was further validated by ELISA. Functional assays of immune cell activation on splenocytes in vitro using conditioned medium from TNBC cells treated with SCD1i or RSL3 revealed a pattern of immune checkpoint inhibitor expression in various immune cell types, suggesting a potential activating phenotype. In vivo assessment of SCD1i in combination with PD-1 inhibition in the E0771 model is revealed a higher response to the combination than single agents. Immune profiling of E0771 tumors treated with SCD1i showed an increase in PD-1⁺ macrophages and PD-1⁺ dendritic cells.

Conclusion

Taken together, our results suggest that the immune-activating markers released by cells undergoing ferroptosis may enhance the efficacy of immune check-point inhibitors in TNBC treatment. The combination of SCD1 inhibitors and PD-1 inhibitors in the E0771 TNBC mouse model resulted in an improved response compared to monotherapy.

EACR25-2102

Overcoming P-glycoprotein-mediated resistance in tumor cells through copolymers for cytotoxic drug delivery

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Introduction

Cancer cells often fail to respond to chemotherapeutic regimens because of either inherent or acquired resistance. Several factors contribute to the development of chemoresistance, including epigenetics, the presence of cancer stem cells, the tumor microenvironment, and changes that affect the cell's signaling pathways. Increased drug clearance by the transporter-facilitated efflux of the drugs from cancer cells is one of the critical mechanisms involved in (multi)drug resistance (MDR). It can be mediated by overexpression of membrane transporters, such as adenosine triphosphate binding

cassette (ABC) pumps, from which P-gp (ABCB1, MDR1) is the most prominent. A significant array of specific inhibitors of individual ABC family transporters exists. However, their effect is burdened with undesirable toxicities and interactions, because ABC transporters also have extensive physiological expression in healthy cells and organs. Some recently developed nanodrug delivery systems may bypass the resistance phenomenon.

Material and method

We used an amphiphilic diblock polymer nano-therapeutics containing a hydrophilic block based on the N-(2-hydroxypropyl)methacrylamide (HPMA) co-polymer and a hydrophobic poly(propylene oxide) block (PPO). The amphiphilic character of the diblock polymer ensures self-assembly into micelles with hydrodynamic radius Rh ~15 nm in aqueous solutions. The polymer conjugates were characterized in vitro using permanent tumor cell lines. In vivo, syngeneic murine tumors, namely CT26 colon carcinoma with elevated P-gp expression, and immunodeficient mice transplanted with clinical samples of head and neck tumors (PDX model) were explored.

Result and discussion

The diblock copolymer and its conjugates with doxorubicin (DB-Dox) significantly increased the intracellular concentrations of Dox and markedly sensitized multidrug resistant tumor cells to chemo-therapy. The underlying mechanisms included inhibition of P-gp-mediated drug efflux, alteration of mitochondrial membrane potential, depletion of intracellular ATP, and increased reactive oxygen species production. Moreover, the DB-Dox conjugates inhibited tumor growth in vivo more effectively when compared to corresponding HPMA-based drug delivery system. Copolymers with additional PPO loaded in the micelle core demonstrated superior efficacy in terms of P-gp inhibition, ATP depletion, and chemosensitizing effect in vitro, as well as antitumor activity in vivo. In primary human tumor cells in vitro and in PDX model in vivo, the diblock copolymers effectively decreased ATP levels, suggesting their capacity to enhance the effectiveness of chemotherapy.

Conclusion

DB-Dox conjugates loaded with PPO possess significant antitumor efficacy demonstrating their potential as an effective drug delivery system in the treatment of chemo-resistant tumors.

EACR25-2108

Dual targeting BRD4-CDK9 transcriptional complex as a potent therapeutic strategy in glioblastoma

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Introduction

Glioblastoma multiforme (GBM) is the most aggressive brain tumor, characterized by a high recurrence rate and resistance to current treatments. Available therapies, such as radiotherapy and chemotherapy with temozolomide (TMZ), offer a median survival of only 15 months after diagnosis. Glioma-initiating cells (GICs) contribute to TMZ resistance, highlighting the need for novel therapeutic strategies. Bromodomain and extraterminal (BET) proteins regulate the transcription of key genes involved in oncogenesis and treatment resistance. BET inhibitors (BETi) have been shown to modulate genes associated with proliferation, metastasis and cancer stem cells and have demonstrated efficacy in other solid tumors such as breast cancer. CDK9 is an essential kinase in gene transcription and its overexpression is associated with poor prognosis in several cancers. CDK9 inhibition induces apoptosis, and studies suggest that the combination of BETi and CDK9 inhibitors (CDK9i) may enhance their antitumor effects, providing new treatment options for GBM. The aim of this study is to evaluate the therapeutic potential of BETi alone or in combination with CDK9i in GBM models.

Material and method

TMZ-resistant cell line (U87R) were generated from TMZ-sensitive immortalized cell line (U87) using pulse treatments. BET and CDK9 expression and prognosis were analyzed using the GlioVis and Roc Plotter databases. In addition, BET and CDK9 protein expression was determined in the GBM cell lines (A172, T98, U87, U87R) by Western blot (WB). The efficacy of BETi and CDK9i alone or in combination was assessed using MTT, flow cytometry and WB assays. Tumor characteristics such as migration, invasion and recurrence were also analyzed. The treatment's efficacy was confirmed in a subcutaneous xenograft model in mice using the resistant model (U87R). RNAseq analyses were performed.

Result and discussion

Increased expression of BRD4 and CDK9 was observed in GBM tissues compared to non-tumor tissues. A positive correlation was found between BRD4 and CDK9, suggesting that both may be effective therapeutic targets. We confirmed by WB that the resistant model showed elevated levels of BRD4 and CDK9 proteins. Combination treatment with BETi and CDK9i induced a strong synergistic effect leading to increased cell death as confirmed by flow cytometry and WB. In addition, a reduction in tumor cell migration, invasion and recurrence capacity was observed with the combination treatment compared to the single treatments. The *in vivo* experiments also showed a reduction in tumor growth with the combination therapy. Finally, RNAseq analyses were performed to elucidate how the combined pharmacological inhibition works, leading to the identification of several deregulated genes and pathways that require further analysis.

Conclusion

Combined inhibition of BRD4 and CDK9 shows promise as a potential therapeutic strategy for the treatment of GBM.

EACR25-2112

Unraveling mechanisms of CDK4/6 inhibitors resistance in metastatic breast cancer

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Introduction

Breast cancer (BC) poses a significant mortality risk when it metastasizes and develops treatment resistance. Hormone receptor-positive (HR+) BC, representing 75% of all BC cases, initially responds to endocrine therapy combined with CDK4/6 inhibitors (CDK4/6i), which has prolonged progression-free survival in patients with metastatic HR+ BC. Yet, a substantial number of these tumors adapt and develop resistance, a process that transcends mere genetic mutations in metastatic cells and demands a more comprehensive view of dynamic cellular adaptation mechanisms. Our study investigates cellular and molecular mechanisms in driving drug resistance and metastatic adaptation in HR+ breast cancer.

Material and method

Using an innovative lineage-tracing system with a bar-coded ZR75 cell line library (CATCH technology), we will define treatment-resistance plasticity, characterizing the phenotypic diversity in HR+/HER2-enriched BC cells at different stages of metastasis. Functional studies, including gene silencing and phenotypic assays, will be conducted to validate the candidate's role in driving resistance.

Result and discussion

To address this challenge, we performed a genome-wide CRISPR/Cas9 knockout library screening in BC cell lines combined with gene expression analysis of two patient cohorts (CDK patient cohort and CORALEEN data set), identifying key candidate genes potentially involved in CDK4/6i resistance (unpublished). Currently, we aim to: i) explore and identify the determinants of metastasis adaptive potential at a single cell resolution, mapping the transcriptomic and epigenomic landscape during metastasis drug resistance; ii) decipher and functionally validate the molecular pathways that drive phenotypic plasticity and thus enhance the adaptive potential of metastatic cells; and iii) develop a novel therapeutic strategy to target plastic metastatic cells using the basis of CAR-T technology.

Conclusion

Understanding the mechanisms that drive CDK4/6 inhibitor resistance in metastatic breast cancer is crucial for improving therapeutic strategies. By leveraging a lineage-tracing system and functional studies, our research aims to uncover key determinants of adaptive resistance and metastatic plasticity. The insights gained from this study will contribute to a more comprehensive understanding of tumor evolution under treatment pressure, paving the way for novel therapeutic approaches to counteract resistance and improve patient outcomes.

EACR25-2121**Targeting BCL-2 proteins as a Strategy Against Therapy Persistency in TNBC**

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Introduction

Triple-negative breast cancer (TNBC) is an aggressive entity lacking hormone receptors, and HER2 expression, limiting treatment to chemotherapy and radiotherapy. However, frequent resistance leads to relapses and metastasis, reducing patient survival, highlighting the urgent need of novel treatment strategies. Cells regulate death through a balance of pro- and anti-apoptotic proteins, and disruptions in this balance contribute to malignancy. In this line, targeting anti-apoptotic BCL-2 family members is a promising strategy, as their over-expression is linked to recurrence and resistance. This study shows that targeting BCL-2 proteins can overcome TNBC resistance, reducing tumor growth and metastasis *in vivo*.

Material and method

Gene expression association with prognosis and response to chemotherapy was analyzed using KM and ROC plotter databases, respectively. BCL2 levels were measured in TNBC patient biopsies via immunohistochemistry. A drug screening with BH3 mimetics was performed on a panel of 8 TNBC cell lines. Resistant TNBC cell models (MDA-MB-231R, BT-549R) were generated using a cisplatin pulse-based strategy. BCL2, MCL1, and BCL-XL expression levels were evaluated by Western blot. Effects on proliferation, colony formation, migration, invasion, and tumor progression were assessed in resistant cells, while apoptosis was analyzed by flow cytometry. Drug's efficacy was validated *in vivo* models, including patient-derived xenografts (PDX) and MMTV-PyMT. A PROTAC based strategy was used to validate the experimental approach.

Result and discussion

Gene expression levels of anti-apoptotic BCL2 family members are associated with a lack of chemotherapy response in TNBC. Moreover, high BCL-2 and BCL-XL levels correlated with poor prognosis in patients with neoadjuvant therapy. A drug screening using BH3 mimetics drugs that directly induce apoptosis by inhibiting anti-apoptotic BCL-2 family members identified Obatoclax, a pan-inhibitor, as the most efficient compound to reduce proliferation in all tested TNBC cell lines, regardless of chemotherapy sensitivity. Functional *in vitro* analysis in chemo-persistent cells demonstrated that Obatoclax effectively reduced pro-

liferation, migration, invasiveness, and clonogenic potential, while also inducing apoptosis in persistent cells. *In vivo*, the drug inhibited tumor progression and metastatic potential in mouse models: an orthotopic xenograft with resistant cells, a syngeneic TNBC model, and a taxane-refractory PDX model. Our results were reproduced using a PROTAC with a dual effect on the targets, showing no cross-resistance

Conclusion

Our findings suggest that the simultaneous targeting of BCL2 anti-apoptotic members could represent a promising therapeutic approach for chemotherapy-resistant TNBC.

EACR25-2193**Targeting Epigenetic Mechanisms to Overcome Drug Resistance in TaxaPlatin-Resistant Prostate Cancer**

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Introduction

In metastatic CR-PCa, treatment typically begins with docetaxel (Dtx) as the first-line chemotherapy due to its ability to prolong survival. However, as most patients eventually develop resistance to Dtx, platinum-based drugs are commonly introduced as the next therapeutic option. To explore the reuse of taxanes and cisplatin in these patients, we generated cisplatin resistance in taxane-resistant PCa cells, naming them "TaxaPlatin" or "super-resistant" cells. Our study focuses on understanding the mechanisms behind multi-drug resistance and investigating methods to overcome resistance through epigenetic modulation.

Material and method

Taxane and cisplatin resistance in a castration resistant cell line, generated by dose-increment method, had been used. Acquired drug resistance was assessed with cell viability and colony formation assays. Our approach involved thoroughly characterizing TaxaPlatin cells phenotypically, followed by transcriptomic analysis. Wound healing assay was performed to measure motility of cells. The apoptotic response of TaxaPlatin-resistant cells to drug treatment was assessed using Caspase 3/7 and Annexin V assays. To characterize lysosomal sequestration and exocytosis in resistant cells, a lysosomal gene panel qPCR and β-hex exocytosis assay were performed. Screening of an epigenetic library helped identify compounds that restore sensitivity to drugs in TaxaPlatin-resistant cells.

Result and discussion

Characterization of TaxaPlatin-resistant cells revealed phenotypic changes and a slower proliferation rate. Wound healing assay demonstrated significantly lower motility in resistant cells compared to parental cells. Apoptosis assays indicated that TaxaPlatin-resistant cells do not undergo apoptosis upon taxane or cisplatin treatment. The β-hexosaminidase assay showed no significant difference in exocytosis between parental and resistant

cells. During the development of TaxaPlatin-resistant cells, the expression levels of ATP7B, TFEB, MITF, TMEM205, TPP1, and PPT1 correlated with increasing cisplatin doses. Additionally, a drug library screen was performed to systematically identify epidrugs that resensitize TaxaPlatin-resistant cells to chemotherapy. The candidates obtained from the epidrug screen will be discussed within the scope of this study.

Conclusion

Our findings highlight the complexity of multidrug resistance in cancer and underscore the role of epigenetic regulation in this process. Notably, lysosomal exocytosis did not appear to be a major contributor to resistance, whereas the upregulation of lysosomal genes suggests a potential link between lysosomal biogenesis and cisplatin resistance. Candidate molecules and target epigenetic regulators will be validated using functional assays and molecular biology techniques. These findings may contribute to the development of novel therapeutic strategies aimed at overcoming multidrug resistance in cancer.

EACR25-2239

CDK12/13 inhibition can counteract Epithelial Ovarian Cancer chemoresistance

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Introduction

Epithelial Ovarian Cancer (EOC) is featured by drug resistant recurrences, especially to platinum (PT) compounds. Alternative Splicing (AS) and transcriptional dysregulations could be ascribed as PT-resistance mechanisms, exerted, among the others, by transcriptional Cyclin Dependent Kinases (CDKs). Here, we identified CDK12 (and the highly homolog CDK13) as possible therapeutic targets in EOC, evaluating their role in AS and DNA damage response (DDR), under the selective pressure of PT-treatment. CDK12 phosphorylates RNA polymerase II, regulating transcriptional elongation at multiple levels. Its depletion impacts on long DDR genes expression, leading to genomic instability.

Material and method

Mutational status of CDK12/13 was assessed using a panel of primary EOC samples and available datasets. CDK12/13 pharmacological inhibition (CDK12i), alone or in combination with chemotherapeutic agents, was addressed in several models, including EOC cell lines and patients-derived primary cultures, both sensitive and resistant to PT. CDK12i-resistant EOC cells were also generated. To explore transcriptional effects on CDK12i, we performed RNA-seq analysis on both sensitive and PT-resistant cells. Finally, we verified CDK12i effects *in vivo* using both syngeneic and PDX models.

Result and discussion

CDK12 is the most and CDK13 the second more frequently mutated and amplified CDK in EOC. CDK12i is effective in a panel of EOC cell lines and patients-derived primary cultures, both sensitive and resistant to PT, as well as in the CRISPR-modified ID8 cells. CDK12i re-sensitizes PT-resistant cells to both PT and PARPi, when used in a sequential treatment schedule, preventing the appearance of PT-resistant clones. The strong antitumor and anti-metastatic activity of the combined regimen was confirmed *in vivo* using both syngeneic and PDX models. Intriguingly, we generated and characterized CDK12i-resistant cells that become extremely sensitive to PT. Mechanistically, CDK12i treatment reduced the expression of the DDR genes ATM, RAD51 and FANCD2 at both mRNA and protein level, likely through AS alteration and/or intronic polyadenylation (IPA) sites usage. Accordingly, RNA-seq revealed that CDK12i modulates mainly Ribosome and Splicing KEGG gene networks expression, impinging on AS patterns. Consistently, we observed a previously undisclosed interaction between CDK12 and the master splicing regulator complex SFPQ/p54nrb, both under PT and CDK12i treatment, suggesting that CDK12 exerts its activity at least in part by regulating SFPQ/p54nrb complex.

Conclusion

We propose that CDK12/13 could represent a valuable actionable therapeutic target alone or in combination therapies, to prevent the onset of PT-resistance or to treat resistant EOC patients, for whom very few therapeutic options are currently available.

EACR25-2246

Immune infiltration is associated with favorable response to neoadjuvant chemotherapy and carboplatin in hormone receptor positive and triple negative breast cancer

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Introduction

The rational selection of chemotherapy regimens is crucial to optimize the clinical management of breast cancer (BC), yet it remains a significant challenge. Carboplatin (Cb) has been shown to improve treatment efficacy in many triple negative breast cancer (TNBC)

patients, but not all patients may need this chemotherapy. Additionally, patients with aggressive hormone receptor positive breast cancer (HR+ BC) may also benefit from Cb treatment, but appropriate selection criteria need to be determined. The I-BCT-1 (Improved Breast Cancer Therapy –1; NCT02546232) study aims to investigate biomarkers for patient stratification, identifying high-risk HR+ BC and TNBC that will benefit from extended chemotherapy with Cb, and to discover novel actionable target in patients showing a lack of response to the current treatment modalities.

Material and method

Tumor samples were collected from patients with high risk HER2-negative BC (TNBC, n = 72; HR+, n = 115) before surgery, and after 3 and 12 weeks of treatment. Patients received paclitaxel (80 mg/m², Q1W), either alone or in combination with Cb (AUC6 Q3W), for 12 weeks, followed by four cycles of EC90 (epirubicin 90 mg/m² and cyclophosphamide 600 mg/m²; Q3W). Bulk RNA sequencing (RNAseq) was performed on pre- and on-treatment biopsies to analyze differential gene expression, pathway alterations and infer immune cell subpopulation changes using CIBERSORT.

Result and discussion

Recognizing distinct response mechanisms due to HR expression, patient data were first separated based on HR status. Significant differences in overall gene expression were observed at baseline when comparing good with poor responders. The number of genes with significantly different expression between the responder groups were notably higher in HR+ BC compared to TNBC. Additionally, higher fraction of activated immune cell subsets (including CD8+ T-cells and dendritic cells (DCs)) was associated with favorable response in both HR+ BC and TNBC at baseline. Notably, these differences were more prominent in TNBC than HR+ BC. We are currently analyzing longitudinal samples, to identify mechanisms associated with treatment-induced resistance. The overall aim is to identify potential actionable targets among patients not responding to treatment.

Conclusion

Using results from RNAseq analyses, we have identified transcriptional differences in BC patients with different responses to NAC +/- Cb. Baseline immune infiltration was associated with a favorable treatment response, while the on-treatment immune alterations are currently under investigation.

EACR25-2270

Inhibition of the RNA-binding protein HuR reduces cell proliferation and promotes apoptosis in EGFR-TKI resistant NSCLC cells

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Introduction

Patients affected by advanced non-small cell lung cancer (NSCLC) with mutant epidermal growth factor receptor (EGFR) are treated with tyrosine kinase inhibitors (TKIs) such as gefitinib and osimertinib, yet most patients develop disease progression due to acquired resistance. Resistance mechanisms include secondary mutations on EGFR and other membrane receptors responsible for cell proliferation and survival, along with epigenetic alterations critical to tumor recurrence. To this end, increasing data on regulation of RNA metabolism by RNA-binding proteins (RBPs) is supporting their pathological relevance. The RBP Hu antigen R (HuR) is an emergent regulator of cancer, currently considered for therapeutic targeting. HuR enhances mRNA stability/translation of key effector genes involved in all cancer traits including sustained cell proliferation, evasion of cell death and drug response. We investigated the impact of gene ablation and pharmacological inhibition of HuR on cell proliferation and survival in EGFR-TKI resistant NSCLC cells.

Material and method

EGFR-TKI-resistant cell lines (HCC827GR/PC9GR/PC9OR/H1975OR) were generated by treating HCC827/PC9/H1975 cells with gefitinib or osimertinib. HuR role was studied using PC9- and H1975-HuR-KO cells generated by CRISPR/Cas9 technology and commercially available HuR inhibitors KH-3 and SRI-42127. Cell proliferation was evaluated by Cell Counting Kit-8 assay. Cell cycle and apoptosis were analyzed by flow cytometry. Protein expression was analysed by immunoblotting.

Result and discussion

In silico analyses of public databases showed significant upregulation of both HuR mRNA and protein levels in primary lung adenocarcinoma tumors compared to normal tissue, with additional increase in samples from patients upon development of EGFR-TKI resistance. Similarly, in vitro generated EGFR-TKI-resistant cell lines showed higher expression of HuR respect to parental cells. Analysis of proliferation of PC9- and H1975-HuR-KO cells indicated that HuR loss significantly reduced cell proliferation with cell cycle arrest in G0/G1 phase associated with p21Cip1/Waf1 upregulation and Cdk2 or Cdk6 downregulation. Pharmacological HuR inhibition by compounds KH-3 and SRI-42127 (2,5 μM, 24 hours) arrested PC9 and PC9GR cells in G1/S phase while in PC9OR, H1975 and H1975OR cells it induced cell accumulation in G2/M phase and modulated the levels of the kinase Aurora A that is a determinant of the acquisition of osimertinib resistance. Regarding cell survival, while HuR gene ablation did not affect cell viability, both HuR inhibitors induced a significant increase of the apoptotic rate in all EGFR-TKI-sensitive and resistant cell lines.

Conclusion

In conclusion, our findings indicate HuR protein as relevant mechanism and potential target in EGFR-TKI

resistant NSCLC given its pro-survival and proliferative functions. Study supporting by AIRC

EACR25-2279

High resolution profiling of complex *in vitro* 3D lung cancer model to monitor resistance to immune checkpoint inhibitors

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Introduction

Lung cancer is the leading cause of cancer related deaths worldwide with non-small cell lung cancer (NSCLC) being the most common type (80-85%). While Immune Checkpoint Inhibitors (ICIs) has revolutionized the treatment of NSCLC, their benefits remain limited due to significant resistance and therapeutic failure. The tumor microenvironment (TME) plays a pivotal role in response to immunotherapies, as it enables cancer cells to evade the immune system. However, accessing tumors after the first lines of treatment remains challenging. The development of alternative models to study this resistance is therefore essential.

Material and method

To overcome this limitation, we propose to characterize the influence of the TME on ICIs resistance by developing a complex 3D co-culture model derived from human NSCLC. This model includes patient-derived NSCLC lung tumor cells (ADCA117), human umbilical vein endothelial cells (HUVEC), fibroblasts (HFF-2), as well as monocytes and T lymphocytes isolated from healthy donor blood. We aim to characterize the 3D model at baseline and monitor the influence of TME on response to treatment. To achieve this, we implemented a longitudinal analysis combining multiple approaches, including single cell and spatial transcriptomics (Visium HD), immunohistochemistry, confocal microscopy, and viability assays.

Result and discussion

Confocal microscopy analysis using CD45, CD3, and CD163 markers revealed a homogeneous distribution of immune cells in the 3D complex model throughout the culture. This infiltration was facilitated by the successive addition of PBMCs to the culture medium, to simulate the arrival of new immune cells at the tumor site. This distribution was further confirmed by immunohistochemistry, revealed with CD68 and CD163 staining and highlighting the model's consistent structure. We demonstrated with confocal microscopy that treatments induced DNA damage within the structure, as evidenced by γH2AX staining, along with a decrease in cell proliferation revealed by Ki67. A higher-resolution analysis of the model using spatial transcriptomics with the Visium HD technology revealed distinct layers inside the structure, with a proliferative signature at the periphery

transitioning to an inflammatory and hypoxic signature at the center, indicating the presence of a hypoxic gradient.

Conclusion

The complex human NSCLC derived 3D co-culture model developed is now used to assess treatment effects on both tumor cells and cells from the TME. Combined with an in-depth analysis of treatment responses, this approach will help identify new targets and biomarkers to overcome ICI resistance and improve patient stratification.

EACR25-2319

EGFR/SHP2-targeted antibody-drug conjugate for colorectal cancer theranostics

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Introduction

Colorectal cancer (CRC) is the 2nd leading cause of cancer-related death worldwide. Cetuximab (Ctx) is an Epidermal Growth Factor Receptor (EGFR)-targeted therapy approved for the treatment of metastatic CRC patients (mCRC). However, in many cases patients develop resistance to this therapy, highlighting the need for the development of new therapeutic strategies to overcome Ctx resistance. Recently, it was demonstrated that tumors with high levels of the EGFR effector phospholipase c gamma 1 (PLC γ 1) are resistant to Ctx, while the inhibition of SH2-containing protein tyrosine phosphatase 2 (SHP2) can sensitize these Ctx-resistant cells. With this in mind, our goal is to build a platform for combined EGFR/SHP2-targeted therapy to improve the therapeutic efficacy and overcome resistance in the treatment of mCRC, while reducing systemic toxicity. Towards this goal, we developed an antibody-drug conjugate (ADC) that combines: i) Ctx as a therapeutic anti-EGFR antibody to confer specificity and therapeutic effect; ii) a specific SHP2-inhibitor (SHP099) for cell sensitization; iii) a DOTA chelator for complexation of an imaging radionuclide (67Ga).

Material and method

The synthesis and characterization of an ADC carrying Ctx and SHP099 via maleimide chemistry was performed. Viability studies and western blot analysis were conducted in cell lines with different PLC γ 1 expression (Caco-2 parental and Caco-2 with knocked-down PLC γ 1). DOTA was conjugated to Ctx and to the ADC, and the conjugates radiolabeled with 67Ga. The radiochemical purity (RCP) of the resulting radioconjugates was assessed by ITLC-SG, and their in vitro stability evaluated in PBS. Cellular uptake and internalization of the radioconjugates were studied in SW48 and

Caco-2 CRC cell lines, and blockade and competitive binding assays were performed using Ctx as the “cold” competitor.

Result and discussion

The ADC was obtained with a drug-to-antibody ratio of 3.5. Cell studies show a decrease in viability of cells expressing high levels of PLCg1, while western blot analysis of the same cells demonstrate that the ADC is able to inhibit EGFR and phosphorylated ERK to a higher extent than Ctx. The Ctx and ADC were radiolabeled with 67Ga, leading to radioconjugates with a RCP > 95% that remain stable in PBS up to 48 h. 67Ga-Ctx presents high cellular uptake and internalization. Blocking of EGFR with “cold” Ctx decreases the uptake, indicating that it is receptor mediated. The ability of 67Ga-Ctx in inhibiting EGFR, determined as IC₅₀, was 3.4 nM in Caco-2 cells. Similar studies are underway with the radiolabeled ADC and will also be presented.

Conclusion

We expect to obtain an ADC with improved biological properties for cancer treatment and radiotools to assess EGFR level in tumors. Overall, the proposed dual targeting strategy should promote synergistic therapeutic effects that might overcome resistance to Ctx therapy, enabling a personalized theranostic approach for mCRC.

EACR25-2322

Targeting PI3K/AKT Pathway

Dysregulation in Triple-Negative Breast Cancer: Overcoming Alpelisib Resistance through Dual Inhibition

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Introduction

Triple-negative breast cancer (TNBC) is an aggressive breast cancer (BC) subtype lacking hormone receptor (HR) and HER2 expression, making it unresponsive to conventional therapies and necessitating novel targeted strategies. The PI3K/AKT signalling pathway is frequently dysregulated in TNBC, with PIK3CA mutations (~15% of cases) promoting uncontrolled proliferation. Alpelisib, a PI3K/AKT inhibitor, is approved for PIK3CA-mutant, HR-positive BC, but resistance mechanisms remain. We hypothesise that combining alpelisib with approved drugs targeting alternative PI3K/AKT activation mechanisms may benefit a TNBC cohort.

Material and method

Differential expression analyses (DEAs) based on TP53 and PIK3CA mutations were performed on TCGA BC patient RNA-seq data (N=981) using R. The 5-day anti-proliferative effects of targeted drugs, alone and in combination, were assessed using acid phosphatase assays. Reverse-phase protein array (RPPA) and western blotting were performed on lysates from 24-hour-treated cells. Mutant p53-expressing cell lines were transduced with lentivirus to express mutant PIK3CA.

Result and discussion

DEAs showed TP53 contributes to genetic dysregulation in TNBC, with distinct patterns in cases harbouring both TP53 and PIK3CA mutations. TP53 mutations down-

regulate PTEN, a negative regulator of PI3K, leading to aberrant PI3K/AKT activation. We hypothesised that TP53/PIK3CA co-mutations contribute to alpelisib resistance. The TP53/PIK3CA-mutant MFM-223 cell line was less sensitive to alpelisib than PIK3CA-only mutated TNBC cells but more sensitive than TP53-only mutated cells, suggesting PIK3CA mutations improve response while TP53 mutations drive resistance via sustained PI3K/AKT activation. Dual PI3K/AKT targeting could overcome this. Given frequent receptor tyrosine kinase (RTK) dysregulation in TNBC, we tested the approved RTK inhibitor crizotinib with alpelisib, identifying a synergistic effect in TP53/PIK3CA-mutated MFM-223 cells. RPPA analysis revealed dysregulated 4E-BP1 expression in cells treated with this combination, which was validated by western blotting, confirming reduced 4E-BP1 expression and phosphorylation. As a key PI3K target, 4E-BP1 is typically phosphorylated by mTORC1, promoting translation. The alpelisib-crizotinib combination may impair translation regulation, disrupting protein synthesis and proliferation. In MCF10A lines expressing different mutant p53 proteins, alpelisib sensitivity varied by mutation, with western blotting revealing differing PI3K pathway marker expression, suggesting TP53 mutations affect PI3K activation independently of PIK3CA.

Conclusion

TP53/PIK3CA co-mutations drive TNBC dysregulation and may confer PI3K inhibitor resistance. The alpelisib-crizotinib combination may overcome resistance in this TNBC cohort. Future *in vitro* and *in vivo* studies will further investigate therapeutic potential.

EACR25-2375

Overcoming PARP inhibitor resistance in ovarian cancer: interest of the HDAC inhibitor belinostat

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Introduction

The clinical management of ovarian cancer has been improved by the introduction of PARP inhibitors (PARPi), such as olaparib, as maintenance therapy after the first-line chemotherapy. PARPi-mediated inhibition of PARP leads to the accumulation of DNA double-strand breaks, which are normally repaired by the homologous recombination (HR) pathway. In HR-deficient tumors (HRD status), PARPi-induced double-strand breaks remain unrepaired, triggering cell apoptosis by a “synthetic lethality” mechanism. Olaparib is therefore indicated for patients with HRD tumors. However, around half of ovarian tumors have a proficient HR pathway (HRP status) and are thus resistant to olaparib. Identifying strategies to sensitize HRP tumors to olaparib constitutes a major challenge for these patients. HDAC inhibitors (HDACi), which modulate gene expression, have been shown to indirectly disrupt the expression and/or function of key components of the HR pathway, thereby impairing its efficiency. In this context, our objective was to evaluate the potential of belinostat, an FDA-approved pan-HDAC inhibitor, to sensitize HRP ovarian cancer cells to olaparib.

Material and method

We assessed the efficacy of the belinostat/olaparib combination in a cohort of a dozen of patient-derived tumoroid models, preclinical models representative of patient tumors. The models included in the study were established from ovarian tumors of different histological subtypes and displayed HRP status.

Result and discussion

Our study showed that belinostat efficiently sensitized half of the tumoroid models to olaparib, highlighting for the first time the efficacy of belinostat/olaparib combination in preclinical ovarian cancer models. This sensitizing effect revealed to persist over the long term and to be associated with apoptosis induction.

Interestingly, analysis of DNA damage using comet assay showed that the combination induced more damage than olaparib in the sensitive tumoroids but not in the resistant ones. This suggests that the sensitizing effect of belinostat may be based on its ability to inhibit the HR pathway. In order to identify predictive biomarkers of response to the combination therapy, a transcriptomic analysis of genes differentially expressed at baseline between sensitive and resistant tumoroids was performed.

Conclusion

Collectively, these results support the use of belinostat as a valuable therapeutic strategy to sensitize HRP ovarian cancers to olaparib, and pave the way for personalized clinical management of HRP patients.

EACR25-2390

Endothelial-Derived CAFs Drive Chemosensitivity via ERCC1-IL-17A Axis: A Novel Therapeutic Target in Tumour Microenvironment

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Introduction

Cancer-associated fibroblasts (CAFs) are key regulators of tumour progression and metastasis. By modulating the tumour microenvironment, CAFs enhance cancer cell invasiveness and survival. Additionally, a growing body of evidence indicates that CAFs contribute to tumour drug resistance through the secretion of cytokines and growth factors that alter cancer cell behaviour.

Material and method

We used endothelial-derived CAFs originating from human microvascular endothelial cells (HMEC-1) and human umbilical vein endothelial cells (HUVEC). Furthermore, we employed colorectal cancer cell lines characterized by high (HT29, SW620, HCT116) and low (LS180, LOVO) IL-17A receptor expression. Protein levels were determined using Western blot, while cytokine secretion was analyzed using ELISA assays. The expression of IL-17A and ERCC1 was silenced via interfering RNA. Cell motility of CAFs was tested using the wound healing assay. Endothelium and CAF morphological changes were evaluated by light microscopy.

Result and discussion

Our findings demonstrate that CAFs derived from endothelial cells exhibited resistance to oxaliplatin compared to parental cells. We identified ERCC1 (an essential component of the DNA repair machinery) as a key factor contributing to this phenomenon. Silencing ERCC1 effectively decreased the chemoresistance of CAFs and restored their proliferation to levels similar to those of their endothelial precursors. Furthermore, ERCC1 knockdown partially reversed the mesenchymal phenotype of CAFs, confirmed by decreased levels of mesenchymal markers and increased endothelial ones. Moreover, CAFs with reduced ERCC1 protein level restore endothelial functions. Those changes were associated with the inactivation of the NF-κB signalling pathway and reduction in IL-17A secretion. Since IL-17A provided by CAFs stimulated colon cancer cells and induced chemoresistance, ERCC1 downregulation impaired tumour cell proliferation and increased cancer cells' sensitivity to oxaliplatin or irinotecan chemotherapy.

Conclusion

Our study offers new insights into the molecular mechanisms of CAF-mediated drug resistance, emphasizing the ERCC1-NF-κB-IL-17A axis as a potential therapeutic target to improve the efficacy of colorectal cancer treatment.

EACR25-2407

Reducing Glioblastoma Treatment Heterogeneity Through Tumor Treating Fields Application Coupled With tmCLIC1 Impairment by Metformin

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Introduction

Glioblastoma (GB) is the most common and lethal tumor of the central nervous system. Despite the multimodal therapeutic approach, patients' survival remains extremely poor. Sensitivity to therapy varies among patients due to high GB heterogeneity. Tumor Treating Fields (TTFields) represent the only breakthrough of the last decades in increasing life expectancy but are not able to counteract tumor relapses. Tumor recurrence is driven mainly by a subpopulation of cells, the GB stem cells (GSCs), characterized by altered ion channel expression and activity. Unfortunately, most of them cannot be viable therapeutic targets due to their involvement in essential physiological cellular functions. A promising exception is the transmembrane form of Chloride Intracellular Channel 1 (tmCLIC1), upregulated in GB stem-like cells and promotes tumor proliferation. It was recently demonstrated that an inhibitor of tmCLIC1 is the antidiabetic drug metformin. tmCLIC1 could serve as a valuable target for a combined antitumoral therapy alongside TTFields to impair glioblastoma development.

Material and method

We run a genomic and proteomic analysis of surgical material after two biopsies from patients treated or not with TTFields. We developed also an in vitro GB relapse model. GB primary cultures have been treated for up to 12 days with the INOVITRO system. Resistant cells were analyzed using patch clamp, metabolic assays, ROS measurement, and for the expression of stemness marker. Moreover, the combination of metformin and TTFields was tested with growth curve assays.

Result and discussion

TTFields-treated tumors exhibited reduced inter-patient heterogeneity and shared alterations in key cytosolic pathways. In vitro transcriptomic analyses of resistant GB cells confirmed the upregulation of ion channel transcripts, stemness markers, and oxidative stress-related pathways. After 288 hours of continuous TTFields application, glioblastoma cells showed an average depolarization of the resting membrane potential, increasing oxidation and acidification of the cytoplasm. Our data have demonstrated that TTFields combined with metformin resulted in extremely efficient in promoting cancer cell death.

Conclusion

Here, we demonstrate that combining TTFields stimulation with continuous metformin treatment, an inhibitor of tmCLIC1, synergistically suppresses GB growth. These findings highlight a novel therapeutic strategy to overcome treatment resistance and reduce GB recurrence by targeting tmCLIC1 in conjunction with TTFields.

EACR25-2410

miR-181a-5p and Caveolin-1 Express Inversely in HCC and Augment the Aggressive Phenotype of Sorafenib-Resistant HCC Cells

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Introduction

Hepatocellular carcinoma (HCC) has one of the highest mortality rates among all cancers, and the most common reasons are metastasis, recurrence, and therapy resistance. As the first multi-kinase inhibitor given in HCC treatment, sorafenib remains the most preferred drug. However, sorafenib resistance develops as a result of prolonged therapy. Although the exact mechanism is yet unknown, non-coding RNA-based mechanisms are thought to be one of the causes of treatment resistance. Previously, we showed that the descending expression of miR-181a-5p causes an aggressive phenotype in HCC. In sorafenib resistance, Caveolin-1 (Cav-1) is overexpressed and enhances the aggressive phenotype. In this study, we wanted to shed light on the mechanism of sorafenib resistance from the perspective of aggressive phenotype triggers that are miR-181a-5p and Cav-1 and their relation.

Material and method

Initially, we examined publicly available transcriptome data of HCC patients treated with SOR/placebo (GEO accession ID: GSE109211) and examined the expressions of MIR181A and CAV1 in patients who are classified as sorafenib responders and non-responders. Then, we generated sorafenib-resistant (SOR-Res) HuH-7, SNU-449 and Mahlavu HCC cell lines, and we determined Cav-1 and miR-181a-5p expression levels compared to their wild-type counterparts. We determined the miR-181a-5p level in SOR-Res cells by overexpressing and silencing miR-181a-5p through transfecting the mimic or inhibitor of miR-181a-5p in biological assays, including MTT, colony formation, stress fiber staining, and motility assays.

Result and discussion

In the patient dataset, MIR181A expression was significantly lower in non-responders compared to responders, whereas CAV1 expression was significantly higher. In SOR-Res cells, Cav-1 expression was increased, and miR-181a-5p expression was decreased; when we overexpressed miR-181a-5p, SOR-Res cells tended to be more sensitized to sorafenib, their colony forming, motility ability and stress fibers were decreased. All the outcomes, including Cav-1 expression, changed to the opposite when we inhibited miR-181a-5p expression.

Conclusion

Since miR-181a-5p and Cav-1 are negatively expressed in SOR-Res cells and patients, miR-181a-5p may be a possible target of Cav-1, and they may contribute to sorafenib-resistance by augmenting the aggressive phenotype in HCC.

EACR25-2458**Overcoming Taxane Resistance Through PRMT5 Inhibition: Targeting Epigenetic Mechanisms**

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Introduction

Castration-resistant prostate cancer (CRPC) is a lethal form of prostate cancer that no longer responds to androgen deprivation therapy. CRPC treatment primarily relies on chemotherapy, particularly taxanes such as docetaxel (Dtx) and cabazitaxel (Cbz). However, the development of taxane resistance remains a significant clinical challenge. Identifying novel targets to overcome resistance is critical for improving therapeutic efficacy. In this context, PRMT5 has emerged as a potential epigenetic regulator associated with cancer progression and drug resistance.

Material and method

To identify epigenetic regulators of taxane resistance, Dtx- and Cbz-resistant CRPC cell lines were established and an epigenetic drug screen with a library of 150 molecules identified PRMT5 as a re-sensitizing modulator. PRMT5 inhibition was assessed for its role in overcoming taxane resistance using pharmacological inhibitors (CMP5, GSK591, HLCL61) and shRNA knockdown, followed by functional assays including cell viability, clonogenic assay, apoptosis (Annexin V/Caspase-3/7 assays) and cell cycle analysis. Western blot assessed PRMT5 substrates (H3R8me2, H4R3me2) and symmetric dimethylarginine levels. ChIP-seq and RNA-seq analyses identified PRMT5-mediated transcriptional regulation and epigenetic modifications. Patient data, including PRMT5 expression, Gleason scores and clinical outcomes were analyzed from public databases.

Result and discussion

PRMT5 inhibition overcame resistance CRPC and restored taxane sensitivity, as demonstrated by decreased IC₅₀ values and Combefit synergy analysis. CMP5, GSK591 and HLCL61 emerged as the most effective PRMT5 inhibitors, reversing taxane resistance in SRB and clonogenic survival assays. PRMT5 pharmacological inhibition led to a reduction in sDMA levels and histone methylation markers (H3R8me2, H4R3me2), confirming on-target activity. Combination of docetaxel and PRMT5 inhibitor induced G2/M arrest in resistant cells, indicating restored taxane susceptibility. Importantly, PRMT5 inhibition no effects on parental cells, reinforcing its selective role in taxane resistance mechanisms. ChIP-seq analysis revealed differential PRMT5 binding sites between parental and resistant cells, while RNA-seq demonstrated significant downregulation of MYC and E2F target genes. Key regulatory factors, including CCND1, RB1, and ABL1, were identified through the integration of ChIP-seq and RNA-seq data, suggesting their potential relevance in taxane resistance.

Conclusion

Pharmacological inhibition of PRMT5 modulates cell cycle and transcriptional pathways, re-sensitizing

resistant cells to taxane-based chemotherapy, with RNA-ChIP integration revealing key mechanisms related to cell cycle regulation. Given that PRMT5 inhibitors are already being evaluated in clinical trials, our findings further support its potential as a therapeutic target for taxane-refractory patients.

EACR25-2495**Elucidating Topotecan-Resistance Mechanism in Human Breast MCF-7 Cancer Cells and Paclitaxel Cross-Resistance by DNA Damage Signaling Pathway Gene Expression**

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Introduction

Gene expression profiling and pathway analyses are a detrimental tool to unravel the pharmacological effect of chemotherapeutics on tumor cells. Moreover, gene expression profiling is an important tool to analyze pathways that may confer resistance to chemotherapeutic drugs commonly used in clinical practice for the treatment of several types of cancer. Topotecan is a clinically active anticancer agent, used as a second-line treatment or in combination with other drugs. Topotecan interacts with topoisomerase I and forms DNA double-strand breaks. On the other hand, Paclitaxel is a natural anti-tumor agent that has become the standardized first-line chemotherapy treatment for several types of cancer. Unfortunately, Paclitaxel is associated with the development of drug resistance. At present, studies suggest that Topotecan and Paclitaxel do not exhibit significant cross-resistance, suggesting that tumors resistant to one drug may still be susceptible to the other.

Material and method

Here, we generated an in vitro Topotecan-resistant breast cancer cell line. MCF-7 cells were treated with 50 nM of Topotecan for two weeks with cell culture media changes every 3 to 4 days and Topotecan concentration increased 1.5-fold every two weeks until cells were resistant to 1.2 μM. Resistant cells were treated with 130 nM of paclitaxel for 15 days and then cell pellets were collected for ARN extraction and cDNA synthesis. A real time PCR array that evaluated 26 genes involved in DNA Damage signaling was performed.

Result and discussion

Surprisingly, DNA damage signaling gene expressions were downregulated between -3-to-10-fold change. Down-regulation was significantly reduced after Paclitaxel treatment, however, DNA damage response signaling gene expression was not increased more than 1.5 fold change upregulation.

Conclusion

This findings suggest cross-resistance in HER2 expressing breast cancer cells and that the use of Paclitaxel in combination with topotecan for the treatment of HER2 breast cancer is not an effective line for treatment.

EACR25-2503**Cisplatin resistance hinders the response to CDK4/6 inhibitors in esophageal squamous cell carcinoma cells**

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Introduction

Esophageal squamous cell carcinoma (eSCC) remains one of the deadliest cancers with an overall survival below 20%. The cyclin D-CDK4/6-pRB axis, frequently deregulated in eSCC, was suggested as target for therapy. However, recent clinical trials testing CDK4/6 inhibitors against advanced EC (including eSCC) reported limited clinical activity. Interestingly, all enrolled patients with eSCC tumors had been previously treated with genotoxic drugs. This study aims to investigate how resistance to genotoxic treatment (e.g. cisplatin) may modulate the response to CDK4/6 inhibitors such as palbociclib.

Material and method

Human eSCC cell lines KYSE-140, KYSE-180, KYSE-410 and TE-6 were rendered resistant to cisplatin with two different approaches, pulsatile or continuous. Pulsatile-resistant (RP) cell lines (KYSE-140-RP2, KYSE-180-RP2, KYSE-410-RP2, TE-6-RP2) received high doses of cisplatin in 24h pulses, while continuous-resistant (RC) cell lines (KYSE-140-RC2, KYSE-180-RC2, KYSE-410-RC3, TE-6-RC1) received lower doses continuously over time. Cisplatin resistance was characterized with MTT assay, qPCR and RNA sequencing. Flow cytometry, clonogenic, qPCR and RNA sequencing assays were conducted to characterize differences in response to palbociclib treatment between resistant and parental cell lines.

Result and discussion

All established cell lines decreased their sensitivity to cisplatin and remained stable treatment-free except for KYSE-410-RP2, which progressively lost its resistance. Resistant KYSE-140 cell lines had the most pronounced shift in IC50. Interestingly, clonogenic assays showed KYSE-140-RP2, KYSE-140-RC2, KYSE-180-RP2 and KYSE-410-RC3 cell lines to be significantly less responsive to palbociclib treatment than their parentals. Oppositely, KYSE-410-RP2 didn't alter its response. Similarly, PCA analyses from RNAseq data showed all resistant cell lines to cluster separately from their parental lines (with or without treatment), except for KYSE-410-RP2. qPCR analyses revealed that KYSE-180, KYSE-410 and TE-6 resistant cell lines had a significant upregulation of the multidrug resistance-associated protein MRP2. Unexpectedly, KYSE-140 resistant cell lines had a significant downregulation of this protein, concomitant with the downregulation of CTR1, an influx transporter. These results suggested that divergent resistance mechanisms may influence differently palbociclib response.

Conclusion

These findings indicate that eSCC cells develop different mechanisms of cisplatin resistance, which may lead to a co-acquired resistance to CDK4/6 inhibitors. A further characterization of these mechanisms with phospho-

proteomics will reveal strategies to rescue the sensitivity to CDK4/6 inhibition and suggest novel combinations for eSCC patients.

EACR25-2546**Unraveling tumor intrinsic responses: IFN-gamma predicts clinical immunotherapy efficacy and exclusively triggers apoptosis in solid tumors while sparing hematologic malignancies**

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Introduction

Over the past lustrum, tumor intrinsic interferon gamma (IFN γ) has emerged as a pivotal factor in the realm of cancer immunotherapy resistance for solid tumors, being determinant for optimal cancer immunotherapy response to both antigen presentation dependent immunotherapies such as immune checkpoint blockade and T cell redirection therapies like CAR T cells and bispecific antibodies (bsAbs) whose recognition do not rely on the expression of the major histocompatibility complex I (MHC-I). However, the requirement of tumor IFN γ for the efficient killing of CAR T cells and bsAbs in hematologic tumors, which has shown remarkable clinical efficacy, remains uncertain, yielding contradictory results. Furthermore, these studies employed genetically modified knock-out (KO) models with complete absence of the pathway, overlooking its potential role in tumors that acquire resistance through transcriptional down-modulation. In addition, it has been demonstrated distinct molecular mechanisms between complete IFN γ non-responders and partial responders, both of whom exhibit resistance. Furthermore, the tumor antigen's biology, the intrinsic differences between bsAbs and CAR T cells, and the precise antitumor mechanism of action exerted by IFN γ has been disregarded.

Material and method

Hematologic cancer cells naturally expressing CD19 or engineered to express HER2, and solid cancer cells expressing HER2 or engineered to express CD19, and bsAbs and CAR T cells targeting these antigens.

Result and discussion

We demonstrate that a transcriptional impairment of tumor IFN γ signaling employing shRNAs targeting the IFNGR1 gene leads to resistance to bsAbs and CAR T cells exclusively in solid tumors. In addition, we show that IFN γ induces apoptosis in solid but not in hematologic cancer cells, and we show that hematologic cancer cells exhibit a higher basal intrinsic IFN γ signaling which result in a suboptimal response to IFN γ , which can explain their lack of response to the pro-apoptotic effects of IFN γ . Importantly, by analyzing publicly available transcriptomic data from hundreds of cancer cell lines of various origins, we confirmed that hematologic cancers express significantly higher levels of IFN γ -related genes, particularly IRF1, the master regulator of the IFN γ signaling pathway. Furthermore, an analysis of tumor fractions from scRNA-seq datasets of patients treated with various cancer immunotherapies – specifically

immune checkpoint blockade (anti-PD1) for solid tumors and CAR T cells or BsAbs targeting CD19 for leukemia patients – demonstrates that a tumor IFN γ signature predicts clinical efficacy exclusively in solid tumors while sparing leukemia cancers.

Conclusion

These findings underscore the clinical need to develop tailored treatments that are able to activate tumor IFN γ signaling to maximize cancer immunotherapy efficacy specifically in solid tumors, thereby minimizing unnecessary toxicities in hematologic cancers.

EACR25-2550

Signaling Pathways affected by RAGE inhibition in vemurafenib treated melanoma cells

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Introduction

The receptor for advanced glycation end products (RAGE) is a pattern recognition receptor that has been shown to participate in melanoma cell proliferation, migration, invasion and tumor growth. Using a subcutaneous xenograft mouse model of melanoma, we previously showed that RAGE also contributes to resistance to the chemotherapeutic drug dacarbazine. Because dacarbazine is not the current standard of care for melanoma anymore, we investigated if RAGE inhibition could enhance the efficacy of vemurafenib, a BRAF mutant inhibitor and a current standard of care for melanoma patients. We also investigated the signaling pathways affected by RAGE inhibition in vemurafenib treated cells.

Material and method

We used two cell lines that express the mutant form of the BRAF kinase. Both cell lines derive from the WM115 human melanoma cell line: the parental WM115 cell line expresses low endogenous levels of RAGE, whereas the engineered WM115-RAGE cell line overexpresses RAGE. The small molecule RAGE inhibitor FPS-ZM1 was used. A synergy assay was performed in vitro and analyzed using the Combenefit software. Signaling pathways related to autophagy and apoptosis were interrogated by Western blot analysis.

Result and discussion

Treatment of WM115 or WM115-RAGE cells with FPS-ZM1 alone did not affect the viability of the melanoma cells. However, when combined with vemurafenib, FPS-ZM1 enhanced the apparent IC₅₀ of vemurafenib by 2- to 3-fold, suggesting that RAGE inhibition enhances the efficacy of vemurafenib in WM115 cells and possibly in other melanoma cells. We are currently investigating the autophagic signaling pathways affected by RAGE inhibition by Western blot analysis.

Conclusion

Our preliminary studies suggest that RAGE inhibition enhances the efficacy of vemurafenib in human melanoma cells. Ongoing studies are currently investigating the signaling pathways affected by RAGE inhibition in melanoma treated cells. Future studies using

a mouse model of melanoma will be performed to determine if the synergistic effect between vemurafenib and the RAGE inhibitor is also observed in vivo.

EACR25-2572

Berberine, a P-glycoprotein inhibitor, shows synergistic anti-cancer effect when combined with Dactolisib in Colorectal Carcinoma HCT116 cell lines

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Introduction

Berberine, also known as a P-glycoprotein inhibitor, is an isoquinoline alkaloid that shows low toxicity and anti-cancer activities. P-glycoprotein is an energy dependent drug efflux transporter that can reduce drug accumulation within cells by pumping out therapeutic agents. Berberine shows antiproliferative effect in colorectal carcinoma and induced apoptosis. The phosphatidylinositol 3-kinase (PI3K)/AKT/mTOR pathway, is critical in tumor growth and metastasis. The therapeutic potential of PI3K/AKT/mTOR inhibitors and their combinations have led to their active investigation in preclinical and clinical studies for colorectal carcinoma treatment. Dactolisib, also known as NVP-BEZ235, is a dual PI3K and mTOR kinase inhibitor. The current study investigates the synergistic anti-cancer effect of Berberine and Dactolisib on colorectal carcinoma HCT-116 cell line.

Material and method

Cell viability assay was conducted by resazurine dye and colony forming assay was conducted to determine the cell growth inhibition in HCT-116 cell line when treated with Berberine and Dactolisib. Migration ability of combination treatment of Berberine and Dactolisib was determined by in vitro scratch assay. Cell cycle assay was conducted by staining with propidium iodide to detect the cell distribution of HCT-116 cells. Intracellular uptake of Berberine was detected by fluorescence microscopy. Kinetic analysis and competition assay of both drugs were monitored by Ligand tracer to detect the real-time interaction measurements on live cells.

Results and discussion

Cytotoxicity of Berberine and Dactolisib was observed in a dose and time dependent manner in colorectal carcinoma HCT-116 cell line. Low doses of Dactolisib (0.25 μ M and 0.5 μ M) showed nearly 50% cell viability when combined with 10 μ M Berberine at 48 hours. Co-treatment of Berberine and Dactolisib showed a significant reduction in colony formation and migration ability in HCT-116 cell line. Treatment with Berberine and Dactolisib led to an increase in SubG0 phase of HCT-116 cells, suggesting possible induction in apoptosis. Intracellular uptake of Berberine was higher in combination groups compared to Berberine alone group. Co-treatment of Berberine and Dactolisib reflects the stabilizing effect of Dactolisib on Berberine binding kinetics in HCT-116 cells and ensure prolonged intracellular retention and activity of Berberine. As Berberine a P-glycoprotein inhibitor, this synergistic approach could be a potential combination to overcome drug

resistance. Resistant HCT-116 cell line will be used to further analyze this combination therapy.

Conclusion

These findings suggest a synergistic anti-cancer effect of Berberine and Dactolisib combination on HCT-116 cell line, demonstrating a potential therapy for colorectal carcinoma to overcome drug resistance.

EACR25-2593

Targeting AURKA Overcomes Gemcitabine Resistance in TP53-Mutated Cancers by Disrupting the Replication Stress Response

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Introduction

Gemcitabine (GEM), a nucleoside analog, has been widely used in the treatment of various cancers. However, its therapeutic efficacy is often compromised by intrinsic or acquired resistance. TP53 mutations have been strongly associated with poor clinicopathological outcomes, reduced survival rates, and significantly enhanced resistance to gemcitabine. Therefore, identifying effective combination strategies to overcome gemcitabine resistance in TP53-mutated cancers is of critical importance.

Material and method

A kinase inhibitor library screen was performed in GEM-resistant TP53-mutated cholangiocarcinoma cells, with synergistic effects assessed using AUC-based scoring. Functional validation was conducted through *in vitro* and *in vivo* studies, including organoid models, patient-derived xenografts (PDX), and clinical dataset analysis. Molecular mechanisms were explored using immunoblotting, immunofluorescence, isolation of proteins on nascent DNA (iPOND), and DNA fiber assays.

Result and discussion

Clinical data analysis revealed that AURKA over-expression correlates with worse survival outcomes in TP53-mutated cancers. Functionally, AURKA inhibition significantly sensitized TP53-mutated cholangiocarcinoma, pancreatic, and colorectal cancer cells to GEM. Mechanistically, AURKA inhibition impaired MRE11-dependent stalled replication fork restart and homologous recombination repair, ultimately compromising S-phase checkpoint activation and inducing mitotic catastrophe in GEM-treated cells. Further investigations demonstrated that AURKA exerts its effects through its kinase activity by regulating PARP1, an upstream modulator of MRE11, thereby promoting replication fork restart and contributing to GEM resistance in TP53-mutated tumors.

Conclusion

Our findings identify AURKA as a key mediator of GEM resistance in TP53-mutated cancers. Targeting AURKA disrupts replication fork restart and DNA repair, thereby enhancing GEM sensitivity. This study provides a strong rationale for the clinical evaluation of AURKA inhibitors in combination with GEM as a promising therapeutic strategy for TP53-mutated cancers.

Epigenetics

EACR25-0049

RASSF1A Methylation and Survival Outcome in Retroperitoneal Leiomyosarcoma

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Introduction

The utilisation of liquid biopsy in the detection of methylation status in the cell-free DNA is a new approach in the field of soft tissue sarcoma. We performed a proof-of-concept experiment utilising the cell-free DNA (cfDNA) of retroperitoneal leiomyosarcoma and aimed to investigate the correlation between methylated RASSF1A in leiomyosarcoma with tumour burden and overall survival.

Material and method

Plasma-derived DNA was extracted and eluted in 50 mL of nuclease-free water (NFW). Buffy coat's and tissue sample's DNA were also extracted using the DNeasy® Blood and Tissue Kit. DNA was eluted in 100 mL of Buffer AE. Every sample was subjected to two ddPCR reactions – one with the MSRE and the other without the MSRE. Droplets were generated using the QX200™ Droplet Generator (Bio-Rad). Incubation and thermal cycling were performed using the C1000 Touch Thermal Cycler (Bio-Rad), with the following program. Following PCR, droplets were read and quantified using the QX200 Droplet reader and QuantaSoft™ Software. Methylation ratio {[RASSF1A (Methylated)]: [RASSF1A (Un-methylated)]} was calculated and correlation with tumour burden and survival analysis was made.

Result and discussion

A total of twenty-four patients (8 males and 16 females) were included in the study, with a mean age of 55.33 years. Cell-free DNA (cfDNA) was successfully detected in all 24 samples. Six plasma-derived DNA samples from patients with LMS, confirmed by immunohistochemistry as positive for either desmin, smooth muscle actin, and/or heavy caldesmon were selected, and these include four samples taken pre-operatively and two sets of paired samples (including post-operative samples) were analysed. Methylated RASSF1A was detected across all samples. We did not observe a consistent reduction in percentage of DNA methylation in the post-operative samples. The correlations between methylation ratio and tumour burden (cm^3) ($p = 0.246$) as well as disease-free survival (days) ($p = 0.108$) were positive but not statistically significant.

Conclusion

DNA methylation can be detected using liquid biopsy and ddPCR approach. We optimised the DNA methy-

lation protocol in detecting methylated RASSF1A. A larger sample is required to improve the sensitivity and specificity of the assay.

EACR25-0077

Investigating the influence of copper homeostasis on epigenetic modulation in the tumour microenvironment

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Introduction

Neuroblastoma, a pediatric cancer originating in the neural crest (Maris JM, 2010), remains a significant challenge in oncology. It predominantly affects young children (Ward E et al., 2014) and exhibits diverse clinical behaviors, ranging from spontaneous regression to aggressive, treatment-resistant forms (Swift et al., 2018; Tolbert et al., 2018). Emerging evidence highlights the multifaceted role of copper in neuroblastoma (Voli et al., 2020), suggesting its involvement in tumor development, progression, and treatment resistance. Copper ions modulate the activity of various enzymes critical for epigenetic regulation, including histone acetyltransferases, histone deacetylases, and DNA methyltransferases. These modifications can alter gene expression patterns, promoting tumorigenesis. Notably, our most recent study showed that high intratumoral copper levels contribute to immunosuppression by impairing neutrophil function (Rouaen et al., 2024). Understanding how neutrophils contribute to the immune landscape of neuroblastoma is critical for developing targeted therapies.

Material and method

Neuroblastoma cell lines were treated with the copper chelator TEPA to evaluate its impact on epigenetic regulation. Expression levels of epigenetic regulators were analyzed using quantitative PCR and Western blotting. The tumor microenvironment was further investigated using co-culture systems comprising differentiated immune cells and neuroblastoma cells. Neutrophil differentiation and activation were assessed in vitro by treating immortalized neutrophil cell lines with varying concentrations of copper and TEPA. Epigenetic changes in immune cells were assessed by flow cytometry and gene expression analysis.

Result and discussion

Treatment with TEPA resulted in significant down-regulation of epigenetic factors EZH2, UHRF1, and DNMT1 in neuroblastoma cells, suggesting that copper depletion alters the epigenetic landscape of these cells. Furthermore, TEPA treatment induced molecular changes in tumor-infiltrating immune cells, including increased expression of genes associated with anti-tumor immunity. Neutrophils treated with TEPA exhibited shifts in their functional phenotype, including enhanced antitumorigenic activity, as evidenced by increased ROS production and expression of immune-activating markers. These findings suggest that copper homeostasis influences not only the cancer cells but also the neutrophil-mediated immune responses within the tumor microenvironment.

Conclusion

Targeting copper-dependent pathways using chelators like TEPA could disrupt tumor growth while enhancing immune-mediated recognition. These strategies hold promise for the development of combinatorial therapies that exploit copper homeostasis to improve patient outcomes in neuroblastoma.

EACR25-0114

An epigenetic shift of CEMIP drives tumor metastasis and 3D spheroid formation in Androgen-resistant prostate cancer

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Introduction

12.5% of men will receive a prostate cancer diagnosis at some point in their lives, making prostate cancer (PCa) one of the main causes of death and morbidity among men worldwide. Our previous population study based on UK Biobank suggested a significant decreased risk of PCa and PCa deaths. Here, we investigate the possible role of cell migration inducing hyaluronidase 1 (CEMIP) and its epigenetic change as molecular marker that drives tumor metastasis and 3D spheroid formation in androgen-resistant prostate cancer.

Material and method

Our analysis from public database from ChIP-Atlas and UCSC Genome Browser revealed the significance of CEMIP among prostate cancer cell lines. To further investigate, Androgen-insensitive (DU145, PC-3) and androgen-sensitive (LNCaP) human prostate cancer cell lines were knock-downed (si-RNA). Total RNA was extracted from the cells using the Trizol reagents before performing RT-qPCR with different cDNA concentration (25 ng, 50 ng and 100 ng) to confirm a successful knock-down. The results from MTT assay, 3D spheroid formation, migration, invasion and cell cycle analysis furtherly confirmed that the knock-down of CEMIP was more significant in androgen-resistant cell lines with statistical analysis from GraphPadPrism software.

Result and discussion

The results from ChIP-Atlas and UCSC Genome Brower showed that there is significant epigenetic shift of CEMIP at promoter region with Androgen-receptor (AR) binding to it. The results from RT-qPCR confirmed the successful knock-down. The knock-down displayed cytotoxicity effects against cancer cells ($p < 0.05$). Moreover, cancer cells form less aggregation (3D spheroid) after si-CEMIP. The MTT assay, migration, invasion, cell cycle analysis furtherly confirmed that the knock-down effect was more significant in androgen-resistant cell lines ($p < 0.05$). Thus, we confirmed that CEMIP drives tumor metastasis and 3D spheroid formation in Androgen-resistant prostate cancer. Our investigations provide compelling and novel evidence that epigenetic shift of CEMIP is a molecular marker in androgen-resistant prostate cancer and serves as a potential therapeutic target in prostate cancer in the future.

Conclusion

The current study provides novel perspectives into the epigenetic modification of CEMIP in prostate cancer. CEMIP can be a crucial player in prostate cancer as

molecular marker that drives tumor metastasis and 3D spheroid formation. The in vitro and in vivo validation of these results are required to examine whether the promising results are clinically translational. Overall, our outcomes reveal that CEMIP may have potential as a future therapeutic target in prostate cancer.

EACR25-0181

Deciphering the epigenetic de-regulation of the stem cell related HOX signature in glioblastoma

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Introduction

Bromodomain and Extra-terminal Tail (BET) proteins are epigenetic transcription regulators and potential therapeutic targets in glioblastoma (GB), the most aggressive primary malignant brain tumor in adults. A differential gene expression analysis of GB-derived sphere (GS) lines demonstrated that the BET inhibitor JQ1 significantly downregulates cancer-relevant gene signatures, including our previously defined HOX signature. HOX genes are crucial for limb segmentation during embryonic development and are associated with treatment resistance in a subset of GB patients characterized by a high-HOX signature. The HOXA gene cluster, located on chromosome 7 (Chr7), is a CpG-rich region and the expression of the HOXA-dominated signature correlates with Chr7 gain and widespread DNA hypermethylation. Previous findings indicated a significant negative correlation between DNA hypermethylation at the alternative HOXA10 promoter and HOX gene expression. This study aims to investigate the oncogenic role of HOXA10 and the epigenetic mechanisms underlying HOXA cluster deregulation in high-HOX GB.

Material and method

To explore these mechanisms, we used various assays: (1) comparative methylation analysis at the HOXA locus in a high-HOX GS line vs original tumor, along with RNA- and ChIP-seq analysis after JQ1 treatment; (2) BETi treatment and modulation of HOXA10/HOXA5 expression using inducible shRNA/ectopic expression systems to examine HOXA gene regulation; (3) functional assays post-HOXA10 modulation to assess cancer-relevant biological impact.

Result and discussion

We confirmed that BETi concertedly decreases HOXA gene expression in high-HOX GS lines. Remarkably, knockdown of HOXA10 in high-HOX GS lines is associated with a concerted downregulation of other HOXA genes; however, ectopic HOXA10 expression suggests that HOXA10 proteins may not be the sole factor contributing to HOX signature expression.

Specificity tests using shHOXA5 models affirmed the unique role of HOXA10, as HOXA5 knockdown did not

alter the expression of other HOXA genes. Functionally, HOXA10 knockdown in high-HOX GS lines results in potently decreased cell proliferation and stemness, accompanied by an emergence of senescence-like traits.

Conclusion

Our results set HOXA10 as a key player of the stem cell-related HOX signature in high-HOX GB, likely enabling tumor progression by its association with proliferative and stem-like features. BETi-mediated downregulation of HOX signature genes presents a promising therapeutic avenue for targeting high-HOX GB. Ongoing research focuses on differential gene expression, aiming to identify HOXA10-regulated genes and reveal its role in BETi-mediated effects. Besides, we are also assessing the potential changes in 3D chromatin architecture and epigenetic landscape around the HOXA cluster that may be involved HOX signature expression.

EACR25-0273

Boosting Pancreatic Cancer Treatments: overcome chemoresistance by targeting N6 methyladenosine modification

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Introduction

Pancreatic ductal adenocarcinoma (PDAC) is a highly aggressive malignancy with poor survival rates due to chemoresistance, clinical heterogeneity, and the absence of reliable early diagnostic markers. A key driver of chemoresistance is the presence of cancer stem cells (CSCs), a small subpopulation with distinct transcriptional profiles regulated by epigenetic and epitranscriptional mechanisms. N6 methyladenosine (m6A), the most abundant RNA modification, plays a crucial role in CSC regulation and cancer progression. Targeting the m6A regulatory machinery presents a promising strategy to enhance treatment efficacy. This study investigates the potential of m6A modulators in reversing epitranscriptomic alterations to promote chemosensitization in patient derived PDAC models.

Material and method

Subtherapeutic concentrations of m6A modulators were tested in circulating tumor cell (CTC)- and patient derived xenograft (PDX)-derived spheroid cultures, both individually and in combination with gemcitabine. m6A immunoprecipitation (Me-RIP) was performed on total RNA, followed by sequencing using the Illumina NovaSeq X Plus platform. Peak calling and transcript identification were conducted using findPeaks tool in HOMER.

Result and discussion

Bioinformatics analysis validated treatment effects, identifying enriched m6A sites and dynamic epitranscriptomic changes. Comparative analyses revealed variability in m6A peak distribution and gene expression, suggesting broad biological impact. Pathway enrichment analysis highlighted significant alterations in proliferation, survival, stress response, and immune regulation. Unique and common m6A peaks, along with differentially expressed genes, indicated both conserved and condition specific regulatory mechanisms.

Conclusion

This study provides preliminary evidence that m6A modifications influence PDAC progression and suggests that targeting m6A could enhance chemotherapy efficacy. Further bioinformatic analysis and GLORI based single base resolution quantification will provide deeper insights into the molecular mechanisms underlying the role of m6A in PDAC.

EACR25-0284

The effectiveness of EZH2 inhibitors in treating human papillomavirus associated cervical cancer and associated pathways

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Introduction

High-risk human papillomavirus (HPV) is a major risk factor in the development of several types of epithelial-origin cancers, including cervical and head and neck cancers and is associated with the aggressive and invasive behaviour of cancer cells. Enhancer of zeste homolog 2 (EZH2), a histone methyltransferase, has been found to downregulate expression of tumour suppressor genes and upregulate oncogenes and has also been observed in HPV-associated epithelial cancers, suggesting the possible role of high-risk HPV. Hence, it could promote cancer cell proliferation, invasion and drug resistance. EZH2 inhibitors have been shown to act as epigenetic regulators in cancer treatment, but there is still no consensus on the mechanisms of their action and their effectiveness on HPV-associated cancers.

Epithelial-to-mesenchymal transition (EMT), which induces phenotypic cellular changes, has been implicated in the pathological processes of epithelial cancer progression and metastasis. Epigenetic reprogramming of EMT via EZH2 inhibitors could promote anticancer effects. This study sets up to investigate the expressions of epigenetic changes and EMT markers in HPV-associated cancers cells *in vitro* and to identify whether EZH2 inhibitors (EPZ6438 and ZLD1039) can be effective in suppressing the EMT process and whether this would be associated with HPV oncogene status.

Material and method

EZH2 inhibitors' drug toxicity was evaluated by cell proliferation assay, cell cycle arrest and cell apoptosis assays using flow cytometry analysis on HPV+ and HPV-cervical cancer cells. Following a cell migration-scratch assay, epigenetic, oncogenic and EMT markers have been examined using immunocytochemistry staining, western blotting, flow cytometry and RT-qPCR.

Result and discussion

Both EZH2 inhibitors demonstrated effectiveness in reducing EZH2 expression on both mRNA and protein level, inducing apoptosis and arresting cells in G0/G1 phase. Results also showed a tendency to upregulate expression of epithelial markers at the mRNA and protein levels, which was supported by slower migration observed following the cell migration-scratch assay. This suggests EMT reversal could be initiated through EZH2 inhibition. Oncogene expression was reduced at both mRNA and protein levels following EZH2 inhibitor treatments.

Conclusion

Data from this study suggests that EZH2 inhibitors might be promising therapeutic agents in treating high-risk HPV-associated cervical cancers by downregulating HPV oncogenes and EMT reversal.

EACR25-0329

The epigenetic regulator TRIM24 controls melanoma cell dedifferentiation and resistance to treatment in melanoma

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Introduction

Cancer cell plasticity plays a key role in tumor progression and the acquisition of resistance to targeted and immunotherapy in melanoma. Although the transcriptional programs enabling the switch between differentiated/melanocytic and undifferentiated/invasive phenotypes are characterized, unravelling druggable epigenetic regulators that sustain melanoma cell adaptation and resistance remains crucial. Herein, we identified TRIM24, a bromodomain protein frequently amplified in human melanomas as a crucial regulator of melanoma cell plasticity.

Material and method

TRIM24 expression was analyzed in melanoma patient cohorts under immunotherapy. We then performed shRNA-mediated knock-down in human melanoma cell lines as well as TRIM24-specific degradation using a PROTAC-based approach. We further performed a comprehensive analysis of the transcriptomic and epigenomic reprogramming by RNA-seq, ATAC-seq, and Cut & Tag for histone marks and TRIM24 itself. Finally, *in vivo* experiments in syngeneic mouse models were implemented.

Result and discussion

We demonstrate that TRIM24 expression is increased in metastatic tumors and is associated with relapse under immunotherapy in melanoma patients. Loss-of-function of TRIM24 decreases the migratory capacities and increases the sensitivity to BRAF inhibitors in BRAF-mutated melanoma models. PROTAC-mediated degradation of TRIM24 resensitizes resistant human

melanoma models to targeted therapy. Integrative analysis of the transcriptional profile and the chromatin landscape reveal that TRIM24 knock-down represses undifferentiated/invasive and promotes differentiated/melanocytic transcriptional programs by inducing an epigenome reprogramming in melanoma cells. By delineating TRIM24 binding profile and histone marks affinity, we identify that TRIM24 recognizes a specific histone mark, it directly binds genes controlling an undifferentiated phenotype and alters their chromatin state. We further define a TRIM24 transcriptomic signature, that is found consistently enriched in treatment-resistant undifferentiated/invasive sub-populations in melanoma single-cell RNA-seq datasets. Finally, TRIM24 knock-down in mouse models induces a remodelling of the immune infiltrate and synergizes with immune checkpoint inhibitors.

Conclusion

Overall, our findings spotlight TRIM24 as a novel epigenetic regulator driving melanoma cell dedifferentiation and resistance to therapy. Targeting TRIM24 could offer a promising therapeutic avenue to reverse phenotype switching and restore tumor sensitivity to treatment.

EACR25-0365

Defining the Epigenetic Landscape regulating a Progenitor Signature in Development and, during Tumour Initiation and Progression

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Introduction

This project investigates the epigenetic regulation of cell-state transitions in melanoma, focusing on mechanisms underlying oncogenic competence and drug resistance. While MAPK-targeted therapies initially show high response rates, resistance emerges rapidly, leading to relapse and disease progression. Drug tolerance is closely linked to cell-state transitions and minimal residual disease, with progenitor-like states playing a key role in resistance. Melanoma initiation and progression are influenced by neural crest developmental programs, regulated by epigenetic factors that control cellular plasticity. Among them, ATAD2 and ATAD2B, highly expressed in progenitor cells and frequently altered in melanoma, may drive both normal progenitor state maintenance and drug resistance acquisition.

Material and method

We will use hPSC-derived neural crest cells and melanoblasts with doxycycline-inducible CRISPR/Cas9 knockouts of ATAD2 and ATAD2B to analyze their role in progenitor maintenance and melanoma adaptation. ATAC-seq and Cut&Run will assess chromatin accessibility and transcription factor binding, while RNA-seq and RT-PCR will evaluate transcriptional

changes in drug-treated melanoma cell lines. For in vivo validation, we generated Atad2 lox/lox and Atad2b lox/lox mouse models for inducible knockout during development and tumorigenesis. NSG mice injected with luciferase+ melanoma cells will undergo chronic therapy, with subsets receiving Bay-850 to assess its effect on resistance prevention.

Result and discussion

We expect ATAD2 and ATAD2B to regulate melanoma plasticity by modulating chromatin accessibility and transcriptional programs. Knockout models will clarify their role in progenitor state maintenance and therapy resistance. Single and double knockouts will assess potential compensatory mechanisms in vivo. Bay-850 efficacy will be evaluated in resistant tumours, with alternative inhibitors tested if needed.

Conclusion

This study aims to elucidate the role of ATAD2 and ATAD2B in melanoma plasticity and drug resistance by integrating hPSC models, patient-derived samples, and in vivo systems. By dissecting how these epigenetic regulators influence cellular state transitions, we seek to identify key mechanisms driving tumour adaptation, minimal residual disease, and therapy resistance. Understanding these regulatory networks will provide insights into the interplay between developmental programs and oncogenic transformation. Defining how ATAD2 and ATAD2B contribute to tumour heterogeneity and therapy-induced adaptation could reveal novel vulnerabilities for therapeutic intervention. Ultimately, this research aims to contribute to more effective strategies to overcome drug resistance, improve patient outcomes, and prevent disease relapse in melanoma.

EACR25-0465

Identification of epigenetic drivers in colorectal cancer from clinical tissue and blood samples

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Introduction

Colorectal cancer (CRC) presents a major health burden worldwide, and there has been a consistent increase in CRC incidence, particularly in younger people (age <50 years) where the disease is often presented at an advanced stage. Prognosis and survival of CRC patients rely heavily on the stage at diagnosis – the earlier, the better. Epigenetic changes are a universal and hallmark feature of cancer and recent studies from ourselves and others have provided strong evidence that epigenetic drivers play a key role in cancer progression and could be used as early markers. Further, the development of cell-free tumour DNA (ctDNA) methylation analysis represents a major step towards improving the diagnosis and management of cancer.

Material and method

We have streamlined and utilised genome-scale DNA methylation analysis methods (using reduced representation bisulfite sequencing or RRBS) for clinical samples along with RNA-Sequencing analysis for functional epigenomic analysis on tissue samples. In addition, we have streamlined modified RRBS technology to generate ultra-low input DNA methylomes in critical clinical samples such as Formalin Fixed Paraffin Embedded (FFPE) samples and cell-free DNA. Further, we are developing novel machine learning based algorithms to analyse large epigenomic data.

Result and discussion

Our study led to the identification of epigenetic (DNA methylation) drivers for both early and late stages of CRC. These putative methylation driver regions were associated with critical cancer cell pathways and function. Our CRISPR-based DNA methylation editing work indicates that many of these drivers can directly control gene expression without any changes to the underlying DNA sequence. In our cell-free DNA methylome work, we are able to generate robust and reproducible data from a small amount of ctDNA. Our results showed discriminatory methylation differences between malignant and non-malignant conditions and demonstrated high sensitivity and specificity.

Conclusion

We were able to identify epigenetic drivers in both tissue and ctDNA from patient samples. For future clinical application, our research will contribute to the development and application of sensitive and non-invasive methylation-based methods for the detection and monitoring of cancer. This work will potentially identify new epigenetic targets for therapeutic benefits, which will contribute to improved patient outcomes.

EACR25-0613

Multomics 6-base sequencing enhances the performance of early colorectal cancer detection from cell-free DNA

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Introduction

Early detection of colorectal cancer (CRC) has the potential to improve treatment outcomes and survival rates. Liquid biopsy for profiling of cell free DNA (cfDNA) in blood holds huge promise for early CRC detection in otherwise asymptomatic patients.

Material and method

Epigenetic biomarkers have already been shown to significantly contribute to cancer detection in liquid biopsies, but traditional DNA methylation sequencing conflates two cytosine modifications, 5-methylcytosine (5mC) or 5-hydroxymethylcytosine (5hmC), with different and opposing biological functions.

Discrimination of these two states could therefore be crucial for increasing the amount of functional information for CRC detection.

Result and discussion

We therefore employed duet evoC, a biomodal technology that provides the 6-base genome (the complete genetic sequence whilst simultaneously distinguishing 5mC and 5hmC), to cfDNA obtained from a cohort of 32 healthy volunteers and 37 patients with colorectal cancer (CRC) at stages I and IV. Through machine learning approaches, we built classifiers to differentiate between cfDNA from patients with stage I CRC and individuals without cancer using features based 5mC alone, 5hmC alone, both 5mC and 5hmC, or the conflated 5mC/5hmC (modC, as it would be generated by traditional epigenetic technologies).

Conclusion

Our findings reveal that, compared to traditional approaches, 5mC and 5hmC behave synergistically for the detection of early-stage CRC, enhancing diagnostic accuracy (AUC = 0.95). Notably, most regions with an increase in 5hmC in stage I CRC correlate with decreased in 5mC in stage IV CRC, suggesting that 5hmC could be an early marker of demethylation that occurs further down the cancer development trajectory. Applying 6-base sequencing to larger clinical cohorts, and across different indications, will evaluate the potential of 6-base data to improve the earlier detection, diagnosis, and treatment of other diseases.

EACR25-0629

MicroRNA-Driven Modulation of TACSTD2 (TROP2) in Colorectal Cancer Progression

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Introduction

The transmembrane protein TROP2, encoded by TACSTD2, has been identified as a key driver in cancer progression. Interestingly, a significant proportion of colorectal cancer (CRC) tumors are TROP2-negative, suggesting a high degree of plasticity during tumor development. To date, only a few studies have reported on the transcriptional regulation of TACSTD2, either through the transcription factor ATF2 or promoter hypermethylation. MicroRNAs (miRNAs) are small, ~22-nucleotide-long regulatory molecules that bind to the 3' untranslated regions (3'UTRs) of target mRNAs, leading to translational repression or mRNA degradation. The objective of our study was to identify and characterize miRNAs that regulate TACSTD2 (TROP2) expression in CRC.

Material and method

We first conducted an in silico analysis to identify potential miRNA candidates targeting TACSTD2.

Selected miRNAs were then overexpressed in CRC cell

lines using miRNA mimic transfections. Protein and RNA expression levels following miRNA modulation were validated using Western blot and qPCR analyses. A luciferase reporter assay was performed to confirm miRNA binding to the 3'UTR of TACSTD2. Additionally, functional assays were conducted to assess the effects of miRNA modulation in CRC cell lines and human colon tumor organoids.

Result and discussion

For our study, we selected CRC cell lines with high (DLD1, SW480) and low (T84, HCT116) TROP2 expression. We identified miR-376c-3p as a promising candidate for TACSTD2 regulation. Following miR-376c-3p mimic transfection, we observed a time-dependent reduction in TACSTD2 expression in DLD1 and SW480 cells. Furthermore, we confirmed miR-376c-3p binding to the 3'UTR of TACSTD2. Treatment of tumor cells with the DNMT1 inhibitor 5-azacytidine led to an increase in TROP2 expression, which could be attenuated by miR-376c-3p in a context-dependent manner.

Conclusion

Our findings suggest that miR-376c-3p is a novel post-transcriptional regulator of the TACSTD2 oncogene. Altered miRNA expression may contribute to the high intratumoral heterogeneity of TROP2 expression in CRC. Using RNAscope analysis, we will further investigate the inverse correlation between miR-376c-3p and TACSTD2 in the *in vivo* CAM model and patient samples.

EACR25-0720

Correlation with DNA methylation changes and chemoresistance in glioblastoma after mitochondrial transfer

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Introduction

Glioblastoma is an aggressive brain tumor with high mortality rate despite treatment with radiotherapy and chemotherapy with temozolomide. This is in part due to the high rate of resistance to temozolomide. It is well known that MGMT methylation is a marker for the prediction of the patients' response to temozolomide. Mitochondrial transfer is recently revealed to cause increased tumorigenicity and chemoresistance in glioblastoma. It is hypothesized that mitochondria transfer may cause increased tumorigenicity and chemoresistance via DNA methylation pathway, which mainly operates via the DNMT proteins.

Material and method

Glioblastoma cells and mitochondria in immortalized normal human astrocytes (NHA) are labelled with fluorescent dye. Cells with or without mitochondrial transfer from NHA are sorted out with a cell sorter after coculture. Methylation specific PCR is performed on bisulfite converted DNA of glioblastoma cells with or without mitochondrial transfer to determine the methylation status of MGMT. Western blotting and qPCR is done to determine the level of MGMT and DNMT in tumor cells with or without transfer. Global methylation

is determined using ELISA methods. Cell viability is assessed on cells with or without mitochondrial transfer after treatment with temozolomide by MTT.

Result and discussion

The glioblastoma cells with mitochondrial transfer show a decrease in global methylation, and global methylation has been shown to be correlated with poor outcome in glioblastoma. The glioblastoma cells with mitochondria transfer is expected to show a decrease in the level of MGMT methylation. The level of DNMT expression is expected to decrease, which may possibly lead to the decreased global methylation and MGMT methylation, and consequently chemoresistance. Cells with mitochondrial transfer is expected to have a higher cell viability after treatment with TMZ when compared to cells without mitochondrial transfer.

Conclusion

Mitochondria transfer in glioblastoma can confer chemoresistance to glioblastoma cells, which may in part be due to MGMT demethylation. Mitochondrial transfer may also contribute to the poor outcomes in glioblastoma by changing the global methylation landscape of the glioblastoma cells.

EACR25-0735

The CTCF - H3K27 methylation axis supports the migration of melanoma cells by regulating cholesterol biosynthesis

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Introduction

Cancer cell migration is critical for metastasis formation and depends on heterochromatin levels. Heterochromatin formation characterized by H3K9me3 and H3K27me3 is important for optimizing the transcriptome to support cell migration. Cancer cell migration also depends on the chromatin architectural protein CTCF by a poorly understood mechanism. CTCF acts as a 3D chromatin regulator by controlling cohesin-dependent loop formation as well as a classical transcription factor. Here, we looked for the molecular mechanism by which CTCF supports migration.

Material and method

Melanoma cells with a partial loss of function (pLoF) of CTCF were analyzed for their migration rate and transcriptome changes. Statins were used to manipulate cholesterol levels. Fluorescent microscope analyses were used to measure the formation of migrasomes and focal adhesions. Chromatin binding and 3D chromatin loops were determined by ChIP and 4C-seq techniques, respectively.

Result and discussion

CTCF pLoF inhibited the cellular migration rate while increasing the expression of multiple enzymes in the cholesterol biosynthesis pathway, which was accompanied by an elevation in cellular cholesterol levels. In parallel, we detected changes in membrane dynamics as measured by reduced formation of migrasomes and focal adhesions. Inhibition of cholesterol

synthesis in CTCF pLoF cells restored both the migration rate and the formation of migrasomes and focal adhesions. Conversely, increasing cholesterol levels in wild-type cells inhibited their migration rate. Thus suggesting that CTCF supports cell migration by suppressing cholesterol synthesis. Detailed analysis of the promoter of Hmgcs1, an early enzyme in the cholesterol biosynthesis pathway, revealed that CTCF prevents the formation of a loop between that promoter and another promoter located 200kb away. Additionally, CTCF supports the recruitment of the histone methyl transferase PRC2, leading to H3K27me3 formation at the Hmgcs1 promoter. H3K27me3 at the promoter of Hmgcs1 prevents SREBP2 binding and activation of transcription.

Conclusion

CTCF helps to recruit PRC2 to methylate H3K27 at promoters of cholesterol biosynthesis enzymes to repress SREBP2 binding and transcription. This leads to optimal cholesterol levels for cancer cell migration.

EACR25-0746

EZH2 regulates cell identity in neuroendocrine-like prostate cancer by driving alternate transcription via activation of translation and RNA processing regulation

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Introduction

Second-generation androgen deprivation therapies (ADT) have provided significant life-extension for patients with metastatic castration resistant prostate cancer (mCRPC); however, tumors eventually progress via therapy resistance as no therapies provide durable response. Approximately between 15-20% of these mCRPC tumors are independent of AR activity via a mechanism termed phenotypic plasticity. The development of phenotypic plasticity is associated with loss of function of the tumor suppressor genes RB1 and/or TP53. These tumors often display altered kinase signaling, chronic inflammation, and multilineage cellular states. Using genetically engineered mouse models (GEMMs) devoid of Pten and

Rb1, it has been previously demonstrated that the chromatin reprogramming factor Enhancer of zeste homolog 2 (EZH2) is an important regulator of alternative transcription programs that promotes phenotypic plasticity. Moreover, the loss of Tristetraprolin (TTP), an RNA binding protein that regulates mRNA stability and increases NF- κ B activation, has been associated with higher rates of aggressive prostate cancer (PCa). Here, our overall goal was to better understand EZH2 role in reprogramming and how this could be exploited therapeutically.

Material and method

To investigate the cell identity control by EZH2, PCa GEMMs were treated with an EZH2 inhibitor. Analysis was performed using a multi-omics approach involving CRISPR/Cas9 functional genomics screen, rapid immunoprecipitation mass spectrometry of endogenous protein (RIME), and snRNASeq. To validate these data, genetic and chemical tools were used with *in vitro* and *in vivo* PCa models.

Result and discussion

We observed that EZH2 regulates alternate transcription programs leading to a multilineage cell states downstream of Rb1 loss that is associated with activation of the mammalian target of rapamycin complex 1 (mTORC1). These data were further supported through RIME and functional genomic analysis validating cross talk between EZH2 and activation of translation. Combined chemical inhibition of EZH2 and PI3K/mTORC1 resulted in superior anti-tumor activity in murine and human phenotypic plastic models and was most significant when this combination was used with castration. Moreover, we elucidated that the regulation of cellular state transition by EZH2 inhibition requires activation of the RNA degrader, TTP.

Conclusion

Together, these data indicate phenotypic plasticity dependence on coordination between EZH2, TTP and mTORC1 signaling that represent novel therapeutic dependencies for this lethal PCa phenotype.

EACR25-0777

Targeting the chromatin remodeler HELLS to Improve Therapeutic Strategies in T-Cell Lymphoma

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Introduction

Chromatin remodelers dynamically modify DNA-nucleosome interactions, playing a crucial role in chromatin structure, accessibility and gene expression. Dysregulation of these proteins is common in cancer and often represents a therapeutic vulnerability. The chromatin remodeler HELLS has emerged as a critical regulator of genome stability and gene expression in aggressive T-cell lymphoma subtypes like ALK- anaplastic large cell lymphoma (ALCL). Selective knock-down of HELLS sensitizes ALCL cells to chemotherapy, suggesting the potential of HELLS as a therapeutic target. This study aimed to explore the role of HELLS in

transcriptional dynamics in ALCL and to assess its therapeutic potential.

Material and method

Formalin-fixed paraffin-embedded sections from diagnostic/relapsed primary lymphoma biopsies were used for retrospective cohort analysis. In vitro experiments combined omics approaches (RNA-seq, ChIP-seq, ATAC-seq and CMap) with functional assays.

Result and discussion

To investigate the transcriptional function of HELLs in ALK- ALCL, we integrated ChIP-seq on the TLBR-2 cell line, the principal model in prior studies elucidating HELLs role in lymphomagenesis, with RNA-seq in HELLs knockdown (HELLs KD) and control cells. We identified 467 deregulated and directly bound by HELLs based on ChIP-seq, termed HELLs-direct genes (HDGs). Their clinical significance was confirmed *in vivo* in a retrospective cohort of 44 ALCL patients (15 ALK⁺ and 29 ALK⁻ ALCL) through the NCounter platform. Merging HDGs with RNAPII-ChIP seq data revealed that about 60% of HDGs showed defects in RNAPII elongation, while 40% exhibited a significant alteration in RNAPII occupancy after HELLs KD. Gene ontology analysis showed that these last HDGs were enriched in T-cell mediated immunity, cytokines signaling and the JAK/STAT pathway, highlighting HELLs' underscored role in immune-related pathways. ATAC-seq analysis showed that HELLs modifies chromatin accessibility mainly at immune-related promoters in T-cell lymphoma cells. This process facilitates RNAP recruitment and transcription factor binding, enhancing transcriptional activity. Immune-related gene promoters were enriched for H3K4me3, further suggesting chromatin structural changes are necessary for their precise gene expression. To assess the therapeutic potential of HELLs, we performed an HDGs-based drug repurposing analysis by interrogating the CMap. We identified PI3K, JAK/STAT, and DNA-PK as top-scored synergistic pathways with HELLs depletion. In vitro experiments confirmed that Ruxolitinib (JAKi) and AZD7648 (DNA-PKi), caused significant synthetic lethality combined with HELLs depletion.

Conclusion

These findings demonstrate the direct role of HELLs in transcriptional regulation and pave the way for the development of targeted and clinically translatable strategies for HELLs inhibition.

EACR25-0800

Unraveling Inflammation-Related microRNAs in Pleural Mesothelioma: Gene Regulation and Pathway Analysis

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Introduction

Pleural mesothelioma (PM) is a rare and highly aggressive malignancy originating from the pleural lining. Approximately 80% of cases result from the

inhalation of asbestos fibers. This exposure triggers a chronic inflammatory response in the pleura, leading to tissue damage, uncontrolled cell growth and genetic mutations that promote cancer. There are three main PM histotypes, epithelioid, biphasic, and sarcomatoid. Recent studies highlight the importance of non-coding RNAs in PM, including microRNAs (miRNAs), as potential diagnostic and prognostic cancer biomarkers.

Material and method

Herein, we investigated the differential expression of inflammation-related miRNAs by RT² Profiler PCR Array Human Inflammatory Response (n = 84 miRNAs) in primary mesothelioma cell lines of different histotypes, epithelioid (n = 2), biphasic (n = 2), and sarcomatoid (n = 2), and normal human mesothelial cell lines (n = 2). miRNA expression analysis was analyzed using the 2- $\Delta\Delta Ct$ method. Log₂ fold change <-2>/2 was considered statistically significant. Algorithm, i.e. miRSYSTEM was used for miRNA target genes prediction using the default setting. Gene ontology (GO)/Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analyses were employed to categorize genes and identify relevant pathway.

Result and discussion

Our results demonstrate distinct miRNA expression profiles in PM compared to control group. A total of 10/84 (12%) miRNAs were differentially expressed when comparing PM to normal cells: of these 6/10 (60%) were upregulated and 4/10 (40%) were downregulated. To determine the potential biological relevance of the differentially expressed miRNAs to PM, we predicted the target spectrum of individual deregulated miRNAs and assessed which biological pathways were enriched with their putative targets. A total of 2,201 targets were predicted for the 4 downregulated miRNAs. GO for biological process showed that regulation of transcription of cell cycle G1/S phase transition were the most enriched processes in PM cell lines. GO for molecular function exhibited that activin receptor activity, protein serine/threonine kinase activity and DNA binding were the most enriched. Moreover, GO indicated that serine/threonine protein kinase complex, activin receptor activity, nucleus and heterochromatin were the most enriched cellular component.

Conclusion

Our investigation highlights that inflammation-related miRNAs are differentially regulated in PM, underscoring their potential as diagnostic biomarkers and therapeutic targets. Future studies will focus on validating these miRNA signatures in a larger panel of cell lines and patient sera, and on elucidating their functional roles in PM pathogenesis.

EACR25-0822

Promoter methylation regulates CDHR5 and UPB1 expression in clear cell Renal Cell Carcinoma with sarcomatoid differentiation

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Introduction

Renal Cell Carcinoma (RCC), particularly clear cell RCC (ccRCC), is one of the most lethal among urological cancers, with 25% of cases diagnosed at metastatic stage and 20% of these exhibiting sarcomatoid features, which drive tumor aggressiveness and poor prognosis. Although it is known that altered DNA methylation occurs during ccRCC progression, its contribution to the emergence of sarcomatoid differentiation remains elusive. This study aims to find new DNA methylation biomarkers that accurately identify sarcomatoid features arising in ccRCC.

Material and method

In silico analyses were conducted to assess differentially methylated genes in sarcomatoid ccRCC (sccRCC) using The Cancer Genome Atlas (TCGA), focusing on specific genes linked to normal cellular function, cell stemness, and metabolism. Promoter methylation and expression were validated for the candidate genes in a tissue sample set (54 ccRCC and 34 sccRCC) using Mann-Whitney U test to compare gene methylation and transcript levels between ccRCC and sarcomatoid ccRCC groups and by plotting Receiver Operator Characteristics (ROC) with the respective biomarker performance estimates. Ethics approval was conceded by the Ethics Committee of IPO Porto (CES. 158/023).

Result and discussion

CDHR5 and UPB1 promoter methylation levels were significantly higher in sarcomatoid ccRCC than in ccRCC without sarcomatoid features, while respective transcript levels were significantly diminished in both independent cohorts (TCGA and IPO Porto). When combining CDHR5me and UPB1me levels in a panel, sccRCC was discriminated from ccRCC with 78.7% specificity and 63% sensitivity. Furthermore, increased CDHR5me levels significantly associated with metastatic dissemination at diagnosis in sccRCC patients.

Conclusion

Promoter methylation of CDHR5 and UPB1 is recognized as a possible hallmark to identify sarcomatoid change in ccRCC and may serve as a biomarker to this end. Validation in liquid biopsies could enhance the clinical utility of these findings.

EACR25-0978

Methylation profiles in cervical swabs: challenges in early diagnosis of ovarian cancer

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Introduction

Developing cost-effective and non-invasive methods for the early diagnosis of ovarian cancer remains an unmet medical need, largely due to the disease's unspecific symptoms in its early stages. In this project, we aimed to identify differentially methylated CpG loci in cervical swabs from patients diagnosed with ovarian cancer and benign pelvic masses.

Material and method

Methylation profiles of 77 cervical swabs were analyzed using the Infinium EPICv2 array. The cohort was divided into training and testing sets to develop a diagnostic signature. Multiple statistical approaches were applied to identify CpG sites distinguishing patients with ovarian cancer from patients with benign ovarian diseases.

Result and discussion

None of the statistical methods identified CpG loci capable of diagnosing ovarian cancer with sufficient specificity and sensitivity. Methylation differences associated with clinical conditions appeared masked by variations in cell composition, the primary source of sample heterogeneity.

Conclusion

Diagnostic strategies should not rely solely on methylation patterns in cervical biology but rather focus on detecting tumor-specific DNA sequences transferred from the ovarian cancer site into cervical swabs.

EACR25-0989

Single-Cell Analysis of Epigenetic Landscapes Highlights Recurrent Chromatin Accessibility Alterations in Colorectal Cancer

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Introduction

Phenotypic variation among cancer cells is shaped not only by genetic heterogeneity but also by distinct epigenetic patterns. Current studies investigating the co-evolution of the genome and epigenome in colorectal cancer (CRC) are largely limited to analyses of bulk samples. Here, we characterise the epigenetic landscape of CRC and adjacent normal tissues at single-cell resolution.

Material and method

Fresh tissue specimens were collected from treatment-naïve CRC cases ($n = 17$) referred for surgery at Fondazione IRCCS Istituto Nazionale dei Tumori (Milan,

Italy). Samples of normal colon tissue ($n = 4$) were obtained as controls to assess epigenetic rewiring in non-malignant cells adjacent to CRC. Bulk whole-genome sequencing was conducted to characterise the genetic landscape of tumours, while single-nucleus 10x multiome assays were performed to assess chromatin accessibility using ATAC-Seq. In the multiome data, cell types were annotated based on canonical cell markers; tumour cells were identified as clusters exhibiting copy number alterations (CNA). Chromatin accessibility peaks in tumour cells were identified for each cancer sample and subsequently compared against a pooled set of normal epithelial cells. Differentially accessible regions ($|LFC| > 1$, $p_{adj} < 0.01$) were annotated, and recurrent somatic chromatin accessibility alterations (SCAAs) in promoters and enhancers were identified and characterised.

Result and discussion

The most recurrent SCAAs affecting promoters and enhancers of cancer-related genes were observed in over 75% of patients, including the activation of promoters for FOXQ1, TOP1, PTCH1, and PLCG1, as well as the inactivation of promoters for TP53, SMAD3, and TSC2. The recurrence of these SCAAs was significantly higher than that of many genetic driver mutations. An analysis of genome-wide chromatin accessibility in tumour cells at transcription factor (TF) binding sites revealed increased accessibility associated with the TCF7/LEF1 family, homeobox TFs (HOX, FOX, SOX families), and SNAI1/2/3 TFs. Distinct epigenetic profiles were observed among CNA clones within the same tumour, although highly recurrent SCAAs were shared across clones. A comparison of normal epithelial cells adjacent to the tumour core with distal normal epithelial cells revealed a subset of SCAAs in cancer-related genes. Intriguingly, some of SCAAs resembled those observed in the tumour cells. Epigenetic footprints affecting extracellular matrix remodelling and antigen presentation were detected in stromal cells adjacent to tumours, compared to stromal elements within normal colon tissue.

Conclusion

A subset of recurrent SCAAs in most CRC samples represents stable, heritable events. Alterations of chromatin accessibility in normal cells adjacent to tumours suggest a spatially dependent epigenetic shift influenced by the tumour microenvironment.

EACR25-1064

IRF2BP2/TRIM28/DNMT1 suppresses transposable elements to maintain AML survival

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Introduction

Acute myeloid leukemia (AML) is an aggressive hematologic malignancy with poor survival rates, driven by genetic and epigenetic alterations. A promising therapeutic approach involves targeting dysregulated chromatin factors. In this study, we applied Perturb-seq to a primary AML patient sample to identify key chromatin regulators involved in the blockade of AML differentiation. Our analysis revealed interferon

regulatory factor 2 binding protein 2 (IRF2BP2) as a critical genetic dependency. However, its function in AML remains poorly understood. Here, we demonstrate that IRF2BP2 loss impairs AML cell survival by reactivating transposable elements (TEs), leading to the induction of an endogenous immune response signature.

Material and method

To define the role of IRF2BP2, we performed a CRISPR/Cas9-based knockout system in human AML cell lines both *in vitro* and *in vivo*. Furthermore, we conducted RNA sequencing, ChIP sequencing, Rapid Immunoprecipitation Mass Spectrometry of Endogenous Proteins (RIME), and CRISPR interference, to investigate the molecular mechanism by which IRF2BP2 regulate in AML.

Result and discussion

We identified IRF2BP2 as a critical regulator in AML. Its expression is significantly higher in AML patient samples compared to normal hematopoietic cells, suggesting a potential therapeutic window for targeting IRF2BP2 in AML treatment. Our study demonstrated that IRF2BP2 depletion in human AML cell lines induces apoptosis, impairs cell proliferation, and enhances immune response signatures. *In vivo*, loss of IRF2BP2 significantly prolongs survival and reduces leukemia burden in AML models. Mechanistically, we identified TRIM28 and DNMT1 as key interaction partners of IRF2BP2. Both TRIM28 and DNMT1 are well-established master regulators of TEs expression, whose reactivation can trigger innate immune signaling. Our findings reveal that silencing TEs partially rescues the phenotypic effects of IRF2BP2 loss in AML. Overall, IRF2BP2, in conjunction with TRIM28 and DNMT1, functions to suppress TE expression, thereby preventing excessive immune responses in AML.

Conclusion

We demonstrated that IRF2BP2 as a critical dependency in AML via Perturb-seq. We next validated this finding in multiple human AML cell lines both *in vitro* and *in vivo*. Our mechanistic investigations reveal that IRF2BP2, in interaction with TRIM28 and DNMT1, suppresses TEs expression and prevents excessive immune responses. This work lays the foundation for developing novel therapeutic strategies targeting IRF2BP2 and its interaction partners.

EACR25-1097

Epigenetic changes on the X chromosome in chronic lymphocytic leukaemia

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Introduction

Chronic lymphocytic leukaemia (CLL) is the most common adult leukaemia. Epigenetic studies have revealed that the CLL epigenome displays widespread

loss of DNA methylation and is shaped by the cell of origin, proliferative history, and selection for gene-specific traits during progression. However, studies performed to date have routinely excluded the X and Y chromosomes, due to challenges in accounting for gender imbalances and X-inactivation in data analysis. Yet, the X chromosome harbours many genes of key relevance to CLL and immune function, including BTK, CD40LG, and chemokine and interleukin receptors. Here, we examined DNA methylation changes on chrX in CLL at diagnosis and during disease progression.

Material and method

DNA methylation at 11,232 loci on chrX were analysed using the Illumina 450k DNA methylation microarray platform. Epigenetic changes present at diagnosis were identified in 115 CLL patients with Stage A disease (78 male, 47 female) compared to CD19+ B-cells from 14 healthy individuals (8 male, 6 female). DNA methylation pre- and post-treatment was analysed in paired samples from 20 patients (12 male, 8 female). Gene expression was examined in 184 CLL patients (105 male, 79 female) and 32 healthy individuals (20 male, 12 female) using the Affymetrix U133 microarray platform.

Result and discussion

We identified 660 differentially methylated loci mapping to chrX ($\Delta\beta > 0.2$, pFDR < 0.05), of which 308 were CLL-specific and not observed during B-cell maturation. These primarily displayed hypomethylation (86%) and were enriched at loci that are biallelically methylated in healthy females. Sex-specific differential methylation was observed, with 137 loci unique to male patients, 68 unique to females, and 103 shared. Highly methylated loci displayed greater variability between male patients in comparison to low and intermediately methylated loci, but not between female patients. Among the differentially methylated genes were the BCL6 corepressor BCOR, pro-survival signalling CD40LG, the growth factor FGF13, the transcription factor KLF8, polycomb group protein SCML1, toll-like receptor TLR7, and the regulator of Wnt signalling ZIC3; we did not observe differential methylation of BTK. Each of these genes was confirmed to be differentially expressed in CLL. In patients undergoing treatment, we observed only moderate changes in DNA methylation ($\Delta\beta > 0.1$) at 110 loci, which were no longer significant following FDR correction.

Conclusion

Targeted analysis of the X chromosome has identified differential methylation and expression of genes with critical relevance to CLL biology, with changes primarily relating to hypomethylation of highly methylated loci. Further work is required to elucidate their impact upon disease progression and patient outcomes.

EACR25-1176

KDM6A Regulates Sex Specific Immune Responses in Melanoma

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Introduction

KDM6A is a lysine demethylase with well characterised roles in tumour suppression across multiple cancer types.

Interestingly, this X-linked gene fails to undergo inactivation of the second gene copy in females, potentially contributing to enhanced immunity and the female survival advantage observed in many cancers including melanoma.

Material and method

We interrogated datasets of melanoma patients treated with targeted or immune checkpoint inhibitor immunotherapy to determine how KDM6A expression influences treatment outcomes. Functional studies using CRISPR-Cas9 depletion of KDM6A was performed in human melanoma cell lines, followed by growth assays.

Chromatin Immunoprecipitation (ChIP)-seq and RNAseq was used to identify downstream target genes and pathways regulated by KDM6A. A syngeneic mouse melanoma model looked at the effects of Kdm6a knockout on targeted therapy responses in mice.

Result and discussion

KDM6A is more highly expressed in female melanomas and is associated with increased tumour infiltrating lymphocytes, decreased melanoma cell proliferation and better overall survival, compared to males. Melanomas from male patients who have relapsed on BRAF inhibitor treatment show a striking loss of KDM6A compared to females. CRISPR-Cas9 depletion of KDM6A in human melanoma cell lines caused increased cell proliferation and colony formation in vitro, consistent with a tumour suppressive function. In mice fed a normal chow diet, YUMMER1.7 tumours depleted of Kdm6a grew significantly larger than control tumours and were devoid of CD8+ T cells. In mice fed chow containing the Braf inhibitor PLX4720, control tumour growth was relatively constrained, however this effect was abrogated by the loss of Kdm6a. Mechanistically, KDM6A binds to genes associated with immune recognition, particularly those implicated in Major Histocompatibility Complex (MHC) antigen presentation.

Conclusion

Collectively our results support a tumour suppressive role for KDM6A in melanoma, that is particularly prominent in women compared to men and may influence responses to treatment. Understanding these subtleties may improve outcomes for both sexes.

EACR25-1181

Higher global DNA methylation levels in tumoral tissue from Mexican patients with Prostate Cancer with different Gleason score

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Introduction

Prostate cancer (PCa) is one of the most prevalent cancers in men worldwide. Its aggressiveness is evaluated using the Gleason score, which assesses histological patterns of differentiation. DNA methylation, a key epigenetic mechanism, involves the addition of a methyl group (-CH₃) to cytosine in CpG dinucleotides, particularly in CpG islands at gene promoters, playing a crucial role in gene expression regulation. In prostate cancer, these epigenetic modifications are recognized as critical contributors to tumor progression and treatment resistance. This study aims to evaluate the global DNA methylation of tumor tissue from Mexican patients with PCa.

Material and method

Formalin-fixed paraffin-embedded tumoral tissue and fresh tumoral tissue biopsies from Mexican patients with PCa were collected from Hospital Civil de Culiacán and Pathology and Nephropathology, Diagnosis and Research Center. The Gleason Score for histopathological analysis was assessed by certified pathologists according to ISUP 2019. The global DNA methylation (5-mC) was determined using a colorimetric by an ELISA kit, where the percentage of methylated DNA was proportional to the optical density (OD) intensity. Statistical analyses were performed using GraphPad Prism version 8.0.1.

Result and discussion

A total of 30 PCa samples were collected. The most frequent Gleason Score was 9 (26.66% [8/30]), followed by Gleason Score 7 (4+3) (23.33% [7/30]). Gleason scores 6 and 8 were observed in equal proportions (20% [6/30] each) while the least frequent was Gleason score 7 (3+4) (10% [3/30]). The results showed that global DNA methylation levels were more than twice as high in PCa samples (5.50%) compared to control samples (2.55%). Additionally, a significant difference in global DNA methylation percentages was observed between Gleason score 7 (4+3) and Gleason score 8 (adjusted p-value = 0.0302).

Conclusion

Mexican prostate cancer patients exhibit higher global DNA methylation levels compared to healthy subjects. Additionally, a potential association between the Gleason Score and Global DNA methylation percentage is suggested.

EACR25-1194

Multi-omics Study Identified Novel Oncogenic Roles of DNA-interacting Circular RNA as MLL1 Regulator for Histone H3K4 Methylation in Pancreatic Ductal Adenocarcinoma

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Introduction

Circular RNAs (circRNAs) play important gene regulatory roles in pancreatic ductal adenocarcinoma (PDAC). Functioning as microRNA sponges is the first identified role of circRNAs. However, emerging evidence suggests that circRNAs may participate in additional gene regulatory mechanisms. We previously identified the dysregulated circRNAs circFOXK2 promoting PDAC growth and liver metastasis. Our current study aimed to uncover the novel and critical gene regulatory mechanisms of circRNAs in PDAC.

Material and method

CircFOXK2-specific pulldown assay with mass spectrometry analysis, and chromatin isolation by RNA purification (ChIRP) with DNA-sequencing, were performed to identify circFOXK2-interacting chromatin complex. Co-immunoprecipitation, electrophoretic mobility shift assay, and three-dimensional structural modeling were performed to study the role of circFOXK2 on peptidylprolyl isomerase E (PPIE)-mixed lineage leukemia protein-1 (MLL1) interaction. Chromatin immunoprecipitation (ChIP) after circFOXK2 knockdown in PDAC cells was performed to investigate the roles of circFOXK2 on histone H3 lysine 4 (H3K4) methylation.

Result and discussion

Mass spectrometry analysis of the circFOXK2-specific pulldown identified circFOXK2-interacting proteins for epigenetics and chromatin remodeling. Particularly, circFOXK2 interacted with PPIE, which is the inhibitor of H3K4 methyltransferase MLL1. Through circRNA-DNA interaction by complementary base pairing, circFOXK2 recognized downstream targets at different genomic locations. At the target genes, circFOXK2 formed a docking pocket by intramolecular base pairing for the N-terminal RNA-recognition motif of PPIE, disrupting the PPIE-MLL1 interaction. This removed the PPIE inhibitory effects on MLL1 for gene-activating H3K4 methylation. Specifically, circFOXK2 promoted thymosin beta 15b (TMSB15B) and brain enriched myelin associated protein 1 (BCAS1) expressions by enhancing H3K4me3 at the promoter. At the gene body, circFOXK2 facilitated transcription elongation of trio rho guanine nucleotide exchange factor (TRIO) and laminin subunit alpha-4 (LAMA4) by intragenic H3K4me3.

At distal intergenic enhancer, circFOXK2 promoted

H3K4me1 of cytochrome p450 family 26 subfamily a member 1 (CYP26A1) and t-complex 11 (TCP11) for gene activation.

Conclusion

The upregulated circFOXK2 promoted oncogenic gene expression through MLL1-mediated H3K4 methylation in cancer. Our study highlighted the importance of circRNAs in gene regulation and the novel mechanism of circRNA-DNA interaction in epigenetics, which may serve as a novel therapeutic target.

EACR25-1336

High-throughput DNA methylation profiling of urological malignancies: defining epigenetic signatures in prostate, bladder and kidney cancers

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Introduction

Epigenetic alterations, particularly DNA methylation changes, are key drivers of tumorigenesis and progression in urological cancers. While prostate cancer (PCa) overdiagnosis highlights the need for better biomarkers, bladder cancer (BICa) relies on invasive procedures for diagnosis and follow-up, and kidney cancer (KCa) remains challenging due to its histological heterogeneity and resistance to conventional therapies. DNA methylation profiling holds promise for biomarker discovery and therapeutic targeting. This study aimed to characterize the DNA methylation landscape of PCa, BICa and KCa, integrating findings with The Cancer Genome Atlas (TCGA) datasets to identify shared and cancer-specific DNA methylation signatures with functional relevance.

Material and method

DNA methylation profiles of 72 fresh-frozen tissues (25 PCa, 5 normal prostate, 14 BICa, 5 normal bladder, 17 KCa, and 6 normal kidney) were analyzed using the HumanMethylation450 BeadChip (Illumina). All samples were classified by an expert uropathologist and staged using the AJCC 8th Edition [1]. Differentially methylated CpG sites were identified and integrated with TCGA datasets [RNAseq and DNA methylation (450k)] to

evaluate their impact on gene expression. Gene ontology (GO) analysis was performed to identify affected biological pathways.

Result and discussion

Distinct methylation landscapes were observed across urological cancers. BICa and KCa exhibited genome-wide hypomethylation, whereas PCa was characterized by predominant promoter hypermethylation. Hypermethylation-associated gene silencing was a common feature in PCa and BICa, particularly affecting genes involved in DNA repair, oxidative stress regulation, epithelial-mesenchymal transition (EMT), and immune evasion. Cancer-specific analyses revealed that PCa hypermethylation predominantly impacted metabolic and detoxification pathways, whereas in BICa, it affected genes regulating cell fate and tissue organization, likely contributing to stem-like phenotypes and EMT dysregulation. These findings highlight both shared and unique epigenetic alterations that may drive cancer-specific pathways.

Conclusion

This study provides new insights into the epigenetic heterogeneity of urological cancers, reinforcing the critical role of DNA methylation in tumor-specific biology. The identification of both shared and cancer-specific methylation patterns underscores their potential as biomarkers for early detection and disease monitoring, and therapeutic targeting. These findings support the development of epigenetic therapies and companion biomarkers tailored to the unique vulnerabilities of urological malignancies, highlighting the relevance of precision oncology approaches in these cancers.

[1] Ethical approval CES-IPOPG-EPE 205/2013

EACR25-1432

Epigallocatechin-3-gallate as a potential epigenetic regulator of ALDH1 expression in colorectal cancer

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Introduction

Aldehyde dehydrogenase 1 (ALDH1) isoforms have recently been identified as crucial molecular markers in colorectal cancer (CRC). Epigallocatechin gallate (EGCG) has been demonstrated as a potential epigenetic-modulating phytochemical agent in cancer prevention and therapy. In this study, we investigated the effect of EGCG on the regulation of the expression of two ALDH1 isoforms, 1A1 and 1A3, in a time- and concentration-dependent manner.

Material and method

We used cell lines with different levels of ALDH1 expression: HT-29, pts80 and LS180 with high expression of ALDH1A1; and FUR, HCT 116, SW480 and LS180 showing high expression of ALDH1A3. After EGCG treatment, the expression of ALDH1 and genes involved in its epigenetic regulation was quantified using RT-qPCR, and protein levels were assessed by Western blot. We employed analyses to monitor DNA methyltransferase (DNMT) activity and pyrosequencing to

detect promoter methylation. Oxidative stress was measured using the CellROX® Orange Reagent.

Result and discussion

After 48 hours, high EGCG concentrations had no significant effect on ALDH1A1 or ALDH1A3 expression. On the other hand, low (2 and 5 µg/ml) concentrations led to a significant ALDH1A3 expression increase at 6 hours, followed by a rapid decline at 12, 24 and 48 hours. ALDH1A1 expression remained unchanged. We also observed that EGCG modulates the expression of Ten-eleven translocation methylcytosine dioxygenase 1 (TET1) and other genes involved in ALDH1A3 expression regulation. These findings suggest that EGCG modulates ALDH1A3 expression through epigenetic mechanisms and may also influence related signalling pathways.

Conclusion

We demonstrated that EGCG selectively affects ALDH1A3 expression, and we suggest that ALDH1A1 and 1A3 are regulated by distinct molecular and epigenetic mechanisms. EGCG influences not only the epigenetic status of ALDH1A3 but also the signalling pathways modulating its expression. Further analyses are required to elucidate the molecular mechanisms underlying EGCG's effect on ALDH1A3 expression.

Acknowledgement: This work was supported by the Slovak Research and Development Agency under contract APVV-21-0296, by VEGA 2/0170/22, and the Slovak Academy of Sciences through DoktoGrant APP0447.

EACR25-1464

Unraveling MLH1 promoter methylation and CpG Methylator Phenotype interplay in colorectal adenocarcinoma

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Introduction

Cooperation between molecular and epigenetic modifications is crucial for colorectal cancer (CRC) carcinogenesis. The main hallmarks in CRC scenario are RAS/BRAF mutations, mismatch repair deficiency (MMRd), microsatellite instability (MSI), and the CpG methylator phenotype (CIMP). Unlike the well-studied aberrant methylation of MLH1 promoter (pMLH1), the role of CIMP and its interaction between the other molecular signature remain not fully understood.

Material and method

To unravel the relationship among these molecular and epigenetic features, we selected a retrospective cohort of 123 CRCs, subdivided in three groups according to their molecular characteristics: 33 cases MMR proficient (MMRp)/BRAF p.V600E mutated (BRAFmut), 46 cases MMRd/BRAFmut, and 44 cases MMRd/BRAF wild type (BRAFwt). We evaluated for the entire cohort the pMLH1 methylation status using pyrosequencing, correlating these data, for 82 sample, with CIMP results assessed with the EpiTect® MethylLight kit.

Result and discussion

The correlation between pMLH1 methylation and MMR protein loss reflected literature data in BRAF mutant CRC, with higher pMLH1 methylation in MMRd/BRAFmut patients compared to MMRp/BRAFmut. Conversely, BRAFwt CRC exhibited mixed pMLH1 methylation status. Overall, our data demonstrated that not always the pMLH1 methylation reflects the widespread genomic methylation. Indeed, only the MMRd/BRAFmut displayed a significantly homo-geneous CIMP-High phenotype (CIMP-H) profile compared to MMRd/BRAFwt and MMRp/BRAFmut groups ($p < 0.0001$), in which this association was not confirmed. In addition, CIMP analysis identified a subgroup of MMRp/BRAFmut patients who exhibited a CIMP-H phenotype without pMLH1 methylation, indicating the presence of alternative pathways linking CIMP and BRAF mutations. Overall, no direct link was established between BRAF mutations and the CIMP phenotype, indicating other mechanisms may influence methylation in CRC.

Conclusion

Our study highlights the heterogeneity of CRC, showing that pMLH1 hypermethylation, MMR deficiency, BRAF mutations, and CIMP do not fully overlap. This aspect warrants further exploration in larger, multi-institutional studies, to better define the correlations among these features and to be able to better exploit them for the clinical management of patients.

EACR25-1489

Glucagon-like Peptide-1 Receptor involvement in breast cancer progression is regulated by epigenetic mechanisms

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Introduction

Breast cancer (BC) is the most common oncological disease affecting women worldwide, which represents approximately 36% of oncological patients. Despite improvement in the survival rate over the past two decades, its incidence continues to rise every year. Among several factors associated with BC risk, metabolic syndrome is involved both in the onset and progression, affecting incidence, prognosis and treatment

response efficacy. Specifically, women with metabolic syndrome show a higher BC-dependent mortality rate. Recently, the repositioning of anti-diabetic drugs for cancer treatment has been investigated with encouraging outcome on cancer prevention especially upon Glucagon-like Peptide-1 Receptor Agonists (GLP-1RAs). Despite the clinical relevance of GLP-1R targeting, its involvement in BC is still poorly investigated. Here, GLP-1R expression regulation in BC will be addressed.

Material and method

GLP-1R contribution in BC tumorigenesis was analyzed in:

- 1) human BC cell line models: MCF-7, MDA-MB-361, SK-BR-3, MDA-MB-231 representing, respectively, the main four BC subtypes;
- 2) human tissue samples derived from biopsy harvested from infiltrating primary tumor of chemotherapy-naïve patients.

Result and discussion

The Cancer Genome Atlas (TCGA) data sets showed an inverse correlation between GLP-1R expression and BC patient life expectancy, highlighting its prognostic value. Interestingly, immunoblotting analysis showed that GLP-1R protein levels are progressively down-modulated in more aggressive BC subtypes associated to worse prognosis. We investigated whether its progressive down-modulation can be attributed to DNA methylation mechanism. First of all, we analyzed global DNA methylation levels in BC subtypes, revealing an increase of 5-methylcytosine (5mC) in more aggressive BC subtypes. To investigate whether DNA methylation increase occurs at GLP-1R promoter regions, we interrogated the Shiny Methylation Analysis Resource Tool (SMART), revealing a higher methylation level of GLP-1R promoter in BC tissue compared to normal one. Specifically, the most methylated CpG islands were found in GLP-1R promoter region of more aggressive BC subgroups. Indeed, the administration of demethylating drugs to BC cell lines was able to restores GLP-1R transcription even in the most aggressive BC subtypes. Interestingly, GLP-1R restoration by gain of function experiments was able to mitigate higher proliferation, migration and invasiveness of aggressive BC.

Conclusion

These findings show that GLP-1R loss could represent one of the molecular players supporting higher aggressiveness in BC, as consequence of a DNA methylation dependent transcriptional regulation. Since GLP-1R restoration counteracts BC aggressiveness, therapeutic approaches preventing its silencing should be developed in a therapeutic perspective.

EACR25-1496

Metabolic deregulation of TET/TDG DNA demethylation complex impairs base excision repair process promoting PDAC tumorigenesis

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Introduction

Pancreatic ductal adenocarcinoma (PDAC) is one of the deadliest cancers with poor prognosis and therapeutic outcome. Several epidemiological studies show that chronic metabolic diseases, including obesity and type II diabetes, are associated with an increased risk of PDAC incidence and worse clinical outcome. In these conditions, the prolonged exposure to altered metabolism induces aberrant metabolite fluctuations, possibly affecting epigenetic enzyme reactions and consequently modifying the epigenome, thus supporting cancer development. Here, we investigated the correlation between metabolism and epigenetics contributing to PDAC development upon dysmetabolic condition

Material and method

The link between dysmetabolism and PDAC was investigated in pre-invasive pancreatic tumour in *in vivo* and *in vitro* models:

- 1) LSL-KrasG12D; PDX-1- Cre (KC) mice fed with high-fat diet (HFD);
- 2) human pancreatic ductal epithelial cells bearing Kras mutation (HPDE-K-RasG12V) exposed to a combination of free fatty acids and high glucose, to mimic metabolic alterations

Result and discussion

Metabolomics analyses of pancreatic samples highlighted free fatty acid level alteration, higher S-adenosyl methionine (SAM) level and a ratio between α -ketoglutarate (α -KG) and succinate (SA) lower than one during PDAC tumorigenesis in HFD mice. Interestingly, metabolite level alterations were paralleled by ten-eleven dioxygenase 1 (TET1)/ thymine DNA glycosylase (TDG) DNA demethylation complex dissociation and consequent accumulation of iterative cytosine modifications, including 5-formylcytosine (5fC). These findings were validated in the *in vitro* model, revealing a decreased α -KG/SA balance, TET1/TDG complex dissociation, 5fC accumulation, and increased levels of apurinic/aprimidinic site (AP) sites, pointing out TDG function derangement. In this light, we investigated the role of SA on TDG activity modulation by different biochemical analyses, revealing that SA directly binds TDG at residue Arg275, inducing its hyperactivation. To investigate whether dysmetabolism-dependent AP site accumulation is consequence of impaired Base Excision Repair (BER) process, we evaluated the expression of proteins involved in this pathway, revealing decreased levels of ligase 1 (LIG1) and 3 (LIG3) due to higher

methylation levels of their promoter regions in HPDE-K-RasG12V cells exposed to dysmetabolic conditions

Conclusion

Our results show that metabolic alterations affect the DNA methylation/demethylation machinery as consequence of increased intracellular SA levels, leading to TDG hyperactivation, accumulation of iterative cytosine modifications and AP sites, due to BER machinery impairment. These metabolic-epigenetic alterations might support PDAC tumorigenesis and need specific attention for diagnostic and therapeutic purposes

EACR25-1546

EZH2 inhibition: A Key to Unlocking the Notch-Driven Tumor Suppression in Acute Myeloid Leukemia

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Introduction

The overexpression of the histone H3K27 methyltransferase has been linked to poor prognosis and drug resistance in Acute Myeloid leukemia (AML) by epigenetically regulating critical gene expression, most of which is unknown. Conversely, Notch pathway activity levels are extremely low in the majority of AML samples, consistent with the accumulation of the H3K27me3 repressive histone mark at the loci of its target genes. Of note, enforced Notch signaling activation induces apoptosis of blasts and cell lines of AML and counteracts leukemia in vivo, suggesting Notch-activating strategies as a viable intervention for this hematological cancer. However, the molecular mechanisms influencing Notch repression in AML and its role in the pathogenesis of this disease remain poorly understood and need further exploration.

Material and method

HL-60, NB4, U937 and ME-1 cells; In vitro pharmacological treatment; Gene silencing; Trypan blue exclusion assays; RT-qPCR; Nuclear/cytosol fractionation; Western blotting.

Result and discussion

We hypothesized that EZH2 exerts part of its oncogenic functions in AML through the repression of the Notch signaling pathway. Confirming our hypothesis, EZH2 pharmacological inactivation and/or gene silencing increased Notch receptors, ligands and its target gene HES1 in HL-60, NB4, U937 and ME-1 cell lines, which represent various AML subtypes. Moreover, EZH2 blockage decreased cell proliferation and increased the expression of the differentiating factor CEBP α across the above-mentioned cell lines. Of note, Notch inhibition partially abrogated the antigrowth effects of EZH2-blockage only in acute pro-myelocytic leukemia cells HL-60 and NB4 cells, suggesting that although the interaction between EZH2 and Notch signaling is transversely conserved in AML, Notch activation might have a subtype-specific anti-leukemic effect. In addition, by exploring EZH2/Notch crosstalk in chemoresistance, we observed that EZH2 inhibition synergized with Cisplatin by enhancing its cytotoxic effect in HL-60 cells.

Conclusion

Overall, our findings reveal that EZH2 mediates epigenetic silencing of Notch signaling in AML and suggest the targeting of the EZH2-Notch axis as a viable therapeutic strategy to overcome chemoresistance and enhance the anti-leukemic efficacy of the treatment with Cisplatin in AML.

EACR25-1686

Promoter methylation identifies potential biomarkers for early detection of colitis-associated cancer

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Introduction

Colitis-associated cancer (CAC), a subtype of colorectal cancer resulting from chronic inflammation in inflammatory bowel disease (IBD), presents a major challenge in identifying high-risk patients. Current biopsy-based surveillance methods are invasive and often insufficient for distinguishing benign inflammation from dysplasia. Consequently, there is a critical need for non-invasive biomarkers to predict CAC progression.

Material and method

In this study, we performed whole-genome bisulphite sequencing (WGBS) on fresh frozen and formalin-fixed paraffin-embedded colonic tissue samples from UC patients at various stages of the disease to explore DNA methylation changes associated with CAC progression. We identified differentially methylated regions (DMRs) and analysed their functional enrichment. Additionally, we assessed copy number alterations (CNAs) to examine genomic changes linked to CAC.

Result and discussion

Our analysis of 10 pilot colonic tissue samples, including cancers, dysplasia, adjacent tissues, inflamed and healthy tissue, revealed significant differences in global methylation patterns across disease stages. Genome-wide CNAs confirmed specific chromosomal alterations in diseased tissues, with losses of chromosomes 22, 4, and 5 in both cancer and dysplastic samples, and gains on chromosome 20. Global methylation analysis indicated significantly lower CpG methylation levels in inflamed and cancer tissues compared to controls, with cancer tissues displaying pronounced CpG hypomethylation. We identified inflammation-associated and cancer-specific DMRs, with cancer samples exhibiting promoter hypermethylation. Importantly, we found differential methylation of approximately 1,000 genes in cancer tissues, including promoters of 25 known driver genes. Promoters hypermethylated specifically in the cancer group were

enriched in pathways related to transcriptional regulation, morphogenesis, and cellular differentiation, underscoring their role in tumorigenesis. Genes involved in DNA binding, transcription factor activity, and chromatin organization were particularly affected, suggesting widespread epigenetic silencing of key regulatory networks.

Conclusion

This study on a pilot set of samples offers critical insights into the epigenetic alterations linked to CAC development and progression. By identifying specific DMRs, we highlight potential targets for early detection. The validation of these findings in a larger cohort, including non-invasive samples like stool and blood, promises to advance effective strategies for CAC surveillance and management, ultimately improving outcomes for patients with IBD.

EACR25-1728

Epigenome-Wide Knockout Screens Reveal Epigenetic Modifiers of Doxorubicin Resistance in Triple-Negative Breast Cancer

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Introduction

Breast cancer is the most frequently diagnosed cancer among women, with triple-negative breast cancer (TNBC) comprising approximately 15% of all cases. It is an aggressive form of breast cancer which is characterized by the absence of estrogen, progesterone, and HER2 receptor expressions. The current treatment for TNBC includes surgical removal of tumor, radiation and conventional chemotherapy which commonly utilizes anthracyclines such as doxorubicin. However, TNBC has a high heterogeneous and aggressive nature which results in development of resistance to chemotherapy and disease relapse. This resistance may emerge from the interplay between genetic and epigenetic mechanisms.

Material and method

This study aimed to investigate the roles of epigenetic modifiers in formation and maintenance of chemo-resistance in TNBC. Epigenetic Knock-Out Library (EPIKOL), a CRISPR-Cas9 based library, is designed to target all chromatin readers, writers, erasers, and associated proteins. This library investigates the function of 779 genes, each targeted by 10 sgRNAs, alongside multiple controls and 35 essential genes to monitor depletion effects.

Result and discussion

To study chemoresistance, doxorubicin-resistant TNBC models were developed by progressively exposing SUM159PT cell line to escalating concentrations of doxorubicin. EPIKOL screens on these resistant models identified SWI/SNF complex members as critical mediators of chemoresistance. RNA sequencing of naïve and chemo-resistant cells revealed key mechanisms implicated in acquired chemoresistance. Our current efforts are directed towards validating the effects of

candidate genes and transcriptome analysis through functional assays.

Conclusion

In conclusion, this study reveals previously unrecognized roles for epigenetic regulators, particularly members of the SWI/SNF complex, in the regulation of chemotherapy resistance, offering a potential foundation for the development of combination therapies targeting these pathways.

EACR25-1754

Multiomics assessment of lung adenocarcinoma subtypes defined through tumor purity-adjusted DNA methylation

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Introduction

Molecular subtypes of lung adenocarcinoma (LUAD), the most common histological subtype of lung cancer, have been proposed based on one or two-dimensional studies, but none are yet implemented in clinical routine. In this study, we set out to refine LUAD subtyping using a multi-omic approach based primarily on the epigenetic landscape of LUAD from a tumor intrinsic perspective. Epigenetic modifications in cancer cells are independent of sequence variants, directly linked to gene and genome regulation, and provide important information to guide subclassification efforts.

Material and method

DNA methylation profiling (Illumina EPIC v1 beadchips or Illumina Infinium HumanMethylation450 BeadChip array) was performed or obtained from previous studies, on a total of 95 primary LUAD samples from our Swedish cohort. DNA methylation data were adjusted for tumor cell content. Unsupervised clustering using non-negative matrix factorization and accounting for genomic context was used to divide the cohort into epigenetic subgroups/entities. The obtained clusters were then contrasted based on clinicopathological, genomic, transcriptomic, proteomic, and metabolomic data. Findings were validated on LUAD samples from The Cancer Genome Atlas initiative and on a previously published cohort of LUAD cell lines.

Result and discussion

Of the four methylation clusters, two were associated with previously proposed mRNA/protein subtypes, showing similar characteristics to what has been published. As an example, one of the clusters was enriched for older patients, non-smokers, lower tumor cell proliferation, and variants in the EGFR gene. The remaining two clusters, M2 and M3, were a combination of other proposed subtypes. Despite showing a similar mRNA/protein subtype composition to each other, clusters M2 and M3 differed through e.g., higher expression in M3 of a napsin A/surfactant metabolism

metagene, a marker of the LUAD histology. Genes included in this metagene showed lower DNA methylation in M3, despite an aggregate promoter hypermethylation phenotype seen in this cluster. Importantly, M2 and M3 cell lines recapitulated the metagene expression patterns seen in samples from those clusters.

Conclusion

Dissecting LUAD based on combined biological characteristics from multi-omics data results in a deeper understanding of the heterogeneity in this complex disease and can lead to treatment options guided by molecular subtypes as is the case in e.g., breast cancer.

EACR25-1838

Arid1a directs lineage specification in mammary epithelial cells

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Introduction

Epigenetic regulation is critical to many developmental processes, including mammary gland differentiation. However, the precise epigenetic regulators governing mammary cell fate remain poorly understood. Mutations in the chromatin regulator ARID1A are associated with metastatic disease, therapy resistance, and the maintenance of luminal cell identity in breast cancer.

Material and method

We investigated the impact of Arid1a loss on morphogenesis *in vivo*. Epithelial lineage dynamics were further characterized in organoid-based functional studies. Multiomic single-cell RNA and chromatin accessibility profiling were performed to identify lineage-specific regulators of the Arid1a deficient state. Furthermore, the transcription factors of interest were validated by CRISPR/CAS9-mediated loss-of-function screening.

Result and discussion

Arid1a knockout disrupts key developmental structures such as ductal branching and terminal end buds in mice. Breast organoids derived from these mice exhibit cystic morphology, impaired differentiation, and atypical granularity. Multiomic single-cell RNA and chromatin accessibility profiling further revealed that Arid1a maintains basal, luminal progenitor, and alveolar cell states. Depletion of Arid1a restricts cells to undifferentiated luminal hormonal state diminishes estrogen receptor responsiveness and decreases chromatin accessibility and SWI/SNF targeting at key lineage-defining transcription factors (TF). CRISPR/CAS9-mediated loss-of-function screening identifies Foxa1 and Meis1 as key TFs, whose deletion recapitulates the Arid1a-deficient phenotype. These findings uncover a specific epigenetic mechanism that governs cell-fate specification in the mammary epithelium.

Conclusion

Our work highlights Arid1a and lineage-specific TFs as essential factors for mammary epithelial cell fate specification with important implications for both normal development and breast cancer.

EACR25-1867

LACTB Promotes SHMT2 Degradation, Modulating Glycine Metabolism and DNA Methylation Patterns in Breast Cancer Cells

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Introduction

β -lactamase-like protein (LACTB) is a recently identified mitochondrial tumor suppressor with broad activity across various tissues. It has been shown to downregulate key pro-survival pathways, including Akt and Wnt/ β -catenin, and to induce cell cycle arrest, apoptosis, differentiation, autophagy, and suppression of epithelial-mesenchymal transition. However, the upstream regulatory mechanisms governing these LACTB-induced effects remain largely unknown.

Material and method

In this study, using proximity biotinylation *in vivo* screening we found that LACTB can interact with another several mitochondrial proteins. Through co-immunoprecipitation and proximity ligation assays we confirm that some of these proteins interact with LACTB in several luminal like and basal like breast cancer cell lines.

Result and discussion

One of the LACTB-interacting proteins found is the serine hydroxymethyl transferase 2 (SHMT2), which catalyzes the conversion of serine to glycine. We found that LACTB can induce a decrease in the expression of SHMT2 protein but not at transcriptional level. Previously, it has been shown that LACTB contain a catalytic domain, so these results suggest that LACTB promotes the degradation of SHMT2 through their interaction. Additionally, we found that the LACTB-induced decrease of SHMT2 promotes a decrease in the levels of intracellular glycine. During the conversion of serine to glycine, SHMT2 generates a byproduct called 5,10 methylenetetrahydrofolate (5,10-MTHF). This byproduct is a methyl donor that can fuel the production of activated methyl groups (S-adenyl methionines), which are essential in the regulation of epigenetic mechanisms, such as DNA methylation. In this way, we found that the decrease in SHMT2 induced by the over-expression of LACTB is able to decrease the global DNA methylation.

Conclusion

Our findings uncover a novel regulatory mechanism in which LACTB modulates SHMT2 protein levels, thereby influencing glycine metabolism and DNA methylation patterns in breast cancer cells. This study provides new insights into the mitochondrial control of tumor suppression and epigenetic regulation.

EACR25-1900

Targeting CBX2 disrupts DNA damage repair and mitosis regulation in glioblastoma – a novel therapeutic opportunity

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Introduction

Glioblastoma (GBM) is the most common and aggressive form of brain tumour. The average length of survival for GBM patients remains low at only 12–18 months following initial diagnosis, partly due to a lack of targeted therapeutics. Identification and validation of novel therapeutic targets is crucial. Chromobox 2 (CBX2), a component of polycomb repressive complex 1 (PRC1), regulates gene expression by altering chromatin accessibility. CBX2 expression is elevated in numerous aggressive cancer types, including GBM. CBX2 has an oncogenic role in other cancers associated with poor prognoses, however, its role in GBM remains largely unexplored. This study aims to determine the functional mechanisms by which CBX2 may promote GBM; thereby determining its potential as a novel therapeutic target.

Material and method

This study uses patient-derived cells (PDCs) and employs a translationally relevant microfluidic perfusion device (“chip”) to maintain micro-dissected GBM patient tissue in a viable state, directly from surgical resection. PDCs and ex vivo maintained GBM tissue were treated with SW2_152F, a selective CBX2 inhibitor. The phenotypic effects of CBX2 inhibition in PDCs were assessed by 5'-bromo-2'-deoxyuridine (BrdU) proliferation assays, flow cytometry, and fluorescence microscopy. Genes and biological pathways differentially regulated following CBX2 inhibition were assessed by RNA-sequencing and Gene Set Enrichment Analysis (GSEA). The molecular and phenotypic effects of CBX2 inhibition in GBM tissue were evaluated by RT-qPCR and immunohistochemical analyses.

Result and discussion

CBX2 inhibition in PDCs caused significant down-regulation of gene signatures associated with G2/M checkpoint signalling, DNA damage repair pathways, and mitosis regulation; concomitant with observations of reduced cellular proliferation, increased double-stranded DNA breaks, and ultimately cell death. Targeting CBX2 induced significant downregulation of the oncogenic transcription factor forkhead box protein M1 (FOXM1) and its target gene polo-like kinase 1 (PLK1). Analysis of patient datasets shows that the expression of both FOXM1 and PLK1 are positively correlated with CBX2 expression in GBM tissue. FOXM1 and PLK1 form a functional axis in regulating mitotic progression and DNA damage repair; overexpression of FOXM1 is linked to tumour growth and chemoresistance in GBM.

Conclusion

We propose that CBX2 inhibition disrupts the FOXM1–PLK1 axis, exploiting the vulnerabilities of GBM by impairing DNA damage repair and checkpoint control, leading to severe mitotic stress and subsequent cell death. Targeting CBX2 may sensitise GBMs to current standard-of-care therapies, offering a promising avenue for novel synergistic treatments.

EACR25-1905

hsa-miR-200c-3p, hsa-miR-25-3p and hsa-miR-301a-3p as potential biomarkers and therapeutic targets for restoration of PTEN expression in clear cell renal cell carcinoma

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Introduction

Clear cell renal cell carcinoma (ccRCC) is an aggressive subtype of renal cancer, with 30% of patients who undergo curative surgery experiencing recurrence within five years. Its highly hypoxic tumor microenvironment confers resistance to chemo- and radiotherapy and, although targeted therapies have improved patient survival, resistance remains a common challenge. Thus, there is an urgent need for accurate biomarkers and novel treatments to improve patient management. In recent years, extracellular vesicles (EVs) have emerged as promising diagnostic and therapeutic tools due to their ability to transport microRNAs (miRNAs) that regulate gene expression and tumor progression. A previous study by our group identified an EV-derived miRNA profile (hsa-miR-200c-3p, hsa-miR-25-3p and hsa-miR-301a-3p) as a post-transcriptional silencing mechanism that promotes ccRCC progression by inhibiting phosphatase and tensin homolog (PTEN). Thus, this study aimed to evaluate these miRNAs' biomarker and therapeutic potential in 2D and 3D ccRCC models.

Material and method

EVs from four renal cell lines (HKC-8, 786-O, Caki-2, and Caki-1) were isolated and characterized for size and concentration by nanoparticle tracking analysis, morphology by transmission electron microscopy, and membrane marker presence by ELISA. The selected miRNA profile and PTEN mRNA was quantified by RT-qPCR in cells and EVs derived from renal cell lines in 2D and 3D models. Then, 786-O cells were transfected with miRNA inhibitors using lipofectamine in both cell culture models. Its effect was evaluated by analyzing miRNA and PTEN expression, through RT-qPCR and western blotting, alongside phenotypic changes, including alterations in proliferative and migratory capacity in 2D culture, assessed by WST-1 and wound healing assay, as well as metabolic capacity and spheroid morphology in 3D models.

Result and discussion

The intracellular expression of hsa-miR-25-3p, hsa-miR-200c-3p and hsa-miR-301a-3p was higher in ccRCC cells compared to HKC-8 cells. All miRNAs were excreted in EVs derived from all cells and their expression, intracellularly and in EVs, increased with the aggressiveness of ccRCC cells, while intracellular PTEN mRNA expression decreased. The combined administration of inhibitors targeting these miRNAs significantly increased PTEN expression, leading to reduced proliferation and

migration of ccRCC cells in 2D models, as well as a decrease in spheroid size and metabolic capacity in 3D models.

Conclusion

This study highlights the oncogenic role of these miRNAs and their potential not only as biomarkers for monitoring disease aggressiveness but also as promising therapeutic targets in ccRCC. The combined inhibition of these miRNAs demonstrated the potential to reduce the pro-tumor phenotype, paving the way for a more personalized therapeutic approach for ccRCC patients.

EACR25-1943

Integrated analysis of longitudinal cell-free DNA and matched tumor profiling data to identify novel breast cancer biomarkers

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Introduction

Breast cancer is the most common malignancy and leading cause of cancer-related death in women, accounting for 15.4% of all female cancers. Despite advancements in diagnosis, standard methods such as imaging, tissue biopsy, and blood tests remain invasive, costly, and variable in accuracy. Most cases are diagnosed at an advanced stage, limiting treatment options and reducing survival rates. Cell-free DNA (cfDNA), fragmented DNA released into the bloodstream by normal and tumor cells, has emerged as a promising non-invasive biomarker for early cancer detection. Several initiatives have adopted targeted multi-cancer methylation-based approaches due to their relative simplicity and high performance. However, breast cancer remains particularly challenging to detect, underscoring the need for improved liquid biopsy biomarkers. This study aims to improve the early detection of breast cancer by leveraging cfDNA methylation profiling and matched tumor profiling data to identify more sensitive and specific genomic and epigenetic biomarkers.

Material and method

We analyzed longitudinal plasma samples collected by the Helsinki Biobank, matched to tumor samples available in the iCAN Digital Precision Cancer Medicine Flagship, a large-scale Finnish biobank study integrating deep tumor profiling with longitudinal health data. The cohort included plasma cfDNA from 26 breast cancer patients at stages III and IV, with samples collected both pre-diagnosis (mean 21 months, range 6–47 months before diagnosis) and post-diagnosis (<2 months after diagnosis), totaling 52 samples. Whole-genome enzy-

matic methyl sequencing (EM-seq) was performed, followed by analysis using DRAGEN and Bismark to obtain DNA methylation profiles and genomic variants. Tumor profiling data, including RNA sequencing (RNA-seq) and whole-exome sequencing (WES), is available on the iCAN Discovery Platform, enabling integrative analysis of epigenetic and genomic alterations.

Result and discussion

cfDNA was extracted with a mean yield of 10.9 ng (range: 2.0–64.4 ng). Whole-genome enzymatic methyl sequencing (EM-seq) yielded an average of 88 GB of sequencing data per sample, with a median coverage depth of 6.5× and a 95% alignment rate. Differential methylation analysis is planned to distinguish breast cancer subtypes and identify informative CpG sites for early detection. Copy number profiling and somatic variant analysis will assess genome-wide alterations and determine concordance between cfDNA-derived CNAs and tumor WES data. We will analyze cfDNA fragmentomic landscapes to explore its potential as predictive biomarkers for breast cancer detection and profiling.

Conclusion

The integration of cfDNA methylation, fragmentation, and genomic data holds promise for advancing biomarker discovery, enabling earlier cancer detection and more precise therapeutic strategies.

EACR25-2049

Hypermethylation of extracellular matrix genes in colorectal adenocarcinoma

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Introduction

Abnormal methylation is a long-recognized feature of colorectal adenocarcinoma tissues. Examination of methylation patterns across the genome in large patient populations aids the identification of novel biomarkers for diagnosis, prognosis, and potentially new therapeutic targets. Through the modulation of proliferative signals and the facilitation of invasivity, extracellular matrix (ECM) proteins play a multifaceted role in tumor progression. This study investigates DNA methylation alterations in extracellular matrix (ECM)-related genes using a large-scale, genome-wide DNA methylation dataset.

Material and method

We analyzed genome-wide methylation data from 2,356 tumor and normal samples (19 GEO series and GDC-TCGA), processed using minfi and watermelon in R. Differentially methylated CpG islands (CGIs) were identified via Kruskal-Wallis test and ROC analysis (AUC > 0.91). Hierarchical clustering of CGI methylation was performed, and correlations with gene expression (TCGA-COAD RNA-seq) were evaluated. For data visualization, we used epigenplot.com, a web platform established based on our database.

Result and discussion

Among 20 ECM genes with hypermethylated promoter CGIs (AUC > 0.91), 19 exhibited tumor-specific hypermethylation ($\Delta\beta \geq 0.2$, $p < 0.001$). The most discriminatory CGIs localized to ITGA4 (chr2: 182321761–182323029; $\Delta\beta = 0.37$, AUC = 0.97) and

COL25A1 (chr4: 110222970-110224257; $\Delta\beta = 0.31$, AUC = 0.97), both showing strong inverse correlations with gene expression (ITGA4: R = -0.33, p < 0.001; COL25A1: R = -0.19, p < 0.001). Hierarchical clustering of HM450K data revealed two distinct clusters: 90% normal (cluster 1) and 98% tumor (cluster 2). EPIC platform clustering showed partial overlap (74% normal vs. 79% tumor clusters). The robust separation of tumor/normal samples by ECM CGI methylation underscores its diagnostic potential. The weaker clustering fidelity in EPIC data highlights technical variability between platforms or intrinsic tumor diversity. ITGA4, an integrin critical for cell-ECM adhesion, and COL25A1, a collagen linked to neuronal pathfinding, are epigenetically silenced in tumors, suggesting their dysregulation may promote microenvironment alterations. Notably, the high AUC (>0.91) of top CGIs positions them as candidate biomarkers, though their clinical utility requires validation in independent cohorts. The inverse methylation-expression correlation supports methylation-driven silencing.

Conclusion

Analysis of extracellular matrix (ECM) gene methylation in large colorectal adenocarcinoma cohorts identifies epigenetic drivers of tumor microenvironment remodeling and promising biomarkers.

EACR25-2096

Integrative Multi-Omic Analysis Reveals a Pax8-Driven Gene Network Linking Tumor Stemness to Therapy Response in Ovarian Cancer

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Introduction

Pax8 is a transcription factor essential for the development of the Fallopian tube epithelium and is aberrantly expressed in most ovarian cancers, where it plays a key role in tumorigenesis. It regulates gene networks associated with cell proliferation, survival, and stemness, contributing to therapy resistance and disease progression. Understanding the Pax8-driven regulatory network in ovarian cancer is crucial for identifying potential therapeutic targets and improving patient outcomes.

Material and method

We use public Pax8 chromatin binding data (ChIP-seq, CUT&Tag, CUT&RUN), ATAC-seq and RNA-seq in control and Pax8 knock-out high-grade serous ovarian cancer cells, as well as transcriptomic databases and survival data of ovarian cancer patients, and ovarian cancer cell lines

Result and discussion

We integrated Pax8 chromatin binding, chromatin accessibility, transcriptomic, and patient data to define the core regulatory network driven by Pax8 in ovarian tumors. Despite heterogeneity in Pax8 binding sites across cell lines, we identified a common set of regulated genes. By combining chromatin accessibility, transcription factor footprints, and gene expression changes following Pax8 knockout, we reconstructed a transcriptional network explaining Pax8-regulated genes. Analysis of patient expression data revealed a Pax8-associated gene signature linked to tumor stemness, a key factor in therapy resistance. Notably, this signature predicts disease outcomes and therapy responses in ovarian cancer patients. Finally, we validated experimentally our results from bioinformatic analyses, thus reassuring their robustness.

Conclusion

Our study identifies a Pax8-driven regulatory network that underlies tumor stemness and therapy resistance, highlighting potential targets for precision therapies in ovarian cancer.

EACR25-2159

SUV39H1 regulates cell adhesion of pediatric high-grade gliomas through modulation of β -catenin

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Introduction

Pediatric high-grade gliomas (pHGGs) are heterogeneous, diffuse, and infiltrative tumors with dismal prognosis. Aberrant post-translational histone modifications with elevated histone 3 lysine trimethylation (H3K9) have been recently implicated in pHGGs pathology. Herein, we investigate the biological role of SUV39H1 methyltransferase in cell proliferation, motility, and gene regulation in pHGG.

Material and method

SUV39H1 mRNA levels were first determined in a public database of pediatric gliomas and protein levels were investigated in a cohort of 24 pHGG, by immunohistochemistry and western immunoblotting. Gene silencing of SUV39H1 was further performed in pHGG cell lines to investigate their functional role in cell proliferation, cell migration and EMT markers.

Result and discussion

SUV39H1 was found enriched in pHGG compared to normal brain and KEGG pathway analysis showed positive correlation with cell adhesion molecules. SUV39H1 protein expression was significantly increased

in pHGG tissues compared to normal brain ($P < 0.001$). Gene silencing of SUV39H1 in pHGG cell lines showed a significant reduction in cell viability ($p < 0.05$) and reduced cell migration ($P < 0.05$) followed by reduced expression of vimentin and β -catenin. Furthermore, SUV39H1 silencing reduced epithelial-mesenchymal transition (EMT) marker genes CDH2, SNAI1 and MARCKS levels.

Conclusion

Our findings demonstrate that increased expression of SUV39H1 in pHGG is involved in regulation of cell adhesion and contributes to epithelial-mesenchymal transition.

EACR25-2204

Functional impact of histone methyltransferase KMT2B in pediatric astrocytomas

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Introduction

Histone modifications coordinate gene expression by regulating transcription factor binding. Although the role of the epigenome is clear in a variety of cancers, in pediatric astrocytomas still remains ambiguous. The Mixed Lineage Leukemia 2 (MLL2/KMT2B) protein, responsible for H3K4 trimethylation (H3K4me3), was shown to mediate pro-oncogenic effects in several cancers. Here, we aimed to investigate the functional role of histone KMT2B and KMT2B-regulated signaling pathways in pediatric gliomas.

Material and method

We first performed in silico analysis of a publicly available microarray dataset of 49 pediatric astrocytoma samples using the R2: Genomic Analysis. Then, we evaluated the mRNA and protein expression levels of KMT2B by qRT-PCR, immunohistochemistry, and Western immunoblotting in 38 archival pediatric astrocytoma tissues (Grade 2-4) and normal brain samples. We proceeded to evaluate the functional role of KMT2B silencing (using siRNA) in pediatric glioblastoma cell lines (SJGBM2, CHLA-200) proliferation by XTT, as well as in cell migration by scratch assay.

Result and discussion

In silico analysis revealed that pediatric astrocytomas exhibit significantly increased KMT2B expression levels compared to normal brain tissues, which was further confirmed by qRT-PCR, and Western immunoblotting in tissue samples. Immunohistochemical analysis showed significantly lower KMT2B immunoreactivity in pilocytic astrocytomas compared to grade 3-4 diffusely infiltrating tumors. Accordingly, H3K4me3 protein levels were detected significantly lower in normal brain

compared to astrocytomas grade 1 and grade 2-4. The univariate survival analysis of the entire cohort showed correlation of reduced patient's survival with increased KMT2B expression, indicating a significant clinical impact. To this end, silencing of KMT2B in pediatric astrocytoma cell lines showed a significant reduction in cell proliferation and p53 expression, as well as in cell migration and at mesenchymal marker vimentin levels. Moreover, exposure of these pediatric cell lines to MM-102, a potent inhibitor of the WDR5/MLL interaction, led to a reduction in cell proliferation and migration.

Conclusion

Taken together, our data show a potential oncogenic role of KMT2B in pediatric astrocytomas, correlating to tumor progression and inferior patients' survival that needs further investigation.

EACR25-2261

Methylation-driven epigenetic regulation of the MIR141 core promoter in bladder cancer short-term progression

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Introduction

Owing to its markedly heterogenous molecular landscape, bladder cancer (BlCa) is still characterized by non-personalized prognosis and treatment decisions necessitating lifelong post-treatment surveillance. Alterations of DNA methylation are considered to be among the earliest events of bladder carcinogenesis, serving as significant predictors of patient survival and treatment response. Herein, we investigated the methylation profiling and clinical values of MIR141 core promoter in BlCa.

Material and method

DNA and RNA were extracted from 135 bladder tissues. miRNA-seq was conducted in 10 non-muscle invasive (NMIBC; Ta Low Grade: n = 5; T1 High Grade [HG]: n = 5) and 10 muscle invasive (MIBC; T2: n = 5; T3-T4: n = 5) bladder tumors, identifying miR-141-3p as one of the most downregulated miRNAs. Next, in silico analysis was performed to confirm its epigenetic regulation. Genomic DNA underwent bisulfite conversion, followed by PCR amplification of MIR141 core promoter and methylation levels were quantified by pyrosequencing. miR-141 levels were measured by RT-qPCR, following 3'-end polyadenylation of total RNA. Disease progression and patients' death were used as clinical endpoint events. TCGA-BLCA (n = 412) was used as institutionally independent validation cohort for MIR141 gene methylation and transcription.

Result and discussion

miRNA-seq highlighted the loss of miR-141-3p in muscle-invasive tumors (T2-T4), while correlation analysis in TCGA-BLCA (rs: -0.748, p < 0.001) and screening (rs: -0.395, p < 0.001) cohorts unveiled MIR141 core promoter methylation as key regulatory region on miR-141 modulation. In line with miR-141 loss, elevated methylation of MIR141 promoter was associated with aggressive phenotype (muscle-invasive, advanced stages and HG tumors; p < 0.001). Notably, MIR141 promoter hypermethylation was linked to higher risk for short-term progression of superficial (TaT1) to muscle-invasive tumors (p < 0.001), resulting in risk-stratification of the clinically relevant T1HG patient subgroup for post-treatment progression to MIBC (p = 0.035). Multivariate models highlighted MIR141 promoter methylation as an independent marker for short-term progression (HR = 5.828, p = 0.002) compared to established disease markers, displaying superior clinical benefit of MIR141-fitted models in BlCa prognostication.

Conclusion

MIR141 core promoter methylation controls miR-141 levels in bladder tumors and emerged as a robust modern molecular marker to address clinical heterogeneity of disease prognosis, enhance risk stratification beyond conventional clinical markers and support personalized treatment/monitoring decisions for BlCa patients.

Acknowledgements: Supported by the Hellenic Foundation for Research and Innovation (HFRRI) under the "2nd Call for HFRRI Research Projects to Support Faculty Members & Researchers" (project no. HFRRI-3765).

EACR25-2323

Exploiting the tumor-suppressive role of the transcriptional repressor SAMD1 in pancreatic ductal adenocarcinoma

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Introduction

Despite extensive research efforts to better understand pancreatic ductal adenocarcinoma (PDAC) and improve therapeutic outcomes, it remains one of the deadliest cancers with limited treatment options. PDAC is particularly challenging to treat due to its early metastasis, pro-tumorigenic microenvironment, and late diagnosis. Epigenetic mechanisms are important for advancing PDAC therapies, but their impact in complex models like 3D cultures and the tumor microenvironment remains less understood. One class of epigenetic mechanisms is the repression by chromatin regulatory proteins. SAMD1 is a transcriptional repressor that binds to unmethylated CpG islands and interacts with histone demethylase KDM1A and the Polycomb-associated proteins L3MBTL3 and SFMBT1. SAMD1 plays a role in various biological pathways and its loss is embryonically lethal in mice. While high SAMD1 levels are associated with poor survival in some cancers, in PDAC and other cancers, high SAMD1 levels are linked to better survival. PDAC cells lacking SAMD1 show

increased expression of genes related to EMT and inflammation, adopting a more mesenchymal phenotype with accelerated migration rates, partly driven by the SAMD1 target gene CDH2.

Material and method

CRISPR-Cas9 was used to generate SAMD1 KO in PDAC cell lines. PDAC 3D cell cultures were established using ultralow attachment plates and analyzed through microscopy, RNA sequencing, and ChIP. Proximity Extension Assay (PEA) was performed on the supernatants of both 2D and 3D cultures, while extra-cellular vesicles (EVs) from 2D cultures were analyzed by mass spectrometry. Bystander cells were treated with conditioned medium (CM) from PDAC cells, and their gene expression was subsequently analyzed.

Result and discussion

We found that the loss of SAMD1 in PDAC 3D cultures results in a smaller and denser structure, partially mediated by the derepression of CDH2. In addition to the upregulation of EMT marker genes, several genes potentially involved in influencing the tumor microenvironment (TME) were differentially expressed in SAMD1 KO PDAC 3D cultures. Proximity Extension Assay (PEA) of the secretome from both 2D and 3D cultures, along with mass spectrometry analysis of EVs from 2D cultures, revealed differential expression of factors impacting EMT and the TME, correlating with RNAseq data in 2D and 3D models. While in PDAC SAMD1 KO CM WT PDAC cells EMT-promoting genes were upregulated, in macrophages and stellate cells changes in subtype marker gene expression were observed.

Conclusion

Our results suggest that SAMD1 not only regulates EMT in 2D cell culture but may also play a role in 3D models and beyond, influencing the composition of the tumor microenvironment by altering the secretome and EV content of PDAC cells. These findings will help gain a deeper understanding of the role of epigenetic mechanisms in PDAC.

EACR25-2545

Investigating the tumor-suppressive role of the transcriptional co-repressor IRF2BP2 in pancreatic ductal adenocarcinoma

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Introduction

Pancreatic ductal adenocarcinoma (PDAC) is a highly lethal cancer with limited treatment options. To identify new therapies, it is crucial to further explore its molecular modes of action. Studying epigenetic mechanisms in PDAC will enable the development of targeted therapies to improve patient vitality. Chromatin-regulating proteins play a key role in the epigenetic regulation of gene expression, influencing processes such as tumor growth via inflammation and resistance. One such protein is the transcriptional cofactor IRF2BP2 (Interferon Regulatory Factor 2 Binding Protein 2). Recent studies demonstrated that IRF2BP2 plays a role in multiple biological

processes, such as proliferation and inflammation. In PDAC, higher expression levels of IRF2BP2 correlate with improved patient survival, highlighting its potential importance as a tumor suppressor.

Material and method

CRISPR-Cas9 was used to generate IRF2BP2 knock out (KO) in PDAC cell lines. The KO were analysed by microscopy, biological assays such as proliferation and migration assays, and RNA sequencing. Mass spectrometry (MS/MS) was used to investigate PDAC-specific interaction partners.

Result and discussion

KO of IRF2BP2 in PDAC cell lines resulted in a more elongated cell shape. This phenotype suggested increased motility of the KO cells. We confirmed the increased motility of IRF2BP2 KO cells by analysing cell migration rates through wound healing. Other biological assays such as proliferation assays, adhesion rates and colony formation assays are planned to further investigate the IRF2BP2 KO phenotype. RNA sequence data from PaTu 898t IRF2BP2 KO cells show an increased expression of epithelial-to-mesenchymal transition (EMT) and inflammation-related genes compared to WT cells. The increased expression of EMT-related genes in the KO cells supports the investigated phenotype and the increased migration rate. The increased expression of EMT-related genes in KO cells supports the observed phenotype and the increased migration rate. The increase in inflammation-related genes in the KO cells suggests that IRF2BP2 KO cells are more sensitive to inflammatory stimuli. Therefore, treatment of the cells with cytokines IL1 α and TNF α is planned. The MS/MS data revealed interesting interaction partners like TAF13, KAT6, and CEBPZ, which are still investigated.

Conclusion

The results suggest that IRF2BP2 plays a role in inflammation and EMT in PDAC cells. This may be important for its tumor suppressive role. This study contributes to a better understanding of the role of epigenetic mechanisms in PDAC.

Experimental/Molecular Therapeutics, Pharmacogenomics

EACR25-0009

Preventive and Therapeutic Effects of Amaranthus hybridus and Corchorus olitorius on Breast Cancer

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Introduction

Considering the projected increase in incidence rates of cancer globally and the burden being higher in Africa, there is a need to find preventive and therapeutic solutions that can be tailored to the African population. Breast cancer is the leading cancer in African females. The willingness to adopt alternative medicine for the

management of diseases is high in Africa. Therefore, this study sought to evaluate the effects of two readily available food plants, Amaranthus hybridus and Corchorus olitorius in preventing and treating breast cancer.

Material and method

Carcinogenesis was induced in laboratory rats by administering a single oral dose of DMBA at 80 mg/Kg body weight. Plant extracts were administered 2 weeks before and 18 weeks after induction of carcinogenesis in the chemopreventive and therapeutic treatments, respectively. RNA was extracted from the mammary tissues at the end of the experimental period, and the expression levels of selected genes including CCND1, APEX1, BAX, and SOD2 associated with anticancer effects were quantified using the RT-qPCR technique.

Result and discussion

No tumor was recorded in the chemopreventive groups. Expression of CCND1 was significantly increased in the groups that developed mammary tumors. APEX1 expression was significantly increased in all the treatment groups that received the plant extracts, while the highest expressions of BAX and SOD2 were recorded in both the chemopreventive and therapeutic groups that received a combination of both plants.

Conclusion

Amaranthus hybridus and Corchorus olitorius leaves contain potent bioactive compounds that prevent tumor development through chemoprevention, and the combination of both leaves possesses better gene amplification effect on the DNA repair gene APEX1, pro-apoptotic gene BAX, and mitochondrial SOD2 gene.

EACR25-0047

Phage display identified claudin 18.2 binding peptide for early pancreatic diagnosis and tumor-target killing

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Introduction

Pancreatic adenocarcinoma is a lethal condition with a rising incidence, predicted to become the second leading cause of cancer-related deaths worldwide by 2030. One major hurdle in the treatment of this disease is the predominantly elderly patient population and clinically silent and aggressive nature. Early detection of pancreatic neoplasms and novel treatment for highly aggressive and metastatic conditions is of great need in pancreatic tumor therapy. Claudins are crucial components of tight junctions. They are transmembrane proteins and are the keeper of the “fence function”. Claudins in tumor are like the fall of the soldiers entrusted to protect the gate. Claudin 18.2 is a member of the claudin family, commonly expressed in multiple cancers, including Gastric cancer and Pancreatic cancer. Claudin-18.2 expression on cancer cells is increased and exposed on surface of the cells. The development of malignant tumors leads to the disruption of tight junctions, exposing the Claudin 18.2 epitope on the surface of tumor cells as a specific target. Also, claudin 18.2 is transcriptionally upregulated with the binding of cyclic AMP-responsive

element binding protein to the methylated CLDN18.2 promoter region. Claudin 18.2 is considered an early stage marker of pancreatic carcinogenesis. Claudin 18.2 is considered a ‘dark horse’ in anti-tumor therapy. Thus, Claudin 18.2 could be a good “target” for early detection and target-specific treatment of pancreatic tumor.

Material and method

Construction of phage peptide library and bio panning for claudin18.2-binding peptides using claudin18.2 over-expressing cells. Clone selection, Sequence analysis and peptide synthesis. Phage cell-binding ELISA. Immunofluorescence, flow cytometry analysis and pull-down assays of cellular binding of peptides on claudin 18.2 high and low expressing cell lines. In vivo tumor targeting and anti-tumor therapy with claudin18.2-binding peptide conjugated with proapoptotic peptide.

Result and discussion

After five rounds of screening, phage titers that bind to transfected cells were enriched higher-fold compared to phage titers isolated in the first round. 2 phage clones displaying peptides that selectively bound to transfected cells were chosen and sequenced for further study. The two peptides showed selectively binding to claudin 18.2 expressing cells as analyzed by Immunofluorescence. Pull down assay further confirmed selective binding of peptide to claudin 18.2. Bioaccumulation study in vivo showed significant accumulation of peptide in tumor tissue. Treatment of KPC transgenic pancreatic tumor bearing mice with claudin 18.2 peptide conjugated to proapoptotic peptide enhanced the survival of the mice.

Conclusion

Claudin 18.2 peptide can specifically target the tumor cells and conjugation with proapoptotic peptide can induce tumor targeted apoptosis with increased survival in pancreatic tumor bearing mice.

EACR25-0065

Nanobody-PROTACs: Method development towards the use of single-domain antibody proteolysis-targeting chimera constructs (sdAb-PROTACs) to target 'undruggable' proteins for degradation

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Introduction

Proteolysis targeting chimeras (PROTACs) are bifunctional molecules that can bring an E3 ligase (e.g. VHL) into close proximity with a protein of interest (POI). The E3 ligase recruits E2 to ubiquitinate the POI, marking it for degradation via a proteasome. Uniquely, PROTACs do not need to inhibit or bind an active site, allowing tailored targeting of the POI, followed by subsequent degradation. This means that PROTACs have the potential to degrade targets previously considered ‘undruggable’, such as the oncogene, KRAS, which is historically difficult to therapeutically target due to lack of deep binding pockets. Typically, PROTACs utilise small molecules to target the POI, however, our work aims to exploit the potential of nanobodies to replace

these small molecules. The gold standard for protein targeting remains monoclonal antibodies, however, nanobodies possess the same antigen binding site specificity, whilst possessing a tertiary structure only a tenth of the size. Our proof of concept work focuses on identifying whether the nanobody-PROTAC has the potential for cellular entry, and thus degradation of its POI.

Material and method

We tagged an anti-GFP nanobody with AZdye647, using the thiol-maleimide Michael addition reaction, SDS-PAGE analysis was used to assess successful conjugation. This conjugate was then used to treat fixed and live cells expressing enhanced green fluorescent protein (EGFP), followed by fluorescent microscopy to confirm cellular entry and POI targeting.

Result and discussion

Initial SDS-PAGE analysis revealed the successful conjugation of AZDye647 to the nanobody. Specific binding within fixed cells was confirmed using fluorescent microscopy, as co-localization of EGFP and the AZdye647-tagged anti-GFP nanobody was revealed. Our data also confirmed that liposomal encapsulation of the AZdye647-tagged anti-GFP nanobody yielded efficient delivery into live EGFP expressing cells.

Conclusion

Together, this data provides confirmation that a nanobody-PROTAC would be capable of passing through the cellular membrane, maintaining its integrity and degrading its POI. Replacement of AZdye647 with an E3 ligase ligand will provide further understanding of whether a nanobody-PROTAC would result in the degradation of ‘undruggable’ oncogenes such as KRAS, *in vitro*, confirming whether this is a viable therapeutic approach to patients with KRAS-mutant cancers.

EACR25-0068

Chromenone Derivatives as Potential CRM1 Inhibitors for Glioblastoma Therapy

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Introduction

Glioblastoma (GBM) is among the most aggressive and lethal cancers, with treatment challenges arising from the complexity and redundancy of its signaling networks. Targeted inhibitors of specific pathways have shown limited success. The nuclear export receptor Chromo-

some Region Maintenance 1 (CRM1) has recently gained attention as a promising therapeutic target, as its inhibition disrupts multiple oncogenic drivers simultaneously. In this study, we investigate whether chromenone derivatives, known for their ability to detect thiol-containing molecules, can act as CRM1 inhibitors.

Material and method

We synthesized various chromenone-based derivatives and demonstrated their ability to inhibit CRM1-mediated nuclear export in a structure- and dose-dependent manner.

Result and discussion

A preliminary structure-activity relationship (SAR) analysis, supported by molecular docking studies, provided insights into selective CRM1 binding. Furthermore, we showed that active chromenone derivatives effectively block the nuclear export of endogenous nuclear export signal (NES)-containing substrates in glioblastoma cells.

Conclusion

Several of these compounds exhibit selective cytotoxicity against glioblastoma cell lines, underscoring their potential as targeted GBM therapies.

EACR25-0084

IQS080 is a novel cytosolic multitargeted kinase inhibitor that simultaneously targets key kinase families, making it a promising candidate for the treatment of pancreatic cancer

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Introduction

Pancreatic ductal adenocarcinoma (PDAC), often called a 'silent cancer,' is one of the most aggressive tumor types, with a poor prognosis and a five-year survival rate of 10–15% after diagnosis. For those patients who present with resectable disease, surgery followed by adjuvant chemotherapy with FOLFIRINOX (fluorouracil, irinotecan, leucovorin, oxaliplatin) or single-agent gemcitabine is the standard treatment. Among such treatments, Erlotinib, a tyrosine kinase inhibitor (TKI) inhibiting EGFR, is used in severe cases. To improve current treatments, we developed a program focused on multitarget TKIs targeting key TK receptors involved in PDAC (EGFR, VEGFR-1, VEGFR-2, PDGFR, and IGFR). We computationally designed, synthesized, and evaluated a series of candidates in vitro against these receptors and in 2D cultures of PANC-1, MiaPaca-2, and BxPC-3 cell lines. Although several compounds exhibited residual activity below 27% for most of the targeted kinases, their IC₅₀ values against PANC-1 ranged between 20 and 50 μM.

Material and method

Surprisingly, during the study, we identified IQS080, a pyrido[2,3-d]pyrimidine with significantly improved metabolic stability (over 85% after 60 minutes of incubation with rat liver microsomes) but presenting almost no interaction with the TKs above but showing the best IC₅₀ against the PDAC cell lines.

Result and discussion

IQS080 shows good inhibitory activities against a group of cytosolic TKs (ERK1, ERK2, JNK1, JNK2, p38alpha, and p38beta). It is remarkable the inhibitory activity against p38alpha, and p38beta with residual activities below 15% both at 5 and 10 μM. Moreover, IQS080 shows IC₅₀ for the inhibition of KRAS wild-type and some of the mutants (G12C, G12V) in the range 17 to 42 μM, revealing a certain degree of interaction with such proteins. The safety profile of IQS080 was tested using the Chick Chorioallantoic Membrane (CAM) assay showing that, even at high doses, does not exhibit significant toxicity or adverse effects on the embryos. Ongoing *in vivo* studies in xenografted subcutaneous PDAC cell lines and Patient-Derived Orthotopic Xenografts (PDOX) are also yielding promising initial results, both as a single agent and in combination therapies.

Conclusion

IQS080 is a novel intracellular multitarget kinase inhibitor that has demonstrated both safety and efficacy as a single agent in preclinical models of PDAC. Its unique mechanism of action could involve the simultaneous inhibition of the three key MAPKs (ERKs, JNKs, and p38s), along with partial inhibition of KRAS (wild-type and major mutant forms). This multitarget approach effectively blocks the activation of compensatory pathways in tumor cells, leading to enhanced antitumor activity. The versatility and favorable safety profile of IQS080 position it as a promising candidate, for further investigation in combination studies with chemotherapy and targeted therapies.

EACR25-0085

Impact of immune checkpoint inhibitors (ICIs) alone or combined with chemotherapy (ICI/chemotherapy) on the prognosis of non-small cell lung cancer (NSCLC) patients with high expression of programmed

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Introduction

Immune checkpoint inhibitors (ICIs) are the standard therapy for patients with advanced non-small cell lung cancer (NSCLC) exhibiting a programmed death-ligand 1 (PD-L1) tumor proportion score (TPS) of ≥50%. However, the efficacy of ICIs alone or in combination with chemotherapy (ICI/chemotherapy), in patients with NSCLC characterized by high PD-L1 expression and cancer cachexia remains unclear.

Material and method

This retrospective, multicenter cohort study involved 250 advanced NSCLC patients with a PD-L1 tumor proportion score of ≥50%. Patients received either

pembrolizumab monotherapy or pembrolizumab in combination with chemotherapy. Cancer cachexia was defined as weight loss exceeding 5% of total body weight or a body mass index (BMI) of 2% within the 6 months prior to treatment initiation. A total of 50 patients (20%) met the criteria for cancer cachexia.

Result and discussion

The overall survival (OS) and progression-free survival (PFS) of patients with cachexia in both treatment groups were significantly shorter than those of patients without cachexia (OS: 18.8 vs. 37.3 months, $p < 0.001$; DFS: 4.8 vs. 11.3 months, $p = 0.004$). After propensity score matching and stratification by cancer cachexia status, no significant differences in PFS and OS were observed between the pembrolizumab monotherapy group and the combination therapy group, regardless of cancer cachexia status.

Conclusion

For NSCLC patients with high PD-L1 expression and concurrent cancer cachexia, the benefits of ICI/chemotherapy appear limited, suggesting that ICI monotherapy may be the preferable treatment option.

EACR25-0086

Engineering Bacteriophages for Targeted Photodynamic Cancer Therapy

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Introduction

Conventional cancer therapies often face challenges such as off-target toxicity, resistance and high costs, requiring the development of innovative, precise and affordable therapeutic approaches. Bacteriophages (phages), viruses that infect bacteria, represent a promising platform for precision medicine due to their ability to be genetically engineered and conjugated with therapeutic agents. This study investigates the potential of engineered M13 phages as nanobiotechnological tools for photodynamic cancer therapy (PDT). The phages are modified to target overexpressed cancer markers and conjugated with photosensitisers (PS) that generate reactive oxygen species (ROS) upon light activation, enabling targeted cancer cell death while sparing healthy tissues.

Material and method

The pIII protein of M13 phages was genetically engineered to display a nanobody specific for the epidermal growth factor receptor (EGFR), a marker commonly overexpressed on various cancer cell lines. Photosensitisers, such as Rose Bengal, were conjugated to the major coat protein pVIII for a high payload delivery. These engineered phages were tested in vitro on 2D cell cultures, 3D spheroids and ex vivo on patient-derived ascites samples. After light activation, ROS-mediated cancer cell killing was assessed by cell viability assays, measuring dose-dependent and specific cancer cell eradication.

Result and discussion

The engineered phages demonstrated excellent specificity

for EGFR-overexpressing cancer cells with no effects on negative control cell lines. Conjugation of PS to the phages significantly enhanced PDT efficiency compared to free PS, primarily by increasing local drug accumulation at the tumour site. Each phage delivered hundreds of PS molecules while maintaining structural integrity and stability. In a dose-dependent manner, this platform demonstrates effective eradication of cancer cells. Moreover, ex vivo studies in patient-derived ascites have validated translational potential of the platform. The low cost of phage production also highlights the scalability and accessibility of this platform for cancer therapy in resource-limited settings.

Conclusion

This study establishes engineered M13 bacteriophages as a versatile and precise nanobiotechnology platform for cancer PDT. Their high specificity, robust payload capacity and low production costs position them as a promising alternative to conventional cancer therapies. Ongoing work will focus on overcoming challenges related to phage immunogenicity and macrophage-mediated clearance to improve in vivo efficacy and facilitate clinical translation. These advances have the potential to revolutionise the targeted treatment of cancer and to address global health inequalities.

EACR25-0087

A potential nanocarrier for Androgen-resistant in prostate cancer: Green synthesis of ZnO nanoparticle

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Introduction

12.5% of men will receive a prostate cancer diagnosis at some point in their lives, making prostate cancer (PCa) one of the main causes of death and morbidity among men worldwide. Our previous population study based on UK Biobank suggested a significant decreased risk of PCa and PCa deaths. The current study is aim to synthesize ZnO nanoparticle and investigate its underline biological mechanisms of anti-tumor effects in prostate cancer cell lines

Material and method

The preparation of ZnO nanoparticle involves the production and full characterization of EGCG, via gamma rays irradiation, followed by peptide conjugation for subsequent use as an effective template toward the synthesis of tumor cell specific ZnO NPs. Androgen-resistant (DU145, PC-3), androgen-sensitive (LNCaP) human prostate cancer cell lines and BPH-1 control cell line were treated with different concentration of 30 $\mu\text{g/mL}$, 60 $\mu\text{g/mL}$, and 90 $\mu\text{g/mL}$. After 48 hours of treatment, the MTT assay, Migration, Invasion and flow cytometry were performed to confirm the phenomenon

Result and discussion

The ZnO NPs polymeric nanoparticles (86 nm) served multiple roles as reducing and stabilizing agents in the overall template synthesis of tumor cell targeted ZnO NPs. The ZnO NPs capped with EGCG exhibited average Au-core diameter of 20 nm with hydrodynamic diameters of 67 nm, respectively. The ZnO NPs showed optimum in vitro stability in biologically relevant solutions. We

observed a significant suppression of the cell growth in all of the four cell lines at 48 hrs. The results from migration, invasion, cell cycle analysis furtherly confirmed that this suppression effect was more significant in androgen resistance cell lines. The ZnO NPs displayed cytotoxicity effects against cancer cells. Our investigations provide compelling evidence that ZnO NPs functionalized with EGCG is an innovative nano-medicine approach for use in molecular imaging and therapy of GRP receptor positive tumors. The template synthesis of EGCG-ZnO NPs serves as an excellent non-radioactive surrogate for the development of the corresponding NPs theragnostic nanoradio-pharmaceutical for use in cancer diagnosis and therapy.

Conclusion

The current study provides fresh perspectives into the underlying mechanisms of action of ZnO NPs, a plant-derived nanoparticle, in prostate cancer. Future *in vitro* and *in vivo* validation will be conducted to examine whether the promising results are clinically translational.

EACR25-0132

The antihelmintic drug rafloxanide promotes oxidative stress and VDAC1 opening, leading to mitochondrial dysfunction in colorectal cancer cells

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Introduction

Functional mitochondria are essential for cancer cells, which rely on oxidative phosphorylation to produce energy and promote tumor growth. Inhibiting this process results in mitochondrial damage and cell death. Therefore, mitochondrial uncouplers, which interfere with oxidative phosphorylation by dissociating ATP synthesis from the electron transport chain, represent a promising class of compounds that can be used as sensitizers in cancer therapy. The halogenated salicylanilide compound rafloxanide (RFX) is an anthelmintic drug that acts as an uncoupler of mitochondria to kill gastrointestinal roundworms. We have recently shown that RFX inhibits cell proliferation and promotes cell death of colorectal cancer (CRC) cells, but the mechanisms underlying these effects remain unclear. We aim to assess whether RFX may influence mitochondrial activity and impact the metabolism of CRC cells.

Material and method

The human CRC cell line HCT116 and DLD1 were treated with RFX at different time points. The oxygen consumption rate (OCR) and the mitochondrial membrane potential (MMP) were assessed using the seahorse XF24 assay and flow cytometry, respectively. Transcriptomic, proteomic and metabolic analysis, as

well as quantification of cytochrome C in the cytosolic fractions, were performed in CRC cells treated with RFX for 24 hours. Oligomerization of the voltage-dependent anion channel (VDAC)-1 was assessed by Western blotting. Reactive oxygen species (ROS) were quantified by fluorescent probe (DCFDA). Balb/c mice received an intraperitoneal (i.p.) injection of azoxymethane (AOM; 10 mg/kg) once every week for 6 weeks. After 20 weeks, mice were treated with RFX (7.5 mg/Kg, i.p.) or vehicle (DMSO) for an additional 8 weeks. Animals were then sacrificed and colonic tumors were collected for proteomic analysis.

Result and discussion

CRC cells treated with RFX showed a significant reduction in basal OCR, ATP production, and maximal respiration, indicating cellular quiescence. Transcriptomic, proteomic, and metabolic analyses revealed a critical impairment in mitochondrial activity following prolonged RFX treatment, along with an increased release of cytochrome C into the cytosol. RFX rapidly enhanced ROS production, promoted the open configuration of VDAC1 - a key regulator of mitochondrial metabolic functions and mitochondrial mediated apoptosis- and induced a progressive decrease in MMP in CRC cells. Treatment with the ROS scavenger N-acetylcysteine significantly limited RFX-dependent VDAC1 opening and mitochondrial membrane depolarization. Proteomic analysis of tumor colonic samples isolated from mice with sporadic CRC revealed a significant mitochondrial dysfunction in those treated with RFX.

Conclusion

Data indicate that RFX-mediated oxidative stress promotes the opening of VDAC1 and mitochondrial dysfunction, leading to the release of cytochrome C and resulting in mitochondria-mediated cell death.

EACR25-0175

Preclinical Evaluation of RAS(ON) Multi-Selective Inhibitors as a Therapeutic Strategy for KRAS mutant Cholangiocarcinoma

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Introduction

Cholangiocarcinoma (CCA) comprises around 3% of all GI cancers and features a highly dismal prognosis. Therapies predominantly rely on chemotherapy, and treatment advancements have been stagnant for the past decades, underscoring the need for innovative therapies. KRAS is among the most prevalent oncogenes in CCA, highlighting a potential role for novel KRAS inhibitors as treatment options. Here, we present preclinical data on

RAS(ON) multi-selective inhibitors as a potential therapy for CCA with KRAS mutations.

Material and method

Human and murine KRAS mutant cell lines, and immortalized cholangiocytes expressing KRAS wild type and mutant alleles (4B, G12, G13, Q61) were used to study the effect of RAS(ON) multi-selective inhibitors, the investigational agent daraxorrasib (RMC-6236) or the preclinical tool compound RMC-7977, in vitro (2D and 3D) and in vivo (CDX, PDX and CDA). RNA-seq and Western blot were conducted to analyse molecular changes and resistance mechanisms. Pharmacokinetic (PK) and pharmacodynamic (PD) analysis were carried out in KRAS mutant xenografts treated with RMC-7977 to establish the PKPD relationship in vivo. The tumor immune microenvironment was assessed by multiplex flow cytometry. Compound combinations to address resistance were tested in vitro and in vivo. Human and murine cell lines resistant to RMC-7977 were generated and characterized by WES and Western blot.

Result and discussion

KRAS mutant CCA cells were dependent on RAS signalling for growth, like other KRAS-driven cancers such as those from the lung and pancreas. RMC-7977 decreased proliferation and viability of mouse and human CCA cells with KRAS mutations and immortalized cholangiocytes expressing various mutant alleles in vitro. RMC-7977 and RMC-6236 effectively inhibited tumor growth of human and mouse KRAS mutant CCA models in vivo, impairing proliferation and inducing apoptosis. Immune phenotyping of RMC-7977-treated CDAs showed a notable increase in NK, CD8, and CD4 infiltration, and a reduction of PMN-MDSCs, indicative of a less immunosuppressive microenvironment. Consistently, addition of RMC-7977 to the standard of care regimen (Gemcitabine+Cisplatin+antiPD1) exhibited a synergistic antitumor effect, leading to deep regressions in vivo. Early adaptive resistance mechanisms to RMC-7977 were mainly driven by MAPK pathway reactivation, including KRAS upregulation, and RTK and SHP2 overactivation. RMC-7977 plus a SHP2 inhibitor had combinatorial benefits in both treatment-naïve and resistant CCA lines in vitro and in vivo. Lastly, genomic changes in resistant CCA cells involved KRAS, MYC and EGFR amplification, and informed additional drug combinations with RMC-7977.

Conclusion

In summary, our preclinical findings support the clinical testing of RAS(ON) multi-selective inhibitors, either alone or in combination with standard of care regimen or KRAS-pathway inhibitors, as a potential therapeutic strategy for CCA treatment.

EACR25-0194

The synergistic effect of sedanolide and 5-fluorouracil on triggering mitochondria-dependent apoptosis in colorectal cancer cells

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Introduction

Sedanolide (SN) is a major component found in celery, and it has the potential to act as an anticancer agent by regulating apoptosis. Colorectal cancer (CRC) is the second most common cause of cancer-related death globally, and 5-fluorouracil (5-FU) is one of the most commonly used medications for treating CRC. However, the high doses and long-term use of this medication can lead to significant side effects for colorectal cancer patients.

Material and method

This study aims to investigate the effects of SN combined with 5-FU on inducing apoptosis in human colorectal cancer cells. We analyzed cell viability, apoptosis, and the levels of several key proteins and indicators, including p53, mitochondrial membrane potential (MMP, $\Delta\psi_m$), Bax, Bcl-2, Cytochrome C, Caspase-9, I κ B, voltage-dependent anion channel 1 (VDAC1), and Hexokinase 2 (HK2) after treatment with the combination of SN and 5-FU.

Result and discussion

Our results showed that the combined treatment with both 5-FU and SN reduced the viability of colorectal cancer cells by inducing apoptosis. The combination treatment activated intrinsic apoptotic genes and proteins, including p53, Bax, Cytochrome C, and Caspase-9, while reducing levels of Bcl-2. Furthermore, this study demonstrated that the combination of 5-FU and SN can enhance NF- κ B-DNA binding activity, which decreases the binding of I κ B to VDAC1 and HK2 complexes in the mitochondrial membrane, leading to mitochondrial dysfunction.

Conclusion

In conclusion, the combination of SN and 5-FU enhances mitochondria-dependent apoptosis by regulating intrinsic apoptotic pathways and NF- κ B pathways, resulting in synergistic effects.

EACR25-0202

The interplay between melatonin and alpha-synuclein on melanoma biomolecular signatures

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Introduction

Malignant melanoma is an aggressive malignancy with high metastatic burden, and therefore, it is difficult to treat. Alternative treatment approaches are extremely urgent, and this requires a deeper understanding of the molecular mechanisms. Melatonin (MEL) is the hormone of sleep, primarily synthesized in the pineal gland but is also produced in skin. MEL has significant antitumor effects and was suggested as an anticancer agent. However, the mechanisms of action of MEL as an anti-cancer agent remain elusive. One hypothesis is that MEL may inhibit detrimental protein aggregation. Alpha-synuclein (aSyn) is a key player in Parkinson's disease (PD) due to its aggregation in characteristic inclusions in patient's brains. In melanoma, increased aSyn expression is associated with the progression of melanoma through unknown mechanisms. This study evaluated the effect of MEL on melanoma cells and investigated its impact on aSyn expression and associated biomolecular signatures.

Material and method

Melanoma cellular models with varying metastatic status were generated and the cytotoxicity of MEL was assessed. The effect of MEL on the biomolecular signatures of melanoma cells was explored using Raman spectroscopy and machine learning. ELISA was used to explore variations on aSyn protein levels. Molecular docking assessed the interaction and binding affinities of MEL to aSyn.

Result and discussion

MEL displayed stronger cytotoxic effects in non-metastatic compared to metastatic melanoma cells grown as monolayers or spheroids. This effect was associated with biomolecular changes observed within cells upon MEL treatment and modulation of aSyn levels.

Melanoma metastatic cells and spheroids which showed greater resistance to treatment, exhibited elevated levels of secreted aSyn upon MEL treatment. Thus, MEL's impact on aSyn aggregation was assessed. Molecular docking revealed that MEL binds to an aSyn intrinsically disordered C-terminal domain. This binding may inhibit β -sheet oligomer and fibril formation, potentially interfering with melanoma progression. Raman spectral changes in melanoma cells with different metastatic burden, including significant differences in intensity peaks upon MEL treatment, indicated protein conformational changes. Biomolecular signature changes, particularly glycosylation, were also identified, suggesting a role in MEL response to treatment.

Conclusion

MEL demonstrates potential as a potent anticancer agent for melanoma by inducing biomolecular changes that may impact aSyn aggregation. These findings suggest novel therapeutic targets for melanoma treatment.

EACR25-0220

The anti-cancer activity of novel diazo-derived compounds on triple-negative breast cancer

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Introduction

Breast cancer is the most common cancer among women and the second leading cause of cancer-related deaths. It is classified into subtypes based on the expression of three key receptors: estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2). Triple Negative Breast Cancer (TNBC) accounts for 15–20% of breast cancer cases and is the most aggressive subtype, as it lacks all three hormone receptors. Azo dyes are organic compounds that contain an azo (-N=N-) group and are highly preferred due to their high reactivity and ease of modification. Azo-derived compounds containing different substituted groups have been found to exhibit high antioxidant and anticancer activities. This study aims to evaluate the potential anti-cancer activity of novel diazo-derived compounds on the TNBC cell line MDA-MB-231.

Material and method

The time and dose-dependent cytotoxic effect of the diazo-hydrazone derivative compound 3 was evaluated using the MTT assay in the MDA-MB-231 and the human fibroblast cell line CCD-1072-Sk. Its impact on colony formation and migration was determined through colony formation assay and wound healing assay.

Apoptosis was assessed by analyzing the expression levels of ten apoptosis-related genes: APAF1, BIM, CASP2, CASP7, CASP9, CFLAR, FASLG, NOL3, XIAP, and TP53 using RT-qPCR, alongside Annexin V-FITC analysis performed through flow cytometry. Additionally, nuclear and mitochondrial staining was performed to examine the effects of the compound on cell morphology.

Result and discussion

The IC₅₀ values of compound 3 in MDA-MB-231 and CCD-1072-Sk cells were determined to be 6.67 ± 0.38 and $22.45 \pm 1.94 \mu\text{M}$, respectively, and the Selectivity Index (SI) was calculated to be 3.41 ± 0.06 . The administration of compound 3 was observed to reduce wound healing and the number and size of colonies formed. Both single colony analysis and cell staining revealed the formation of giant multinucleated cells, suggesting that this condition may lead to apoptosis. We observed an increase in the expression of apoptosis-related genes (APAF1, BIM, CASP2, CASP7, CASP9, FASLG, TP53), while there was a decrease in the expression of NOL3, which is known to promote escape from apoptosis in breast cancer. Flow cytometry analysis also revealed that the administration of compound 3 enhanced both early and late-stage apoptosis.

Conclusion

In conclusion, the novel diazo-hydrazone derivative compound 3 was shown to inhibit cell proliferation, colony formation, and migration in MDA-MB-231 cells. It also resulted in the upregulation of apoptosis-initiating and regulatory genes, ultimately inducing apoptosis in cancer cells. The compound may be a good starting template for clinical drug design.

EACR25-0228

Enhanced Efficacy and Targeted Delivery System of a pH-sensitive ARV-825 Nanomedicine for Glioblastoma Treatment

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Introduction

Glioblastoma (GBM) is an aggressive brain tumor with limited treatment options due to tumor heterogeneity and the blood-brain tumor barrier (BBTB). Proteolysis-targeting chimeras (PROTACs) offer a novel therapeutic strategy by degrading target proteins. ARV-825, a BET degrader PROTAC, has shown promise against GBM, but faces challenges such as poor solubility, limited BBTB permeability, and potential off-target toxicity. To address these limitations, we developed a pH-sensitive nanomedicine encapsulating ARV-825 for enhanced delivery and efficacy against GBM.

Material and method

We formulated ARV-825 into nanomedicines using PEG-PBLA polymers and pH-sensitive linker via self-assembly and cross-link. The nanomedicine was characterized for size, stability, and drug encapsulation efficiency. In vitro cytotoxicity was assessed in GL-261 glioblastoma cells by measuring the IC-50. In vivo efficacy was evaluated in orthotopic GL-261-luc glioblastoma models in C57BL/6 mice. We monitored tumor growth using in vivo imaging and assessed treatment efficacy by analyzing survival rates.

Result and discussion

pH-sensitive ARV-825-loaded nanomedicines exhibited enhanced drug potency with an in vitro IC-50 of 0.013 μM compared to 0.1 μM for free ARV-825. In vivo imaging revealed significant tumor growth inhibition in mice treated with ARV-825 nanomedicines. Furthermore, the nanomedicine significantly prolonged survival time and improved survival rates compared to controls.

Conclusion

This study demonstrates that PROTAC-based nanomedicine represents a promising approach for treating glioblastoma. Our ARV-825-loaded pH-sensitive PBLA-PEG micelles enhanced drug potency, improved tumor targeting, and extended survival in preclinical GBM models. This nanomedicine approach warrants further investigation for clinical translation. Future research should focus on detailed mechanistic studies, safety evaluations, and strategies to further enhance tumor-specific delivery and minimize off-target effects.

EACR25-0295

Development and Validation of a Novel Multi-Functional Iron Oxide Nanoparticle for the Targeted Induction of Ferroptosis in Cancer

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Introduction

Ferroptosis is a recently discovered form of iron-driven cell death, wherein intracellular ferrous iron results in lipid peroxidation (LPO) and oxidative stress. Due to the inherent iron-dependency of cancer, ferroptosis may be an exploitable mode of cell death in the treatment of cancers which are resistant to conventional chemotherapeutics.

Material and method

We have developed a novel iron oxide nanoparticle (IONP) for the targeted induction of ferroptosis in cancer. IONPs were coated in FDA-approved PEGylated phospholipids and MC3-DLin-DMA. MC3-DLin-DMA (used in clinically-approved lipid nanoparticles (LNPs)) provides two polyunsaturated fatty acid (PUFA) chains to initiate ferroptosis. These PUFA-rich, PEGylated, phospholipid-stabilised Fe₃O₄-filled micelles were characterised by size, shape, and charge. Uniquely, the pro-fertopto activity of this IONP is initiated by spontaneous homolysis of hydroperoxides and the reaction of H₂O₂ with Fe(II) ions within the micelles. The IONP self-generates hydroxyl and alkoxyl radicals that ultimately cause LPO in cells.

Result and discussion

The efficacy of IONPs in inducing ferroptosis was measured in vitro by viability and LPO assays. The potency of IONPs could be enhanced with the use of RNA interference for a key ferroptosis defence gene (AIFM2). In vivo studies demonstrated that IONPs administered by subcutaneous injection or transdermal delivery using fast-dissolving microneedle patches provide high efficacy in the reduction of tumour volume at low doses.

Conclusion

This study has developed and validated an effective and innovative cancer drug which uses a multi-functional approach to induce ferroptosis. It is intended for this drug to progress to further pre-clinical trials and be evaluated in combination with pro-fertopto drugs.

EACR25-0301

Innovative Cyclic Peptide Disrupts IL-17RB/MLK4 Interaction for Targeted Pancreatic Cancer Therapy

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Introduction

The IL-17B/IL-17RB signaling axis promotes pancreatic cancer progression by interacting with mixed-lineage kinase 4 (MLK4). This interaction activates downstream oncogenic signaling pathways contributing to tumor growth and metastasis. Disrupting the IL-17RB/MLK4 interaction is a promising pancreatic cancer therapeutic

strategy. We previously developed a linear peptide, TAT-IL17RB403-416, designed to interfere with this interaction. In this study, we aimed to improve the therapeutic efficacy of this peptide by converting it into a cyclic form.

Material and method

A cyclic analog of TAT-IL17RB403-416 was designed based on the conserved IL-17RB loop region and synthesized using solid-phase peptide synthesis with disulfide bond cyclization. In vitro experiments, including cellular uptake, soft agar colony formation, and spheroid formation assays, were performed using the CFPAC1 human pancreatic cancer cell line. An orthotopic mouse model was used to assess in vivo efficacy. The IL-17RB/MLK4 interaction was analyzed by co-immunoprecipitation and biolayer interferometry. Computational modeling and docking predicted the cyclic peptide's binding mode to MLK4. Site-directed mutagenesis and co-immunoprecipitation experiments assessed the role of key residues in IL-17RB and MLK4.

Result and discussion

Novel cyclic peptide significantly enhanced cellular uptake and stability compared to the linear form. This improved uptake and stability translated to superior inhibition of pancreatic cancer cell growth in vitro. Furthermore, treatment with the cyclic peptide significantly reduced tumor growth and metastasis in an orthotopic mouse model. Mechanistically, we identified key residues mediating the IL-17RB/MLK4 interaction, including cysteine 408 in IL-17RB and arginine 216 in the MLK4 kinase domain, which are critical for the cyclic peptide's activity. Additionally, we found that lysine 410 in IL-17RB is essential for maintaining the structural integrity and function of the cyclic peptide as a protein-protein interaction disruptor. These findings highlight the therapeutic potential of cyclic peptides targeting the IL-17RB/MLK4 interaction in pancreatic cancer and provide a deeper understanding of the molecular basis of this interaction.

Conclusion

This study highlights the potential of cyclic peptides as effective protein-protein interaction disruptors in pancreatic cancer. The enhanced efficacy observed with the cyclic peptide analog emphasizes the importance of rational design and optimization in peptide drug development. This promising therapeutic approach warrants further investigation for the treatment of pancreatic cancer.

EACR25-0326

A genetic signature predicts the response of aggressive paragangliomas to dual PI3K and CDK4/6 inhibition

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Introduction

Surgery is the first-line and only curative treatment for localized paragangliomas (PPGLs). However, in metastatic cases, surgical intervention is rarely feasible, and effective therapeutic options remain limited, resulting in a poor prognosis for patients with aggressive or metastatic (m)PPGL. This underscores the urgent need to identify novel treatment strategies. In this study, we investigate the antitumor potential of targeting two key dysregulated pathways in PPGL: PI3K signaling and cell cycle regulation.

Material and method

We assessed the efficacy of buparlisib, a PI3K α inhibitor (PI3Ki), and ribociclib, a CDK4/6 inhibitor (CDK4/6i), as monotherapies and in combination, using functional assays in representative in vitro models of PPGL. These models included two rodent cell lines (MPC and PC12) as well as rat- and patient-derived primary PPGL cells. The combination therapy was further evaluated in vivo in PC12-derived mouse xenografts. Transcriptomic analysis of treated PC12 cells identified a mitotic gene signature uniquely dysregulated by combination treatment, which was validated in PPGL cells through qRT-PCR and functional assays assessing mitotic spindle defects. Additionally, RNA-Seq analysis of human PPGLs revealed an upregulation of this gene signature in mPPGLs compared to non-metastatic cases, a finding further confirmed by qRT-PCR in independent patient samples.

Result and discussion

Dual inhibition of PI3K and CDK4/6 resulted in superior suppression of cell proliferation and invasion compared to monotherapies, while also inducing apoptosis in both 2D and 3D organotypic cultures of PPGL cell lines. Synergistic effects were observed in primary organotypic cultures derived from both rat and patient PPGLs, as well as in PC12-derived xenografts, where tumor growth was significantly reduced, even at lower drug doses. RNA-Seq analysis identified key mediators of the combination treatment response, including mitotic spindle pathways and FOXM1 signaling, both of which were experimentally validated. Bioinformatics analyses further confirmed the activation of the mitotic gene network in human mPPGLs.

Conclusion

Combined inhibition of PI3K and CDK4/6 targets mitotic spindle regulation and exerts potent antitumor effects in PPGL models. Given the upregulation of mitosis-associated genes in mPPGL, this combination therapy holds promise as a novel approach for treating aggressive forms of these tumors.

EACR25-0375

Rationally designed cholesterol-coated peptide nanospikes as a novel therapeutic strategy in the management

of Adrenocortical Carcinoma

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Introduction

Management of adrenocortical carcinoma (ACC) is challenging, with diagnosis often occurring at advanced stage. Surgical resection of localized disease offers potential cure, but recurrence is high (75-85%). Mitotane is the only approved drug for advanced ACC, but has a narrow therapeutic window, is poorly tolerated, and effective in less than 30% of cases. Nanotheranostics rationally designs nanomaterials for simultaneous diagnosis, drug-delivery, and treatment. Most FDA-approved nanomedicines rely on the enhanced permeability and retention (EPR), where nanoparticles passively accumulate in tumors. This improves drug delivery while reducing adverse effects but is subject to variability between patients and tumor types, which can be improved by specific nanoparticle-targeting to tumors. ACC cells rely on cholesterol, for steroid hormone synthesis, as a key component of cell membranes, and as fuel. Exploiting this, we designed tri-maleimide peptide-based nanospikes (NS) functionalized with two cholesterol molecules and a fluorescent tag (Rhodamine B). These rationally designed NS were tested for uptake and cytotoxicity on three ACC cell lines: H295R (chemotherapy-sensitive), HAC-15 (chemoresistant), MUC-1 (chemoresistant).

Material and method

Cell death was assessed using SYTOX Blue, and the role of SR-B1 in NS uptake was evaluated using BLT-1. Caspase-dependent/independent cytotoxicity was investigated using z-VAD (pan-caspase inhibitor), and measuring markers of apoptosis, ER stress and autophagy (Caspase 3 & 8, CHOP, LC3). LC-MS/MS was used to measure steroidogenesis.

Result and discussion

NS were rapidly taken up by all ACC cell lines, with significant cell death observed within 2 hours in the H295R cell line at the physiological concentration of 20 μ g/ml ($p < 0.01$), at 50 μ g/ml in HAC-15 ($p < 0.01$) and at 100 μ g/ml in MUC-1 ($p < 0.5$). NS uptake was not prevented by the Scavenger Receptor-B1 (SR-B1) inhibitor BLT-1, suggesting direct-membrane or LDL-R

mediated uptake. Confocal microscopy and TEM revealed that NS accumulated in mitochondria. ACC cell death occurred through a caspase-independent pathway: (i) cleaved caspase-3 protein was not upregulated (ii) zVAD-fmk had no significant effect on NS-induced cytotoxicity, while (iii) autophagy-related markers (LC3II/LC3I ratio) were upregulated. Forskolin-stimulated cortisol production was also reduced in H295R cells after NS treatment.

Conclusion

Rationally designed cholesterol-functionalized nanospikes (NS) demonstrate rapid uptake and potent cytotoxicity in ACC, inducing caspase-independent cell death and disrupting steroidogenesis. These findings highlight the potential of NS as a novel theranostic approach for ACC, offering targeted drug delivery with reduced toxicity and improved efficacy compared to conventional therapies. Future studies will load NS with lipid-soluble cytotoxic drugs e.g. mitotane.

EACR25-0410

PI3K-dependent GAB1/Erk phosphorylation renders head and neck squamous cell carcinoma sensitive to PI3K α inhibitors

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Introduction

Phosphatidylinositol 3-kinase alpha (PI3K α) inhibitors are currently evaluated for the therapy of head and neck squamous cell carcinoma (HNSCC). CYH33 is a novel PI3K α -selective inhibitor discovered by our group, which is currently in clinical trials for the treatment of advanced solid tumors including HNSCC. However, there is an urgent need to elucidate its mechanism of action and improve its efficacy against HNSCC.

Material and method

Tandem-Mass-Tag (TMT) phosphoproteomics was performed to reveal the comprehensive regulation of kinase by CYH33. Cell proliferation was evaluated by Sulforhodamine B (SRB) assay. Western blotting was employed to detect protein levels. Xenografts derived from HN4 or SUNE-1 cells were utilized to evaluate the therapeutic efficacy *in vivo*.

Result and discussion

We found CYH33 displayed promising but variable therapeutic activity against HNSCC. Inhibition of PI3K/Akt pathway by CYH33 was not sufficient for its activity against HNSCC. Tandem-Mass-Tag (TMT) proteomics and phosphoproteomics were performed to reveal comprehensive regulation of kinase by CYH33. Among them, phosphorylation of MAPK1/Erk at

activation sites (T185/Y187) were significantly attenuated by CYH33 in sensitive HNSCC cells. CYH33 significantly suppressed phosphorylation of Erk in sensitive but not resistant cells. Further inhibition of Erk phosphorylation enhanced the activity of CYH33 against HNSCC. Signaling network of differentially phosphorylated proteins illustrated that phosphorylation at multiple sites (Y659, Y406, S651, S648) of the adaptor protein GAB1, which integrates the signaling from PI3K and receptor tyrosine kinases, were significantly down-regulated upon CYH33 treatment. CYH33 dose-dependently suppressed the phosphorylation of GAB1 at Y659 in sensitive HNSCC cells, whereas it had little effect in resistant cells. We found that CYH33 attenuated the membrane localization and phosphorylation of GAB1, resulting in reduced Erk phosphorylation and ultimately inhibiting the proliferation of sensitive cells. Meanwhile, activation of EGFR induced GAB1 phosphorylation independent of PI3K in HNSCC. Concurrent inhibition of EGFR synergistically potentiated the activity of CYH33 against HNSCC.

Conclusion

In conclusion, we found that PI3K α -selective inhibitor CYH33 displayed potent activity against HNSCC, which was associated with inhibition of Akt signaling as well as attenuation of PI3K-dependent GAB1/Erk phosphorylation. Simultaneous inhibition of GAB1 phosphorylation independent of PI3K potentiated the anti-HNSCC activity of CYH33. These findings revealed the insight mechanism of CYH33 against HNSCC and provided rational combination regimen for HNSCC.

EACR25-0411

Targeting Estrogen-Related Receptor Gamma (ERR γ) in Ovarian Carcinoma: Therapeutic Potential of DN200434 and MK2206 Combination in-vitro and in-vivo

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Introduction

Ovarian carcinoma (OC) is a leading cause of cancer-related mortality in females, necessitating the development of novel therapeutic strategies. Estrogen-related receptor gamma (ERR γ), an orphan nuclear receptor, has been implicated in cancer progression; however, its precise role in OC remains to be elucidated. This study evaluated the effects of DN200434, an ERR γ inverse agonist, alone and in combination with the Akt inhibitor MK2206 in OC models. By investigating ERR γ modulation in vitro and in vivo, this study aims to provide insights into its role in OC progression and resistance, offering a foundation for novel targeted therapies.

Material and method

ERR γ expression in ovarian cancer (OC) cell lines (SKOV3, OVCAR3, IGROV, and PA-1) was analyzed using western blotting. The effects of DN200434 on cell proliferation, apoptosis, and cell cycle arrest were examined in SKOV3 and OVCAR3 cells. For combination treatment, the half-maximal inhibitory

concentration (IC50) values of DN200434 and MK2206 were determined using a CCK assay following a 24-hour treatment. OC cells were treated with either drug alone or in combination to evaluate the inhibitory effects on apoptosis, cell cycle progression, colony formation, migration, and spheroid formation. The expression of key molecular markers associated with these processes was validated using western blot analysis. Calcein-AM staining was performed on the spheroids to assess the impact of the drug combinations. To evaluate the in vivo therapeutic efficacy, SKOV3 tumor-bearing xenografts were established, and apoptosis within the excised tumor tissues was assessed using TUNEL staining.

Result and discussion

ERR γ was overexpressed in ovarian cancer (OC) cells, and DN200434 treatment effectively reduced its expression. DN200434 inhibited OC cell proliferation in a dose-dependent manner by inducing cell cycle arrest, followed by apoptosis. Furthermore, DN200434 in combination with MK-2206 exhibited a synergistic cytotoxic effect, significantly enhancing OC cell death. Combination treatment (DN200434/MK-2206) suppressed cell proliferation through cell cycle arrest and apoptosis, while also markedly reducing colony formation efficiency, migratory capacity, and spheroid formation. Additionally, combination therapy diminished the stemness of OC cell-derived spheroids. The antitumor efficacy of DN200434/MK-2206 was further validated in an in vivo OC model, where ex vivo TUNEL staining of tumor tissues confirmed a significant increase in apoptosis in the combination-treated mice.

Conclusion

Our findings indicate that ERR γ acts as a positive regulator of ovarian cancer (OC) progression and is a promising therapeutic target. The combination of DN200434 and MK-2206 significantly inhibited cancer cell growth. This dual therapy holds substantial potential for further investigation and progression to clinical trials.

EACR25-0414

Oxyresveratrol inhibits the tumorigenesis and metastasis of human HCC cells by targeting the CCDC19/PLK1/CCNB1 pathways

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Introduction

Oxyresveratrol (OxyR), a natural polyphenolic phytoalexin found in grape skins, mulberries, peanuts, and red wine, has been reported to exhibit various biological activities, including anticancer, antioxidant, anti-inflammatory, free radical scavenging, and neuroprotective effects. However, its role and molecular mechanisms in hepatocellular carcinoma (HCC) remain unclear.

Material and method

Cell viability, colony formation and in vitro migration and invasion assays were performed to assess the effects

of OxyR on HCC cell proliferation and metastatic potential. RNA sequencing (RNA-Seq) was conducted to identify differentially expressed pathways. The protein and mRNA expression levels of CCDC19, PLK1, and CCNB1 were validated using qRT-PCR and western blotting. Additionally, the expression levels of CCDC19, PLK1, and CCNB1, along with overall survival analysis, were examined by the GEPPIA database and Kaplan-Meier (KM) plot. To further investigate the anti-invasive role of CCDC19 and PLK1 in OxyR-treated HCC cells, siRNA-CCDC19 knockdown and PLK inhibition were employed.

Result and discussion

Our results demonstrated that OxyR treatment inhibited the proliferation, migration, and invasion of HCC cells. RNA-Seq analysis revealed that OxyR downregulated the expression of CCDC19, PLK1, and CCNB1 in HCC cells, which was further confirmed by qRT-PCR and western blotting. Additionally, we found that CCDC19, PLK1, and CCNB1 were significantly upregulated in HCC tissues, and their expression was associated with tumor stage and poor survival outcomes in HCC patients. Silencing CCDC19 or treatment with a PLK1 kinase inhibitor in combination with OxyR significantly suppressed cell migration and invasion. Furthermore, we found that OxyR reduced the expression of the transcription factor FoxO4 in HCC cells.

Conclusion

Our study provides new insights into the anti-tumor effects of OxyR by targeting the CCDC19, PLK1, and CCNB1 expression. These findings suggest that OxyR may serve as a promising anticancer agent for HCC treatment.

EACR25-0450

Integrative Pharmacogenomics in RCC Tumoroids Uncovers Biomarker-Driven Combination Therapies

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Introduction

Effective treatment for renal cell carcinoma (RCC) remains critical for improving survival outcomes, as conventional therapeutic approaches exhibit limited response rates ranging from 30% to 60%. Personalized RCC therapy requires models capturing tumor heterogeneity and drug resistance mechanisms. This study aimed to identify novel anticancer drugs and synergistic drug combinations for RCC through ex vivo pharmacogenomic profiling of patient-derived tumoroids.

Material and method

All patients' samples were obtained from Renji Hospital, with written informed consent and Research Ethics Board approval in accordance with the Declaration of Helsinki. Primary RCC tumoroids were generated from fresh

surgical specimens using a scaffold-free 3D culture system. Histopathological validation was performed through hematoxylin-eosin (H&E) staining and immunohistochemical (IHC) analysis of lineage-specific biomarkers. Whole exome sequencing and RNA-seq were used to characterize the mutation profile, copy number variation and gene expression. Validated tumoroids underwent high-throughput screening against 81 anti-cancer drugs at serial dilutions. Dose-response curves were modeled via the R package GRmetrics to calculate area under the curve (AUC), half-maximal inhibitory concentration (IC50), and maximal efficacy (Emax). Drug-gene associations were assessed through Spearman correlation coefficients between log2-transformed gene expression values and AUC-derived sensitivity metrics (Benjamini-Hochberg adjusted $p < 0.05$). Synergy quantification was performed via SynergyFinder.

Result and discussion

52 RCC tumoroids were successfully cultured and screened from 75 surgical specimens (69.3% success rate). Histopathological and biomarker analyses confirmed retention of parental tumor architecture and pathological types. Molecular concordance was observed between tumoroids and matched primary tissues in mutational landscapes and transcriptomic profiles. Pharmacotyping revealed 15 agents with high potency ($AUC < 0.8$ in $\geq 70\%$ samples), including proteasome inhibitors and HDAC inhibitors, alongside 33 compounds demonstrating heterogeneous responses. Transcriptome-drug sensitivity integration identified 400 significant associations, notably linking elevated INSR expression to resistance against proteasome inhibitors. Combinatorial testing in INSR-high tumoroids revealed synergistic interaction between Ixazomib and the dual IGF-1R/INSR inhibitor BMS-754807.

Conclusion

Scaffold-free tumoroids faithfully recapitulate inter-patient heterogeneity in RCC, serving as robust platforms for drug discovery. Our pharmacogenomic framework identifies actionable therapeutic vulnerabilities and predictive biomarkers, providing a rationale for personalized combination therapies.

EACR25-0490

Targeted Delivery of Extracellular Vesicles to Metastatic Breast Cancer Cells Using the Tumor-Targeting Peptide p28

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Introduction

Conventional cancer therapies induce severe side effects and have limited therapeutic windows. Nanoparticles emerged as a potential solution to overcome some of these limitations. However, the most widely applied carriers are synthetic and often show low biocompatibility and biological barrier crossing. On the other hand, extracellular vesicles (EVs) are phospholipid bound spherical structures that mediate cell to cell communication allowing engineering and loading with therapeutic cargoes. While EVs are promising, they may need to be engineered with target ligands to accumulate into the target tissues at clinically relevant numbers. We investigated the tumor targeting peptide p28 as an EV targeting moiety. p28 is derived from the bacterial protein azurin, and both the full protein and its derived peptide demonstrated selective uptake by cancer cells. In fact, in two phase I clinical trials, p28 showed safety and anti-tumor activity, suggesting a favorable therapeutic index.

Material and method

EVs were isolated from clinically relevant sources, mesenchymal stromal cells (MSCs, a promising therapeutic EV source) and human platelet lysate (hPL, derived from clinically expired platelet concentrates), through a scalable and good manufacturing practice (GMP) compatible method, comprising tangential flow filtration (TFF) for MSC-EVs and size exclusion chromatography (SEC). A characterization using nanoparticle tracking analysis (NTA), total protein quantification, western blotting (WB), and transmission electron microscopy (TEM) was performed.

Result and discussion

The EVs were isolated with a particle-to-protein ratio (PPR) of $(7.8 \pm 0.3) \times 10^9$ and $(5.7 \pm 5.1) \times 10^{10}$ particles/ μg protein for MSC-EVs and hPL-EVs respectively, demonstrating their high purity. These particles showed the expression of EV markers (CD63 and CD9), the classic morphology in TEM, and an average size of 131 nm (for MSC-EVs and hPL-EVs). Concerning particle numbers, MSC and hPL yielded $(2.5 \pm 2.6) \times 10^{10}$ and $(1.1 \pm 0.9) \times 10^{11}$ particles/mL of concentrated starting material, respectively. Both EVs were successfully functionalized with the tumor targeting peptide p28 (p28-EVs). Flow cytometry analysis demonstrated an enhanced uptake of p28-EVs into metastatic triple negative breast cancer MDA-MB-231 cells compared to non-functionalized EVs. MSC-EV uptake increased 1.6 ± 0.7 ($n = 3$), whereas hPL-EV uptake increased 2.2 ± 0.6 ($n = 3$) fold, highlighting the promise of p28 to boost EV penetration in tumor cells.

Conclusion

We demonstrated that p28, when attached to the EV surface, functions as a targeting moiety for cancer cells. We envision the use of this system in targeted cancer therapy with fewer side effects. Acknowledgments: AventaCell Biomed Corp. for providing hPL.

EACR25-0516

Modulation of GPR55 and PINK1 in Glioblastoma Multiforme Models by Cannabis sativa and Piper nigrum

Metabolites

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Introduction

Glioblastoma multiforme (GBM) is the most aggressive primary brain tumor, with poor prognosis and limited treatment options. Resistance to standard therapies, such as temozolamide and radiotherapy, remains a major challenge, necessitating novel therapeutic strategies. Emerging evidence suggests that targeting mitochondrial dysfunction and non-canonical cannabinoid receptors could help overcome resistance in GBM and other cancer subtypes. This study investigates the potential of cannabichromene (CBC), cannabigerol (CBG), and piperine in modulating PINK1 and GPR55, key regulators of mitochondrial homeostasis and tumor progression, as a strategy to enhance treatment efficacy

Material and method

In silico molecular docking was performed to evaluate the binding affinity of CBC, CBG, piperine, and related compounds to PINK1 and GPR55. In vitro assays included cell viability (MTT), lactate dehydrogenase (LDH) release, Western blot (WB), PCR analysis, mitochondrial potential assays, and experiments using both established glioblastoma cell lines (U87MG, T98G) and a primary culture derived from a glioblastoma patient. The expression profiles of PINK1 and GPR55 were also analyzed using a database of 6,010 glioblastoma cases in Colombia spanning the last 10 years. From this database, 20 samples were recovered for immunohistochemical (IHC) analysis and compared against normal cortical tissue, and public transcriptomic datasets were also examined.

Result and discussion

CBC and CBG exhibited high binding affinity to GPR55 and PINK1. Cytotoxicity assays showed that both compounds, along with piperine, reduced glioblastoma cell viability in a dose-dependent manner, with IC₅₀ values comparable to or lower than temozolamide. WB and PCR analyses confirmed that cannabinoid treatment modulated PINK1 and GPR55 expression, suggesting a role in mitochondrial homeostasis and tumor progression. In some cases, cell death was linked to mitochondrial potential alterations, an indirect measure of PINK1 modulation. These findings align with IHC data from patient samples and transcriptomic datasets, indicating

differential biomarker expression in GBM vs. normal tissue.

Conclusion

CBC and CBG exhibit anticancer potential through PINK1 and GPR55 modulation, highlighting novel therapeutic targets for glioblastoma. Their influence on mitochondrial dynamics and oncogenic signaling suggests a role in overcoming therapy resistance in GBM and other cancers. Moreover, PINK1 emerges as a key molecular target given its differential expression in glioblastoma samples and correlation with survival outcomes in Kaplan-Meier analysis. The agreement between in silico predictions and in vitro validation reinforces these findings and underscores the value of integrating computational and laboratory approaches in drug discovery. Further studies are needed to elucidate their mechanisms and enhance their translational applicability.

EACR25-0521

canSERV – providing cutting edge cancer research services across Europe

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Introduction

canSERV is a €15 Mio. project offering cutting-edge research services, enabling innovative R&D projects and fostering precision medicine for patients benefit.

CanSERV involves 18 leading organizations across Europe including Research Infrastructures, key organisations and oncology experts.

Material and method

canSERV's main objectives are:

- (i) offer at least 200 different unique Personalised Oncology relevant and valuable cutting-edge services;
- (ii) establish a single, unified, transnational access platform to request services and trainings;
- (iii) ensure oncology-related data provided will be fully compliant with FAIR principles and complement and synergise with other EU initiatives and
- (iv) ensure long-term sustainability beyond project duration. Furthermore, canSERV establishes the European Molecular Tumour Board Network (EMTNB) that is open for anyone to join. The EMTNB develops Molecular Tumour Board (MTB) consensus guidelines, an MTB outcome registry, and provides advice to scientists, clinicians, and MTBs.

Result and discussion

canSERV offers a series of open and challenge calls for access to services in the amount of ~€9 Mio. The calls are designed to support researchers to develop innovative research projects that explore cutting-edge methodologies and target critical gaps in cancer research and care by providing funding to services. Over the past two years, canSERV launched 3 open calls and 4 challenge calls that resulted in 336 research proposals submissions from 41 countries worldwide. These proposals address a diverse array of important frontiers in cancer research, including biomarker discovery and validation, novel therapeutics, advanced diagnostics, understanding cancer biology, cutting-edge AI/ML models for oncology and the advancement of precision medicine.

Conclusion

canSERV presents an unparalleled opportunity to accelerate cancer research, drive innovation, and improve patient outcomes.

canSERV is granted by the EU Horizon programme under #101058620.

EACR25-0532

Repurposing the antifungal piroctone olamine as a targeted treatment for pancreatic ductal adenocarcinoma

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Introduction

Pancreatic ductal adenocarcinoma (PDAC) is an intractable disease and its incidence is continuously rising in underdeveloped and developing countries including in South Africa where 84% of patients diagnosed die from the disease. This highlights an urgent need for cheap and effective therapeutic agents to reduce the burden of PDAC. We hypothesise that repurposing commercially available non-cancer drugs that inhibit key drivers of PDAC may facilitate the rapid identification of cost-effective drugs. In PDAC the expression of the homologous TBX2 and TBX3 transcription factors correlates with distant metastasis and poor patient survival. Here, we investigated whether TBX2 and TBX3 can serve as targets for piroctone olamine, an antifungal previously shown to inhibit TBX2 and TBX3 in TBX2/3-dependant melanoma and rhabdomyosarcoma cells, for treating PDAC.

Material and method

Human PDAC cell lines (BxPC-3, CFPAC-1, PANC-1, and SW1990) were used in this study. To investigate the role of TBX3 in PDAC as well as the anti-cancer effects and mechanisms of action of piroctone olamine numerous assays were performed in 2D and 3D spheroid and organoid PDAC cell culture models. These included, MTT and clonogenic assays, SA-β-galactosidase activity, Annexin-V/Propidium Iodide staining, immunocytochemistry, western blotting, scratch motility and transwell invasion assays, as well as 3D spheroid formation and invasion assays. The CellTiter-Glo® 3D Cell Viability kit was used to assess the effect of

piroctone olamine in a panel of PDAC patient-derived organoids.

Result and discussion

When TBX3 was stably knocked down in PDAC cells, they underwent senescence and had reduced proliferative ability and spheroid growth. Interestingly, TBX2 levels increased in the TBX3-knocked down PDAC cells, and depleting TBX2 in these cells inhibited their migration and invasion. These results thus demonstrated that TBX2 and TBX3 have distinct oncogenic functions in PDAC and that any effective anti-PDAC drug will need to inhibit them both. Using a high throughput drug repurposing screen we identified piroctone olamine to inhibit TBX2 and TBX3 levels and we show that it is a promising drug candidate to be repurposed for the treatment of TBX2/3-dependant PDAC. Indeed, treatment with piroctone olamine inhibited the levels of both TBX2 and TBX3 and recapitulated the phenotypes observed when TBX2 and TBX3 were knocked down in 2D and 3D PDAC cell culture models. Impressively, treatment with piroctone olamine significantly reduced the size and viability of PDAC patient-derived organoids.

Conclusion

Together, our data suggest that piroctone olamine has great potential to be repurposed for the targeted treatment of TBX2/3-dependant pancreatic cancer.

EACR25-0539

Targeting PDGFRA in Pediatric High-Grade Gliomas: Insights from Brazilian Preclinical Models

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Introduction

Pediatric high-grade gliomas (pHGG) are highly aggressive brain tumors associated with poor prognosis. The development of reliable preclinical models to advance personalized therapeutic strategies is essential. The PDGFRA gene plays a critical role in gliomagenesis, with alterations linked to tumor progression and resistance mechanisms. In this study, we established and characterized patient-derived primary cultures and xenografts (PDX and PDOX) from Brazilian pediatric HGG cases with PDGFRA alterations, aiming to evaluate their biological features and responses to targeted therapies. Two pHGG models were derived from pediatric patients. Case 1 harbored PDGFRA (c.1977C>G) and TP53 (c.637C>T) variants, leading to the generation of HCB570 primary culture, PDX, and PDOX OXP9. Case 2, associated with CMMRD syndrome and

an MSH6 germline mutation, exhibited PDGFRA amplification, resulting in the establishment of HCB589, PDX, and PDOX OXP22.

Material and method

Cell characterization included xCELLigence proliferation assays, doubling time assessment, colony formation, and determination of temozolomide (TMZ) IC50. Patient and the PDX models underwent histopathological and EPIC methylation profiling to classify their molecular subtypes. Targeted sequencing (SOPHiA Solid Tumor and RNA Fusion Panels) was also performed to identify genomic alterations. In vitro drug treatment with Dasatinib, Imatinib, and Regorafenib was performed to assess therapeutic vulnerabilities. Dasatinib was further evaluated in vivo using PDX models.

Result and discussion

Both primary cultures demonstrated colony-forming capability and TMZ resistance with IC50 approximately 750 μM. The PDX models closely resembled patient tumors in histopathological and molecular profiles, confirming their classification as pHGGs IDH wild-type H3 wild-type. Importantly, PDGFRA alterations were detected in both cases, with one harboring a point mutation (c.1977C>G) and the other showing an amplification. Targeted drug treatment indicated that all three PDGFR inhibitors (Dasatinib, Imatinib, and Regorafenib) reduced cell viability in vitro. Dasatinib, selected for further validation in vivo, significantly inhibited tumor growth in the PDX models, reinforcing PDGFRA as a key therapeutic target in pediatric gliomas.

Conclusion

The successful establishment of pediatric pHGG preclinical models with PDGFRA alterations provides valuable tools for understanding glioma biology and testing targeted therapies in the admixture Brazilian population. Our pre-clinical findings highlight Dasatinib as a potential treatment for PDGFRA-driven pHGG, supporting the further clinical trials. Furthermore, including rare genetic backgrounds, such as CMMRD, contributes to diversifying glioma research models and expanding translational oncology efforts.

EACR25-0549

Patient-Derived Glioma Organoids Resist Standard Therapies but Respond to Targeted Inhibition

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Introduction

Gliomas are the most common primary brain malignancies in adults, classified by aggressiveness into low-grade gliomas (LGG, grades II–III) and high-grade gliomas (HGG, grade IV). Glioblastoma (GBM), a grade IV IDH1 wild-type astrocytoma, is the most prevalent and aggressive form, with an incidence of 3.22 cases per 100,000 people. GBM's high inter- and intra-tumoral heterogeneity contributes to its poor prognosis, with a median overall survival of 15 months and a 5-year survival rate of ~5%. Despite this, the standard of care (SOC) – maximal surgical resection, radiotherapy, and temozolomide (TMZ) chemotherapy – has seen little advancement. LGG research faces challenges due to low proliferation rates and tumor heterogeneity, limiting conventional preclinical models. In this context, patient derived *in vitro* models like organoids provide a promising platform for personalized medicine.

Material and method

In this study, we generated patient-derived organoids from over 50 glioma patients, characterized them based on histology, microstructure, expression patterns, and growth, and evaluated their response to SOC therapies. Subsequently, RNA-seq data was used for de novo drug discovery via the DiSCoVER platform, with candidate compounds validated *in vitro*.

Result and discussion

Organoid establishment was successful in most cases, with a higher success rate for GBM-derived organoids compared to LGG. Organoids proliferated and mimicked parental tissue in histology, microstructure, and expression patterns. However, biobanking proved unsatisfactory. Screening of common chemotherapeutic compounds revealed that SOC TMZ and other commonly used treatments had little effect on viability, whereas gefitinib showed a notable impact. Our de novo *in silico* approach identified alectinib, ruxolitinib, and dabrafenib as promising candidates, which were confirmed to significantly reduce viability, impair migration, and alter key molecular pathways.

Conclusion

In conclusion, while patient-derived glioma organoids present limitations in proliferative capacities and biobanking efficiency that must be addressed for clinical implementation, they remain an invaluable preclinical tool. As demonstrated in this work, the combination of organoids with bioinformatics tools like DiSCoVER establishes a potent platform for testing novel therapeutic strategies in the context of untreatable cancers such as gliomas.

EACR25-0559

Combining *in silico* and *in vitro* approaches to repurpose candidate drugs for oral squamous cell carcinoma

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Introduction

Oral Squamous Cell Carcinoma (OSCC) remains a significant public health concern. Despite advances in treatments, including surgery, and chemotherapy, choices remain limited, underscoring the need for innovative therapeutic strategies. Developing new drugs is both costly and time-intensive. Drug repurposing offers a compelling solution by leveraging existing, well-characterized medications that are already approved and toxicologically safe. In this context, aiming to identify promising candidates for repositioning in OSCC, we combined integrated *in silico* analyses tools based on molecular and genetic insights with *in vitro* cytotoxicity screening based on bi- (2D) and three-dimensional (3D) models.

Material and method

A total of 237 drug candidates were retrieved from DrugBank to identify druggable targets. Ranking was based on cancer-related pathways and protein-protein interaction centrality. The *in vitro* cytotoxicity of selected compounds was evaluated in OSCC cell lines (HSC-3, H357) following the National Cancer Institute (NCI) Developmental Therapeutics Program (DTP) guidelines, with CellTiter-Glo® as the viability assay. Homotypic OSCC spheroids were generated using optimized magnetic 3D bioprinting. After ranking, 21 compounds were selected and underwent single-dose screening (10 µM). H357 cells were more sensitive than HSC3, showing lower viability across multiple compounds. Three most promising candidates (Daunorubicin, Erlotinib, and Romidepsin), which displayed growth inhibition properties (at least a 35% reduction), advanced to five-dose screening assays in both 2D and 3D models. In 2D cultures, Romidepsin exhibited the strongest cytotoxic effect, with the lowest IC50 values in both cell lines. In 3D assays, all three drugs demonstrated cytotoxic activity, with Romidepsin once again emerging as the most potent compound.

Result and discussion

The ranking strategy accelerated candidate selection and increased the chances of success by focusing on targets that exert greater influence on pathways relevant to OSCC. HSC-3 cells, known for higher tumorigenic potential, exhibited greater drug resistance than H357. Different mechanisms of action that maximize the therapeutic applications of the selected drugs were found. They include epidermal growth factor receptor (EGFR) inhibition, topoisomerase II inhibition, and a potent class I histone deacetylase (HDAC) blocker. Differences in 2D and 3D responses highlight the need for physiologically relevant models to improve translational relevance.

Conclusion

These findings reinforce the value of drug repurposing as an efficient strategy for expanding OSCC treatment options. By integrating computational approaches with physiologically relevant 3D models, this approach paves the way for more precise and personalized cancer therapies.

EACR25-0618**Discovery of DAC-1522, a novel Trop2-targeting degrader-antibody conjugate for precision oncology**

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Introduction

Antibody-drug conjugates (ADCs) have revolutionized cancer therapies by enabling the targeted delivery of cytotoxic payloads to tumor cells. However, their efficacy and safety are constrained by the limited repertoire of payloads. Notably, all FDA-approved Trop2-targeting ADCs currently rely on topoisomerase inhibitors as payloads, which may lead to both intrinsic and acquired drug resistance. Degrader-antibody conjugates (DACs) have emerged as a next-generation approach, combining the precision targeting of ADCs with the diverse options of protein degraders to overcome these challenges. Our study aimed to develop a Trop-2 targeting DAC with the potential to surpass the limitations of traditional ADCs.

Material and method

We screened a library of BET degraders using MDA-MB-231 and BT-474 cell lines using the CCK8 assay and confirmed their degradation efficacy via Western blot analysis. The selected payload candidates were subsequently converted into linker-drug conjugates and attached to antibodies to generate DACs. The drug-to-antibody ratio (DAR) and aggregation levels were characterized using reversed-phase liquid chromatography (RPLC) and size-exclusion chromatography (SEC). The in vitro potency and selectivity of representative DACs were assessed across a panel of cancer cell lines, including MDA-MB-231, JIMT-1, BXPC-3, KP-4, and NCI-N87. Additionally, their in vivo anticancer efficacy was investigated in JIMT-1, MIA PaCa-2, and BXPC-3 cell-derived xenograft (CDX) models.

Result and discussion

Several BET degraders demonstrated significantly enhanced cytotoxicity and degradation efficacy. The corresponding DACs achieved the designated DAR without aggregation. In vitro studies showed that DAC-1522 exhibits potent antiproliferative activity against multiple human cancer cell lines. Its efficacy is strongly correlated with Trop2 expression, ensuring selective cytotoxicity in Trop2-positive cells while sparing Trop2-negative cells, thereby offering a favorable therapeutic window. Notably, DAC-1522 retains uncompromised antiproliferative activity in an ADC-induced acquired drug resistance model, underscoring its potential to overcome resistance following ADC treatments. In vivo, a single intravenous injection of DAC-1522 achieves complete tumor regression in a human pancreatic cancer

BXPC-3 xenograft model, significantly outperforming Trodelyv® in efficacy. No obvious safety concerns were observed.

Conclusion

Our findings strongly support the further development of DAC-1522 as a highly promising therapeutic candidate for Trop2-expressing cancers.

EACR25-0622**Punicalagin-mediated suppression of oncogenic properties in ovarian epithelial adenocarcinoma cells**

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Introduction

Ovarian cancer ranks among the most prevalent cancers in women, presenting considerable obstacles to effective treatment. Chemotherapy, frequently employed as adjunct post-surgery or as neoadjuvant therapy, is fundamental in the fight against the disease. Phytochemicals may serve as prospective chemo-therapeutic drugs to alleviate these limitations. Punicalagin, a polyphenolic molecule in pomegranates, has attracted interest in its significant therapeutic capabilities, especially its anticancer effects. This study aims to investigate the anti-proliferative and anti-migratory effects of Punicalagin on ovarian cancer utilizing the OVCAR-3 and SKOV-3 cells.

Material and method

Cell proliferation was measured by six distinct concentrations of punicalagin (6.25, 12.5, 25, 50, 100, and 200 µM) during 24-, 48-, and 72-hour incubation durations. Furthermore, a colony formation experiment was conducted to assess the capacity of cells to establish colonies following treatment with punicalagin. Moreover, wound healing and transwell migration experiments were performed to evaluate the migratory capacity of ovarian cancer cells. The MitoSOXTM assay was employed to identify mitochondrial reactive oxygen species (ROS). The rate of apoptosis was determined using the flow cytometry (Annexin V/PI staining), while the autophagy was confirmed using the acridine orange staining. Western blot assay was used to verify the findings obtained.

Result and discussion

Our findings demonstrated a substantial suppression of tumor cell proliferation and colony formation capacity in a dose- and time-dependent manner. Wound healing and transwell migration experiments indicated that Punicalagin inhibits the migratory capacity of ovarian cancer cells. Additionally, the MitoSOXTM assay demonstrated the involvement of reactive oxygen species in the effectiveness of Punicalagin in ovarian cancer cells. Acridine orange staining demonstrated autophagy in OVCAR-3 cells only, while flow cytometry analysis confirmed apoptosis in both cell lines. An increase in the E-cadherin, BAX, and a decrease in N-cadherin were confirmed in both cell lines. At the same time, the conversion of LC3-I to LC3-II was noted in the OVCAR-3 cells.

Conclusion

Punicalagin exhibits promising anticancer properties and may be a valuable chemotherapeutic agent in treating ovarian adenocarcinoma.

EACR25-0623

Anti-cancer effects of Amiodarone via cell cycle arrest, mitochondrial dysfunction, excessive autophagy, and increased apoptosis in thyroid cancer cells

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Introduction

Amiodarone (AMD), an antiarrhythmic drug approved by the World Health Organization in 2005, inhibits various ion channels, including sodium, potassium, and calcium channels. These ion channels play a crucial role not only in normal cells but also in cancer cells. Recent studies have suggested the potential anticancer effects of AMD due to its pharmacological properties. This study aims to confirm the anticancer effect of AMD and its underlying mechanism in thyroid cancer cells.

Material and method

In this study, the thyroid cancer SNU-790 cells were used. WST assay was performed to determine whether AMD affects cell viability in SNU-790 cells.

Subsequently, a trans-well migration assay was conducted to evaluate the migratory effects of AMD. A cell cycle arrest assay using PI staining and an Annexin V/PI staining assay were performed to evaluate the proliferative ability and apoptotic cell population rate. MitoSOX™ staining and 2',7'-dichlorodihydrofluorescein diacetate (DCF-DA) staining assay were used to measure mitochondrial and cytosolic ROS levels, respectively. The change in mitochondrial membrane potential (MMP) was detected by JC-10 staining assay. Additionally, acridine orange (A/O) staining was performed to investigate whether AMD may induce autophagy.

Result and discussion

WST assay revealed a decrease in cell viability with increasing AMD concentrations. Subsequent experiments were conducted using concentrations based on IC₅₀ value (12 µM). Trans-well migration assay demonstrated that higher AMD concentrations inhibited cell migration. MitoSOX™ and DCF-DA staining assay indicated that AMD treatment led to increased mitochondrial and cytosolic ROS levels. Similarly, JC-10 staining assay was able to depict a concentration-dependent decrease in MMP, indicating mitochondrial dysfunction in SNU-790 cells. Furthermore, Annexin V/PI staining revealed an increase in the rate of apoptosis with increasing AMD concentrations. Additionally, since AMD is widely recognized as an autophagy inducer, A/O staining was performed to assess autophagy induction in thyroid cancer cells. The results demonstrated that autophagy activation increased with higher AMD concentrations.

Conclusion

Amiodarone induces cell cycle arrest, mitochondrial dysfunction, excessive autophagy, and increased

apoptosis in SNU-790 thyroid cancer cells by increasing cytosolic and mitochondrial ROS levels.

EACR25-0627

UD-086 overcomes trastuzumab deruxtecan-resistance and has a best-in-class therapeutic index in MMAE-based antibody drug conjugates

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Introduction

Although many antibody drug conjugates (ADCs) have been currently approved, they still face the problem of difficulty in having sufficient therapeutic index due to off-target toxicity caused by linker instability. To solve this problem, we developed a novel UBE's versatile hydrophilic (UVH) linker and auristatin E (AE) prodrugs. UVH linker contains a novel, highly hydrophilic amino acid derivative enables high stability in the blood with the maximum drug to antibody ratio (DAR) of 16. This linker is cleaved by Cathepsin B. AE prodrug is significantly less toxic than AE due to its lower cell membrane permeability. On the other hand, AE prodrugs released from ADCs taken up by cancer cells are converted to AE in the cancer cells and exhibit potent cytotoxic activity. Using this state-of-the-art linker and prodrug, we generated UD-086, a next generation HER2-directed ADC consisting of trastuzumab, UVH linker, and AE prodrugs with a DAR of eight and evaluated in vitro and in vivo efficacy and the safety profile.

Material and method

The drug-linkers were conjugated to trastuzumab via their maleimide groups to prepare UD-086. Linker stability of UD-086 in human serum was evaluated for up to 168 h of incubation. The amounts of released payloads were measured by LC-MS/MS. In vitro cytotoxicity of AE prodrug and AE was tested in NCI-N87 cells. In vitro bystander killing effect of UD-086 was evaluated in a co-culture system of NCI-N87 (HER2-positive) and MDA-MB-231 (HER2-negative). The pharmacokinetic (PK) profile of UD-086 was determined in BALB/c mice. In vivo efficacy of UD-086 was evaluated in NCI-N87, DXd-resistant NCI-N87 and JIMT-1 cells xenograft mouse models. Toxicity study of UD-086 was conducted in cynomolgus monkeys.

Result and discussion

UD-086 showed much higher linker stability in human serum in vitro than trastuzumab deruxtecan (DS-8201a). AE prodrug was stable in human serum, and it was approximately 300 times less toxic than AE in NCI-N87 cells. UD-086 indicated in vitro bystander killing effect as well as DS-8201a. UD-086 demonstrated a comparable total antibody PK profile to trastuzumab. In NCI-N87 xenograft model, UD-086 showed three to 10-fold potent efficacy than DS-8201a and 10-fold potent efficacy than disitamab vedotin, respectively. In NCI-N87 xenograft model, the minimum effective dose of UD-086 was 1 mg/kg. In DXd-resistant NCI-N87 and JIMT-1 xenograft models, DS-8201a resistance was

exhibited, and UD-086 showed more than 30-fold potent efficacy than DS-8201a. The maximum tolerated dose of UD-086 was 6 mg/kg in cynomolgus monkey (Q3W×3, non-GLP).

Conclusion

The therapeutic index of UD-086 is six. UD-086 shows strong efficacy in the DS-8201a resistance tumor models. Despite the conjugating AE with DAR of eight, UD-086 shows very high blood stability and has a significantly improved best in class therapeutic index compared to previous MMAE-based ADCs.

EACR25-0640

Risk and benefit for basket clinical trials in oncology: a systematic review with meta-analysis

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Introduction

Basket clinical trials (BCTs) are human research studies commonly used in precision medicine. BCT simultaneously test a therapeutic intervention for multiple malignancy types under the same study protocol. Patients with different cancers recruited to BCT share specific molecular characteristics which are predictive of clinical benefit from the experimental treatment. Our aim was to evaluate risks and benefits in cancer BCTs.

Material and method

The study protocol was prospectively registered in PROSPERO [1]. We systematically searched Embase, PubMed, and ClinicalTrials.gov for interventional cancer BCTs published between 1 January 2001, and 14 June 2023. We included targeted therapy and/or immunotherapy studies of all cancer types. We measured risk using drug-related grade 3 or higher adverse events (AEs), and benefit by objective response rate (ORR), progression-free survival, and overall survival.

Result and discussion

We identified 126 arms of 75 BCTs (7,659 patients) that met our eligibility criteria. The overall fatal treatment-related AE rate (grade 5) was 0.7% (95% confidence interval [CI] 0.4–1.0), and 30.4% (95% CI 24.2–36.7) of patients experienced grade 3–4 drug-related toxicity. The overall drug-related grades 3–5 rate was higher for targeted therapies than immunotherapies: 31.7% (95% CI 25.6–37.8) vs 7.9% (95% CI 0.0–23.1), $p = 0.004$. The pooled overall ORR was 18.0% (95% CI 14.8–21.1) and was lower in solid tumors, 17.5% (95% CI 14.3–20.8), compared with hematological malignancies, 63.6% (95% CI 36.3–90.9); $p = 0.004$. The median progression-free survival was 3.1 months (95% CI 2.6–3.9), and the median overall survival was 8.9 months (95% CI 6.7–10.2).

Conclusion

Cancer patients should be properly informed about the benefits and risks in BCTs. Our data suggest that nearly four out of five participants did not respond to treatment, one in 135 died because of drug toxicity, and nearly one

third experienced grade 3–4 treatment-related AEs. While we acknowledge that our systematic analyses do not cover all aspects related to benefits and risks in cancer BCTs, we note that efforts should be made to improve the data reporting that allows for such assessments.

[1] CRD42023406401

EACR25-0642

Exploring the Cytotoxic Potential of Calcite on MCF-7 Cells: A Promising Approach for Breast Cancer Treatment

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Introduction

Breast cancer is the most common malignancy in women and a leading cause of cancer-related mortality worldwide. Despite therapeutic advancements such as chemotherapy, radiation, and biological medications, challenges like recurrence and metastasis remain, highlighting the need for novel treatment strategies. While calcium carbonate has been shown to inhibit the progression of lung cancer and induce oxidative damage in HeLa cells, its effects on breast cancer cells remain inadequately studied. This research demonstrates cytotoxic impact of calcite, the most abundant crystalline form of calcium carbonate, on MCF-7 human breast adenocarcinoma cells *in vitro*.

Material and method

Cellular metabolic activity was evaluated using an MTT assay, and live-dead cell viability was determined by Hoechst-Propidium Iodide (PI) staining. The interaction between calcite and cells was visualized via Scanning Electron Microscopy (SEM). Cell proliferation was assessed using Ki-67 immunofluorescence. Additionally, clonogenic assay evaluated the ability of MCF-7 cells to form colonies. Scratch wound assays were performed to assess cell migration, while apoptosis was analyzed through AO-EtBr staining and cell cycle progression was examined by flow cytometry.

Result and discussion

The cytotoxic effects of calcite on MCF-7 cells were assessed across various concentrations (0.01–1 mg/ml) and time points. MTT assay results showed a significant, dose- and time-dependent decrease in cell viability. At day 3, 0.1 mg/ml calcite resulted in a 21% reduction in viability, while 1 mg/ml caused a 60% inhibition, indicating calcite's potent effect on cell viability. Calcite also reduced cell proliferation. At 0.1 mg/ml, Ki-67 expression was decreased by 59%, and at 1 mg/ml, by 36%, compared to the control (61.75%). This correlated with a reduction in colony formation, where calcite-treated cells showed a 31% and 83% reduction at 0.1 mg/ml and 1 mg/ml, respectively, highlighting calcite's potential to inhibit proliferation and colony formation. The effect on migration showed significant inhibition, with a 41% reduction at 0.1 mg/ml and 80% at 1 mg/ml, suggesting calcite interferes with cell motility. Apoptosis analysis revealed a 13-fold increase in necrotic cells at 1 mg/ml and 8-fold at 0.1 mg/ml compared to controls, indicating calcite induces cell death through necrosis,

particularly at higher concentrations. Additionally, calcite treatment caused dose-dependent G0/G1 phase arrest, reducing cells in S and G2/M phase suggesting that this cell cycle arrest may contribute to observed reductions in proliferation and migration.

Conclusion

In summary, calcite effectively inhibits MCF-7 cell growth by decreasing viability, limiting proliferation and migration, and inducing necrosis and cell cycle arrest. These results support further investigation into calcite as a potential therapeutic agent in the treatment of breast cancer.

EACR25-0653

Selective degradation of MLK3 by novel CEP1347-VHL-02 PROTAC compound limits oncogenic potential of TNBC

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Introduction

Triple-negative breast cancer (TNBC) accounts for 20% of all breast cancer (BC) cases and is associated with the worst prognosis because of the lack of effective therapies. Mixed-lineage protein kinase 3 (MLK3, MAP3K11) is a member of the MLK family of serine/threonine kinases, which belongs to mitogen-activated protein kinase kinase kinases (MAP3Ks). MLK3 expression is significantly elevated in TNBC cell lines compared with epithelial non-tumorigenic breast cell lines and other BC subtypes and involved in the high tumorigenic potential of TNBC cells. Proteolysis-targeting chimeras (PROTACs) represent a major targeted protein degradation technology that allow for the selective targeting of protein of interest (POI) in cancer cells. PROTACs are chemically synthesized heterobifunctional compounds consisting of three elements: a ligand for the POI that we want to degrade, ligand for recruiting an E3 ligase and an appropriately designed linker between those two ligands. PROTAC molecules simultaneous bind the POI and E3 ligase together forming a ternary complex, and due to proximity of target protein to E3 ligase complex, a lysine residues within the POI are ubiquitinated and protein is targeted to proteasomal degradation.

Material and method

Here, we present a novel, potent, and selective MLK3 degrader, CEP1347-VHL-02, based on the pan-MLK inhibitor CEP1347 and ligand for E3 ligase von Hippel-

Lindau (VHL), by employing proteolysis-targeting chimera (PROTAC) technology.

Result and discussion

Our synthesized compound effectively targeted MLK3 for degradation via the ubiquitin-proteasome system in several cell line models but did not degrade other members of the MLK family. Furthermore, we showed that CEP1347-VHL-02 robustly degraded MLK3 and inhibited its oncogenic activity in TNBC, measured as a reduction of clonogenic and migratory potential, cell cycle arrest, and the induction of apoptosis in MDA-MB-468 cells.

Conclusion

In conclusion, we present first-in-class PROTAC that induces the rapid and selective degradation of MLK3 in cancer cell lines. Our study provides new opportunities for the selective targeting of MLK3 in cancer and paves the way for the development of next-generation degraders against MLK3 and related kinases. Notably, MLK3 degradation by CEP1347-VHL-02 significantly reduced the oncogenic potential of TNBC cells, suggesting that the targeted degradation of this kinase may be a feasible treatment strategy for this subtype of breast cancer.

EACR25-0660

Design and synthesis of CDK9/EZH2 dual-target inhibitors to achieve synergistic antitumor effects

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Introduction

Cyclin-dependent kinase 9 (CDK9) plays a pivotal role in regulating transcriptional elongation, and has emerged as a promising target in cancer therapy. However, our previous research has demonstrated that CDK9 inhibitors induce abnormal upregulation of H3K27me3 in diffuse large B-cell lymphoma (DLBCL) cell lines, impairing the inhibitory effect on tumor cells proliferation. Interestingly, EZH2 inhibitors can reverse the upregulation of H3K27me3, leading to synergistic antitumor effects.

Material and method

Based on this observation, we designed and synthesized a series of dual inhibitors targeting both CDK9 and EZH2 through fusion based strategies to achieve enhanced antitumor activity.

Result and discussion

Among the compounds developed, the potent CDK9/EZH2 dual inhibitor D16 exhibited impressive and balanced inhibitory properties of both targets, with IC50 values of 67 nM for CDK9 and 84 nM for EZH2.

Notably, compound D16 induced greater DNA damage and exhibited stronger inhibitory effects on DLBCL proliferation compared to the single-target inhibitor SNS-032 or C24. In addition, D16 showed potent anti-proliferative activities in various solid tumor cell lines, which may provide an innovative strategy for the treatment of cancer.

Conclusion

All these findings highlight the potential of dual CDK9/EZH2 inhibitors as a novel approach for cancer therapy.

EACR25-0667

EZH2 inhibitors dramatically sensitize breast cancers to HER2 kinase inhibitors through cooperative effects on YAP and pro-apoptotic regulators

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Introduction

The receptor tyrosine kinase HER2 is overexpressed and/or amplified in around 20% of breast cancers and functions as an oncogenic driver. While HER2 inhibitors have dramatically improved the clinical outcome of HER2+ breast cancer, metastatic disease remains incurable due to intrinsic and acquired resistance. Therefore, there is an urgent need to develop more effective therapeutic strategies that will induce deeper initial responses and delay or prevent relapse. To this aim, we sought to identify additional oncogenic targets that when inhibited, potentiate the effects of HER2 inhibitors. We considered the epigenetic regulator EZH2, which represses transcription by depositing trimethyl marks at H3K27, as such co-target since 1) it is over-expressed in 84% of HER2+ breast cancers, including stage III and stage IV tumors, and 2) has been shown to contribute to breast cancer development and metastasis.

Material and method

We investigated the therapeutic effects of combined EZH2 and HER2 inhibitors in multiple human HER2+ breast cancer cell lines in vitro and in xenograft models in vivo. To identify the molecular mechanism by which these agents cooperate we performed RNA-seq and ChIP-qPCR experiments. The molecular mediators identified were further validated with genetic knockdown or chemical inhibition experiments.

Result and discussion

We have found that EZH2 inhibitors shift the epigenetic state of HER2+ tumors, dramatically enhancing baseline responses to HER2 kinase inhibitors and re-sensitizing drug resistant tumors in vitro and in vivo. Specifically, we report that EZH2 normally silences the pro-apoptotic gene, BMF, by methylating H3K27 at the BMF locus. EZH2 inhibitors promote the release of H3K27me3, but this stimulates the binding of repressive YAP/TEAD complexes which still restrict its expression. However, in the presence of EZH2 inhibitors, HER2 kinase inhibitors trigger the dissociation of repressive YAP/TEAD

complexes, potently upregulate BMF, and kill resistant cells. Accordingly, EZH2 inhibitors similarly cooperate with genetic or chemical inhibitors of YAP/TEAD, which also induce BMF expression and trigger apoptosis.

Conclusion

Together these studies show how EZH2 and YAP coordinately insulate the BMF locus and demonstrate that EZH2 inhibitors can be used to reprogram HER2+ tumors, resulting in a dramatic sensitization to HER2 kinase inhibitors and enhanced killing of residual disease.

EACR25-0683

Pre-clinical Evidence for Ivacaftor as a Potential Repurposed Therapy for High-Grade Serous Ovarian Cancer and Endometrial Cancer

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Introduction

The receptor tyrosine kinase-like orphan receptor 1 (ROR1) has been identified as an attractive target for ovarian and endometrial cancer therapy. Using the pipeline established from a large collaborative drug repurposing project, we identified Ivacaftor, an FDA-approved cystic fibrosis medication, as a candidate predicted to interact with ROR1. This study aimed to provide preclinical evidence supporting the potential repurposing of Ivacaftor for HGSOC and endometrial cancer treatment using 2D preclinical models as well as 3D patient-derived organoid models in vitro.

Material and method

Dose response analysis was undertaken in ROR1 expressing HGSOC cell lines OVCAR4, KURAMOCHI, COV362 and COV318 as well as endometrial cancer cell lines Ishikawa and ARK1 using a serial dilution of eight concentrations of Ivacaftor (3.9 μM to 100 μM). Cell viability was assessed at 72 hours post drug treatment using the MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide) assay. Cells were treated with 15uM ivacaftor and 30uM carboplatin for 24, 48, and 72 hrs for flow cytometry analysis using Apoptosis, DNA Damage and Cell Proliferation Kit (BD Biosciences) which stained for fluorophores against cleaved PARP, H2Ax, and BrdU. Additionally, ROR1-expressing HGSOC and molecularly classified endometrial cancer patient-derived organoids (n = 3 for HGSOC and n = 6 for endometrial cancer) underwent dose-response analysis, with CellTiter Glo-based viability measurements determined at 72 hours post-treatment. Finally, the mechanisms associated with Ivacaftor treatment were

explored in the cell lines and organoids through Western blotting, flow cytometry and real-time Annexin V assays.

Result and discussion

The IC₅₀ for Ivacaftor ranged from 6.5 to 13.2 μM in 2D HGSOC cell lines cultured in 2D and 0.004 to 1.5 μM in endometrial cancer cell lines. In organoid models, IC₅₀ values ranged from 11.6 to 15.1 μM for HGSOC and from 0.4 to 18.6 μM for endometrial cancer. Notably, mismatch repair deficient (MMRd) endometrial cancer models exhibited the highest sensitivity, with IC₅₀ values lower than 1 μM, in contrast to p53 wildtype and p53 abnormal models. Ivacaftor treatment induces cell apoptosis similar to carboplatin treatment, but does not increase DNA damage in the cells, as opposed to carboplatin treatment. Ivacaftor treatment does not cause an increase in cells stalling in S phase. In addition, Ivacaftor treatment suppressed tumour stemness and modulated ROR1 signaling associated oncogenic pathways including the PI3K/AKT pathway and epithelial to mesenchymal transition (EMT).

Conclusion

In conclusion, Ivacaftor demonstrated significant single agent anti-tumour potential in preclinical HGSOC and endometrial cancer models, supporting its further investigation as a repurposed therapy for ROR1-expressing ovarian and endometrial cancers.

EACR25-0701

Synergistic antineoplastic Effects of *Jania rubens* Extracts with Conventional Chemotherapy in Colorectal Cancer

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Introduction

Colorectal cancer (CRC) remains a major global health burden, driving the need for novel, less toxic therapeutic strategies. This study evaluated the potential of bioactive compounds from the Mediterranean red coral seaweed, *Jania rubens*, as adjuvants to standard chemotherapies (Doxorubicin, Capecitabine, and Irinotecan) in CRC treatment.

Material and method

MTT and colony formation assays were used to assess CRC cell viability following treatment with *J. rubens* extracts, alone and in combination with chemotherapeutic agents. Cell cycle analysis and reactive oxygen species (ROS) measurements were performed to determine mechanisms of cytotoxicity. Wound healing and zymography assays were conducted to evaluate cell migration and metastatic potential, respectively. Epithelial-mesenchymal transition (EMT) was assessed by analyzing the expression of N-cadherin, Twist, and Snail

Result and discussion

Our findings demonstrate a synergistic anticancer effect. *J. rubens* extracts, when combined with chemotherapeutic agents, significantly reduced CRC cell viability, as evidenced by MTT and colony formation assays. This enhanced cytotoxicity was associated with the induction of G0/G1 cell cycle arrest and a marked increase in intracellular ROS production. Furthermore, wound healing and zymography assays revealed a dose-dependent reduction in CRC cell migration and metastatic potential, demonstrated by decreased MMP-2 and MMP-9 enzymatic activities. Importantly, the extracts effectively suppressed EMT, as indicated by the downregulation of N-cadherin, Twist, and Snail

Conclusion

These results underline the significant potential of *J. rubens* bioactives as adjuvants to conventional CRC chemotherapy and provide a strong rationale for further preclinical investigations to explore the therapeutic potential of *J. rubens* extracts in CRC management

EACR25-0709

1959-sss/DM4 Antibody-Drug Conjugate targeting extracellular LGALS3BP Exhibits Potent Antitumor Activity in orthotopic Glioblastoma Models

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Introduction

LGALS3BP is a hyperglycosylated secreted protein overexpressed in various human malignancies. Recently, it has been identified as one of the most abundant proteins in extracellular vesicles secreted by cancer cells. Furthermore, studies of biological fluids such as blood, urine, and saliva highlight LGALS3BP as a promising biomarker and therapeutic target. Our group has developed a specific antibody-drug conjugate (ADC) targeting LGALS3BP, named 1959-sss/DM4, demonstrating potent antitumor activity across multiple models, including glioblastoma. In this study, we explored its potential as both a biomarker and therapeutic target using an orthotopic patient-derived glioblastoma model.

Material and method

Orthotopic glioblastoma (GBM) models were established using both commercially available LUC-U87MG cells

and patient-derived cell lines. Tumor tissues were analyzed by IHC and IF to assess antibody penetration into the brain and evaluate ADC anti-tumor efficacy. Circulating tumor-derived LGALS3BP in mice plasma samples was quantified using a sandwich ELISA with the therapeutic 1959 antibody. Additionally, PE-conjugated 1959 therapeutic antibody was used in flow cytometry to specifically detect LGALS3BP-positive extracellular vesicles (EVs) in patients' blood samples.

Result and discussion

We first established an orthotopic GBM preclinical model using U87MG-LUC cells and observed a significant reduction of tumor mass in treated animals, as revealed by LMI signals. Interestingly, an ELISA assay on serum from tumor-bearing mice quantified human circulating LGALS3BP protein, suggesting its potential as biomarker for the disease in this model. We then transitioned to a patient-derived orthotopic model and, in an initial study, we confirmed by immunofluorescence staining that 1959 antibody successfully reached the brain lesion and accumulated within the tumor 72 hours post-injection. Furthermore, we observed that ADC treatment induced mitotic catastrophe in GBM tissues and enhanced tumoral immune infiltration, particularly by B lymphocytes and macrophages. Next, we employed liquid biopsy to detect brain tumors in injected mice in order to design a mouse trial experiment. Indeed, following brain tumor detection via liquid biopsy, mice were treated with either PBS or 1959-sss/DM4 (10 mg/kg). Two weeks after the final treatment, we observed a significant reduction in tumor mass and the complete absence of circulating LGALS3BP, as confirmed by ELISA analysis of plasma samples. Finally, we developed a polychromatic flow cytometry method based on the use of 1959-PE antibody able to identify and count LGALS3BP + circulating EVs in GBM patients' blood samples.

Conclusion

Our findings strongly suggest that circulating LGALS3BP plays a critical role in GBM as both a potential biomarker and therapeutic target, warranting further preclinical and clinical validation.

EACR25-0718

Investigating the Antitumour Potential of Fucoidan with Everolimus: An *in vitro* and *in vivo* Study Focusing on ER α /Akt/mTOR Pathway Suppression

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Introduction

Breast cancer (BC) remains one of the most prevalent cancers worldwide, with resistance and relapse posing significant challenges despite advances in therapy. This

study explores the chemomodulatory effect of fucoidan, a sulfated polysaccharide abundant in edible seaweeds, in combination with everolimus, a clinically approved mammalian target of rapamycin (mTOR) inhibitor.

Material and method

MCF-7 human BC cell line was used *in vitro*. MTT cell viability assay was used to calculate the IC₅₀ of the drugs while the scratch wound assay assessed the effect of the drug combinations on cancer cell motility. Flow cytometry was used for cell cycle analysis and Annexin V/PI apoptosis assays. *In vivo*, forty female Swiss Albino mice (23 ± 2 g) were injected intramuscularly with Ehrlich Ascites Carcinoma (EAC) cells into the hind limb. After tumour development, mice were randomly divided into four equal groups ($n = 10$): untreated control, fucoidan, everolimus, or their combination (E+F). After sacrifice, ELISA was used to evaluate various markers in the tumour tissues [1].

Result and discussion

In vitro, fucoidan synergistically reduced the IC₅₀ of everolimus from >500 nM to 76.6 nM. The scratch wound assay showed significantly lower percentage wound closure in the combination group (19.07%) compared to everolimus alone (28.13%) and control (75.65%) by day 3 ($p < 0.05$). Cell cycle analysis revealed that E+F treated cells had a significantly higher percentage of S-phase arrest (77%) and a lower percentage in G2/M (19.4%) compared to the control ($p \leq 0.0001$). The Annexin V/PI apoptosis assay showed a significant reduction in viable cells (95.56% in control versus 87.7% in E+F). Furthermore, the percentage of late apoptotic and necrotic cells were significantly higher in E+F group compared to the control group (2.8% versus 1.9% and 9.15% versus 1.97%, respectively). *In vivo*, the combination therapy significantly reduced tumour progression. Hematoxylin and eosin staining of control tumours showed extensive sheets of pleomorphic viable cells, whereas the E+F group exhibited large necrotic masses with abundant fragmented and pyknotic cancer cells. No significant weight loss was observed, indicating minimal toxicity from the given therapies. ELISA analysis of tumour tissues revealed a significant reduction in survivin, ER α , VEGF, and the downstream oncoproteins p-mTOR and p-AKT in the E+F group.

Conclusion

This study provides the first preclinical evidence supporting the efficacy of fucoidan with everolimus in BC. The promising anticancer effects appear to be mediated, at least in part, by fucoidan-induced suppression of the ER α /Akt/mTOR axis. Further investigations are required to validate these findings.

[1] All animal experimental procedures were approved by the research ethics committee of the Faculty of Pharmacy, Cairo University, in compliance with EU Directive 2010/EU/63.

EACR25-0741

Bufalin Targets Protein Translation and Cell Metabolism to Overcome Therapy Resistance in Melanoma

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Introduction

Melanoma is an aggressive skin cancer with frequent resistance to targeted therapies against the mutant BRAF pathway, limiting treatment options. Persister cells, an intermediate state between sensitivity and resistance, contribute to therapy escape through translation re-programming and metabolic adaptation. Bufalin, a cardiotonic steroid, has shown anti-cancer properties in melanoma by targeting the Na⁺/K⁺ ATPase α-subunit (ATP1A1). Our study investigates Bufalin effects on translation (c-MYC/eIF4A loop) and metabolism (β-oxidation, phospholipid remodeling, mitochondrial function) to evaluate its therapeutic potential.

Material and method

Bufalin effects were assessed on a panel of melanoma cell lines representing key genetic backgrounds (BRAF-sensitive and resistant to targeted therapies, NRAS, and WT). Cell viability was measured using Crystal Violet staining. ¹H-NMR spectroscopy was used to analyse global metabolic alterations, with a focus on phospholipid and amino acid metabolism. Polysome profiling was performed to evaluate translation dynamics, particularly the regulation of the c-MYC/eIF4A loop. Finally, RT-qPCR and Western Blot were used to assess the expression of β-oxidation enzymes and lipid transporters.

Result and discussion

Bufalin significantly reduced the viability of all tested melanoma cell lines. ¹H-NMR spectroscopy revealed major metabolic alterations, including amino acid depletion (alanine, glycine, asparagine, leucine), phospholipid remodeling, and mitochondrial stress. Notably, we observed a 4-fold increase in glycerophosphocholine (GPC) level and a depletion of phosphocholine and choline, suggesting a disruption in membrane homeostasis and choline metabolism. This correlated with differential expression of GPD5 and GPD6, two key regulators of the phospholipid turnover. Polysome profiling showed that Bufalin induces a global reduction in translation, driven by c-MYC down-regulation (RT-qPCR, Western Blot) and decreased eIF4A transcription (RT-qPCR), impacting the eIF4F complex. Consequently, translation of CREBBP, a key eIF4F target, was also reduced. Bufalin further inhibited β-oxidation by downregulating key enzymes (CPT1A, ACADS, ACOX) and the lipid transporter CD36. Mitochondrial function was significantly altered, as indicated by a complete depletion of pantothenate (a precursor of Coenzyme A, CoA) and a reduction in spare respiratory capacity (Seahorse analysis), suggesting a loss of metabolic flexibility and impaired energy production.

Conclusion

Buflin disrupts translation and metabolic adaptability, two key mechanisms of therapy resistance. By inhibiting c-MYC/eIF4A, it reduces protein synthesis, while its effects on phospholipid homeostasis, β-oxidation, and mitochondrial metabolism impair metabolic flexibility. These findings suggest Buflin could limit persister cell survival and help counteract resistance mechanisms in melanoma.

EACR25-0762

Novel Tamoxifen Derivatives Suppress Breast Cancer Growth and Alter Cancer-Related Gene Expression

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Introduction

Breast cancer (BC) is the most commonly diagnosed cancer and the leading cause of cancer-related mortality among women worldwide. In 2022, approximately 2.3 million new cases were reported, resulting in 665,684 deaths and accounting for 15.4% of all cancer-related deaths in women. Tamoxifen, a selective estrogen receptor modulator (SERM), is a cornerstone in the treatment of hormone receptor-positive (HR+) BC. However, its therapeutic efficacy is often compromised by resistance and adverse effects. Structural modifications, such as the introduction of novel functional groups, have the potential to enhance its anti-cancer properties. This study investigates the cytotoxic and anti-cancer effects of newly synthesized tamoxifen derivatives on breast cancer cells, aiming to improve therapeutic strategies for BC treatment.

Material and method

We synthesized six novel tamoxifen derivatives and assessed their cytotoxicity using the sulforhodamine B (SRB) assay in four human breast cancer cell lines (MCF-7, T47-D, SK-BR-3, MDA-MB-231) and two non-tumorigenic human breast epithelial cell lines (MCF-10A, MCF-12A). The most potent compound, identified through preliminary screening, underwent further in vitro characterization to elucidate its mechanism of action. This included cell cycle analysis via propidium iodide (PI) staining, apoptosis evaluation through Annexin V/7-AAD staining, and quantitative PCR (qPCR) analysis of key cancer-related genes.

Result and discussion

Cytotoxicity screening identified GG009 as the most potent tamoxifen derivative, exhibiting significant anti-proliferative activity in all four breast cancer cell lines, particularly MCF-7 and T47-D, while demonstrating lower cytotoxicity in normal breast epithelial cells. Further mechanistic studies revealed that GG009 induced cell cycle alterations and increased apoptotic cell populations in MCF-7 and T47-D cells. Additionally, qPCR analysis of nearly 90 genes associated with cancer progression showed that GG009 treatment downregulated several oncogenic genes linked to aggressive cancer phenotypes and poor prognosis in breast cancer.

Conclusion

Our findings highlight the promising anti-cancer role of the novel tamoxifen derivative GG009 against breast cancer cells. Gene expression analysis also suggests that GG009 may modulate key cancer-related pathways, demonstrating the need for further investigation into its mechanism of action to enhance breast cancer therapy.

EACR25-0771**Targeting Copper Availability to Enhance Thymoquinone's Cytotoxicity in Colorectal Cancer***D. Ismail¹, H. Muhtasib¹*¹*Faculty of Arts and Sciences, American University of Beirut, Biology, Beirut, Lebanon***Introduction**

Cancerous tumors have been shown to display higher levels of copper (Cu) than normal tissues. Previous studies have demonstrated that Cu can transform thymoquinone (TQ), an antioxidant phytochemical exhibiting a safe profile and remarkable antineoplastic effects against many types of cancer, into a prooxidant agent, generating reactive oxygen species (ROS) and promoting cellular death. However, the mechanism by which Cu improves the sensitivity of cancer cells to TQ remains unclear. In particular, this mechanism has not yet been elucidated in colorectal cancer.

Material and method

In this project, we investigated TQ's effect on HT-29 and HCT-116 colorectal cancer cell lines enriched with Cu to mimic in vivo conditions. We have performed MTT viability assays, Cell-cycle analysis, Apoptosis assay, Intracellular ROS measurement, and in vivo studies in NOD-SCID mice to examine the effect of TQ on Cu-associated cancer progression.

Result and discussion

In this project, we investigated TQ's effect on HT-29 and HCT-116 colorectal cancer cell lines enriched with Cu to mimic in vivo conditions. Our findings indicate that Cu sensitized HT-29 cells to TQ by enhancing its inhibitory actions leading to an improved reduction in cell viability compared to Cu-free conditions. After 24 hours of treatment, the IC₅₀ of TQ in Cu-free HT-29 cells was 111 µM which decreased significantly to 42.8 µM in their Cu-treated counterparts. In addition, under Cu-treated conditions, TQ treatment led to an enhanced G2/M arrest, a significant increase in intracellular ROS levels at 40 and 80 µM, and a higher percentage of HT-29 cells undergoing late-stage apoptosis at 40 µM, compared to Cu-free conditions. TQ also induced a time and dose-dependent inhibitory effect on HCT-116 cells, yet no difference was observed between Cu-free cells and those enriched with Cu. For that, we further aim to study the role of Cu in HCT-116 cells treated with TQ in the presence of the Cu chelator TTM. Notably, in non-cancerous FHs74Int cells, Cu supplementation did not enhance TQ cytotoxicity, suggesting a cancer-selective effect. To gain deeper insights into the Cu-TQ interaction, we will examine the impact of TQ on Cu uptake machinery and downstream signaling pathways by assessing DNA damage and evaluating the induction of apoptosis and oxidative stress. Additionally, ongoing in-vivo experiments using NOD-SCID mice aim to examine the effect of TQ on Cu-associated cancer progression.

Conclusion

Our findings demonstrate that Cu supplementation will enhance the cytotoxic effects of TQ on colorectal cancer, thereby increasing their sensitivity to this natural product. In conclusion, our study could provide an innovative therapeutic approach against colorectal cancer where we

can use the increased level of Cu to target cancer cells while sparing the normal ones.

EACR25-0779**FAM46C expression sensitizes multiple myeloma cells to PF-543-induced cytotoxicity***F. De Grossi¹, A. Miluzio², M. Mancino², S. Biffo¹, N. Manfrini¹*¹*Università degli studi di Milano, Milan, Italy*²*Istituto Nazionale Genetica Molecolare, Milan, Italy***Introduction**

FAM46C is a tumor suppressor protein initially identified in multiple myeloma (MM) and increasingly recognized for its role in other cancers. Despite its importance, research on FAM46C therapeutic potential in combination with targeted treatments remains limited. Sphingosine kinases (SPHK1 and SPHK2) are key regulators of sphingolipid signaling, a crucial pathway for maintaining cell structure and function, which is frequently deregulated in tumors. Recent evidence from our lab suggested that FAM46C expression synergized with treatment with SK1-I, a pan-SphK inhibitor. Here, we concentrated on SPHK1, the sphingosine kinase primarily associated to cancer, and explored the synergistic impact of induced FAM46C expression alongside treatment with PF-543, a specific SPHK1 inhibitor.

Material and method

We first conducted data mining in the DepMap database to determine a potential correlation between FAM46C expression and sensitivity to PF-543 in multiple myeloma (MM) cell lines. Upon identifying a correlation, we validated our findings *in vitro* by assessing cells' sensitivity to PF-543 in presence or absence of functional FAM46C expression. The results we obtained were further validated in an *in vivo* xenograft model established by injecting NOD scid gamma with OPM2 cells harbouring either wt FAM46C or the loss-of-function D90G variant in presence or absence of PF-543 administration.

Result and discussion

We found a correlation between expression of a functional FAM46C and cell sensitivity to PF-543. *In vitro*, MM cells re-expressing functional FAM46C exhibited greater sensitivity to PF-543 compared to cells expressing the loss-of-function D90G variant.

Conversely, down-modulation of FAM46C lead to a reduction of the cytotoxic efficacy of PF-543 in MM cell lines. Using our *in vivo* xenograft model we found that FAM46C-expressing MM tumors were sensitive to PF-543 while tumors harboring the D90G loss-of-function variant of FAM46C were not. Our results indicate that expression of a functional FAM46C can indeed sensitize cells to PF-543 treatment. Future studies should explore whether wt FAM46C synergizes with other SphK1 inhibitors or combination therapies, as well as the precise molecular mechanisms underlying this effect.

Conclusion

Overall, our findings indicate that FAM46C plays a crucial role in drug response, enhancing sensitivity to PF-543 in both *in vitro* and *in vivo* settings. Our results

reveal a novel synergistic interaction between FAM46C expression and SPHK1 inhibition, presenting a promising therapeutic approach for MM treatment.

EACR25-0795

An intracellular cytidine deaminase biodegrader to overcome cancer chemoresistance

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Introduction

Cytidine deaminase (CDA) is an enzyme that catalyzes the deamination of (deoxy)cytidine into (deoxy)uridine, thereby maintaining intracellular pyrimidine homeostasis. Through its enzymatic activity, CDA contributes to the resistance of cancer cells to deoxycytidine (dC) analogue-based chemotherapies, such as gemcitabine and cytarabine, in pancreatic ductal adenocarcinoma (PDAC), lung cancer, and acute myeloid leukemia (AML). While catalytic inhibitors of CDA have been developed, they exhibit off-target effects and have failed in clinical trials. Thus, alternative therapeutic strategies to inhibit CDA and overcome resistance to dC-based chemotherapies are needed. Here, we leverage the specificity of intracellular antibodies (i.e., antibodies expressed within cells) to develop an innovative approach for targeted CDA degradation.

Material and method

We designed intracellular antibody-based degraders (also known as biodegraders or bioPROTACs) to induce targeted protein degradation via the proteasome. Upon transfection or transduction, the expressed biodegrader selectively depletes its intracellular target. To develop these biodegraders, we performed a phage display selection to isolate antibodies binding to CDA, followed by a cell-based screening assay using flow cytometry to identify potent CDA biodegraders. Two lead candidates were further characterized for their efficacy, specificity, and affinity using mass spectrometry, Western blot, and surface plasmon resonance.

Result and discussion

The selected CDA biodegraders efficiently depleted endogenous CDA protein in multiple PDAC, lung cancer, and AML cell lines. Functionally, CDA biodegrader sensitized cancer cells to gemcitabine and cytarabine, reducing their IC₅₀ values and inhibiting cell proliferation through increased apoptosis. In vivo, using a cell-derived xenograft (CDX) mouse model, we demonstrated that the combination of CDA biodegrader and gemcitabine significantly inhibited pancreatic and lung tumour growth. Remarkably, we have begun developing an intracellular delivery approach for this CDA biodegrader and have demonstrated its successful in vitro delivery, effectively sensitizing PDAC and lung cancer cells to gemcitabine.

Conclusion

CDA biodegrader represents a promising and innovative strategy to overcome chemoresistance in dC analogue-

based cancer therapies. Future work will focus on optimizing the intratumoral delivery of CDA biodegrader in experimental PDAC and lung cancer models *in vivo*, with the ultimate goal of advancing these therapeutic protein towards clinical application.

EACR25-0823

dTAG-based degradation of CDK5 as a therapeutic strategy in colorectal cancer

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Introduction

Colorectal cancer (CRC) remains a critical challenge due to limited therapeutic options. CDK5 has emerged as a potential target; however, its structural similarity to other CDKs complicates direct inhibition. Targeted protein degradation (TPD), which utilizes the ubiquitin-proteasome system, offers a novel approach. The dTAG system, based on PROTACs, enables specific protein degradation for functional studies *in vitro* and *in vivo*. Our aim was to lay the foundation to generate a PROTAC drug against CDK5 as a novel treatment for CRC.

Material and method

HT-29 and LoVo CRC cell lines were knocked out of endogenous CDK5 using CRISPR-cas9. FKBP12F36V-tagged CDK5 was reintroduced using lentiviral vectors. Degradation via dTAGv-1 was validated by western blot (WB) (D1F7M, Cell Signaling). Degradation of CDK5 *in vivo* was measured in subcutaneous (SC) HT-29_Nluc_CDK5dTAG and WT mock cells in BALB/c (nu/nu) ($n = 3/\text{group}$). Tumours were treated with vehicle (5% DMSO in 20% solutol/saline) or dTAGv-1 via intraperitoneal (IP) (40 or 80 mg/kg), SC (40 mg/kg), or intratumoral (IT) (40 mg/kg) administration for 3 days. Tumours were harvested on day 4, snap-frozen, and analysed for protein degradation via WB. To assess liver metastasis, HT-29_Nluc_CDK5dTAG cells were injected into the spleen of BALB/c (nu/nu) ($n = 10/\text{group}$), and metastases were tracked via bioluminescence imaging over 4 weeks (IVIS Illumina II). Mice were treated daily with 40 mg/kg dTAGv-1 or vehicle intraperitoneally. At endpoint, ex vivo fluorescence was measured, and liver samples were paraffin-embedded. Statistical analysis used was one-way ANOVA with Tukey's multiple comparison for SC and bioluminescence data, and Mann-Whitney U test for fluorescence.

Result and discussion

CRISPR/Cas9 efficiently knocked out CDK5, and FKBP12F36V-tagged CDK5 was successfully reintroduced in both cell lines. From 500 nM down to 10 nM of dTAGv-1 induced a dose-dependent degradation of CDK5, confirmed by WB. In vivo, IT administration achieved the highest degradation (5.27% \pm 2.58%, $p <$

0.01), outperforming IP and SC routes. Degradation rates for IP (40 mg/kg: 59.46% \pm 10.36%; 80 mg/kg: 40.23% \pm 8.4%) and SC (48.15% \pm 20.37%) routes showed no significant differences. All treatment groups exhibited significant protein degradation compared to vehicle ($p < 0.001$) (mean \pm SD). Metastases were significantly reduced in dTAGv-1-treated mice vs. vehicle controls as measured by bioluminescence ($3.73 \times 10^7 \pm 1.13 \times 10^7$ vs. $3.65 \times 10^8 \pm 1.15 \times 10^8$; p/s/cm²/sr, mean \pm SEM; $p < 0.05$) and fluorescence [$4.45 \times 10^8 \pm 1.59 \times 10^8$ vs. $3.68 \times 10^9 \pm 2.67 \times 10^9$; (p/s/cm²/sr)/(μ W/cm²) mean \pm SEM; $p < 0.01$], respectively.

Conclusion

The dTAG system effectively degraded CDK5 in vitro and in vivo, reducing tumour burden and metastases without observed toxicities. These findings highlight CDK5 TPD's potential as a therapeutic strategy in CRC.

EACR25-0863

Setup of a patient-derived organoid biobank from NSCLC patients

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Introduction

In lung cancer patients (LCPs), patient-derived organoids (PDOs) offer a valuable platform for predicting treatment responses (1). However, the successful establishment of PDO cultures, their multiomic profiling, and high-throughput drug-testing remain challenging due to the difficulty in obtaining pure tumor organoids, highlighting the need for further optimization (2).

Material and method

In this study, PDOs were generated from both primary lung cancer tissues and matched non-neoplastic tissues derived from 22 LCPs. Pathological and multiomic profiling, including methylomic analysis with Epic Array technology, was performed to assess the concordance of genetic and epigenetic landscapes, as well as histopathological features, between primary tissues and PDOs. To improve the success rate of PDO establishment, we evaluated the effects of Nutlin-3a and pan-ERBB inhibitors in eliminating outgrowing normal organoids while selectively enriching specific mutant PDOs. Furthermore, potential therapeutic vulnerabilities and resistances of PDOs were assessed through high-

throughput drug screening using a panel of 142 compounds.

Result and discussion

We generated 12 pairs of lung cancer organoids (LCOs) and matched healthy lung organoids, each validated through histopathological and molecular analyses and exhibiting diverse mutational landscapes. Histopathological and epigenetic profiling demonstrated a strong concordance between LCOs and their corresponding primary tumors. Copy number variation analysis confirmed high concordance of major mutational patterns in PDOs with primary tumors. Deconvolution of latent methylation components revealed tumor-specific epigenetic states for individual patients, which were recapitulated in the LCOs. Furthermore, epigenomic profiling allowed clear discrimination between healthy and tumor organoids, reinforcing genomic findings. Optimization with Nutlin-3a and pan-ERBB inhibitors improved the selection of mutant PDOs. High-throughput drug-screening proved feasible and led to the identification of novel therapeutic targets.

Conclusion

This study demonstrates for the first time that LCOs recapitulate the distinct epigenomic landscapes of primary tumors in addition to genomic features. By refining PDO selection and integrating multiomic analyses with high-throughput drug screening, our approach enhances the utility of LCOs as a robust platform for advancing personalized therapy in precision oncology.

EACR25-0867

Sesquiterpene Lactones Cynaropicrin and Ludartin as Potential Therapeutic Agents in Ovarian Cancer

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Introduction

Ovarian cancer is a heterogeneous malignancy responsible for over 200,000 deaths worldwide annually. Among its subtypes, high-grade serous carcinoma (HGSC) is the most common epithelial ovarian cancer, often diagnosed at advanced stages. While platinum-based chemotherapy initially achieves high response rates, approximately 75% of patients develop resistance within five years, highlighting the urgent need for novel therapeutic strategies. This study investigates the cytotoxic potential of two natural sesquiterpene lactones, cynaropicrin and ludartin, in ovarian cancer cell lines, including chemoresistant models. We aim to evaluate their efficacy and elucidate their mechanisms of action to overcome chemoresistance.

Material and method

To identify potent natural compounds, we performed an NCI-SRB assay on ovarian and breast cancer cell lines. Cynaropicrin and ludartin were selected based on their significant cytotoxic effects. Their mechanisms of action were further explored using PI staining for cell cycle analysis, MUSE Annexin-V staining for apoptosis detection, and Western blotting for protein expression analysis.

Result and discussion

The NCI-SRB assay identified cynaropicrin and ludartin as highly effective against ovarian cancer cell lines, exhibiting low IC₅₀ values. Further analyses demonstrated that both compounds induced cell death and affected cell cycle progression.

Conclusion

Our findings suggest that cynaropicrin and ludartin promote cell death and alter cell cycle dynamics in ovarian cancer cells. Further studies are needed to elucidate their precise molecular mechanisms and therapeutic potential. These results support their development as promising candidates for ovarian cancer treatment.

EACR25-0883

Unlocking the Therapeutic Potential of Platelets in B-Cell Malignancies – Preliminary Study

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Introduction

Platelets are small anucleated blood cells derived from the cytoplasmatic fragmentation of megakaryocytes in the bone marrow. Platelets play a crucial role in various physiological and pathological processes, such as hemostasis, thrombosis, tumor progression, and metastasis. Platelets and other cells have been considered attractive

drug delivery systems for cancer therapy mainly due to their superior biocompatibility, better natural targeting, high drug-loading capacity, lower immunogenicity, and good ability to cross biological barriers. Dysregulation of the NF-κB pathway is common in hematological cancers. The inhibition of this pathway by parthenolide (PRT) has shown great potential, however, its low solubility and lipophilicity have prevented its further approval for cancer treatment. This study aimed to evaluate the potential use of platelets as a drug delivery system of PRT for the treatment of B-cell malignancies in vitro.

Material and method

We used six different cell lines: 697 [B-acute lymphoblastic leukemia (B-ALL)], HG-3 [chronic lymphocytic leukemia (CLL)], Raji [Burkitt lymphoma (BL)], Farage [diffuse large B-cell lymphoma (DLBCL)], H929 and U-266 [multiple myeloma (MM)]. Fresh leuko-reduced human platelets were obtained from health volunteers at a local hospital after obtaining informed consent. PRT and doxorubicin (DOX) loading into platelets was achieved after 1 h incubation with agitation. Drug incorporation was confirmed by HPLC and immunofluorescence (IF) assays. Metabolic activity was evaluated using resazurin assay. Cell death was assessed by flow cytometry (FC) using Annexin V/7AAD double staining. Statistical significance was considered when $p < 0.05$.

Result and discussion

Our results showed that all B-cell malignancy cell lines are sensitive to PRT alone, in a time-, dose- and cell line-dependent manner, with Farage being the most sensitive ($IC_{50} = 1.5 \mu M$) and U-266 the least ($IC_{50} = 22.1 \mu M$). Additionally, PRT induced cell death by apoptosis through oxidative stress and decreased mitochondrial membrane potential. After drug incorporation, platelets were CD61+ (platelet marker), CD45- (leukocyte marker), and CD62P- (P-selectin, platelet activation marker). IF and CF proved the internalization of DOX and HPLC the internalization of PRT. Cell lines were then incubated with DOX- and PRT-loaded platelets in a 1:100 ratio for 48 h. Non-loaded platelets did not reduce the metabolic activity of cells. DOX- and PRT-loaded platelets reduced metabolic activity depending on the cell line. Specifically, PRT-loaded platelets induced more significant metabolic activity reduction in H929 cell line ($71.6\% \pm 8.4\%$, $p = 0.03$).

Conclusion

Our study showed that platelet loading might provide a new therapeutic strategy for PRT delivery, specifically to B-cell malignancies. However, more studies are needed to better understand the relevance of PRT-loaded platelets in hematological cancers.

EACR25-0889

Designer proteins to drive the targeted degradation of tankyrase

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Introduction

The poly(ADP-ribose) polymerases (PARPs) Tankyrase 1 (TNKS1) and Tankyrase 2 (TNKS2) (TNKS1/2) are key regulators of Wnt/β-catenin signalling and a promising drug target in cancers with hyperactive Wnt/β-

catenin signalling. TNKS1/2 contain five ankyrin-repeat clusters (ARCs) (four of which mediate substrate binding), a sterile alpha motif (SAM) domain to allow polymerisation and a catalytic PARP domain. TNKS1/2 promote Wnt/β-catenin signalling via their polymerisation and PARP activities. The consensus-designed tetratricopeptide repeat (CTPR) protein is an ultra-stable modular scaffold protein onto which new functions can be engineered by peptide ‘grafting’. Previously, a CTPR protein was functionalised by grafting a tankyrase-binding peptide (TBP) onto a loop between two CTPR units (1TBP-CTPR2). Then, a series of nTBP-CTPR2n proteins with increasing numbers of TBP loops and CTPR units (nTBP-CTPR2n) were generated. As small-molecule TNKS1/2 inhibitors and other therapeutic strategies to inhibit Wnt/β-catenin signalling have met with limited success to date, we aim to design chimeric proteins containing nTBP-CTPR2n to drive the targeted degradation of TNKS1/2 in cancer cells with hyperactive Wnt/β-catenin signalling.

Material and method

Competition fluorescence polarisation (FP) assays were carried out to measure the binding of His6-tagged nTBP-CTPR2n proteins to TNKS1 (ARC2-3) and TNKS2 (ARC4) in the presence of a fluorescently labelled TBP. Our E3 chimeras were designed with the aid of AlphaFold2 and generated by molecular cloning. HiBiT-TNKS2 and each of the E3 chimeras were transiently expressed in HEK293T cells for 24 hours before the levels of HiBiT-TNKS2 in cells were quantified using the Nano-Glo® HiBiT Lytic Detection System.

Result and discussion

His6-tagged nTBP-CTPR2n proteins are shown to bind TNKS1 (ARC2-3) and TNKS2 (ARC4), and the binding affinity increases as the number of TBPs increases. Following this, a series of E3 chimeras is created by fusing nTBP-CTPR2n to (a) speckle-type POZ protein (SPOP) which lacks its natural substrate binding domain and (b) the RING domain of tripartite motif-containing protein 21 (TRIM21). Transient expression of these E3 chimeras proved that the constructs are not cytotoxic. Co-transfection experiments demonstrated that the level of HiBiT-TNKS2 in HEK293T cells decreased by approximately 50% in the presence 2TBP-CTPR4-SPOP and TRIM21 RING-1TBP-CTPR2.

Conclusion

Chimeric E3 proteins containing nTBP-CTPR2n induce the reduction of HiBiT-TNKS2 in cells, suggesting degradation.

EACR25-0915

Use of the Novel Molecular Glue, MRT-2359 for Targeting MYC-dependent Breast Cancer

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Introduction

Brain cancer is a leading cause of cancer-related mortality in children, with medulloblastoma (MB) being the most common malignant type. Surgery, radio- and chemotherapy are the current standard of care (SoC); however, 30% of patients relapse or develop secondary tumors, and survivors present debilitating neurocognitive impairments from SoC. To address this existing unmet

Since MYC is one of the most frequently altered driver genes in cancer formation, it is a highly attractive target for new anti-cancer therapies. Historically, however, MYC has proved difficult to target due to the absence of a suitable crevice for binding of potential low molecular weight drugs. A promising strategy for targeting “difficult to drug” cancer targets is the use of protein degraders such as PROTACs or molecular glues. A molecular glue currently being evaluated in a clinical trial is the GSPT1 degrader, MRT-2359. Here, we show that MRT-2359 also degraded MYC and inhibited the growth of breast cancer cells in culture.

Material and method

Cell viability was determined with the MTT assay using 14 breast cancer cell lines. Induction of apoptosis was analyzed by flow cytometry using annexin V and propidium iodide (PI) staining.

Result and discussion

Using 3 breast cancer cell lines, MRT-2359 was found to degrade MYC in a concentration-dependent manner. Consistent with its ability to degrade MYC, MRT-2359 was found to inhibit the proliferation of breast cancer cell lines with IC₅₀ (half maximal inhibitory concentration) values ranging from 36.7 nM to 2 μM. Similar inhibition of cell growth was found irrespective of whether the cells were HER2-positive, estrogen receptor-positive or triple-negative. IC₅₀ values for MRT-2359 were significantly lower than those for 2 previously described MYC-MAX antagonists (for MYCi975, p < 0.0001; for MYCMI-6, p < 0.0002), i.e., MRT-2359 was a more potent inhibitor of breast cancer cell growth than either MYCi975 or MYCMI-6. In addition to the inhibition of cell growth, MRT-2359 was found to induce apoptosis in a time and concentration-dependent manner.

Conclusion

Our results suggest that MRT-2359 is a potential treatment for MYC-dependent breast cancer. Furthermore, our data provide a rationale for extending the ongoing clinical trial with MRT-2359 to breast cancer.

EACR25-0925

Targeting MYC as a promising therapeutic strategy for pediatric medulloblastoma

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Introduction

Brain cancer is a leading cause of cancer-related mortality in children, with medulloblastoma (MB) being the most common malignant type. Surgery, radio- and chemotherapy are the current standard of care (SoC); however, 30% of patients relapse or develop secondary tumors, and survivors present debilitating neurocognitive impairments from SoC. To address this existing unmet

medical need, we must develop novel therapeutic approaches. MYC is a master regulator of cellular processes and one of the most dysregulated proteins in cancer, including MB. When overactivated, it drives tumorigenesis and tumor maintenance, and until recently, MYC has been considered undruggable.

Material and method

Omomyc is a MYC dominant negative inhibitor, currently in clinical trials for adults with advanced solid tumors as an Omomyc-based mini-protein (OMO-103). With this project, we investigate the use of Omomyc as a potential therapy for pediatric MB. We examine the effect of Omomyc on inhibiting proliferation and self-renewal in vitro in patient-derived MB cells and neural stem cells, and investigate its effects on extending overall survival in vivo. In addition, we perform RNA-sequencing on Omomyc-treated MB cells to further investigate the mechanism of action, and we explore MB patient samples transcriptomic datasets to identify combinatorial treatment strategies.

Result and discussion

We show that Omomyc selectively targets MB brain tumor initiating cells by impairing proliferation and self-renewal, while sparing the human neural stem cells. Omomyc treatment induces downregulation of MYC regulated genes, cell cycle pathways, and other oncogenic pathways specific to MB. Moreover, the half-maximal inhibitory concentration was maintained between primary and matched SoC-recurrent samples. We further observed a significant survival advantage in our MB patient-derived orthotopic xenograft murine model. Lastly, we identify a suitable synergistic target for combinatorial treatment strategies.

Conclusion

Overall, our work discovers that Omomyc may be a promising therapy for a subset of MB patients with high-MYC expression, particularly at recurrence. Moreover, we have identified a combinatorial treatment strategy with synergistic effect in vitro, and next steps include its validation in vivo.

COI: LS is CEO and employee of Peptomyc, a company developing MYC inhibitory peptides. JW and LS are shareholders in Peptomyc.

EACR25-0930

A novel ribosome biogenesis inhibitor, PMR-116, displays broad spectrum efficacy in preclinical tumour models

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Introduction

Inhibition of RNA Polymerase I, the enzyme responsible for the synthesis of ribosomal RNAs, the nucleic scaffold of ribosomes, has been proven as a novel therapeutic target as most cancer cells are “addicted” to ribosome biogenesis.

Material and method

In collaboration with Pimera Inc, we developed and tested the efficacy of a second generation RNA

polymerase I inhibitor, PMR-116, in various pre-clinical cancer models. On-target activity of PMR-116 was confirmed in peripheral blood cells isolated from patients with solid tumours enrolled in a phase I trial of PMR-116.

Result and discussion

PMR-116 potently inhibits Pol I transcription with ~200 fold higher selectivity towards Pol I compared to Pol II transcription. PMR-116 inhibits rRNA synthesis by stalling the Pol I complex at the rDNA promoter preventing promoter escape and consequently elongation. PMR-116 anti-cancer activity was evaluated against a panel of over 100 cancer cell lines representative of 20 major types of solid and haematological malignancies. This new Pol I inhibitor exhibited broad anti-proliferative and cytotoxic activity with an IC₅₀ ranging from 32–4500 nM and a median IC₅₀ of 300 nM. In contrast, cells derived from normal tissues were significantly less sensitive (IC₅₀ ranging from 6–33 μM). In vivo, PMR-116 significantly improved survival and reduce tumour burden in several preclinical models such as V_k*MYC transgenic model of indolent multiple myeloma, CT26 xenograft model of colorectal cancer, MMTV-PyMT transgenic model of metastatic breast cancer, mixed-lineage leukemia (MLL)-eleven nineteen leukemia (ENL)+Nras acute myeloid leukemia (+/-p53 mutation) and patient-derived xenografts of prostate cancer and AML. In a phase I trial of PMR-116, on-target activity of PMR-116 was observed in cells derived from the peripheral blood.

Conclusion

PMR-116 significantly reduced cancer growth in a wide range of tumor models, including GEMM, syngeneic and PDX models. PMR-116 is undergoing clinical testing in phase I in solid tumors with the objective to determine maximum tolerated dose and identify a phase II dose.

EACR25-0967

Inhibition of Translesion Synthesis (TLS) activity enhances cell death and prevents tumor growth

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Introduction

DNA-damaging chemotherapy agents such as cisplatin have been a first line approach in cancer treatment for decades; however, their long-term success is often reduced by intrinsic and acquired drug resistance. Although the mechanisms causing drug resistance are quite distinct, they are directly connected to mutagenic translesion synthesis (TLS). The TLS pathway promotes DNA damage tolerance by supporting replication opposite to a lesion and inaccurate single strand gap filling. It has been shown that inhibiting TLS enhances cell death, reduces cisplatin resistance and the appearance of secondary tumors. Therefore, small molecule inhibition of the TLS pathway is a new promising strategy for improving cancer treatment. We recently discovered a small molecule named C#3*. C#3 directly binds to the TLS protein - MAD2L2, and inhibits TLS activity by blocking the binding site of Rev1. Therefore, the active TLS complex composed of MAD2L2-Rev1 cannot be formed.

Material and method

To evaluate c#3 synergistic activity, we used both non-transformed cell lines and cancer lines such as lung and melanoma. All cells were co-treated with cisplatin and c#3, and cell survival, DNA damage levels and TLS complex formation were evaluated. We also used purified GST-MAD2L to determine the binding affinity between MAD2L2 and c#3. Finally, we validated c#3's synergistic activity using two syngeneic mice models.

Result and discussion

In cell-based experiments, we found that co-treatment with cisplatin and c#3 sensitized various cancer cell lines, reducing cisplatin's IC₅₀ by twofold or more. Co-treatment caused a significant elevation in DNA damage levels, explaining the sensitization effect. Using diverse biochemical methods, we demonstrated that c#3 directly binds to MAD2L2 and prevents the formation of the TLS complex. Importantly, c#3 is not cytotoxic when administered alone. Using a syngeneic mouse model, we demonstrated that combined treatment of cisplatin and c#3 significantly prevented tumor growth and prevented lung metastasis.

Conclusion

Therefore, c#3's activity underlines its potential as a lead compound for developing novel TLS inhibitors for improving chemotherapy treatment, reducing the appearance of metastasis, and alleviating patients' side effects.

EACR25-0983

Optimizing Gemcitabine Delivery: HPMA Copolymer Conjugates with Controlled Drug Release and Immunomodulatory Activity

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Introduction

Gemcitabine (Gem) is a nucleoside analogue widely used in clinical practice for treating various malignancies, including breast, lung, pancreatic, and ovarian cancers. However, its therapeutic efficacy is limited by its rapid metabolism in the bloodstream, which leads to a short half-life. To address this, we developed N-(2-Hydroxypropyl)methacrylamide (HPMA) copolymer-based Gem nanotherapeutics (P-Gem1–4) designed to protect Gem from degradation, prolong its circulation time, and enable controlled drug release. The nature of the spacer between Gem and the HPMA backbone dictates the drug release kinetics, which significantly influences the anticancer efficacy.

Material and method

We synthesized four P-Gem conjugates with different spacers: P-Gem1 (tetrapeptide GFLG), P-Gem2 (β -alanine), P-Gem3 (valeric acid), and P-Gem4 (amino-caproic acid). To evaluate their in vitro antitumor

activity, we performed [³H]-thymidine incorporation assay, Annexin V assay and Caspase-3 assay. The in vitro studies were conducted on 4T1, LL2, Panc02, Panc1, and MiaPaca2 cell lines. Furthermore, we analyzed changes in gene expression related to apoptosis, cell cycle regulation, proliferation, angiogenesis, metastasis, and immune response in 4T1 tumors using TaqMan qPCR arrays. To further investigate immune modulation, we studied the impact of treatment on immune cell populations in the spleen and tumors of Balb/c mice bearing 4T1 tumors.

Result and discussion

Our in vitro assays revealed a correlation between cytostatic and cytotoxic effect and drug release kinetics, with IC₅₀ values increasing as the drug release rate decreased. These findings confirm that the drug release rate decreases from P-Gem1 to P-Gem4. Ex vivo qPCR analysis of 4T1 tumors treated with P-Gem conjugates showed upregulation of pro-apoptotic and cyclin-dependent kinase inhibitor genes, along with down-regulation of immune-suppressive molecules. Notably, the most significant gene expression changes were observed in tumors treated with P-Gem4. Furthermore, in the spleens of treated mice, we detected a decrease in the levels of MDSC and Treg cells, particularly in those treated with the slow-releasing conjugates P-Gem3 and P-Gem4. These findings indicate that slow-release P-Gem conjugates exhibit superior anticancer activity compared to free Gem and the fast-releasing conjugates P-Gem1 and P-Gem2.

Conclusion

P-Gem conjugates demonstrated drug release kinetics-dependent cytostatic and immunomodulatory activity in vitro and ex vivo. Our results suggest that slow-release P-Gem conjugates have the potential to improve the therapeutic effectiveness of Gem, especially in hard-to-treat malignancies such as triple-negative breast cancer and pancreatic cancer.

Acknowledgement: This work was supported by the project National Institute for Cancer Research (Programme Exceles, ID Project No. LX22NPO5102) – Founded by the European Union – Next Generation EU.

EACR25-0993

Discovering ERBB signaling as therapeutics target for β -catenin mutation driven hepatocellular carcinoma using mouse organoid platform

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Introduction

Multiple drugs have been approved for the treatment of hepatocellular carcinoma (HCC) in recent years. However, the clinical benefit is far from optimal, extending the survival of patients for a few months only. This is due to the complex, heterogeneous nature of different HCC patients, in which cancer cells harbor a mix of driver and passenger mutations. Understanding the characteristics of driver-specific liver cancers is fundamental in the development of effective therapeutics in the era of precision medicine. Therefore, we aim to establish a platform to overcome the current limitation of cell lines and animal models, which the effects of individual driver mutations cannot be studied in detail in the absence of passenger mutations.

Material and method

We utilized HCC tumors generated by the hydrodynamic tail vein injection (HTVI) of proto-oncogene combinations in mice together with the tumor organoid platform to study the characteristics of HCC driven by different driver mutations. Paired tissues and tumor organoids were characterised by whole exome sequencing, transcriptome sequencing, phosphoproteomics and ATAC-seq. Tumor organoids established were tested against an FDA approved anti-cancer drug library comprising over 600 drugs to identify drugs that are effective in killing HCC driven by specific driver mutation.

Result and discussion

We successfully established HCC tumor organoids with specific driver mutations (loss of TP53, Axin1, and PTEN, and CTNNB1 activating mutation in the background of MYC overexpression) and validated their gene mutations at both genomic and proteomic levels. Using a high-throughput screening approach for drug repurposing, we identified ERBB family inhibitors as potential therapeutic targets for CTNNB1 mutation-driven HCC ($\Delta 90$ CTNNB1/MYC). Multi-omics analysis through chromatin accessibility, transcriptomics, and phosphoproteomics revealed activation of the ERBB signaling pathway in CTNNB1 mutation-driven HCC via increase transcription of related genes, Egf and Tgfa, driven by transcription factor Foxa2. Activation of ERBB signaling pathway in HCC patients with CTNNB1 activating mutation was further confirmed with clinical data extracted from publicly available phosphoproteom data. Finally, using a preclinical animal model, we validated the sensitivity of CTNNB1 mutation-driven HCC towards ERBB family inhibitors.

Conclusion

In conclusion, we successfully established an HCC organoid platform that allows the study of driver-specific liver cancers in the absence of passenger mutations. We discovered ERBB family inhibitors as novel therapeutics for CTNNB1 mutation-driven HCC and elucidated the distinct pathways that potentially drive the sensitivity towards ERBB family inhibitors in CTNNB1 mutation-driven HCC. Our findings provide the foundation for future repurposing of FDA-approved drugs for the treatment of HCC patients with CTNNB1 mutation.

EACR25-1022

Nanobiotechnological engineering of M13 bacteriophage for EGFR-targeted theranostics

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Introduction

Bacteriophages, especially the M13 filamentous phage, are gaining attention as nanomedicine platforms due to their low immunogenicity, efficient penetration of biological barriers, safety profile and targeting capabilities. Here, we present the development of a novel theranostic platform using the M13 phage.

Material and method

The acquired tropism of M13D12 was demonstrated through single and co-culture experiments using EGFR-positive and EGFR-negative cell lines. The targeting specificity of the engineered phage was assessed by fluorescence microscopy and flow cytometry. To functionalize the phage for photodynamic therapy (PDT), photosensitizers (Rose Bengal - RB) or fluorescent dyes (CF594) were chemically conjugated onto the pVIII proteins of M13D12. Conjugation efficiency was confirmed via spectrophotometric analysis, SDS-PAGE, and fluorescence spectroscopy. The biological activity of M13D12-RB was evaluated in both 2D and 3D cell culture models. The cytotoxic effect following light irradiation was assessed using standard viability assays. Tumor penetration and cell viability after PDT was evaluated also on ex-vivo sample derived from patient with ovarian cancer. For in vivo validation, EGFR-positive tumor-bearing mice were injected intratumorally with M13D12-RB, followed by laser irradiation and tumor regression was monitored over time.

Result and discussion

Chemical conjugation of photosensitizers and fluorescent dyes onto the pVIII proteins of M13D12 was successfully achieved without altering the phage's targeting properties. In single and co-culture experiments, the engineered phage demonstrated selective binding and internalization in EGFR-overexpressing cells, confirming its acquired tropism. Upon irradiation, M13D12-RB exhibited potent photodynamic killing activity in both 2D and 3D cell cultures, effectively eliminating EGFR-positive cells at picomolar concentrations of nanovector. In 3D tumor spheroids, the phage successfully penetrated the core and induced complete disaggregation of the cytoarchitecture upon light exposure. Ex vivo tumor models further confirmed the deep penetration and selective cytotoxicity of M13D12-RB. In vivo studies demonstrated significant tumor regression following laser irradiation, validating the therapeutic potential of the phage-based PDT approach.

Conclusion

This study demonstrates the potential of engineered M13 bacteriophages as precise and versatile platforms for

targeted cancer therapy, addressing key limitations of PDT through enhanced tumor targeting. While the system was specifically developed for EGFR-overexpressing cancers, its modular design allows for adaptation to other tumor markers, offering a powerful and customizable solution to overcome side effects and drug solubility challenges, enabling the targeted treatment of diverse cancer variants.

EACR25-1025

CircRNA-mediated inhibition of miRNA-454 as a novel therapeutic strategy in prostate cancer

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Introduction

Prostate cancer is one of the most common cancers, causing high mortality rates in men. Since existing drugs are associated with side effects and often do not show long-term efficacy, there is an eminent need for novel therapeutic approaches. Certain miRNAs have been shown to be aberrantly up- or downregulated in tumors and to exert oncogenic or tumor suppressive functions. This may provide a basis for therapeutic intervention, by aiming at miRNA inhibition or miRNA replacement. The role of miR-454 in prostate cancer has been described ambivalently so far, with both, tumor suppressive and promoting functions.

Material and method

Our study aimed to comprehensively explore the role of miRNA-454 in prostate cancer. Two approaches were investigated: miR-454 replacement through reintroduction of miR-454 mimics, and miR-454 inhibition by using circRNAs as miR-454 sponge.

Result and discussion

Especially miR-454 inhibition by introducing a miR-454-specific circ-RNA led to a significant induction of cell death and reduced proliferation in different 2D and 3D models. Moreover, colony formation assays (CFA) revealed substantial effects on the cells' ability to form colonies, indicating decreased tumorigenic potential. In some cases, miR-454 replacement exerted tumor cell-inhibitory effects as well. In an *in vivo* therapy study in prostate xenograft-bearing mice, the therapeutic application of miR-454-specific circRNAs formulated in polymeric nanoparticles for RNA delivery led to tumor inhibitory effects.

Conclusion

These results demonstrate the functional relevance of miRNA-454 and establish miR-454 as a promising potential target in prostate cancer. The dual approach of either replacing or inhibiting miRNA-454 provides flexibility in treatment design and may offer personalized

options based on individual patient profiles. Finally, the circ-RNA mediated miR-454 sponging was identified as the more powerful strategy for miRNA inhibition and treatment of prostate cancer cell *in vitro* and *in vivo*.

EACR25-1032

KA103, a novel targeted toxin for Head and Neck Cancer

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Introduction

Head and neck squamous cell carcinoma (HNSCC) is the sixth most common cancer in the world, with over 878,000 new cases diagnosed and over 440,000 deaths in 2020. Despite the heterogeneity of HNSCCs, it has been found that the transmembrane protein epidermal growth factor receptor (EGFR) is overexpressed in nearly 90% of cases, making it a potential therapeutic target. The EGFR monoclonal antibody cetuximab is the only targeting drug approved for this indication. However, intrinsic and acquired resistances provide considerable limitations for treatment efficacy.

Material and method

The anticancer properties of targeting molecules can be enhanced by linking them to a toxin, generating a targeting toxin (TT). Moreover, we have designed and recombinantly produced KA103, a novel EGFR-targeted fusion protein based on EGF and gelonin, a ribosome inactivation protein toxin (RIP).

Result and discussion

Here, we show that KA103 has similar efficacy as gelonin in a cell-free reticulocyte system but exerts targeted cytotoxicity, as shown in EGFR-positive and negative cell lines. Moreover, KA103 is highly effective against EGFR-expressing HSCC *in vitro*, especially against cetuximab-resistant SCC040 cells, indicating KA103 to overcome cetuximab resistance. In addition, *in vivo* data depicts that KA103 induces SCC040 tumor growth delay without detecting severe toxicity. Furthermore, we have established that fluorescently labeled KA103 can be used to evaluate *in vivo* biodistribution, showing the highest labeled KA103 uptake retention at the tumor.

Conclusion

In conclusion, our findings show that KA103 production is highly reproducible and induces EGFR-targeted toxicity in HNSCC cell lines. In addition, KA103 promotes tumor growth delay in HNSCC models without general toxicity and with high KA103 uptake at the tumor. The presented results warrant further preclinical evaluation of KA103.

EACR25-1052

Evaluation of the antineoplastic effects of imipridone derivatives in prostate cancer: Novel therapeutic opportunities

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Introduction

Prostate cancer (PCa) remains one of the most diagnosed and life-threatening malignancies in men, despite advances in therapy. High recurrence rates and drug resistance in PCa have been attributed to a subpopulation of slowly dividing and dormant cancer cells known as PCa stem cells (PCSCs). Consequently, developing novel therapeutic strategies to target PCSCs and improve PCa management is a critical priority. Imipridones are a class of structurally related compounds with demonstrated anticancer activity across various solid and hematological cancers in preclinical models and clinical trials (phase I–III). Among these, ONC201, the first-in-class imipridone, has shown promising in vitro and in vivo efficacy and progressed to a phase I clinical trial for PCa. However, the anticancer potential of its derivatives, ONC206 and ONC212, remains unexplored in the context of PCa. Thus, this study investigates the antitumor effects of ONC206 and ONC212 at the functional and molecular levels using two-dimensional (2D) and three-dimensional (3D) in vitro PCa models. Furthermore, it evaluates the potential synergistic effects of imipridones with conventional PCa therapies, focusing on patient-derived organoids (PDOs) for clinically relevant insights.

Material and method

The effects of ONC206 and ONC212, relative to ONC201, were assessed using 2D in vitro assays for cell proliferation, viability, and migration on two human PCa cell lines, DU145 and PC3, representing different disease stages. Moreover, the 3D sphere formation and propagation assays were employed to evaluate the potency of these imipridone derivatives in targeting PCSCs.

Result and discussion

ONC212 was the most potent imipridone in inhibiting 2D PCa cell growth, viability, and migration. Notably, ONC212 more effectively targeted PCSCs in 3D assays compared to ONC201.

Conclusion

This study highlights ONC212 as a promising therapeutic candidate for targeting PCSCs, potentially improving PCa management by overcoming tumor resistance and recurrence.

EACR25-1056

Incorporation of disulfide bond into interleukin-2 for enhancing stability, solubility and therapeutic efficacy

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Introduction

Interleukin-2 (IL-2) is a prototypically pleiotropic cytokine that promotes T-cell proliferation, survival and differentiation. It plays a crucial role in regulating immune responses and maintaining immune homeostasis and regulates natural killer (NK) cell natural lethality. In this study, we showed that the introduction of disulfide

bonds and human serum albumin (HSA) into IL-2 can significantly improve its receptor specificity, thermal stability, and solubility.

Material and method

IL-2 mutants and variants were expressed in Pichia pastoris and CHO systems and purified to homogeneity. CTL-L2 cell proliferation and CIK assays used to study their activities. DSC was used to determine the thermostability of IL-2 and its variants. X-ray crystallography was used to determine 3D structures of IL-2 mutants.

Result and discussion

We showed that the incorporation of disulfide bond into IL-2 exhibited 2.2-fold increase in activity, and HSA-cytokines expressed in ExpiCHO exhibited higher activity than that expressed in Pichia pastoris. Differential scanning calorimetry was used to analyze the thermostability of IL-2 and its C125S, C125A, C125L, C125S/K43C/T111C, C125A/K43C/T111C, and C125L/K43C/T111C mutants, and their Tm values were 57.12, 48.36, 49.39, 64.64, 60.61, 66.47 and 82.24 oC. The results showed that the C125S and C125A mutations caused the decrease in their thermostability. In contrast, the incorporation of the K43C/T111C disulfide bond into C125S, C125A, C125L mutants increased the Tm values of 12.25, 17.08, and 17.60 oC, leading to higher stability. To confirm the formation of disulfide bond, 3D structure of the C125S/K43C/T111C mutant was determined by X-ray crystallography. The analysis showed that two disulfide bonds (C43-C111 and C58-C105) were formed. IL-2 mutant was found to be effective in inducing the cytotoxicity effect of PBMCs against HepG2-GFP cell line.

Conclusion

In summary, we have successfully engineered IL-2 by the introduction of disulfide bond, mutation and HSA fusion protein. We anticipate that IL-2 variants from this study can be used for autoimmunity and cancer therapies.

EACR25-1060

In vivo delivery of gemcitabine through HPMA copolymer conjugates: slow drug release kinetics is crucial for potent antitumor activity

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Introduction

Gemcitabine (Gem) is the anticancer drug with potent cytostatic activity causing inhibition of DNA synthesis through so called masked chain termination. Gem is used alone or in combination with paclitaxel bound to albumin nanoparticles for the first-line treatment of pancreatic carcinoma, for the treatment of metastatic or inoperable breast carcinoma (in combo with paclitaxel), advanced or metastatic non-small cell lung carcinoma (in combo with cisplatin), ovarian carcinoma (in combo with carboplatin) and advanced or metastatic bladder cancer (in combo

with cisplatin). The most unfavorable pharmacological features of Gem are very extremely short half-life and possible metabolic inactivation via cytidine and deoxy-cytidine deaminases.

Material and method

We have designed, synthesized and characterized a set of linear N-(2-hydroxypropyl)methacrylamide (HPMA) copolymer conjugates (Mw ~40–45 kDa, D <1.1, HD ~8 nm) bearing Gem (12–15 wt%) covalently linked through amide bond using primary amine group of Gem to extend its half-life in circulation and protect it from cytidine and deoxycytidine deaminases degradation. Four conjugates with different spacers between the HPMA copolymer backbone and Gem were prepared with the aim to study the effect of Gem release kinetics on the in vivo drug half-life and antitumor activity in several mouse tumor models.

Result and discussion

Our HPMA copolymer-gemcitabine conjugates showed different rate of Gem release: P-Gem1 > P-Gem2 > P-Gem3 > P-Gem4 in PBS and in mouse sera, though the release rate was generally faster in sera comparing to PBS. The cytostatic activity in vitro correlated with drug release kinetics, i.e. the faster release the lower IC₅₀. P-Gem conjugates provided also much longer half-life in the circulation upon i.v. administration. Maximum tolerated dose in mice perfectly correlated with Gem release kinetics with fastest conjugate being the most toxic one. Particularly P-Gem3 and 4 showed superior antitumor activity in mouse 4T1 breast, LL2 lung and MiaPaca2 pancreatic carcinoma models in comparison to Gem.

Conclusion

P-Gem conjugates extensively prolonged the half-life of the Gem in the circulation. Our in vivo results suggest that slow-release P-Gem conjugates have the potential to improve the therapeutic efficacy of the Gem in several hard-to-treat carcinomas.

Acknowledgement: This work was supported by the project National Institute for Cancer Research (Programme Exceles, ID Project No. LX22NPO5102) – Founded by the European Union – Next Generation EU).

EACR25-1072

Zebrafish Avatar-test predicts tumor response to chemotherapy in Breast Cancer: A co-clinical study for personalized medicine

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Introduction

Breast cancer (BC) is highly heterogeneous at clinical, histological, and molecular levels. Tumors are classified

into subtypes based on receptor status (ER, PR, HER2), along with stage and grade, guiding targeted treatments and improving outcomes. Despite these advances, chemotherapy remains the mainstay for high-risk early BC and metastatic disease. However, with multiple equivalent options available, treatment efficacy varies, and no test exists to determine the most effective therapy for an individual. As a result, treatment selection relies on physician experience, toxicity, and patient preference rather than direct tumor sensitivity, potentially leading to unnecessary toxicities and ineffective treatment.

Material and method

We developed a fast in-vivo BC therapy screening test using the zebrafish patient-derived xenograft model (zAvatars), where in-vivo results can be obtained in just 10 days. This assay relies on the injection of fluorescently labeled tumor cells from core needle biopsies, surgical specimens, pleural effusions and ascites into zebrafish embryos. Tumor behavior, and therapy response are then assessed after 2–4 days of treatment. To determine the predictive value of the BC zAvatars we performed a pre-clinical study, where zAvatars were treated with the same therapy as the donor-patient and their response to therapy was compared.

Result and discussion

We successfully established zAvatars across all five molecular BC subtypes, at different disease stages, and with varying tumor aggressiveness. Notably, zAvatars derived from ER-positive tumors retained ER expression and key BC biomarkers such as ER, PR, and Gata3, while preserving the tumor microenvironment. Our data show a 100% concordance (18/18 cases) between zAvatar-test and the corresponding patient's clinical response to treatment. Additionally, we observed that the majority of zAvatars derived from patients with early-stage BC did not form micrometastases, whereas all zAvatars from advanced-stage BC displayed micro-metastases. This suggests that zAvatar metastatic potential reflects donor tumor stage. However, given the small sample size and the long latency of BC recurrence, further studies are needed to determine whether the zAvatar model can reliably predict metastasis risk. If validated, this model could significantly enhance BC treatment management by refining risk stratification. The presence of micrometastases in zAvatars may help identify patients requiring more intensive adjuvant therapy and closer surveillance, whereas their absence could suggest a lower risk of recurrence.

Conclusion

The zAvatar model accurately predicted patient treatment responses across diverse therapeutic regimens (single-agent and combination therapies), disease stages and BC subtypes. These findings highlight its potential as a powerful, versatile and personalized in-vivo assay for optimizing BC treatment strategies.

EACR25-1110

Effect of RID-A proteins in cachexia and tumor metabolism

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Introduction

RID-A proteins play an important role in tumor metabolism and cachexia, which is a complex multi-factorial syndrome characterized by a progressive decrease in muscle mass in cancer patients. In particular, these proteins exhibit unique enzymatic properties related to amino acid metabolism and play a vital role in accelerating the hydrolysis of amines and imines. One of the important functions of RID-A is to neutralize the highly reactive metabolite 2-aminoacrylate, which has the potential to disrupt various metabolic pathways, and hydrolyze it into pyruvate. Additionally, alterations in tumor metabolism significantly contribute to the progression and severity of cachexia. Therefore, understanding the detoxifying power of RID-A proteins may offer insights into modulating tumor metabolism and alleviating cachexia symptoms.

Material and method

We used an *in vivo* cachexia model in BALB/c mice using a subcutaneous injection of C26 murine colon carcinoma cells. Mice were treated once a week for two weeks with 2.5 mg/kg of UK114 protein, belonging to RID-A family, synthesized from mouse (MS UK) sequences. Mice were sacrificed at >10% weight loss. At the time of sacrifice, tumors and organs were weighed. The gastrocnemius was analyzed for muscle fibers area. Further analysis was carried out for the expression of atrogens (atrogin-1, musa-1, murf-1, overexpressed in case of cachexia). Furthermore, an analysis of tumor metabolism was carried out, quantifying the levels of pyruvate and lactate on tumor specimens. A trichrome staining was performed to assess tumor angiogenesis, and the expression of vascular endothelial growth factor (VEGF) was evaluated through real-time PCR.

Result and discussion

UK114 treatment induced a slowdown in tumor growth and an improvement in cachectic symptoms, with less weight loss. Furthermore, an improvement in the cachectic state was observed at the level of the muscle fibers as well as at the molecular level. Pyruvate levels were increased and could possibly be implicated in the inhibition of HDAC, whose levels slightly decreased. In parallel, lactate levels decreased. A reduction in the number of intratumoral vessels was also observed, which was confirmed at the molecular level by the reduced VEGF levels.

Conclusion

Our study evaluated the detoxifying effect of UK114 on neoplastic cachexia and demonstrated how UK114 induces a reduction of tumor volume, angiogenesis and an amelioration of muscle fibers. Further studies will better assess the effect on tumor metabolism.

EACR25-1112

Targeting BCKDK to Overcome Paclitaxel Resistance in Breast Cancer: A Novel Therapeutic Approach

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Introduction

Breast cancer is one of the most common malignancies globally, with chemotherapy, including paclitaxel, being a cornerstone of treatment. However, paclitaxel resistance is a major clinical challenge, limiting treatment success and leading to relapse. Branched-chain keto-acid dehydrogenase kinase (BCKDK), a key regulator of branched-chain amino acid metabolism, was identified in siRNA screens as a potential contributor to paclitaxel resistance. This study evaluates BCKDK as a novel therapeutic target to overcome chemotherapy resistance in breast cancer.

Material and method

Paclitaxel-resistant MCF7 breast cancer cells were cultured under standard conditions and treated with paclitaxel alone or in combination with experimental BCKDK inhibitors, CMVA and DCBC. These inhibitors were identified through prior siRNA screening and chemical design to specifically target BCKDK activity. Protein extraction was performed using RIPA buffer, followed by quantification using the BCA assay. Western blotting was used to confirm BCKDK overexpression in resistant cells. For functional evaluation, BCKDK protein was purified using His-tag affinity chromatography, followed by ion-exchange chromatography, and characterized via HTRF kinase assays to assess enzymatic activity and inhibitor potency. HTRF assays measured the ability of CMVA and DCBC to block ATP binding to BCKDK. Cell viability was assessed using the SRB assay and CellTiter-Glo, comparing paclitaxel monotherapy versus combination treatments. Statistical analysis was performed to evaluate synergy using Combination Index (CI) calculations.

Result and discussion

BCKDK was overexpressed in paclitaxel-resistant MCF7 cells compared to sensitive cells. Combining paclitaxel with BCKDK inhibitors significantly reduced cell viability compared to either treatment alone. HTRF assays confirmed potent inhibition of BCKDK kinase activity by novel inhibitors, interfering with ATP binding. The data suggest that BCKDK promotes survival mechanisms that contribute to chemoresistance. By blocking BCKDK, cells become more susceptible to paclitaxel-induced apoptosis, indicating a synergistic therapeutic potential.

Conclusion

BCKDK is a promising therapeutic target in paclitaxel-resistant breast cancer. Dual targeting of BCKDK and microtubule dynamics via paclitaxel offers a novel strategy to overcome chemotherapy resistance. This approach could improve treatment outcomes for patients with resistant breast cancer.

EACR25-1129**Preclinical Evaluation of T-DXd and AZD1390 in HER2-Low, Triple-Negative Breast Cancer Models**

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Introduction

Triple-negative breast cancer (TNBC) is defined by its deficiency of oestrogen-, progesterone-, and HER2 receptors; Antibody-drug conjugates (ADCs) provide a novel treatment strategy. Trastuzumab deruxtecan (T-DXd), a HER2-directed ADC with a topoisomerase I-inhibiting payload, is approved in both HER2-amplified and HER2-low (HER2 IHC1+ and IHC2+, without amplification) breast cancer. 30–40% of TNBC tumours show HER2-low status and may benefit from T-DXd. Additionally, disruption of the DNA damage response pathway is a promising approach. Ataxia-telangiectasia mutated (ATM) kinase is a major player in double-strand break repair response (DSB). Inhibitors of ATM kinase enhance the effect of DSB inducers, like topoisomerase inhibitors, resulting in DNA damage accumulation. Therefore, there is strong rationale for investigating T-DXd plus ATM inhibition in TNBC.

Material and method

We investigated T-DXd plus the ATM inhibitor AZD1390 in eight TNBC cell lines with HER2-low expression. Target expression patterns of the TNBC panel were examined via Western Blotting. Proliferation was assessed by 5-day matrix based acid phosphatase assay in 2D conditions. Synergy scores were calculated with Combenefit Software. 3D cell culture models were created with the RASTRUM Bioprinter. Cell viability was assessed after 7-day drug treatments in 3D cell models via CellTiter-Glo® 3D Cell Viability Assay. Synergistic combinations were further characterised by investigation of Caspase 3/7 apoptosis induction via live-cell imaging and assessment of DNA damage induction via high-resolution fluorescence microscopy.

Result and discussion

All tested cell lines were sensitive to T-DXd and AZD1390 single agents (SA) below the achievable peak plasma concentration. Both drugs were combined across clinically relevant concentrations. No antagonism was observed. Moreover, strong synergy was observed across concentrations in the entire cell line panel. Three cell lines were chosen for further investigation, MDA-MB-468, MDA-MB-231, and HCC1937, which all showed high responsivity to the combination treatment. 3D-cell models of these cells were created using the RASTRUM Bioprinter. T-DXd [5 µg/ml] and AZD1390 [2.5 µM] were given as SA or in combination over a 7-day treatment. T-DXd plus AZD1390 synergistically reduced cell growth in 3D models, and cell viability was significantly reduced in combinations compared to single-agent ADC ($p \leq 0.05$). Response to treatment in 3D culture conditions mirrored 2D cultures, with no difference in drug sensitivity. Live-cell imaging revealed that combination treatment synergistically increased apoptotic

cell death compared to SA (T-DXd [5 µg/ml], AZD1390 [2.5 µM]).

Conclusion

T-DXd plus AZD1390 shows significant synergy in growth reduction of cancer cells in 2D and 3D culture conditions, even at low nanomolar concentrations. This combination shows promising results to enhance target therapies for TNBC patients.

EACR25-1144**Datopotamab deruxtecan plus PARP inhibition is highly synergistic in 2D and 3D ovarian cancer models**

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Introduction

Ovarian cancer (OC) is the 5th leading cause of cancer deaths in women worldwide. Datopotamab deruxtecan (Dato-DXd) is a novel TROP2-directed antibody-drug conjugate (ADC) with a topoisomerase I inhibitor payload that is FDA approved for the treatment of hormone receptor-positive, HER2-negative breast cancer that has been previously treated with systemic therapy. TROP2 is commonly overexpressed in OC, making it an attractive therapeutic target. PARP inhibitors (PARPi) target the DNA damage response (DDR) and are used to treat different cancer types, especially cancers with DDR deficiency. Olaparib is a PARPi approved for the treatment of several different cancer types. Saruparib (AZD5305) is a PARP1-selective inhibitor in clinical development. This preclinical study examines the efficacy of Dato-DXd alone and in combination with PARPi olaparib and saruparib in OC cell lines in 2D and 3D culture conditions.

Material and method

This study utilised a panel of six OC cell lines with different BRCA2 mutation and HR deficiency status. The anti-proliferative effects of Dato-DXd and olaparib/saruparib were determined using a 5-day acid phosphatase assay. Synergism was assessed using Combenefit software with the Loewe additivity model. Induction of caspase 3/7 activation, a marker of apoptosis, was tested with single agents and combinations using kinetic, fluorescent microscopy with the Incucyte S3 imaging system. Internalisation rates of Dato-DXd were monitored using the Incucyte. Single agent and combination anti-proliferative effects were assessed in 3D bioprinted culture conditions utilising the RASTRUM platform. Comparison of image-based analysis and metabolic readout with Cell Titer Glo 3D was carried out.

Result and discussion

The combinations of Dato-DXd + olaparib/saruparib were highly synergistic in the TROP2-high cell line models tested with no antagonism observed (concentration range 0.008–5 µg/mL and 0.625–10 µM, respectively). Lower levels of synergy were still observed in TROP2-low cell lines. Treatment response of PEO1 and PEO4 cells was further functionally characterised.

Dato-DXd in combination with PARPi synergistically induced apoptosis. Combinations were effective in 3D models of cancer growth with comparable efficacy to 2D models. Dato-DXd is internalised in a time- and concentration-dependent manner in TROP2 expressing cells. Internalisation assays revealed increased levels of Dato-DXd internalisation when combined with olaparib.

Conclusion

This study provides a preclinical rationale for the clinical investigation of Dato-DXd in combination with PARPi olaparib or saruparib in OC.

EACR25-1165

Reactivating the crosstalk between natural killer and tumour cells in non-small cell lung cancer (NSCLC) patients with no actionable mutations

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Introduction

Significant progress has been made in treating non-small cell lung cancer (NSCLC) over the past two decades, with the discovery of oncogenic driver mutations facilitating personalized targeted therapies. However, managing advanced NSCLC without targetable drivers remains a clinical challenge and, for most patients, chemotherapy is still the standard of care. Advanced-stage tumours seem to have lost the crosstalk with the immune system, leading to poor responses to therapies. Natural killer (NK) cells act as a first line of defence against tumours and recent studies have shown that chemotherapy can enhance the innate immune system activity. Moreover, it is known that NK infiltrating solid tumours can improve patients' survival. Notably, NK cells expressing the P2X7 receptor (P2X7R) within the hypoxic tumour core exhibit an anergic state, which is hypothesized to be reversible through modulation of P2X7R expression. Our study investigates the potential of antimetabolite drugs to reactivate the NK-mediated immune response in NSCLC, focusing also on the combination of standard chemotherapy with P2X7R antagonists.

Material and method

A549 and NK-92 cell lines were acquired from ATCC and maintained following manufacturer's protocol, either for 2D and 3D experiments. Cytotoxicity and apoptosis were investigated through MTS assay (CellTiter 96® AQueous One Solution Cell Proliferation Assay, Promega) and Annexin V assay (Annexin V-FITC Apoptosis Detection Kit). Gene expression profile was determined by qRT-PCR (Applied Biosystems™ 7500 Real-Time PCR System) and Droplet Digital PCR (QX600 Biorad). NKs infiltration was evaluated on 3D tumour spheroids using confocal microscopy. Digital

Spatial Profiling (GeoMx®, Nanostring) was performed on FFPE tissues to study the immune microenvironment.

Result and discussion

We set up a co-culture model with A549 and NK cells to study the crosstalk after treatment with gemcitabine, pemetrexed and cisplatin. In addition we combined the antimetabolite drugs with AZ10606120, a P2X7R antagonist. Our results showed that gemcitabine and pemetrexed enhance NK-mediated cytotoxicity against A549 cells: we observed augmented apoptosis rate and increased expression of NK activating ligands, suggesting a significant NKs activation. Concerning the P2X7R role in modulating NKs activity, we observed an increased NK cell infiltration into 3D tumour spheroids treated with AZ10606120. Moreover, the combination of AZ10606120 with gemcitabine and pemetrexed demonstrated enhanced cell killing efficacy compared to chemotherapeutics alone. Finally, the combination resulted also in the activation of NK receptors, which was not observed with the drugs alone.

Conclusion

Our findings suggest that standard chemotherapy may indirectly enhance anti-tumour immunity by reactivating NK cells, also in combination with P2X7R antagonists, providing a potential avenue for improving therapeutic outcomes in NSCLC.

EACR25-1173

Harnessing the impacts of a G-quadruplex ligand in liposarcoma: Implications for MDM2 oncogene targeting and DNA damage-mediated immunomodulatory effects

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Introduction

Well-differentiated (WD)/dedifferentiated (DD) liposarcomas (LPS), which account for ~60% of all liposarcomas, are characterized by the amplification of the 12q13–q15 region, with consequent aberrant expression of MDM2. Patients have limited therapeutic options, particularly in advanced disease, and their outcomes remain largely unsatisfactory. Hence, there is an unmet need to identify and validate WD/DDLPS-specific actionable targets for developing novel biology-driven therapies. G-quadruplexes (G4s), non-canonical secondary structures that can form in G-rich nucleic acid sequences, have recently been identified and characterized within the inducible MDM2 P2 promoter. In addition, G4 ligand-induced DNA damage can lead to the formation of cytoplasmic micronuclei (MN). Spontaneous rupture of the MN envelope allows its recognition by receptor cyclic GMP-AMP synthase (cGAS), initiating the cGAS-STING-Interferon axis downstream of DNA damage. Therefore, we explored the

potential of inhibiting MDM2 expression by targeting its G-quadruplex structures with small-molecule G4 ligands while also assessing the immunomodulatory effects of the G4 ligand in DDLPS cell lines.

Material and method

We used in-house developed patient-derived LPS cell lines and normal pre-adipocytes. CUT&Tag and RNA-seq were performed to analyze the abundance of G4s in the different cell lines. Cell viability, RT-PCR and Western blot assays, siRNA inhibition and nascent transcript analysis were used to assess the effects of QN-302 treatment on different patient-derived DDLPS cell lines.

Result and discussion

A G4 ligand, QN-302, significantly impaired the growth of DDLPS cells in a dose-dependent manner. We found that DDLPS cell lines showed enhanced G4 formation and that QN-302 activity strikingly paralleled cell-specific G4 abundance. QN-302-mediated MDM2 G4 stabilization at the P2 inducible promoter prevented polymerase progression from the constitutive P1 promoter in DDLPS cells, thereby inhibiting the formation of full-length MDM2 transcripts. This resulted in the accumulation of the tumor suppressor protein p53. In addition, QN-302 induced DNA damage in DDLPS cells and increased MN, with a significant fraction marked by γ -H2AX. Concomitantly, exposure to QN-302 led to the upregulation of a subset of interferon-stimulated genes (ISGs), including IFI27, IFI44L, IFI6, ISG15, and MX2, along with an increased release of certain cytokines, particularly IL-6.

Conclusion

We have identified a novel therapeutic strategy to inhibit MDM2 expression and promote p53 reactivation in DDLPS. Additionally, QN-302-induced DNA damage may activate the cGAS-STING pathway in DDLPS cell lines. The G4 multiple-target modality holds potential for further development through the rational design of drug combinations with standard chemotherapy, targeted therapies, and immunotherapy.

EACR25-1186

Targeted Treatment of Solid Tumors with BRAF Fusions

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Introduction

BRAF fusions are uncommon driver alterations in a range of cancers. While the BRAF V600E mutation is a recognized target for the BRAF inhibitors, BRAF fusions have not yet been investigated as potential targets for treatment in most cancers, and there is limited literature available to help guide treatment for patients with these fusions with targeted therapies, including direct BRAF inhibitors and downstream MEK inhibitors. This systematic review aims to summarize the available literature on the targeted treatment of solid tumors harboring a BRAF fusion.

Material and method

We conducted a systematic review to delineate the reported efficacy, response rates and survival outcomes, of targeted treatment in patients with a solid cancer harboring a BRAF fusion. PubMed, Embase, and Cochrane CENTRAL were searched between 2014 and 2024. Data were extracted to assess the details of efficacy.

Result and discussion

A total of 37 studies involving 113 patients with BRAF fusion-positive cancer who underwent targeted therapies were included. Most of these studies were case reports and case series ($n = 31$, 84%), with four clinical trials (11%) that were either phase I or phase II. The review included various cancer types, with central nervous system (CNS) cancers, including gliomas and pilocytic astrocytomas, being the most common ($n = 66$, 58%), followed by melanoma ($n = 20$, 18%) and non-small cell lung cancer (NSCLC) ($n = 9$, 8%). Other cancers ($n = 18$, 16%) included sarcoma ($n = 7$), genitourinary ($n = 2$) and colorectal ($n = 1$). Among the BRAF fusion gene partners, KIAA1549 was the most frequent ($n = 62$, 55%), followed by SND1 ($n = 6$, 5%) and AGK ($n = 4$, 4%). The targeted therapies used were varied, with MEK inhibitors being used in most patients, selumetinib ($n = 36$, 32%), followed by trametinib ($n = 34$, 30%), as well as, binimetinib and cobimetinib. BRAF and pan-RAF inhibitors used included dabrafenib, vemurafenib, tovotafenib and belvarafenib. The response rates were reported for 103 patients, with 2% achieving a complete response ($n = 2$), 46% showing a partial response ($n = 47$), and 22% exhibiting stable disease ($n = 23$). Objective response rate in NSCLC, sarcoma, melanoma and CNS tumors was 88%, 57%, 52% and 41% respectively. Data on survival outcomes was limited.

Conclusion

In conclusion, there is limited data on the efficacy of targeted therapy in solid tumors with a BRAF fusion, with most evidence coming from case reports/series, alongside a few phase I/II trials. While BRAF fusion-positive cancers show varying yet promising responses to BRAF and/or MEK inhibitors, the effectiveness may vary with histology. Given the rarity of these fusions, there is a clear need for international collaboration and innovative trial designs, such as basket and platform trials, to assess targeted treatment strategies more effectively and improve outcomes for these patients.

EACR25-1193

Synthetic Danshen Compound Inhibits Adipogenesis, Promotes Adipocyte Browning, and Induces G1 Cell Cycle Arrest While Suppressing Tumor Cell Migration and Invasion in Prostate Cancer Cells

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Introduction

Prostate cancer (PCa) is the most prevalent cancer among men in developed countries, with its increasing incidence strongly linked to the rising prevalence of obesity and metabolic syndrome. Our previous studies have shown that the synthetic Danshen compound (SDC) can enhance ATF3 expression, inhibit adipogenesis and lipogenesis, and promote adipocyte browning. While both native and synthetic Danshen compounds have been observed to influence the growth and metastasis of PCa cells, the precise mechanisms underlying their actions remain unclear.

Material and method

This study aimed to investigate whether SDC affects the growth, migration, and invasion of PCa cells by modulating cell cycle and epithelial-to-mesenchymal transition (EMT)-related pathways. Androgen-dependent LNCaP cells and androgen-independent DU-145 and PC-3 cells were used as PCa models. We assessed the effects of SDC on these processes.

Result and discussion

Our findings revealed that SDC significantly reduced cell proliferation in all three PCa cell lines in a dose-dependent manner, as measured by the BrdU assay. The colony formation assay demonstrated a dramatic decrease in colony numbers following SDC treatment, further indicating its anti-tumor effects on PCa cells.

Mechanistically, SDC induced G1 cell cycle arrest by inhibiting the expression of key regulators, including CDK4/CDK6, Cyclin D1/Cyclin D3, and Myc. Moreover, SDC suppressed cell migration and invasion and reduced matrix metalloproteinase (MMP) activity in PCa cells. These effects were associated with the inhibition of AKT/S6 signaling and a reduction in N-cadherin protein expression, highlighting its impact on EMT-related pathways.

Conclusion

In conclusion, this study demonstrates that SDC exhibits potent anti-tumor activity by targeting multiple pathways involved in cell cycle regulation, migration, and invasion. In addition, SDC can promote periprostatic adipose tissue browning via suppressing the microenvironment of cancer progression. These combined effects thus suggest that SDC could serve as a promising therapeutic agent for the treatment of prostate cancer.

EACR25-1204

Long-term organoids derived from head and neck and ovarian cancer patients closely recapitulate tumor of origin and predict clinical outcome

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Introduction

Current approaches to precision oncology have been dominated so far by genomics. However, studies have generally shown that cancer patients who receive genomic testing do not always benefit from a genomic precision medicine strategy. This revealed the interest to explore additional approaches, such as functional precision medicine, a strategy by which living patient tumor cells, represented in patient-derived tumor-derived model, are exposed to therapies and response is measured to predict clinical response. In this context, the recent advent of Patient-Derived Tumor Organoids (PDTO) offers promising opportunities to facilitate personalized treatment strategies. Here we studied the feasibility of performing PDTO-based functional assays in ovarian and Head and Neck Squamous Cell Carcinoma (HNSCC) patients to evaluate their predictive potential for clinical purposes.

Material and method

We established a panel of 37 long-term PDTO models from ovarian cancer patients and 24 long-term PDTO from HNSCC patients. Histological and molecular profiles of PDTO were compared to patient tumor using immunohistochemical analyses and global approaches (CGH array and transcriptomic profiling). PDTO models were exposed to standard treatments for ovarian cancer and HNSCC, and response was assessed using viability assay. To further define the Homologous Recombination (HR) status of ovarian PDTO, we performed a functional assay evaluating the ability of PDTO to initiate HR (RECAP test) using automated histo-imaging quantitative analysis of RAD51 foci, as well as an NGS analysis based on the sequencing of an HR-related genes panel to obtain a genome instability score (GIS).

Result and discussion

We demonstrated that PDTO mimicked histological and expression of tumor markers of paired tumors. Moreover, non-negative matrix factorization approach revealed that PDTO recapitulated the transcriptomic profile of the cancer component from their sample of origin. Exposure of PDTO to the different treatments showed that PDTO exhibit heterogeneous responses. Interestingly, response of PDTO to cisplatin was correlated to prognosis of HNSCC patients while response of PDTO from high-grade serous ovarian carcinoma to carboplatin recapitulated patient response to first-line treatment. Additionally, the detection of HRD phenotype of PDTO using functional assay was associated with the results of the HRD test Genomic Instability Scar (GIScar).

Conclusion

These results provide further evidence of the potential interest of PDTO as relevant models for preclinical research as well as for functional precision medicine.

EACR25-1242**Assay development for addressing drug resistance mediated by fibroblasts in ER+ breast cancer***E. Rodriguez-Tomás¹, F. Massai², M. Vallin², A. Östman¹*¹*Karolinska Institutet, Department of Oncology-Pathology, Stockholm, Sweden*²*Karolinska Institutet, Department of Medical Biochemistry and Biophysics, Stockholm, Sweden***Introduction**

Identifying new drug targets to overcome therapy resistance in ER+ breast cancer is essential for enhancing patient survival and quality of life. Cancer-associated fibroblasts have been suggested as therapeutic targets for overcoming treatment resistance in ER+ breast cancer, given their ability to influence cancer cells via the secretion of signaling molecules, matrix proteins, and immune evasion mechanisms. Here, we establish a co-cultured-based high-throughput screen format to identify drug candidates that either inhibit the pro-tumoral effects of fibroblasts or overcome fibroblast-mediated treatment resistance.

Material and method

This assay utilizes a co-culture format incorporating MCF-7 and BJhTERT cell lines to effectively model ER+ breast cancer cells and human immortalized fibroblasts, respectively. Both cell lines were transfected with lentiviruses to express fluorescent proteins – mCherry in MCF-7 cells and GFP in BJhTERT cells. The cells were cultured in DMEM and seeded at different densities and ratios in mono- and co-culture into 384-well plates using a Multidrop liquid dispenser. At 5, 16, and 24 hours post-seeding, some plates were fixed with 4% PFA and stained with Hoechst nuclear dye, while others were treated with a selective estrogen receptor modulator (SERM) or left untreated as controls. Twenty-four hours after treatment, the plates were fixed, imaged, and analyzed using an Operetta CLS high-content analysis system. The analyses were conducted by quantifying MCF-7 and BJhTERT cell numbers based on Hoechst, GFP, and mCherry signals.

Result and discussion

Optimal cell densities and ratios in 384-well plates were identified as 500 MCF-7 cells and 1000 BJhTERT cells per well in both mono- and co-culture. Sixteen hours post-seeding was found to be the most suitable time to initiate treatment and observe fibroblast-driven growth effects. During this period, MCF-7 cells in monoculture exhibited a fold change of 1.3, indicating a 30% increase in cell numbers relative to the initial count. In co-culture with fibroblasts, the fold change was 1.9, reflecting a 90% increase and significantly greater cell growth than in monoculture, highlighting the growth-promoting effect of fibroblasts on MCF-7 cells. Tamoxifen administration at a concentration of 20 µM led to a significant reduction in MCF-7 cell numbers, with a 63% decrease in monoculture compared to 38% in co-culture. This indicates that the drug was more effective at inducing cell death in MCF-7 cells grown alone than in those co-cultured with fibroblasts, demonstrating the protective influence of fibroblasts.

Conclusion

Our co-culture-based assay format is well-suited to reveal the influence of fibroblasts on drug resistance in ER+ breast cancer cells. Furthermore, this model system will enable us to identify new fibroblast-related drug targets through high-throughput screening.

EACR25-1246**Anisomelic Acid Suppresses HPV-Driven Tumor Growth with Potential TEAD2 Targeting: Evidence from a Xenograft Model and Proteomics***N. Delshad¹, P. Paul¹, J. Rantala², J. Eriksson¹*¹*Åbo Akademi University and Turku Bioscience Centre, Cell Biology, Turku, Finland*²*Misvik Biology, Turku, Finland***Introduction**

Despite widespread vaccination programs, human papillomavirus (HPV) remains one of the leading causes of cancers, with a rising incidence of head and neck squamous cell carcinoma, emphasizing the need for new approaches. In our previous studies, we have shown that treatment of HPV-positive cells with Anisomelic Acid (AA) led to the degradation of the E6 and E7 onco-proteins of HPV16, the primary drivers of HPV-mediated oncogenicity. In the current study, we aimed to evaluate the in vivo efficacy of AA and identify its targets.

Material and method

We evaluated the efficacy and safety of AA in a murine xenograft model using HPV16-positive SiHa cells inoculated in Hsd Nude Foxn1nu mice. Following tumor establishment, mice received daily subcutaneous AA injections or placebo for 10 days. Tumor size was measured weekly during the study and ex vivo, and toxicity was assessed through body weight monitoring, histopathological analysis of liver, kidneys and spleen, complemented by blood biochemical analysis. To identify potential AA targets, we conducted a proteome integral solubility alteration (PISA) assay coupled with multiplexed quantitative proteomics via tandem mass spectrometry (MS/MS) on AA treated SiHA cells. Additionally, we performed computational docking simulations (Schrödinger, Glide) to explore AA's potential binding modes within target proteins. We also treated hippo pathway-dependent cell lines, (NCI-N87 and NCI-H23) with AA, and analyzed their dose-response curves.

Result and discussion

AA treatment inhibited tumor growth compared to the placebo group, without any observable toxicity. Body and organ weights remained within normal range, and histological analyses of the liver, kidneys, and spleen revealed no pathological changes despite minimal changes in alkaline phosphatase (ALP) and Globulin. Quantitative proteomic analysis identified TEAD2 as a potential target of AA. In addition, computational docking simulations on potential targets suggested that AA binds to TEAD2 with a docking score comparable to existing TEAD inhibitors. The binding appears to be stabilized by a salt bridge between AA carboxylate group and LYS389, along with a hydrogen bond involving ASN405. The dose-response curves for NCI-N87 and

NCI-H23 cell lines treated with AA yielded IC₅₀ values of 5.8 μM and 3 μM, respectively, showing the effectiveness of AA and providing more evidence supporting its TEAD inhibitory effects.

Conclusion

Anisomelic Acid (AA) effectively inhibited tumor growth in an HPV positive xenograft model without observable toxicity. Proteomics, computational analyses and in vitro screening suggest TEAD2 as a potential target. Further validation of AA's mechanism of action, particularly its role in TEAD inhibition and E6 and E7 degradation, is necessary. Future studies should explore AA's efficacy, alongside existing TEAD inhibitors in additional HPV positive models to strengthen its potential for clinical translation.

EACR25-1285

Investigating BCL3 as a therapeutic target to treat prostate cancer

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Introduction

Metastatic prostate cancer (mPC) remains the leading cause of mortality among prostate cancer patients, largely due to resistance to conventional treatments, including androgen/AR-targeted therapy (ARTT). The NF-κB signalling pathway is frequently up-regulated in PC/mPC, but current inhibitors face tolerability issues. As a key NF-κB regulator, BCL3 represents a promising therapeutic target. Our well-tolerated BCL3 inhibitor (BCL3i), TNAT-101, has shown potential in modulating NF-κB signalling, making BCL3 a strong candidate for novel therapeutic interventions. This study aims to investigate the role of BCL3 in tumour progression/ARTT-resistance and assess the therapeutic potential of TNAT-101.

Material and method

To examine BCL3 in prostate homeostasis and tumorigenesis, we employed genetically engineered mouse models (GEMMs) using both functional genetic (Bcl3 deletion) and pharmacological (BCL3i) approaches in high-risk/locally advanced PC (hrlPC) GEMMs. To assess the impact of BCL3 loss on ARTT sensitivity, we utilised a panel of human hrlPC/mPC cell lines (AR+/AR-/ARmut/ARamp) and GEMM-derived organoids with varying degrees of enzalutamide sensitivity.

Functional assays and molecular characterisation were performed to determine BCL3's role in ARTT response. Additionally, TNAT-101 efficacy was evaluated in multiple mPC patient-derived xenografts (PDX), and RNA-Sequencing was conducted to elucidate BCL3i

mechanisms and identify potential biomarkers of treatment sensitivity.

Result and discussion

Our findings show that BCL3 is dispensable for normal prostate homeostasis in vivo, yet its involvement in NF-κB signalling correlates positively with tumour progression in PC GEMMs, reinforcing its potential as a therapeutic target. TNAT-101 significantly reduced tumour burden in two castration-resistant mPC PDX models (CU-PC01 and PC2400), whereas PC-3 xenografts remained resistant. Molecular characterisation of PC2400 and CU-PC01 tumours, including RNA-Sequencing, revealed significant transcriptional alterations, including changes in WNT-related signalling genes. Additionally, TNAT-101 treatment in PTEN-deficient hrlPC GEMM-derived organoids ex vivo reduced proliferation and increased apoptosis, supporting its therapeutic potential in early-stage hrlPC. So far, BCL3i or BCL3 knockdown in our panel of cell lines revealed no impact on proliferation or viability but significantly affected stem cell function.

Conclusion

These results indicate that BCL3 drives PC/mPC growth and highlight BCL3-targeted therapy as a promising treatment for advanced PC. Ongoing investigations are exploring the role of BCL3 in prostate homeostasis, tumorigenesis and ARTT resistance to determine when TNAT-101 treatment would be the most effective, ultimately aiming to improve survival outcomes and patient care.

EACR25-1302

PTPN2/PTPN1 inhibition induces tumor eradication in ALK-positive lymphoma by triggering a combination of ALK-induced oncogenic stress and immune responses

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Introduction

Oncogenic fusions of the anaplastic lymphoma kinase (ALK) gene drive a subtype of anaplastic large cell lymphoma (ALCL), leading to the constitutive activation of ALK that triggers downstream oncogenic pathways such as MAPK/ERK, JAK/STAT, PI3K/AKT to promote cellular proliferation and survival. We recently discovered PTPN2 and PTPN1 phosphatases as two direct regulators of ALK phosphorylation, that is crucial to provide an optimal signaling in lymphoma cells: any alteration of ALK activation may reduce the cellular fitness. In this context, PTPN2 and PTPN1 are dependence genes in ALK+ ALCL with a mechanism that is not fully elucidated yet.

Material and method

ALK+ and ALK- ALCL cell lines were treated in vitro with the ALK inhibitor crizotinib (TKI) or the PTPN2/PTPN1 phosphatase inhibitor ABBV-CLS-484 (AC484) with increasing concentrations at various time points. Viability and cell cycle were analyzed by flow cytometry and CellTiter-Glo. ALK and downstream oncogenic signaling activation were assessed by Western Blotting.

In vivo studies were performed by injecting murine ALK+ ALCL cells into syngeneic BALB/c mice or by engrafting human ALK+ ALCL cell lines or patient-derived xenografts (PDXs) in NSG immunodeficient mice. Mice were treated with TKI or AC484. Tumor growth was evaluated by caliper or imaging, and residual tumor cells were assessed by histology and immunohistochemistry. Anti-ALK CD8+ T cell immune responses were measured by flow cytometry.

Result and discussion

AC484 induced a rapid hyperphosphorylation of ALK and its downstream mediators in all murine or human ALK+ ALCL cell lines, an effect that is opposite to the blockade of ALK and downstream signaling obtained with ALK TKIs. AC484 promoted apoptotic and DNA damage responses, although with kinetics delayed compared to TKI. ALK- ALCL and PTPN1/PTPN2 knock-out ALK+ lymphoma cells were insensitive to AC484, highlighting the specificity of AC484 for ALK and PTPN1/PTPN2. AC484 induced apoptosis in ALK+ ALCL cells that became resistant to TKI, suggesting its use as a therapeutic option for untreatable TKI-resistant ALCL. In vivo, AC484 promoted a marked reduction of tumor growth in human ALK+ ALCL grafted into NSG mice. Remarkably, AC484 induced a complete eradication of ALK+ lymphoma cells injected s.c. or i.v. in immunocompetent mice, concomitant to a potent increase of the anti-ALK CD8+ T cell immune response.

Conclusion

We propose AC484 as one of the first drugs that exploits oncogenic signaling amplification, instead of blockade, as a novel therapeutic approach. AC484 markedly amplifies ALK signaling resulting in oncogenic stress and anti-tumor activity selectively in ALK+ ALCL even if resistant to ALK TKI. In addition, AC484 strongly boosts anti-ALK immune responses suggesting that the immune modulating activity of AC484 could contribute to its curative potential.

EACR25-1318

The Superior Therapeutic Potential of Curcumin-Loaded Zinc Oxide Nanoparticles Against Bladder Cancer

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Introduction

Bladder cancer (BC) is one of the most common cancers globally, and while cystectomy remains the gold standard for treatment, it significantly impairs patients' quality of life. Bladder-preserving therapies like chemotherapy, immunotherapy, and novel agents are promising but are limited by severe side effects and high recurrence rates. The latter are mainly encountered due to the non-selective targeting of conventional treatments and tumor heterogeneity. Turmeric curcumin has been proven to have strong anticancer activity, but its clinical efficiency

is hindered by poor bioavailability. Nanotechnology in zinc oxide nanoparticles (ZnO-NPs) has shown to be a great approach to enhance drug delivery and therapeutic efficiency. This study aims to emphasize the possible application of zinc metal oxide in conjugation with curcumin in the eradication of BC cells.

Material and method

The study aims to synthesize and optimize ZnO-NPs with curcumin for improved therapeutic activity against BC. Anticancer activity of ZnO-NPs, curcumin, and their conjugates will be compared *in vitro* by utilizing 2D and 3D BC cell models including human bladder transitional cell carcinoma cell lines UC3 and T24, immortalized uroepithelial cell line SV-HUC-1, and BC patient-derived organoids. Cytotoxicity will be assessed by MTT and Trypan Blue assays, while migration, invasion, and oxidative stress effect will be assessed by wound healing, transwell invasion, and nitroblue tetrazolium assays. Apoptosis and cell cycle distribution will be monitored by flow cytometry. Additionally, cancer stemness will be explored by sphere formation assays, while immunofluorescence, qRT-PCR, and Western blot analysis will reveal important molecular alterations.

Result and discussion

ZnO-NPs were synthesized using the co-precipitation process and the formation was confirmed using X-ray diffraction. Scanning electron microscopy revealed aggregation of nanoparticles, and Energy-dispersive X-ray showed the presence of Zn and O. Additionally, we succeeded in establishing BC patient-derived organoids indicating their feasibility for use in future cancer research.

Conclusion

This study suggests that ZnO may enhance the anticancer activity of curcumin, potentially improving therapeutic outcomes in BC patients.

EACR25-1321

The RUVBL1/2 Complex: An Actionable Molecular Target in Bladder Cancer

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Introduction

Bladder cancer is a common malignancy in urology, marked by a high recurrence rate and limited treatment strategies. This highlights the need for novel biomarkers and therapeutic targets. The RUVBL1/2 complex, consisting of RUVBL1 and RUVBL2 and functioning as a AAA+ ATPase, plays a crucial role in various cellular mechanisms. Dysregulation of RUVBL1/2 has been implicated in cancer, with studies showing that both genetic and pharmacological inhibition can suppress tumorigenic properties. However, the specific contribution of the RUVBL1/2 complex to bladder cancer remains largely unexplored.

Material and method

A genome-wide CRISPR/Cas9 screening using the Brunello library in J82 and RT4 cell lines identified

RUVBL1 and RUVBL2 as druggable vulnerabilities in bladder cancer. To investigate the role of the RUVBL1/2 complex in tumor progression, CRISPR/Cas9-mediated depletion and pharmacological inhibition were employed. Functional analyses included proliferation, BrdU incorporation, cell cycle and apoptosis, 2D clonogenic and 3D soft agar anchorage-independent growth assays, migration and invasion, and DNA Damage and Replication Stress assays. Additionally, the effects of pharmacological inhibition were assessed across these assays, emphasizing its therapeutic potential.

Result and discussion

Genetic depletion or pharmacological inhibition of the RUVBL1/2 complex using the selective inhibitor CB-6644 significantly impaired cell proliferation, survival, and 3D anchorage-independent growth, leading to cell cycle arrest and increased apoptosis. Mechanistically, these effects were accompanied by a reduction in migration and invasion capacities, supported by elevated DNA damage, replication stress, and alterations in key molecular markers, further highlighting the therapeutic potential of targeting RUVBL1/2 in bladder cancer.

Conclusion

Our findings indicate a significant reliance of bladder cancer on the RUVBL1/2 complex, emphasizing its potential as both a biomarker and a therapeutic target. The functional and mechanistic roles of RUVBL1/2 in tumor progression warrant further investigation, particularly in the context of its cellular and molecular interactions. Future studies exploring these pathways will provide deeper insights into the contribution of RUVBL1/2 to bladder cancer pathogenesis and may aid in the development of targeted therapeutic strategies.

EACR25-1325

Cold-pressed lingonberry (*Vaccinium Vitis-idaea*) extract inhibits proliferation, invasion and glycolysis of oral carcinoma cells

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Introduction

Berry phytochemicals, such as polyphenols, show anti-cancer effects and may prevent or inhibit progression of oral cavity carcinoma. Our aim was to investigate the anticancer activity of cold-pressed lingonberry extract (LBE) on oral cancer.

Material and method

Four different cell lines were tested for proliferation and energy metabolism: primary oral tongue carcinoma (SCC25), lymph node metastatic oral tongue carcinoma (HSC-3), HPV-16 transfected, dysplastic oral keratinocytes (DOK) and normal gingival fibroblasts (NGF). Cell

cultures were treated with lingonberry extract (LBE, 0.1%, 1% and 2%) in culture media, with and without pH-adjustment. Cell proliferation was measured after 72 hours with CyQuant direct assay. Cellular energy metabolism, including glycolysis (extracellular acidification rate) and mitochondrial respiration (oxygen consumption rate), were measured after 48 hours of LBE treatment. Additionally, proliferation and apoptosis of the malignant cell lines were investigated over 48 hours with live cell imaging assays. Invasion length and area were measured over 72 hours in a spheroid model of the metastatic HSC-3 cell line.

Result and discussion

LBE inhibited proliferation of malignant and dysplastic cells, but not NGF. Similarly, LBE inhibited glycolysis in the malignant and dysplastic cell lines but had no effect on NGF. The pH-adjusted LBE inhibited cellular proliferation of oral carcinoma cells (SCC25 and HSC-3) but did not affect proliferation of NGF or DOK. LBE increased mitochondrial respiration in SCC-25 and HSC-3 but decreased it in the non-malignant cells. LBE and pH-adjusted LBE inhibited similarly the invasion of metastatic HSC-3, but LBE had a pronounced effect on proliferation compared to the pH-adjusted extract.

Conclusion

Cold-pressed lingonberry extract inhibited proliferation and invasion of oral carcinoma, possibly by targeting cellular energy metabolism.

EACR25-1327

Discovery of a novel 1,2-epoxysteroid oxime with potent anti-pancreatic cancer activity

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Introduction

Pancreatic cancer (PanC) remains one of the most lethal malignancies, with a 5-year survival rate of only 5%, highlighting the urgent need for novel treatment strategies. The introduction of epoxide and oxime functions in the steroid backbone has been shown to have increased cytotoxicity in several types of cancer cell lines. Given the significant burden of PanC, we aimed to assess the anticancer potential of newly synthesised steroid derivatives with oxime and epoxide groups in several PanC cell lines.

Material and method

The compounds OX1, EP2OX and OX4, along with their corresponding parent compounds P1, OX2 and P4 were designed, synthesised and evaluated for their cytotoxicity in three PanC cell lines, MIA PaCa-2, PANC-1, and Hs 766T through the SRB assay, 48h after treatment with the compounds (1 to 75 μ M). Additionally, cell viability, cell death profile, cell cycle alterations and mitochondrial membrane potential were evaluated by flow cytometry.

Result and discussion

The compounds were successfully synthesised following known methodologies for the synthesis of oximes and epoxides. In general, all steroid derivatives decreased MIA PaCa-2, PANC-1 and Hs 766T cancer cells proliferation in a dose-dependent manner. The most sensitive cell line to the three compounds was MIA PaCa-2 with IC₅₀ values of 7.70 μ M (OX1), 2.36 μ M (EP2OX) and 15.01 μ M (OX4). In contrast, in general, the parent compounds exhibited significantly lower cytotoxicity in all the PanC cell lines studied, compared to their corresponding derivatives. Such fact proves that the introduction of epoxide and/or oxime functions increased the cytotoxic potential of these compounds. Additional flow cytometry studies with EP2OX (the most active compound) revealed that it was able to decrease MIA PaCa-2 cancer cell viability, causing cell death by necrosis at higher doses. This was accompanied by a cell cycle blockage at phase G2/M and mitochondrial membrane dysfunction.

Conclusion

EP2OX exhibited a cytotoxic effect driven by necrotic cell death. These findings highlight the need for further investigation into its mechanism of action and selectivity, paving the way for the development of novel therapeutic candidates for PanC treatment.

Funding: CIBB strategic projects: 10.54499/UIDB/04539/2020 and 10.54499/UIDP/04539/2020 and Associated Laboratory funding 10.54499/LA/P/0058/2020. CERES strategic projects: 10.54499/UIDB/00102/2020, 10.54499/UIDP/00102/2020. All projects were supported by FCT.

EACR25-1329

Unveiling the Therapeutic Potential of Tick Salivary microRNAs in Cancer Treatment

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Introduction

MicroRNAs (miRNAs) are key regulators of gene expression, influencing diverse cellular processes including tumorigenesis. While endogenous miRNAs have been extensively studied in cancer, exogenous miRNAs from non-human sources remain largely unexplored. Recent findings suggest that tick salivary miRNAs possess regulatory potential in host cells, with possible implications in cancer therapy. Here, we investigate the impact of a set of tick-derived miRNAs (designated miR-X, miR-Y, miR-Z, and miR-W) on melanoma and pancreatic cancer models, aiming to elucidate their functional role and therapeutic potential.

Material and method

Synthetic miRNA mimics were transfected into A-375 (melanoma), BxPC-3, and MIA PaCa-2 (pancreatic cancer) cells. RNA sequencing (RNA-Seq) and small RNA sequencing (small-RNASeq) were performed in A-375 assessing miRNA stability and differential gene expression. In silico target prediction and gene enrichment analysis identified miRNA targets and affected pathways. Functional assays, including viability (alamarBlue™), apoptosis (Annexin V/PI staining), and cell cycle analysis (PI staining by flow cytometry), RT-qPCR, and Western Blotting were performed in all three cell lines.

Result and discussion

Small-RNASeq confirmed successful miRNA delivery, with stable expression over 24 hours. RNA-Seq identified over 7,000 DEGs, with 81.4% of stable downregulated DEGs corresponding to predicted miRNA targets. Functional enrichment analysis highlighted pathways related to MAPK signaling, apoptosis, and cell cycle regulation. Both A-375 and BxPC-3 (BRAF-mutated) showed moderate metabolic inhibition, while MIA PaCa-2 (KRAS-mutated) exhibited the strongest response, with miR-Y reducing metabolic activity by 90% and increasing apoptosis by 1.83-fold. Cell membrane damage observed via electron microscopy suggested a non-classical cell death mechanism. Molecular validation confirmed Rcc-1, a nuclear factor linked to cell cycle regulation, as a direct miR-Y target, with 93.4% transcript and 16.4% protein reduction.

Conclusion

Our findings demonstrate that tick salivary miRNAs exert regulatory effects on cancer cells, impairing tumor cell survival through modulation of oncogenic pathways. The strong cytotoxicity of miR-Y in KRAS-mutated MIA PaCa-2 cells, along with the moderate metabolic inhibition observed in BRAF-mutated A-375 and BxPC-3 cells, suggests a potential interaction with the MAPK signaling pathway, a key regulator of proliferation and survival in many cancers. These results position exogenous miRNAs as promising candidates for innovative cancer therapies, warranting further exploration in preclinical models.

EACR25-1331

Boronated derivatives with a carborane cage, sulfamido group, and Gd-DOTA for B/Gd-NCT with Carbonic Anhydrase IX inhibition

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Introduction

Mesothelioma is a malignant neoplasm of mesothelial cells primarily caused by asbestos exposure and is highly aggressive. A multi-therapeutic approach is essential to improve treatment outcomes and prevent recurrence. Boron/Gadolinium Neutron Capture Therapy (B/Gd-NCT), combined with Carbonic Anhydrase IX (CA)

inhibition, offers a promising strategy. NCT is a binary radiotherapy using thermal neutron capture by nuclides (mainly 10B and 157Gd) to target malignant tumors, aiming to destroy cancer cells while sparing nearby healthy tissue. Moreover, the use of Gd containing compounds enhances treatment by allowing monitoring through MRI.

Material and method

2 types of compounds were synthesized and evaluated as 10B and 157Gd delivery agents to perform at the same time NCT and CA inhibition: 1-Boronated derivatives (CA-USF) containing a carborane cage conjugated with sulfamido and ureido groups. 2- Boronated derivatives (Gd-CA-SF) conjugated with sulfamido and a Gadolinium complex. BNCT and B/Gd-NCT experiments conducted on AB22 murine mesothelioma cells. Phantom imaging and uptake assessments were performed by incubating AB22 and Met-5A (normal mesothelium cell line) with Gd-CA-SF. In vivo experiments were then carried out using CA-USFs.

Result and discussion

In vitro studies demonstrated that CA-USFs exhibited stronger CA inhibition than Acetazolamide. BNCT experiments on AB22 cells incubated with CA-USFs showed significant proliferation inhibition, with a complete suppression observed 20 days post-neutron irradiation. In vivo experiments showed a significant tumor reduction in mice treated with 10B-CA-USFs, especially with BNCT and CA IX inhibition. The uptake of Gd-CA-SF by AB22 cells was higher than that of Met-5A cells, consistently with the results of phantom imaging, thus demonstrating the selectivity of the system. In vitro B/Gd-NCT results showed that combining the effects of 157Gd and 10B with CA-SF, followed by irradiation, achieved the lowest proliferation rate in AB22 cells.

Conclusion

This study highlights the potential of combining BNCT with CA inhibition for mesothelioma treatment. CA-USFs showed strong CA inhibition, enhancing BNCT efficacy and completely suppressing AB22 cell proliferation in vitro while significantly reducing tumors in vivo. The selective uptake of Gd-CA-SF by AB22 cells, confirmed by phantom imaging, underscores the precision of this approach. The synergy of 157Gd, 10B, and CA inhibition achieved the lowest proliferation rates, demonstrating its therapeutic promise.

EACR25-1333

Investigating the cytotoxic effects of rhenium (V) complexes with apigenin and derivatives

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Introduction

Apigenin is an aglycone of many natural glycosides and is known chemically as 4,5,7-trihydroxyflavone.¹ The biological and pharmacological activities of apigenin are diverse, which include anti-inflammatory, antioxidant, anticancer, anti-proliferative and anti-spasmodic.² The chemistry of oxorhenium(V) complexes is receiving increasing attention because of the potential application in medical and chemistry.

Material and method

Three rhenium complexes with apigenin and its derivatives were synthesized (Complex 1: C₃₇H₂₉Cl₂O₉PRe, Complex 2: C₃₇H₃₇Cl₂O₉PReSi₂, Complex 3: C₃₄H₂₉Cl₂O₇PRe and acetylated C₁₉H₁₄O₇ and silylated apigenin C₂₇H₃₈O₄Si₂), fully characterized and used for biological studies. Cytotoxicity assessments were conducted using the resazurin reduction assay across multiple cancer cell lines, including HT29 (colon-rectal adenocarcinoma), Jurkat (T-cell leukemia), LNCaP (prostate cancer), and MCF7 (breast cancer). Cisplatin was used as a standard of care for this experiment.

Result and discussion

All three complexes showed significant cytotoxic potential toward Jurkat cell line and moderate activity against other cell lines. Furthermore, IC₅₀ values of all complexes were less than 5 μM, and demonstrated enhanced cytotoxic activity compared to the parent ligands in most cases, particularly in leukemia cells (Jurkat), while solid tumor cell lines showed more variable responses. Additionally, two Re compounds were selected for further investigation in dedicated anti-migration and anti-invasion assays.

Conclusion

Taking everything into consideration, rhenium plays a crucial role in disrupting leukemia cell biology. On the other hand, isolated ligands have milder effects, suggesting the complexes' bioactivity is enhanced by rhenium coordination. These findings will elucidate the molecular mechanisms and identify potential biomarkers. This comprehensive assessment of their physicochemical properties and cytotoxicity has provided valuable data to support the continued development of rhenium compounds as potential anti-cancer agents.

EACR25-1342

Biological Basis for the Development of CPL410005 as an Antibody-Drug Conjugate

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Introduction

CPL410005 is a potent UBA1 inhibitor with strong anti-cancer activity in vitro. However, its systemic toxicity and poor tumor selectivity limit its therapeutic potential as a standalone small-molecule drug. To overcome these limitations, this study explores the conjugation of CPL410005 to an anti-HER2 antibody to enhance tumor specificity and reduce off-target effects. Targeting the Ubiquitin-Proteasome System (UPS) with Antibody-Drug Conjugate (ADC) for directed UBA1 inhibition represents a promising strategy for cancer therapy.

Material and method

CPL410005 cytotoxicity was assessed across a broad panel of cancer cell lines. Its pharmacokinetic properties, including solubility, metabolic stability, and hepatotoxicity, were evaluated in vitro. To improve tumor-specific targeting, CPL410005 was conjugated to an anti-HER2 antibody via a selectively cleavable linker. ADCs were characterized in vitro for stability, HER2 binding affinity (SPR), and cytotoxic effects in HER2-positive and HER2-negative cell lines.

Result and discussion

CPL410005 exhibited potent in vitro cytotoxicity on a panel of 130 cell lines, with IC₅₀ values of 15.3 nM in U937 and 22.7 nM in HCT116 cell lines. However, systemic administration in xenograft models resulted in dose-limiting toxicity at 10 mg/kg. ADC conjugation significantly improved tumor-specific uptake, with conjugation yields above 85%, as confirmed by RP-HPLC. HER2-ADC conjugates retained high HER2 binding affinity (KD = 1.2 nM) and displayed selective cytotoxicity in HER2-overexpressing SK-OV-3 and SK-BR-3 cells, with IC₅₀ values of 1.8 nM and 2.5 nM, respectively. No significant cytotoxicity was observed in HER2-negative HEK293 cells at concentrations up to 1 μM. Pharmacokinetic studies showed prolonged plasma half-life (*t*_{1/2} = 18.2 h) compared to free CPL410005 (*t*_{1/2} = 2.9 h), while linker cleavage-dependent drug release ensured selective cytotoxicity in target cells.

Conclusion

CPL410005's potent UBA1 inhibition utilized in the form of ADC technology enhances tumor selectivity and reduces systemic toxicity. These findings support further preclinical development of HER2-targeted UBA1 inhibitors as a novel therapeutic strategy for HER2-positive cancers.

Project co-financed by NCBR, POIR.01.02.00-00-0009/17

EACR25-1347

Arginine deprivation, mTOR signalling and DNA repair response in models of ovarian cancer

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Introduction

High-grade serous carcinoma (HGSC) is the most frequently diagnosed and aggressive form of ovarian cancer that presents with poor clinical outcomes long-term. Despite the initial success of current treatments, such as platinum-based chemotherapies and poly (ADP-ribose) polymerase (PARP) inhibitors, patients often relapse, highlighting the need for more effective maintenance therapies. Arginine deprivation therapies, such as administration of the enzymatic drug ADI-PEG20, target tumour metabolism by removing extracellular sources of arginine that cancer cells rely on for growth and proliferation and has been shown to be especially effective in cells deficient in the rate-limiting enzyme of arginine biosynthesis, argininosuccinate synthase (ASS1).

Material and method

Analyses were performed on CRISPR/Cas9-edited ID8 murine ovarian epithelial models with TP53-loss, PTEN-loss and ASS1-deficiency. The bulk metabolomes of ADI-PEG20-treated or arginine-starved ID8 lines/ omental tumours were analysed by liquid chromatography-mass spectrometry (LC-MS). Treatment-related growth inhibition was assayed using SRB and compared between PTEN-wild type/loss models. The effects of arginine starvation on mTOR signalling and DNA repair response proteins were measured by western blotting analysis.

Result and discussion

Arginine deprivation via ADI-PEG20 treatment or medium starvation induces global metabolic changes impacting several pathways including polyamine biosynthesis, one-carbon metabolism, choline metabolism and glutamine metabolism. Arginine starvation induced suppression of mTOR signalling and DNA repair response factors, suggesting mechanisms by which arginine deprivation may inhibit tumour growth and induce cell death differentially according to PTEN status when combined with the PARP inhibitor Olaparib.

Conclusion

These findings highlight the potential for arginine deprivation therapies in treating genetically defined subsets of ovarian carcinoma, particularly in combination with clinically approved agents that interfere with DNA repair such as PARP inhibitors.

EACR25-1359

Co-targeting of EGFR and Sp1 to inhibit TKIs resistance and improve immune response in NSCLC tumors

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Introduction

Hindering resistance to EGFR tyrosine kinase inhibitors (TKIs) is a major challenge in oncology. Many NSCLC

patients show high sensitivity to EGFR TKIs but under drug pressure, drug-resistant clones emerge, and new therapeutic strategies are needed to avoid treatment failure. Sp1 is a well-recognized transcription factor implicated in cancer progression and here we want to demonstrate that concurrent inhibition of EGFR kinase domain and Sp1 may hinder proliferation of cancer stem cells (CSCs), impair cell migration, improve immune response and enhance cell toxicity in NSCLCs.

Material and method

We used H1975, HCC827, PC9 and PC9 osimertinib resistant (PC9/OR) cell lines growth in adhesion or as tumor-spheres (TS). Cells were screened for Sp1 levels before and after 1 μM osimertinib treatment. Cell toxicity assay was performed using osimertinib (0.1–1 μM) alone or in combination with Sp1 inhibitor Mithramycin A (Mit A). We integrated our data with cBioPortal analysis for SP1 gene CNV expression in lung tumors and with computational studies to identify new Sp1 inhibitors. In silico methods along with HDOCK software were used to identify new compounds that show lower toxicity than Mit A but with similar ability to impair DNA binding activity of Sp1. Wound healing and TS assays were also conducted to detect cell migration ability and stem-like cells survival after 72h treatment. Furthermore, modulation of the immunosuppressive receptor CD47 in treated and untreated cells were also assessed along with screening of PDL-1 levels in exosomes isolated from sensitive and OR cells.

Result and discussion

Treatment with osimertinib 1 μM for 72h was able to strongly reduce cell viability and increase cell death markers in NSCLC cells. However, concomitant increase of Sp1 levels was also observed in treated cells along with activation of EMT, CSC signaling and CD47 expression. Similar results were obtained by western blot analysis of nuclear extracts and protein content of exosomes released from PC9 and PC9/OR cells. We found the highest Sp1 nuclear localization along with the highest PDL-1 exosomes levels in resistant cells. Bioinformatic analysis of SP1 gene CNVs revealed a lower overall survival (OS) in tumors with SP1 amplification. Interestingly, combined treatment using osimertinib along with Mit A was able to improve cell toxicity, reduce cell migration and inhibit the TS formation. We also found that drugs combination caused a reduction of CD47 levels on NSCLC cells surface. In addition, our computational studies showed that Hedera-saponin B, similar to Mit A, binds to the GGGCGG DNA box with high affinity thus representing a new potential inhibitor of Sp1 activity and reported to be a well-tolerated compound.

Conclusion

Co-targeting of EGFR and Sp1 may inhibit migration and survival of resistant cells with stem-like phenotype. In addition, such therapeutic strategy may hinder immune evasion and may be a tool for preventing treatment failure in NSCLC patients.

EACR25-1363

Characterisation of bicyclic drug conjugate tool molecules using complementary 2D and 3D cellular

models in vitro

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Introduction

Bicycle Therapeutics is developing a chemically synthesised bicyclic peptide (Bicycle®) class of medicines characterised by their high affinity and selectivity, small size enabling fast tissue penetration, modularity for conjugation and favourable pharmacokinetics. Bicycle molecules are currently being explored in the clinic as Bicycle® Drug Conjugates (BDCs™) for targeted delivery of cytotoxic payloads into tumours.

Material and method

A panel of cell lines with a range of target cell surface expression levels were used to characterise tool BDC molecules designed to target membrane type 1 matrix metalloproteinase (MT1-MMP). Cell binding and internalization were determined by flow cytometry and confocal microscopy, respectively, using fluorescently labelled peptides. Cytotoxicity assays were performed in 2D and 3D cellular models with Bicycle Drug Conjugates (BDCs).

Result and discussion

Assays have been developed to demonstrate that cell binding correlates with target expression. Additionally, target mediated gradual internalisation was observed within a 6-hour period for binder molecules. By contrast, no cell binding or internalisation was observed with non-binder molecules. Target-mediated cell killing can also be characterised in 2D and 3D with the BDC molecules.

Conclusion

Complementary use of 2D and 3D cellular models for pharmacological characterisation represents the most complete approach to study Bicycle molecules in vitro. As 3D cell models closer mimic the 3D structure and cell-to-cell interaction, these assays may be used to support the translation to *in vivo* models.

EACR25-1373

Diphyllin, a SPP1/DKK1 inhibitor, restricts hepatocellular carcinoma growth by modulating sugar and bile acid metabolism

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Introduction

Hepatocellular carcinoma (HCC) is a prevalent solid tumor with high global incidence and mortality rates. The overexpression of tumor-derived SPP1 and DKK1 is implicated in the malignant progression of HCC, poor prognosis and resistance to chemotherapy. Inhibitors targeting tumor-derived SPP1 and DKK1 thus represents a promising strategy for cancer therapy.

Material and method

Molecular docking was used to screen out the inhibitors of SPP1/DKK1 from herbal databases. *In vitro* and *in vivo* experiments were performed to determine the anticancer effect of diphyllin on HCC. To further

elucidate the underlying mechanism of Diphyllin against HCC, RNA-Sequencing and metabolomics were performed to analyse the alteration of genes and metabolic pathways.

Result and discussion

We discovered a natural molecule, Diphyllin, can suppress the expression of both SPP1 and DKK1. Diphyllin was found to induce apoptosis in HepG2 and Hepa1-6 while suppressing cell proliferation, migration, and invasion. Mechanistically, Diphyllin appears to reprogram sugar metabolism and inhibit bile acid synthesis by regulating the HIF-1 pathway in liver cancer cells, leading to the enhancement of the cellular stress response. These mechanisms collectively promote apoptotic cell death and reduce cell proliferation. Furthermore, the combination of Diphyllin with sorafenib demonstrated significant synergistic anti-tumor activity *in vitro*.

Conclusion

In summary, our findings highlight Diphyllin as a promising natural inhibitor of SPP1/DKK1, effectuating robust anti-tumor effects potentially through the inhibition of bile acid synthesis and the reprogramming sugar metabolism, which enhances the cellular stress response and promotes apoptotic cell death, leading to the suppression of growth on HCC.

EACR25-1374

HSPGs targeting and multimeric cytotoxic conjugates as a novel PDC strategy against pancreatic cancer

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Introduction

Heparan sulfate proteoglycans (HSPGs) are membrane-bound and extracellular matrix glycoproteins playing critical role in cell physiology. Together with fibroblast growth factors (FGFs) and their receptors (FGFRs), HSPGs form ternary signaling complexes regulating numerous cellular processes e.g., differentiation, migration and proliferation. Moreover, HSPGs have been identified as a potential cancer-related biomarkers, overexpressed in several cancer types, especially in pancreatic cancer, underlining the importance of discovery of new drug delivery agents targeting HSPGs.

Material and method

We confirmed successful production of GFPp_FGF1_HS and CC3_FGF1_HS using SDS-PAGE and Western-Blot. Different oligomeric states of GFPp_FGF1_HS were isolated using FPLC. Later, using Native-PAGE, calibrated SEC column and DLS we confirmed the designed oligomeric state of produced MLAs. BLI and FPLC elution profile analyses were performed for estimation of GFPp_FGF1_HS and CC3_FGF1_HS affinity for heparans. We analyzed and compared endocytosis of different oligomeric states of MLAs using fluorescent microscopy. Sortase E-mediated ligation was performed for attachment of cytotoxic drug – mono-methyl auristatin E (MMAE), to GFPp_3x_FGF1_HS and CC3_FGF1_HS, confirmed with Western Blot and mass spectrometry. Finally, we estimated EC50 of

multimeric cytotoxic conjugates using cytotoxic assays with healthy and pancreatic cancer cell lines.

Result and discussion

Here, we designed and tested multimeric ligand assemblies (MLAs) based on engineered FGF1 variant with abolished recognition of FGFRs, selectively interacting with HSPGs. We used two different oligomerization scaffolds – coiled coil (CC) motifs and GFPpolygons (GFPp) for controlled oligomerization of FGF1. Importantly, BLI and FPLC elution profile analyses showed that oligomerization increases MLAs affinity for heparans in comparison to its monomeric form. In addition, fluorescent microscopy revealed that oligomerization of FGF1 increases its endocytosis via HSPGs due to multivalency-dependent clustering of HSPGs on the cell surface, demonstrating the remarkable potential of the studied MLAs as an effective targeting moiety. Finally, we conjugated designed MLAs with monomethyl auristatin E (MMAE), generating HSPG-specific protein drug conjugates (PDCs). Synthesized GFPp-based and CC-based multimeric cytotoxic conjugates showed high cytotoxicity against panel of pancreatic cancer cell lines with EC50 ranging from 4.15 nM to 6.83 nM.

Conclusion

Conclusively, this data suggests that targeting HSPGs with multimeric PDCs is an effective strategy for selective and precise drug delivery into pancreatic cancer cells.

EACR25-1376

Teaching cells the drug uptake – rational modulation of endocytosis for precise delivery of novel HSPGs targeting conjugate into pancreatic cancer cells

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Introduction

Pancreatic cancer has the highest mortality rate of any major cancer. Heparan sulfate proteoglycans (HSPGs) are cell surface glycoproteins involved in the regulation of cell-to-cell communication and modulation of cell signaling. HSPGs are overexpressed in pancreatic cancer, making them an attractive molecular target in precision medicine approaches. The natural ligands for HSPGs are fibroblast growth factors (FGFs) that together with the fibroblast growth factor receptors (FGFRs) transduce signals across the plasma membrane. Here we constructed an FGF1-based HSPG-specific oligomeric ligand, TriFHS, by uncoupling FGF1 from FGFRs and increasing its valency by controllable oligomerisation. We have successfully attached a cytotoxic drug MMAE to TriFHS and proved cytotoxicity of the conjugate against pancreatic cancer cells. Since effective endocytosis is key to the action of PDCs (protein drug conjugates), our goal was also to increase the selectivity and efficiency of delivery of TriFHS-vcMMAE to cancer cells. We decided to do so by manipulating cellular signaling, which ultimately controls endocytosis.

Material and method

Using confocal microscopy and immunofluorescence staining, we showed co-localisation of TriF with a molecular target (proteoglycan) and with endosomes. To find signaling cascades that inhibit internalization of TriFHS-vcMMAE into healthy cells, and at the same time promote endocytosis into pancreatic cancer cells, we employed high content screening (HCS) microscopy using the Opera Phenix Plus high-throughput confocal microscopy platform and the library of kinase inhibitors (2653 compounds). Finally, by studying different cell lines (physiological and pancreatic cancer cells) and selected in the HCS assay chemical compounds, using confocal microscopy, we showed differences in the cytotoxicity of TriFHS-vcMMAE.

Result and discussion

Initially, with HCS we identified kinase inhibitors that reduce the efficiency of TriFHS-vcMMAE endocytosis into healthy pancreatic cells. Based on this, we performed another HCS to study the effect of selected inhibitors on pancreatic cancer cells, and identified nine compounds that can block endocytosis into healthy cells and not affect or promote internalization into tumor cells. With the results gathered in this screen, we are now rationally modulating endocytosis to increase the selectivity and efficiency of cytotoxic drug penetration into pancreatic cells.

Conclusion

We show for the first time that it is possible to teach healthy and cancer cells specific behaviors and thus improve selectivity of ADC uptake, which can help to revolutionize the ADC (Antibody Drug Conjugate) approach.

This work was supported by OPUS grant (2021/43/B/NZI/00245) from the National Science Centre.

EACR25-1382

Apoptotic Effects of a Flavone Combination on Brain Metastatic Triple-Negative Breast Cancer Cells

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Introduction

Breast cancer (BC) is the most common malignancy in women, with approximately two million new cases diagnosed annually worldwide. Triple-negative breast cancer (TNBC) is particularly aggressive and lacks molecular targets, limiting treatment options and worsening prognosis. A major challenge in TNBC is its high tendency to metastasize to the brain, where therapeutic options are further restricted due to the blood-brain barrier and the limited efficacy of conventional treatments. Polyphenols have emerged as promising candidates for cancer therapy due to their ability to regulate key cellular processes such as proliferation, apoptosis, and metastasis. Previous studies identified diosmetin, apigenin, and luteolin as the primary flavones responsible for the antiproliferative effects of olive leaf extracts. This study evaluates the cytotoxic and cytostatic effects of a flavone combination (DAL511), composed of diosmetin, apigenin, and luteolin, in the brain metastatic TNBC cell line MDA-MB-231-BR. Human astrocytes

served as a non-tumoral control to assess treatment selectivity. Additionally, we analyzed apoptotic markers and key signaling pathways involved in TNBC progression.

Material and method

Cell models: MDA-MB-231-BR (TNBC, brain metastasis variant) and human astrocytes (non-cancerous control). Clonogenic assays were performed using crystal violet staining. Cell migration and invasion were assessed through wound healing and Matrigel GFR-based assays. Cell cycle, mitochondrial membrane potential and apoptosis analysis were performed using a Muse Cell Analyzer (Millipore) and their respective kits. ROS generation was analyzed using dichlorofluorescein diacetate staining. Western blotting investigated apoptotic markers and related signaling pathways.

Result and discussion

DAL511 had a stronger antiproliferative effect on MDA-MB-231-BR cells than on human astrocytes. Treatment significantly reduced clonogenic survival, migration, and invasion. Cell cycle assays showed an increase in the subG0 cell population, indicating cytotoxic activity correlated with enhanced apoptosis and mitochondrial membrane depolarization. ROS levels also increased after DAL511 treatment. Western blot analysis confirmed activation of apoptotic markers, including cleaved caspase-3 and PARP, along with alterations in key signaling pathways such as MAPK and PI3K/AKT.

Conclusion

The flavone combination DAL511 induces apoptosis in MDA-MB-231-BR breast cancer cells by disrupting mitochondrial function and increasing ROS production. Additionally, it modifies apoptosis-related protein expression and signaling pathways, supporting its potential as a therapeutic candidate for TNBC.

EACR25-1400

Integrating Proximity Ligation Assay for PD-1-PDL1 Immunoassay with Multiplex Immunofluorescence Imaging in Urothelial Carcinoma

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Introduction

The PD-1/PD-L1 axis is a critical regulator of immune checkpoints and a primary target for immune checkpoint inhibitors (ICIs) in solid tumor treatment. However, despite its promise, PD-1/PD-L1 blockade has limited efficacy, with only 20–30% of bladder cancer patients responding to therapy. Currently, patients receive ICIs based solely on PD-L1 status, overlooking the spatial

complexity of tumor-immune interactions. We propose that direct measurement of PD-1/PD-L1 interactions between immune and tumor cells provides a more accurate predictor of treatment response. The *in-situ* Proximity Ligation Assay (isPLA), commercialized by Navinci Diagnostics, enables the visualization of protein-protein interactions between cells by using paired antibodies. If the target proteins are within 40 nm of each other, a rolling circle amplification signal is generated and detected via fluorescence microscopy. When combined with multiplex immunofluorescence, this method can further distinguish interactions among specific cell populations, such as tumor and immune cells, while providing insights into the tumor microenvironment. By integrating isPLA with multiplexed immunofluorescence, we aim to refine patient stratification and improve the predictive accuracy of immunotherapy, contributing to the development of more personalized cancer treatments.

Material and method

FFPE tissue samples from a retrospective cohort of bladder cancer patients were stained using the Naveni® protocol on a Leica Bond RX system, followed by multiplexed immunofluorescence with Akoya Bioscience's PhenoCycler technology. isPLA detection oligos were incorporated to visualize PD-1/PD-L1 interactions alongside a 14-plex antibody panel.

Result and discussion

We successfully established an automated isPLA workflow on the PhenoCycler Fusion imaging platform, enabling precise detection of PD-1/PD-L1 interactions while simultaneously profiling single and co-expressed markers across immune, stromal, and tumor cells. Preliminary data from a proof-of-concept cohort of 13 patients revealed a significantly higher number of PD-1/PD-L1 interactions in complete responders compared to non-responders, suggesting that the frequency of these interactions may serve as a potential predictor of response to ICI treatment. Building on this, we have implemented the interaction assay with a 40-plex panel to achieve deeper spatial profiling, allowing for more comprehensive characterization of the tumor microenvironment in patient samples.

Conclusion

In conclusion, we have created a method that enables simultaneous detection of PD-1/PD-L1 interactions while spatially profiling the tumor microenvironment. Our findings suggest that differences in the frequency of isPLA spots correlate with response to ICI treatment. This approach provides a valuable framework for refining patient stratification and improving predictive biomarkers for immunotherapy response.

EACR25-1412

Type I and II BIR domains as targets for cancer therapy

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Introduction

Inhibitors of Apoptosis Proteins (IAPs) are oncological targets whose over-expression enhances cell survival and resistance to anticancer agents. IAPs regulate NF-κB pathway acting as E3 ligases; furthermore, they sequester caspases to prevent apoptosis. Such IAPs functions are mediated by type I and II BIR (Baculovirus IAP repeat) domains, respectively. Type II BIRs are validated targets for cancer therapy. The biophysical and structural analysis (crystallography, SAXS) of a specific cavity on type II BIRs allowed the rational design of Smac-mimetics, which we patented as potent anti-cancer agents; however, chemoresistance events depending on type I BIRs have been reported.

Material and method

We, thus, virtually screened libraries of commercial compounds (Chembank, DrugBank, LOPAC) targeting type I BIRs, identifying candidates with promising in vitro profiles, both on isolated proteins and in cell-based assays. In particular, one candidate modulated NF-κB activation in TNBC (Triple Negative Breast Cancer) models, inducing cell death, both as single agent and in combination with Smac-mimetics. Integrating cell-viability assays, virtual docking, NMR and techniques to determine drugs affinities towards the target proteins a library of 50 derivatives has been characterized and a selection of derivatives has been further investigated in cell-based and protein-based assays.

Result and discussion

The selection of derivatives demonstrated to reduce cell viability in TNBC and in NSCLC (Non-Small Cell Lung Cancer, NCI-H1299, cell line smac-mimetics-resistant). Further investigations revealed that the cytotoxic effect of these derivatives perturbs the NF-κB pathway and induces caspase-mediated cell death, increasing ROS production. The effect of drug candidates has been evaluated also on target proteins, including single BIR domains and full length IAPs. In fact, the selected candidates present low micromolar affinities for the protein targets and produce thermal shifts of the proteins tested.

Conclusion

The hypothesis of IAPs-engagement in the function of this new class of derivatives is now under investigation and further studies on different cellular models and on pro-apoptotic pathways is ongoing. The objective is to propose a candidate for *in vivo* tests. The modulation of pro-survival complexes regulating the NF-κB pathway, as the ones mediated by IAPs, can be the strategy to overcome cases of resistance to current IAPs-targeting chemotherapics, but also to better define IAPs roles/functioning and find accurate/selective therapies.

EACR25-1416

Alpha-1 adrenergic antagonists to sensitize neuroblastoma over isotretinoin therapy

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Introduction

High-risk neuroblastoma (NB) is an aggressive pediatric malignancy with poor prognosis, as the 5-year disease-free survival rate remains below 50% despite intensive therapy. Developing novel combination treatments is crucial to improving outcomes. Isotretinoin (13cRA), a key agent in post-consolidation NB therapy, exhibits anti-proliferative and pro-differentiative effects. To identify compounds that enhance its efficacy, we screened a library of 169 natural polyphenols and found that isorhamnetin (ISR) synergizes with 13cRA, reducing cell viability by 80%. Mechanistically, this effect correlates with upregulation of the adrenergic receptor α 1B (ADRA1B), a member of the catecholamine receptor superfamily. This study investigates ADRA1B's role in mediating the synergistic effects of 13cRA and ISR, as well as the broader impact of adrenergic receptor modulation on NB growth in combination with 13cRA.

Material and method

We generated ADRA1B-knockout (KO) NB cell lines using CHP134 (13cRA-sensitive) and SK-N-AS (13cRA-resistant) cells. Cellular responses to 13cRA and isorhamnetin, alone or in combination, were assessed via metabolic assays, apoptosis assays, differentiation markers, and gene expression analysis. Additionally, a high-throughput screen using an adrenergic receptor ligand library evaluated cell viability upon co-administration with 13cRA. Finally, the combinatorial approach was tested *in vivo* using NB xenograft models.

Result and discussion

CHP134 ADRA1B-KO cells treated with 13cRA showed reduced viability and increased Caspase-3/7 activation, comparable to wild-type cells treated with the combination of 13cRA and ISR. Furthermore, 13cRA exposure in CHP134 KO cells induced neurite outgrowth and upregulation of neural differentiation. In contrast, SK-N-AS cells (WT or ADRA1B-KO) showed no changes in viability or differentiation marker expression following 13cRA treatment. Pharmacological inhibition of α 1B-AR and 13cRA administration to the cells, mirrored the genetic KO results. Treating a panel of 11 NB cell lines with α 1/ α 1B-AR antagonists revealed that the presence of MYCN-amplification is essential to observe the sensitization over the cell viability reduction and neural differentiation induced by 13cRA, thus mimicking the ISR activity. As expected, the addition of α 1/ α 1B-AR agonists led to opposite effects. *In vivo*, co-treatment with 13cRA and the FDA-approved α 1 AR antagonist doxazosin significantly slowed tumor progression in NB xenograft models.

Conclusion

Our findings suggest that α 1-AR antagonism sensitizes NB cells to 13cRA by facilitating the pro-differentiative and pro-apoptotic effects, potentially disrupting an auto-

crine catecholamine-driven pro-survival circuit. Given the druggable nature of ARs, α 1-AR inhibition emerges as new a promising pharmacological therapeutic strategy for neuroblastoma treatment.

EACR25-1419

Transcriptional profiling and ex vivo drug pre-screen for personalized therapy selection

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Introduction

Pancreatic ductal adenocarcinoma (PDAC) is associated with rapid progression, therapy resistance, and a highly complex tumor microenvironment (TME). Our research focuses on developing patient-derived models to test investigational drug compounds' effects and elucidate the interplay between epithelial cells and TME elements in PDAC therapy response and resistance. To this end, we established a PDAC patient-derived xenograft (PDX) model, along with PDX-derived organoids (PDXO) and ex vivo tissue slice models, to enable personalized drug testing and therapeutic optimization.

Material and method

The PDAC-PDX was established by subcutaneous implantation of metastatic PDAC tissue in immunocompromised SCID mice. Bulk RNA sequencing of the PDX tissue was analyzed using a drug prediction algorithm (Darwin OncoTarget™/OncoTreat™) PDXOs were generated in an extracellular matrix gel, while ex vivo tissue slices from PDX tumors were cultured at the liquid-air interface in a transwell system for four days. Drug testing was performed on the PDXOs, with viability assessment post-treatment using an ATP-based viability assay. Additionally, selected therapeutic agents are applied to ex vivo PDX slices to evaluate drug effects in a more complex setup where tissue architecture and stroma components are partially preserved.

Result and discussion

The established PDAC-PDX model preserves key genomic characteristics of its original patient-derived tissue while maintaining consistent histological features of PDAC across *in vivo* passages. PDXOs retained molecular and phenotypical features of the PDX tissue, as confirmed by IF and genomic analysis. Transcriptomic analysis of PDX tissue identified both FDA-approved and investigational drug candidates. PDXO drug treatment experiments determined the efficiency of PDAC standard-of-care combinations gemcitabine & nab-paclitaxel and folfirinox. Additionally, predicted compounds showed reduced organoid viability, such as AT9283, plicamycin, belinostat, lapatinib, trametinib,

olaparib, or bosutinib. These compounds are being evaluated in an ex vivo tissue slice model, to better capture PDAC complexity and evaluate the effect also on TME elements.

Conclusion

Drug resistance remains a major challenge in PDAC treatment, demonstrating the need for new therapies. Our findings highlight organoids as a robust model for testing tumor epithelial cell-targeting compounds, offering a key advantage in personalized drug screening due to in vitro expansion potential. Using a newly developed PDX model and derived PDXOs, we demonstrated the feasibility of testing SOC drugs and therapies predicted from transcriptomic data. We are extending this approach to a larger cohort of patient-matched tissue and organoids.

EACR25-1421

Remodeling the Tumor Microenvironment through TSP-1/CD47 Antagonization: Non-Clinical Characterization of the TAX2 drug candidate

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Introduction

Thrombospondin-1 (TSP-1) is a matricellular glycoprotein overexpressed in a wide range of cancers and a poor prognosis marker. Among its receptors, CD47 is mainly implicated in tumor angiogenesis and immune evasion. While most efforts focused up to now on disrupting CD47 binding to its macrophage counter-receptor SIRPa (“don’t eat me signal”), the TSP-1/CD47 axis is emerging as promising target for treating solid tumors. We have developed TAX2, a 12 aa cyclic peptide acting as an orthosteric antagonist for TSP-1/CD47 interaction.

Material and method

The binding properties of TAX2 peptide to TSP-1 from various species were characterized in vitro while the anti-cancer activity and biodistribution of TAX2 were evaluated in tumor-bearing mice. The stability of TAX2 was tested in plasma and whole blood from different species before pharmacokinetic (PK) profiling following a single intravenous (IV) administration. The potential impact of TAX2 on CD47/SIRPa interaction was investigated using molecular modeling and a cell-based reporting assay. Potential off-target interactions were assessed across a panel of 44 targets, while potential unintended immunogenicity of TAX2 was evaluated in cytokine release assays. Toxicology studies were conducted in rats and dogs to characterize the toxicity profile of TAX2 after IV injection. Last, PK data obtained in rodents and dogs were used for prediction of Human PK parameters using allometric scaling.

Result and discussion

In silico and in vitro studies showed that TAX2 binds selectively to human, rat, mouse and dog TSP-1, and

disrupts the TSP-1/CD47 interaction without affecting CD47’s interaction with SIRPa. The pharmacologically active dose (PAD) of TAX2 in mice was determined at 30 mg/kg based on efficacy studies in several cancer models. In a highly aggressive ovarian cancer model, TAX2 demonstrated its efficacy by improving mouse survival, while offering clinically relevant combination potential with PARP inhibitors. TAX2 PK profile determined in rodents and dogs showed rapid plasma clearance over 1 to 4 hours, while exposure increases proportionally with the dose. Biodistribution studies revealed preferential accumulation in tumors and TSP-1-expressing tissues. TAX2 peptide showed no significant off-target interaction or functional effect across a panel of 44 human targets and did not induce uncontrolled cytokine release in human whole blood, supporting a favorable safety profile. Toxicology studies indicated that TAX2 was well tolerated with NOAEL (No Observable Adverse Effect Level) values of 400 mg/kg in rats and 100 mg/kg dogs. According to PK predictions, the PAD in humans ranges between 9 and 33 mg/kg.

Conclusion

TAX2 peptide, a first-in-class TSP-1/CD47 antagonist, is being developed as an injectable product for the treatment of cancers. These non-clinical data support this concept and TAX2 is now expected to enter in Phase 1/2a clinical trial in advanced solid tumors.

EACR25-1429

A Novel High-Throughput Assay for Targeted Protein Degradation: Application to BCL-xL in Cancer Cells

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Introduction

Cancer is driven by genetic alterations that disrupt normal cellular regulation, leading to uncontrolled proliferation and evasion of apoptosis. One crucial mechanism in tumor development is oncogene addiction, where malignant cells become highly dependent on a single oncogene for survival. Additionally, synthetic lethality – where the simultaneous loss of two genes results in cell death, while the loss of a gene alone is tolerated – is a common feature in cancer pathology. Targeting either the key oncogene or its synthetic lethal partner can induce tumor regression when cancer-specific mutations are identified. In solid tumors, BCL-xL, an anti-apoptotic BCL-2 family member, is often over-expressed, promoting survival and resistance. The small molecule BCL-2 inhibitor, venetoclax, was approved for the treatment of hematopoietic tumors, highlighting the therapeutic potential of targeting BCL family in cancer. Targeted protein degradation, including BCL-xL degraders, has emerged as a promising strategy to restore apoptosis in cancer cells. A cell-based assay was developed to assess BCL-xL protein degradation, offering a sensitive and reliable tool for studying protein

turnover dynamics and to evaluate the potency of BCL-xL degraders in cancer cells.

Material and method

To enable precise tracking of BCL-xL degradation, a gene editing approach was applied to introduce a HiBiT sequence into the endogenous BCL-xL gene expressed in a relevant cancer cell line. This modification allows for the direct detection of BCL-xL protein levels through a luminescent signal. In the presence of LgBiT, the reconstituted NanoLuc luciferase produces a luminescent signal directly proportional to protein levels, enabling accurate quantification of degradation. The assay was developed in 384-well plate format, allowing for high-throughput screening and scalability.

Result and discussion

The assay was validated using DT2216, a well-characterized BCL-xL degrader, which effectively diminished the luminescent signal by promoting target degradation. After optimization for high-throughput screening, the assay demonstrated robust performance in evaluating candidate degraders acting on HiBiT-tagged proteins. Further validation through western blot analysis in cancer cells confirmed that the assay faithfully represents the behavior of the native, untagged protein, underscoring its suitability for extensive screening campaigns.

Conclusion

This study introduces a robust cell-based assay for monitoring targeted protein degradation in cancer cells. The method streamlines the screening process for novel degraders and can be adapted to other oncogenic targets. Ultimately, this approach holds great promise for the development of targeted cancer therapies that selectively eradicate tumor cells while minimizing collateral damage to normal tissues.

EACR25-1434

Muscle-invasive bladder cancer organoids as a functional system to address therapy response: Perspective in advancing precision medicine

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Introduction

Muscle-invasive bladder cancer (MIBC) exhibits high inter-patient heterogeneity, driving recurrence, treatment failure, and poor prognosis, underscoring the need for precision oncology. Patient-derived organoids (PDOs) are robust preclinical models that preserve parental tumors' (PT) molecular and cellular complexity. This study developed a PDO-based drug screening platform to improve therapeutic precision.

Material and method

MIBC PDOs were derived from resected tumors (44 samples) and expanded under optimized 3D culture.

PDOs were treated for 48h with the reported human Cmax values of both standard-of-care (SOC) chemotherapy and selected FDA-approved compounds, including Tyrosine kinase inhibitors (TKIs), mTOR inhibitors, PARP inhibitor, and other non-SOC drugs. Cell viability was assessed using Cell Titer-Glo® luminescent assay. Differences between treatment groups and the respective controls were analyzed using ordinary one-way ANOVA. Morphology alterations were observed before and after treatment using CQ1 confocal imaging cytometer.

Result and discussion

Our study confirms that PDOs retain most of the characteristic markers of PT. The success rate of PDO establishment was around 84% within 6–14 days of culture. MIBC PDOs exhibited two distinct morphological types: mixed (65%) and solid (35%). SOC chemotherapy – cisplatin/gemcitabine (78%, 25/32) and MVAC (88%, 14/16) – showed strong efficacy, significantly reducing PDO-cell viability, aligning with widely observed clinical response of patients, which reinforced the model's predictive reliability. Notably, in some non-SOC compounds like daunorubicin (50%, 10/20) and epirubicin (84%, 16/19), the cytotoxic effect was equal to or even better than SOC. Nonetheless, lapatinib (30%, 6/20) and olaparib (31%, 5/16), dual TKIs (EGFR/HER2) and PARP inhibitor, respectively, also showed to be promising for MIBC treatment. We unexpectedly discovered a highly aggressive MIBC during the process, exhibiting a cell line-like growth pattern with rapid, sustained proliferation and morphological stability after multiple passages. It provides a novel model for studying aggressive MIBC. Ongoing research is in progress to explore therapeutic vulnerabilities.

Conclusion

In this study, we established a valuable MIBC biobank for drug discovery and validation. Inter-sample drug variability reflects MIBC heterogeneity, emphasizing the need for molecular profiling and *in vitro* screening to guide personalized treatment and informed clinical decisions.

EACR25-1449

Novel Titanocene-derived complex with high albumin affinity: Evaluation of antitumor activity in ovarian cancer cells

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Introduction

Chemotherapy resistance in ovarian cancer remains a current issue. Ti(IV)-derivatives represent a new alternative for platinum-resistant tumour treatment. They have demonstrated low toxicity and high efficacy *in vitro* and *in vivo*. However, their low stability and solubility in biological media have impeded its clinical application. Therefore, we have developed Ole-Ti, a new titanium complex with an oleic-like aliphatic chain, improving stability and solubility through non-covalent binding with

serum albumin. In this study, we report some results about in vitro and in vivo effects of Ole-Ti.

Material and method

Ole-Ti cytotoxicity was tested on A2780 and A2780Cis (cisplatin resistant) ovarian tumour cells lines by MTT assays. Intracellular Ti(IV) accumulation was quantified by TXRF. DNA interaction was studied by electro-phoretic mobility assays. Apoptosis and oxidative stress were evaluated by flow cytometry with AnnexinV and DCFH-DA cell staining. Moreover, Caspase-3 protein level was assessed by western blot. PCR and qPCR were used to analysed the expression of endoplasmic reticulum stress mediators. For in vivo studies, A2780 cells were subcutaneously injected into nude-Foxn1 mice model. Mice were treated with Ole-Ti for two weeks and tumors volume were measured daily.

Result and discussion

Ole-Ti showed greater cytotoxic activity than its precursor (TiCp2Cl₂) on A2780 and A2780Cis cells. Based on IC₅₀ and resistance factor values, we determined that Ole-Ti had high potency on A2780Cis cells. This seems to be related to elevated Ole-Ti uptake into cells. Thus, apoptotic cells increased significantly after Ole-Ti treatment. As well, some molecular mechanisms proposed for other titanium derivatives were evaluated with Ole-Ti. DNA does not seem to be the main target of Ole-Ti since no conformational change was observed in the electrophoretic mobility assay. In addition, we inspected Ole-Ti ability to induce cellular stress. The endoplasmic reticulum stress pathway was not activated in tumour cell lines after Ole-Ti treatment for 48 hours. Although Ti(IV) is a non-redox metal, oxidative stress production was significant in Ole-Ti treated cells. Taking in further, the addition of N-acetyl-L-cysteine significantly decreased the cytotoxic activity of Ole-Ti, suggesting that ROS could be responsible for cell death. Finally, the in vivo Ole-Ti potential was demonstrated as treatment with Ole-Ti reduced tumour size more effectively and earlier compared to cisplatin, leading to an increase in the mice survival.

Conclusion

Overall, Ole-Ti displays strong antitumor activity in ovarian cancer cells both in vitro and in vivo. Nevertheless, the cellular mechanism of Ole-Ti is still uncertain. We anticipated that oleic-like aliphatic chain might play a key role in Ole-Ti molecular activity. Hence, further research is required to identify additional pathways and molecular targets.

EACR25-1450

Mesoporous Silica Nanoparticles for the Selective Delivery of Doxorubicin to Folate Receptor-Expressing Cancer Cells

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Introduction

Cancer is a significant global health issue, responsible for nearly 20% of deaths in 2021. One of the major challenges in cancer treatment is the development of therapeutic strategies that selectively target tumor cells while minimizing harm to normal tissues. Localized drug delivery can help achieve this goal. A mesoporous silica-based nanodevice, designed to carry the chemotherapy drug doxorubicin (DOXO), was developed. This device, referred to as FOL-MSN-DOXO, releases the drug in response to the acidic environment of tumors and incorporates folic acid (FOL) for targeting purposes. The effectiveness of FOL-MSN-DOXO was tested in vitro against folate receptor-expressing (FR+) cancer cells, as well as against folate receptor-negative (FR-) normal (healthy) cells.

Material and method

The endocytosis of mesoporous silica nanoparticles (MSNs) was monitored through transmission electron microscopy (TEM). The efficacy of FOL-MSN-DOXO was evaluated using growth experiments, Annexin V and TUNEL assays, reactive oxygen species (ROS) measurements, Western Blot analysis, and immuno-staining on rat Dorsal Root Ganglion (DRG) cells.

Result and discussion

FOL-MSN-DOXO effectively killed FR+ cancer cells while sparing FR- normal cells. In contrast, free DOXO was toxic to all cell lines, regardless of FR expression. The uptake of MSNs occurred exclusively in FR+ cells via FR-mediated endocytosis, with no uptake observed in FR- cells. Both FOL-MSN-DOXO and free DOXO significantly increased ROS production, leading to apoptosis. However, only free DOXO induced ROS and triggered apoptosis in FR- normal cells. Importantly, the vehicle alone (FOL-MSN) showed no toxicity in any tested cell lines, and immunostaining on DRG cells demonstrated significantly lower neuronal toxicity for FOL-MSN-DOXO compared to free DOXO

Conclusion

The FOL-MSN-DOXO nanosystem specifically targets cancer cells that express FR, offering enhanced safety and comparable effectiveness against tumors when compared to conventional doxorubicin formulations. As a result, it presents a promising strategy for the targeted and safe delivery of chemotherapy in the treatment of cancers that express folate receptors.

EACR25-1458

Targeting UBE2N with Novel Non-Covalent Inhibitors: A Drug Discovery Approach to Combat PARPi Resistance in Ovarian Cancer

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Introduction

Ovarian cancer poses a major global health challenge, resulting in approximately 200,000 deaths annually. While Poly ADP-ribose polymerase inhibitors (PARPi) offer a promising treatment strategy, their effectiveness is primarily limited to patients with deficiencies in the homologous recombination (HR) pathway, affecting only about half of the patient population. This highlights a critical need for novel approaches to enhance PARPi efficacy and broaden its applicability in ovarian cancer. UBE2N, an E2 ubiquitin ligase, emerges as a promising target to improve the efficacy of PARPi therapy. This enzyme plays a crucial role in the HR pathway, a vital mechanism for DNA repair. By inhibiting UBE2N and disrupting the HR pathway, we aim to render ovarian cancer cells sensitive to PARPi treatment, allowing it to potentially benefit a wider range of patients. Our lab research demonstrated how UBE2N inhibition enhances ovarian cancer cell sensitivity to DNA-targeting drugs, particularly PARPi. While research on UBE2N inhibitors is in its early stages, there are promising options of covalent inhibitors like NSC697923 and BAY 11-7082 and non-covalent inhibitors like ML307 as well as Variabine B. However, none of these molecules have the necessary properties for preclinical or clinical development. This research aimed to develop novel, noncovalent UBE2N inhibitors to enhance PARPi effectiveness in ovarian cancer.

Material and method

To identify potential UBE2N inhibitors, we utilized a combined structure- and ligand-based virtual screening approach, targeting the 19,000-compound CERMN Chemolibrary. The screening was based on three available UBE2N structures (PDB codes: 4ONM, 6UMP, and 3HCU), which capture distinct conformations of the flexible 114–124 loop. This loop, located near the active site residue Cys87, is critical for defining the shape and volume of the active site cavity. Conformational changes within the loop can therefore directly impact binding and potentially modulate the UBE2N activity. From the virtual screen, 22 compounds were selected based on interaction energy calculations and assessments of the stability of the compound-UBE2N complexes. These compounds, all targeting the UBE2N active site, underwent *in vitro* evaluation using SKOV3 ovarian cancer cells.

Result and discussion

Preliminarily, cytotoxicity assays demonstrated that 5 compounds significantly reduced cell viability. Further investigation using PARPi sensitization assays and clonogenic assays for colony formation identified 2 novel non-covalent UBE2N inhibitors that exhibited significantly higher potency than the known inhibitor ML307, particularly when combined with the PARPi Olaparib.

Conclusion

This study demonstrates, for the first time, successful PARPi sensitization using non-covalent UBE2N inhibitors, highlighting their potential to broaden PARPi therapy eligibility in ovarian cancer by enhancing tumor cell sensitivity to Olaparib.

EACR25-1465

Lysosomal Stress as an Off-target Effect of UNC0642 in Pancreatic Cancer Cells

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Introduction

Many emerging and established anticancer therapies accumulate in lysosomes due to their physicochemical properties, independent of their intended targets. However, the relevance to anticancer activity remains understudied. Here, we use UNC0642, a G9a/GLP inhibitor, as a tool compound to investigate the impact of chronic lysosomal accumulation of a therapeutic molecule in pancreatic ductal adenocarcinoma (PDAC) cells.

Material and method

We generated a transient G9a/GLP double knockout (DKO) in a murine PDAC cell line using CRISPR-Cas9 and validated the loss of protein expression and enzymatic activity via Western blot. This DKO cell line was used to differentiate the on-target versus off-target effects of UNC0642. Immunofluorescence was performed to assess the expression of lysosome-associated proteins. Additionally, RNA-Seq followed by quantitative real-time PCR was used to examine transcriptional changes induced by lysosomal-damaging agents.

Result and discussion

UNC0642 was originally designed as a catalytic inhibitor of the methyltransferase G9a and its paralog GLP. However, using a G9a/GLP DKO PDAC cell line, we demonstrate that its primary anti-proliferative effect is mediated predominantly by an off-target mechanism. Lysotracker co-staining confirmed UNC0642 accumulation in lysosomes, leading to lysosomal swelling, as evidenced by increased LAMP-1 puncta and altered lysosomal morphology. Furthermore, UNC0642 induced chronic lysosomal damage, as demonstrated by increased galectin-3 puncta, a well-established marker of lysosomal membrane damage. Notably, UNC0642 triggered lysosomal damage to a similar extent as L-Leucyl-L-Leucine methyl ester (LLOMe), a known lysosomal detergent. Chronic lysosomal damage induced by UNC0642 and LLOMe correlated with reduced PDAC cell viability. Additionally, RNA-Seq revealed a novel transcriptional signature indicative of lysosomal damage, including upregulation of Serpinb9 family members, Car6, and Rgs1.

Conclusion

Our findings suggest that chronic lysosomal damage is a significant off-target effect that can create vulnerabilities in PDAC cells for targeted therapeutic treatment. These effects should be considered when evaluating the mechanisms of action of current and emerging anticancer therapies.

EACR25-1466

Multimodal targeting of Ephrin A2 in solid tumors using bi-cyclic drug conjugates

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Introduction

Bicycle® molecules are short linear peptides stabilized into a bi-cyclic conformation by a chemical scaffold. They are characterized by high affinity, selectivity, and small size, enabling fast tumor penetration and favourable pharmacokinetic. Bicycle® molecules can be readily conjugated to a variety of payloads, or to other Bicycle® molecules providing a toolkit of building blocks to create novel precision guided medicines. Ephrin A2 (EphA2) is a member of the Ephrin superfamily of receptor tyrosine kinases, which regulates cell migration, adhesion, proliferation and differentiation during development. It is highly expressed in different solid tumors while relatively low in healthy tissue but its targeting by antibody-drug conjugates has been unsuccessful.

Material and method

EphA2-specific binders were identified using phage display, and structure guided chemical optimization led to the development of a molecule with improved affinity, physicochemical properties and metabolic stability. This molecule was then used as the targeting moiety for the development of a set of three EphA2-specific therapeutic agents characterized by different mechanism of action: 1) a Bicycle® Drug Conjugate (BDC™) composed by the EphA2-targeting molecule, a valine-citrulline cleavable linker, and a monomethyl auristatin E (MMAE) cytotoxin payload (BT5528). 2) A Bicycle® Radionuclide Conjugate (BRC®) obtained by radiolabelling the EphA2-targeting molecule with 68Ga, for imaging purposes and with 177Lu, as a therapeutic agent. 3) A Bicycle® tumor-targeted immune cell agonist (TICA®) designed as a heterodimer composed of an EphA2- and CD137-targeting molecules (BT7455). Preclinical characterization of these molecules was evaluated in a variety of assays across both in vitro and in vivo settings.

Result and discussion

BT5528 demonstrated EphA2-dependant robust anti-tumor activity in preclinical models, including both cell- and patient-derived xenografts that are resistant to current treatments. In addition, imaging studies performed with an EphA2-targeting BRC, showed high tumor uptake and renal-mediated clearance, with minimal systemic exposure. Finally, BT7455 exhibited highly potent EphA2-dependent stimulation of CD137 and robust in vivo anti-tumor activity against EphA2-expressing tumors in preclinical models.

Conclusion

The Bicycle Platform enables the development of a multimodal therapeutic approach for solid tumor treatment, including the targeting of previously intractable proteins for other agents such as ADCs, therefore offering the opportunity to develop both best and first in class therapies. This approach is exemplified herein with EphA2 targeting Bicycle® containing molecules, with BT5528 currently in Phase 1/2 clinical trials

EACR25-1474

Tackling Hepatocellular Carcinoma with novel PROTAC Degraders of eIF6

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Introduction

eIF6, a translation initiation factor that operates downstream of the insulin pathway, has recently emerged as a potential drug target. eIF6 levels have been shown to increase throughout the progression from Non-Alcoholic Fatty Liver Disease (NAFLD) to hepatocellular carcinoma (HCC), a major cause of cancer-related mortality worldwide. Furthermore, depletion of eIF6 has been observed to reduce tumorigenesis in Myc-induced lymphomagenesis and to decrease white fat accumulation in the liver. On these premises, modulating eIF6 activity could represent an effective strategy to prevent the pathological progression of NAFLD to HCC, as well as to target existing HCC. To this end, we are leveraging the PROTAC (Proteolysis Targeting Chimera) technology, which exploits the ubiquitin-proteasome system for targeted protein degradation.

Material and method

To explore the feasibility of targeting eIF6 for degradation, we are developing selective eIF6 PROTAC degraders based on previously identified eIF6 binders, along with new hits obtained through a virtual screening campaign. A computational study was conducted on a set of eIF6 degraders, utilizing molecular docking, molecular dynamics simulations (MDs), and ligand binding free energy calculations (MM-GBSA) to identify the most promising binders. These compounds are currently under investigation in biophysical and cellular assays to evaluate binding affinity and degradation efficiency.

Result and discussion

Several putative eIF6 binders were identified through computational studies, and biophysical assays confirmed their ability to bind the translation factor. The lead PROTAC candidates exhibited strong binding interactions in docking and MD simulations, supporting their potential to induce eIF6 degradation. These results suggest that selected compounds could serve as effective degraders. Further experimental validation is ongoing to assess their efficacy in cellular models.

Conclusion

The top-scoring eIF6 degrader candidates are currently under study. Preliminary findings support the potential of the PROTAC technology in targeting eIF6 for the treatment of HCC. The design, synthesis, and characterization of novel eIF6 PROTACs will be presented and discussed.

EACR25-1484

A titanocene-derivative complexed with a myristic-like aliphatic chain exhibits antitumor activity in cisplatin-resistant models both in vitro and in vivo

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Introduction

Coordination compounds are among the main chemotherapeutic treatments used for several cancer types. Although widely used, platinum-based therapies have limitations such as low selectivity, leading to side effects, reduced efficacy, and drug-resistance. Titanium (Ti) coordination compounds have emerged as promising agents in the treatment of cisplatin (CP)-resistant tumors. To solve some instability and solubility problems of these compounds, we synthesized a new titanocene-derived complex, $[\text{TiCp}_2(\text{OOC})_2\text{py-O-myrr}]$ (Myr-Ti), with improved stability and high albumin affinity. We explore the antitumor activity of Myr-Ti in CP-resistant models both *in vitro* and *in vivo*.

Material and method

Cytotoxicity of Myr-Ti in A2780CIS and COR-L23CPR (both CP-resistant tumor cells) and their sensitive variants was assessed by MTT assays. Intracellular Ti accumulation was measured by ICP-MS. Apoptosis and cell cycle progression were analyzed by flow cytometry with Annexin-V and PI staining. ROS production was evaluated using the DCFHDA probe by flow cytometry. Interaction of Myr-Ti with DNA was assessed by spectroscopic studies and DNA mobility assays. Proteins involved in ER stress were analyzed by western blot and PCR. For *in vivo* studies, nude-Foxn1 mice were subcutaneously xenografted with A2780CIS cells. Mice were treated for three weeks with Myr-Ti, and tumor volumes were measured daily.

Result and discussion

Myr-Ti was more cytotoxic than its precursor titanocene dichloride in all cell lines tested. IC₅₀ and resistance factor values pointed that this greater cytotoxicity is maintained in CP-resistant cells and correlated with increased Myr-Ti uptake. In addition, Myr-Ti led to an increase of apoptotic population and G2/M cell cycle arrest, which was not accompanied by an increase in ROS levels. Myr-Ti does not appear to interact with DNA, as no conformational changes were observed in electrophoretic mobility or by spectroscopic techniques. Furthermore, ER stress pathways were not activated in cells after Myr-Ti treatment, indicating that the

mechanism of action of Myr-Ti differs from those proposed for other Ti-derivatives. Finally, CP-resistant tumor-bearing mice treated with Myr-Ti showed greater tumor volume reduction and increased overall survival. Histological and Ki67 staining confirmed that Myr-Ti caused cell death in tumor tissue with minimal damage to other primary organs.

Conclusion

Together, these data support that Myr-Ti has significant antitumor activity in CP-resistance models both *in vitro* and *in vivo*. The underlying mechanisms seem to differ from those proposed for other coordination compounds, although further research is required. Myr-Ti showed improved therapeutic efficacy for overcoming CP-resistance, with limited adverse systemic side effects, highlighting its potential for further studies and clinical translation.

EACR25-1518

Targeting CITK in high-grade brain tumors: a comparison of polypharmacological and specific inhibitors

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Introduction

Medulloblastoma (MB) and glioblastoma (GBM) are the most frequent high-grade brain tumors (HGBT) in children and adulthood, respectively. Despite the improvements in patient survival, many patients still die and those who survive suffer from neurological and endocrine disorders. Therefore, more effective therapies are needed. Citron Kinase (CITK), the primary product of the CIT gene, mutations of which are responsible for the MCPH17 primary microcephaly syndrome, is essential during neurodevelopment for proper cytokinesis and genomic stability in normal neural progenitor cells. CITK is validated as target for MB treatment as its depletion leads to cytokinesis failure and DNA double strand breaks (DSBs) accumulation in MB cells. Moreover, loss of CITK induces apoptosis and reduces tumor growth *in vivo*.

Material and method

After discovering that Lestaurtinib is an inhibitor of CITK, we tested it on different MB and GBM patient derived cell lines and *in vivo* injecting the drug in MBs arising in SmoA1 transgenic mice. In parallel, we conducted a Hit Discovery Program, which resulted in the identification of specific molecules targeting citron, which we subsequently tested on GBM patient-derived cell lines.

Result and discussion

Similar to CITK knockdown, treatment with Lestaurtinib leads to cytokinesis failure, accumulation of DNA double strand breaks, impairs cell proliferation and increases cell

death in MB and GBM cells. Also, Lestaurtinib treatment reduces tumor growth and increases mice survival. The Hit Discovery Program resulted in the identification of molecules that inhibit CITK in the low-nanomolar range. When tested on GBM cells, some of these molecules produced phenotypes characteristic of CITK loss, although their effects were weaker compared to the polypharmacological compound Lestaurtinib.

Conclusion

Compared to a polypharmacological inhibitor, more specific CITK catalytic inhibitors may recapitulate the effects of protein knockdown, but to a lesser extent and in a cell line-dependent manner. Given that the data on different cell lines show a varied response to CITK inhibition, we have begun to explore the genes and pathways that make a cell line sensitive to CITK, with the aim of predicting in the future which lines would be more responsive to treatment.

EACR25-1520

Development of Lipid-Based Gene Delivery Systems for CART Cell Therapy

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Introduction

Chimeric Antigen Receptor (CAR) T cell therapy has revolutionised cancer treatment by engineering T cells to recognise and eliminate tumour cells. While viral vectors are the standard method for gene delivery in CAR T cell production, they present challenges, including high costs and safety concerns. Lipid-based vectors, particularly liposomes, have emerged as a promising alternative due to their biocompatibility, and ability to protect and efficiently deliver genetic material. Taking advantage of these characteristics, this study aims to develop and characterise liposome formulations to overcome current limitations in gene delivery and improve CAR T cell production.

Material and method

Five formulations (F1-F5), with different molar ratios of selected lipids, were prepared using the thin-film hydration method followed by extrusion. Lipid quantification was performed using the Liebermann-Burchard method for formulations F1, F2 and F5 and Fiske-Subbarow for F3 and F4. Lipoplexes were formed by mixing the liposomes with a DNA plasmid, encoding the green fluorescent protein, at different (+/-) charge ratios (1/1; 2/1; 4/1; 8/1). Dynamic light scattering characterised the lipoplexes for size, polydispersity index and zeta potential. Transfection efficiency and cytotoxicity were evaluated 48h post-transfection by flow cytometry.

Result and discussion

Prepared liposomes were large unilamellar vesicles (98.3-152.7 nm) with positive surface charges (34.2-47.8

mV), confirming their cationic nature, which can facilitate plasmid DNA complexation. Electrostatic interactions, established between the positive charges of liposomes and negative charges of DNA, induced the formation of liposome/DNA complexes, also called lipoplexes, as indicated by size and zeta potential shifts. Lipoplexes prepared at lower charge ratios (1/1; 2/1) displayed larger particle sizes, suggesting less DNA complexation, while higher ratios (4/1; 8/1) resulted in smaller, more stable complexes. Lipoplexes prepared at the charge ratio 1/1 revealed a negative surface charge, while the remaining formulations (2/1; 4/1; 8/1) exhibited a positive surface charge. Lipoplexes prepared with liposomes F4, at the charge ratio of 4/1, achieved the highest transfection efficiency likely due to the balance between particle size, surface charge and lipid composition, potentially enhancing membrane interaction and endosomal escape. Lipoplexes prepared at the charge ratio 8/1 promoted an increase in cytotoxicity, suggesting that while higher charge ratios may stabilise lipoplexes, they can also compromise cell viability.

Conclusion

Although liposomes show promise as a safer, more effective alternative for CAR T cell obtention, further optimisation, such as incorporating targeting ligands to improve lipoplexes internalisation, is needed to enhance transfection efficiency.

EACR25-1553

Development of GD2-Targeted Herpes simplex vectors for OncoVirotherapy

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Introduction

GD2 is a ganglioside gaining therapeutic relevance due to its high expression in tumors like glioblastoma, melanoma and neuroblastoma (NB), and minimal presence in normal tissues. It plays a role in cell signaling and tumor progression, making it an attractive target for cancer immunotherapy. Among these tumors, NB is particularly significant; it accounts for 7-8% of childhood cancers and 15% of cancer-related deaths. High-risk NB remains a major therapeutic challenge due to its heterogeneity, plasticity, and immunosuppressive micro-environment. Despite advances in multimodal treatments, survival rates for high-risk NB remain suboptimal, underscoring the need for novel strategies. A recent FDA-approved therapy targets GD2 with monoclonal antibodies (mAbs) and GM-CSF. However, GD2's low-level expression on neurons and peripheral nerve fibers leads to off-target effects, causing severe pain in treated patients. To address these limitations, we developed GD2-targeted oncolytic Herpes virus for intratumoral therapy, based on attenuated versions of HSV-1. Improved safety and specificity of the engineered vectors was obtained by replacing a key region of HSV glycoprotein D (gD) with

a single-chain variable fragment (scFv) from anti-GD2 mAbs, ensuring selective infection of GD2-positive cells.

Material and method

We modified the HSV-1 backbone using recombineering in *E. coli*. Positive clones were validated by sequencing and expanded. For optimized viral production, producing cells were transfected with constructs carrying GD3 and GD2 synthase coding sequences, and stable clones were selected via antibiotic resistance.

Result and discussion

We engineered the HSV-1 genome to obtain a viral construct characterized by high safety profile, possessing the ability to selectively target GD2-expressing tumor cells; in order to optimize viral production, we generated a stable producer cells with an improved feature of GD2 exposure on cell surface. Flow cytometry confirmed GD2 upregulation in these cells. Preliminary data show that the engineered virus preferentially infects GD2+ cells compared to GD2-negative controls, demonstrating selectivity and potential therapeutic relevance.

Conclusion

Our GD2-targeted oncolytic HSV-1 combines attenuation with de-targeting and re-targeting strategies to enhance safety and efficacy. By integrating tumor-specific targeting with immunostimulatory effects, this approach maximizes anti-tumor activity while minimizing off-target effects. Furthermore, our GD2 upregulation strategy provides a versatile platform adaptable to other cell lines, broadening its potential in targeted virotherapy. These findings lay the groundwork for further preclinical validation, offering a promising avenue for improving treatment of GD2-positive tumors, including neuroblastoma.

EACR25-1579

Therapeutic deep eutectic systems as promising selective therapeutic agents in the anticancer battle

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Introduction

Considering cancer is the second leading cause of death worldwide, and colorectal cancer (CRC) ranks among the most incident and deadly ones, with conventional therapeutics lacking on selectiveness with severe consequent undesired side-effects. The main aim of this work was to design a selective anti-CRC agent aligned with pharmaceutical industry sustainable goals.

Following eutectic systems spotlight due to their remarkable physicochemical and biological properties, this work sought to push forward their reported promising cytotoxicity towards cancer cells, by unravelling eutectics specific interaction with CRC cells.

Material and method

For that, therapeutic deep eutectic systems (THEDES) based on the combination of a terpene with an anti-inflammatory drug, were prepared embodying liquid formulations that could leverage both individual components inherent therapeutic traits. Subsequently, an integrated approach was employed to evaluate

THEDES effect on several indicators of potential therapeutic value towards CRC.

Result and discussion

In a first step, combining menthol:ibuprofen (3:1), thymol:ibuprofen (3:1), perillyl alcohol:ibuprofen (3:1) and (8:1), limonene:ibuprofen (4:1) and (8:1), revealed promising anti-CRC effect by enhancing ibuprofen bioavailability, reduce reactive oxygen species, and inducing cell death via apoptosis. Secondly, THEDES specific interaction with CRC cellular metabolome showed alterations in the metabolite landscape, with deleterious effect on essential metabolic pathways, such as lipid and anaerobic glycolysis energy production. Preliminary *in vivo* systemic toxicity assessments using a Zebrafish animal model indicated non-relevant toxicity of these THEDES within their therapeutic window concentration range. Finally, a controlled drug delivery system based on liposomes (LPS) was developed foreseeing THEDES further therapeutic application. LPS with particle size within the range of 200 nm, mono-dispersed and with negative homogeneous zeta potentials were obtained, showing 27% of Me:Ibu (3:1) encapsulation efficiency.

Conclusion

In summary, this work aimed to provide a better understanding of the specific anticancer activity of THEDES, towards their establishment as an alternative or complementary therapeutic agent towards CRC.

EACR25-1583

Identification and Validation of new In-Silico Designed MYC Binders

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Introduction

The MYC protein, as a transcription factor, is involved in the regulation of 70% human genome in response to proliferation signals. The C-terminal region plays an essential role in transcriptional activity with its obligatory binding partner-MAX by binding to E-box region of DNA. MYC deregulation has been found in various types of cancers with high prevalence, such as lung cancer, breast cancer, and ovarian cancer. In the past several decades, the MYC protein as a priority target for cancer therapies has been intensively studied due to its importance in tumorigenesis. However, due to its intrinsically disordered nature, no drug against MYC passed clinical trials in the past twenty years. Targeting MYC is challenging, however, small-molecule inhibitors have been shown promising potential recently by disrupting of MYC-MAX heterodimerization or preventing of DNA binding. However, most known MYC inhibitors have limitations, including low binding affinity, low potency, or poor tissue penetration. While many researchers still focus on small-molecule MYC inhibition, proteolysis targeting chimeras (PROTACs) are an advanced tool to reduce MYC over-expression by hijacking the ubiquitin-proteasome system (UPS) and inducing cellular protein depletion rapidly and long-lastingly. The design of PROTACs requires a ligand binding to the protein of interest, which is MYC, and a ligand recruiting UPS. Computer-aided drug discovery

has been used as a useful tool for hit compound identification, including structure-based virtual screening (SBVS) and ligand-based virtual screening (LBVS). The aim of this study is to identify and validate new MYC binders via SBVS and LBVS based on known small-molecule MYC inhibitors for the design of PROTACs against MYC.

Material and method

Ligand-based virtual screening (LBVS) and structure-based virtual screening (SBVS) were performed in parallel in three selected chemical libraries, Specs, ChemDiv, and Vitas, to identify potential MYC binders. Potential binding sites of the MYC protein in MAX-bound form were predicted by Glide in the putative active sites. Docked chemicals were ranked in GlideScore for the prediction of binding affinities and validated in auto-blind docking and GOLD docking due to the lack of concaved cavities of MYC for strong interactions. The binding abilities and the effects on MYC stability were assessed in *in vitro* and *in cellulo* assays including SPR and HiBiT system.

Result and discussion

Nine novel compounds have been identified as potential MYC binders from *in silico* screening, and three of them have been shown to bind to MYC C-terminal protein and destabilize MYC in *cellulo*. NC04 only had binding ability with no apparent effect on endogenous MYC stability whereas NC05, NC06, and NC09 showed to destabilize MYC only.

Conclusion

The work in this study offers prospective POI ligands for the construction of PROTACs against the MYC protein, although further biological evaluations are required.

EACR25-1585

Investigation of novel anti-leukemic compounds uncovered through high-throughput screening of human model leukemias

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Introduction

Acute myeloid leukemia (AML) is a hematologic malignancy that represents ~20% of pediatric leukemias, but accounts for most of the disease-related mortality in children. Given the genetic heterogeneity of pediatric AML that impacts patient survival rate and relapse, a single treatment strategy does not exist for all AML subtypes. This underlines the urgent need for novel targeted (genotype specific) therapies. Through a high-throughput drug screen comparing the growth inhibition of 37 AML samples and normal cord blood stem cells (CB CD34+) treated with approximately 11,000 compounds, we identified compound 280 which is structurally related to a previously reported proteasome inhibitor.

Material and method

In collaboration with the Drug Discovery Unit at the Institute for Research in Immunology and Cancer (IRIC), we synthesized and characterized over 80 analogs of compound 280. We determined the half-maximal inhibitory concentrations (IC₅₀) for these analogs in cell viability assays using three AML cell lines and conducted purified proteasome inhibition assays to further evaluate their proteasome inhibitory activities. To assess the potential mechanisms of action of these analogs, we performed a CRISPR chemogenomic screen in the B-ALL cell line NALM-6 using the top two analogs and Bortezomib (BTZ). To evaluate the behavior of these compounds *in vivo*, we have conducted pharmacokinetic (PK) studies, as well as metabolic stability in animal models.

Result and discussion

Several analogs exhibited lower proteasome inhibition compared to BTZ based on the enzymatic IC₅₀ values, but demonstrated greater efficacy in inhibiting AML cell growth, as indicated by cell-based IC₅₀ values. CRISPR chemogenomic analysis confirmed that both our analogs and BTZ target the proteasome. However, the loss of genes linked to resistance mechanisms, including those associated with drug efflux, increased cell sensitivity to the analogs, but did not alter sensitivity to BTZ. This finding suggests that while both BTZ and our novel compounds target the proteasome, they likely rely on different molecular mechanisms of resistance. PK studies indicated that the selected analogs, when administered intraperitoneally (IP), showed favorable stability, prolonged systemic exposure, and appropriate PK parameters, supporting their potential for further development.

Conclusion

Our novel proteasome inhibitor shows promising potential for clinical application in pediatric AML. By understanding how compound 280 and its analogs impact AML cell growth and resistance mechanisms, we aim to develop targeted therapies tailored to specific genetic alterations in AML patients. Furthermore, these therapies may extend their applicability to other hematologic

disorders and solid tumors, addressing a broader spectrum of cancers.

EACR25-1587

Combined siRNA targeting of oncogenic NRAS and BRAF drivers as a strategy for mole reversal and melanoma prevention

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Introduction

An estimated 30% of melanomas arise from a pre-existing melanocytic naevus (mole), the majority expressing an activating variant in BRAF or NRAS, and naevus count is one of the major risk factors for melanoma development. Mole reversal – or induction of complete regression in naevi – is therefore highly desirable as a novel strategy in the prevention of melanoma. Recent data demonstrated siRNA targeting of NRASQ61K induces intrinsic apoptosis in primary naevus cells, that variant-allele specificity reduces off-target effects by 90%, and that it is easily deliverable into the skin *in vivo*. We sought to extend the therapeutic potential of mole reversal therapy by combining siRNAs against the three commonest melanoma drivers into a single product.

Material and method

Antisense siRNAs to siNRASQ61K (NM_002524.5 (NRAS):c.181C>A,p.(Q61K)), siNRASQ61R (NM_002524.5(NRAS:c.182A>G,p.(Q61R)) and siBRAFV600E (NM_004333.4(BRAF):c.1799T>A,p (V600E) were designed and optimised to be variant allele-specific. Melanoma cell lines, each expressing a single driver, were transfected with the relevant single and the triple combination siRNAs. Allele preferential knockdown was determined by RT-qPCR. Live cell imaging monitored cell viability proliferation and caspase 3/7 activation over 5 days post-transfection. Dose-response relationships between siRNA dose and apoptotic activity were determined.

Result and discussion

We show here that single driver variant-targeted knockdown reduces proliferation and induces intrinsic apoptosis in multiple melanoma cell lines, in a dose-dependent manner, compared to non-targeting siRNA. Combination siRNA therapy was equally effective at inducing knockdown of the relevant driver, and induction of apoptosis. Furthermore, no significant pro-apoptotic effect of individual or combination siRNAs was observed in wild type expressing cell lines, highlighting the specificity of the therapy.

Conclusion

A single combination siRNA therapy is a viable therapeutic option for mole reversal and melanoma prevention and would target 80% of naevi without the need to biopsy and genotype. The efficacy across multiple melanoma cell lines also supports exploration of this as a therapy for melanoma.

EACR25-1595

Potential of inhibition of AR to enhance the sensitivity of OVCAR-3 cells to PARP inhibitor

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Introduction

Ovarian cancer (OVCA) is among the most lethal gynecological malignancies with a variety of cellular origins and has a variety of histological characteristics and therapeutic responses. The ovarian cancer treatment involves surgical de-bulking, followed by chemotherapy based on platinum drugs or taxane. The treatment challenge represents the appearance of relapse and developed drug-resistant disease. Estrogen and progesterone receptors (ER and PR) are widely expressed in normal and tumor ovarian tissues, but interestingly, the androgen receptor (AR) is more widely expressed in ovarian cancer than either ER or PR in up to 85% of cancers. The AR plays although unclear, important role in OVCA progression and lately represents an interesting potential therapeutic target for OVCA treatment. In ovarian cancer, approximately 50% of tumors display defective HR repair (homologous recombination pathway of DNA repair). This can be used to create drug-drug synthetic lethality scenario with an inhibitor of PARP. In the present study, we investigated the effect of non-steroidal, second generation, potent AR signaling inhibitor, enzalutamide (MDV-3100), in combination with the potent and selective PARP1 and PARP2 inhibitor AZD2461, an olaparib analog, in concomitant or lead-in treatment in OVCAR-3 cells. OVCAR3 cells are selected for this study, as one of the most widely studied and highly representative model of High grade serous ovarian cancer (HGSOC) cells.

Material and method

The effect of MDV-3100 in combination with the AZD2461 in concomitant or lead-in treatment in OVCAR-3 cells was determined on cell viability by MTT assay, apoptotic potential by dual Annexin/PI staining and flow cytometry and cell cycle perturbations was analyzed by flow cytometry

Result and discussion

Our results in OVCAR-3 cells showed that 3 days pre-treatment with MDV-3100 (30 μ M concentration) followed by PARP inhibitor AZD2461 treatment (30 μ M concentration) for additional 3 days, more efficiently inhibited cell viability (up to 40,73% cell survival) compared to concomitant administration (up to 54,31% cell survival) and AZD2461 alone (80,38% cell survival). This effect was result of induced cell cycle perturbations and apoptosis. Investigation of apoptotic potential confirmed higher efficiency of pre-treatment with MDV-3100 compared to the concomitant administration of MDV-3100 and AZD2461.

Conclusion

Investigation of presented treatment modality had for task to find treatment strategy with non-cytotoxic concentrations of agents in order to enhance efficacy. The pre-treatment with AR inhibitor MDV-3100 followed by PARP inhibitor AZD2461 *in vitro* is more efficient in growth inhibition and induction of apoptosis in OVCAR-

3 cells, compared to the concomitant administration. This treatment strategy may expand the benefits of using PARP inhibitors in OVCA treatment.

EACR25-1600

Oral liquid crystalline dispersion effect in chemotherapy-induced intestinal mucositis

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Introduction

Intestinal mucositis (IM) is a common adverse effect of oncological treatments, significantly impacting the outcome of the cancer treatment. Unfortunately, treatments are often limited to palliative care. As a result, there is an urgency for effective therapies to prevent and treat chemotherapy-induced mucositis in the clinic. β -caryophyllene (BCAR) is a terpenoid with anti-inflammatory properties found in some plants such as rosemary and copaiba. One of the challenges in treating IM orally is the short residence time of the active ingredients in the intestine, which reduces their action. Sustained release systems such as liquid-crystalline (LC) dispersions can gel in contact with gastrointestinal fluids, increasing AI retention in the site and thus enhancing its therapeutic effect. Therefore, this work aims to develop *in situ* gelling LC dispersions containing BCAR and evaluate the BCAR release properties. In addition, their mucoadhesive effect was studied in a model of IM induced by chemotherapy.

Material and method

LC dispersion containing 2.5% of BCAR was prepared by a simple mixture of monolein, oleic acid, and water. BCAR release study in simulated gastric (SG) and intestinal media (SI) was performed, and its quantification was determined by high liquid chromatography. IM was induced in Balb/C male mice (20–25 g) by an intraperitoneal injection of 300 mg/kg of 5-fluorouracil. After 72 h, the IR780 free dye or the LC-BCAR-IR780 were administered orally in IM-bearing mice ($n = 3$). After four hours, animals were anesthetized using 2 % isoflurane, and whole-body images were taken using the IVIS® fluorescent optical imaging technique. Total radiant efficiency was acquired through the ROI of the whole animal and each tissue [1]. The data were analyzed by ANOVA (GraphPad Prism 5) and were expressed as mean values \pm standard deviations. P-values < 0.05 were considered significant.

Result and discussion

The LC formulation was successfully obtained for mucosal administration, resulting in an increased viscosity upon contact with gastric and intestinal fluids. The release of BCAR from LC in SI media was higher than in SG. The opposite was observed for free BCAR. Four hours after oral administration, fluorescence was seen in the gastrointestinal tracts of the animals. Ex vivo results demonstrated increased intestinal retention of IR780 associated with LC-BCAR compared to the free IR780 ($p < 0.05$) confirming the mucoadhesive properties of LC-BCAR in the intestine.

Conclusion

These results indicate that LC-BCAR can represent a potential therapeutic advantage for patients with IM once it exhibits gelling properties upon contact with gastrointestinal media and mucoadhesive characteristics, facilitating a greater presence of BCAR at the target site. [1] This work was approved by the Ethics Committee on Animal Use of the Federal University of Ouro Preto (protocol 1719080322).

EACR25-1691

Targeting Invasive Behavior in 2D, 3D, and In Vivo Models with Pentamethinium Salts as Migrastatic Agents

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Introduction

Metastasis remains a major cause of cancer-related death, underscoring the need for therapies targeting cancer cell migration and invasion. Migrastatic therapy – strategies specifically designed to disrupt cancer cell migration or invasion – represents a promising approach to preventing metastatic progression. Pentamethinium salts (PMS), compounds initially developed as mitochondrial-targeted probes, bind specifically to the inner mitochondrial membrane and inhibit oxidative phosphorylation, a critical energy source required for invasive behavior. This study evaluates the migrastatic efficacy of PMS across 2D, 3D, and *in vivo* cancer models.

Material and method

In vitro assays utilized breast cancer and melanoma cell lines. Standard wound healing and invasion assays were conducted to evaluate migratory and invasive capacities in 2D cultures. For 3D assays, spheroids were generated by culturing cancer cells under ultra-low attachment conditions. Spheroids were embedded in a collagen matrix and subsequently treated with varying concentrations of PMS. *In vivo* studies utilized syngeneic mouse models of breast cancer and melanoma. Tumor-bearing mice received intraperitoneal injections of PMS to assess their impact on metastatic dissemination. Primary tumor growth was monitored over time, and metastatic lesions in distant organs were quantified histologically.

Result and discussion

PMS significantly inhibited the invasive behavior of cancer cells across 2D, 3D, and *in vivo* models. In 2D assays, treatment with PMS markedly reduced cancer cell migration and invasion. Similarly, in 3D spheroid assays, PMS effectively prevented spheroid invasion into the collagen matrix compared to untreated controls. *In vivo*, PMS treatment resulted in a notable reduction of metastatic spread in syngeneic mouse models, without affecting primary tumor growth. These findings demonstrate the robust migrastatic activity of PMS across multiple experimental platforms and highlight their therapeutic potential in preventing metastatic dissemination.

Conclusion

PMS exhibit strong migrastatic efficacy, effectively suppressing invasive behavior in 2D, 3D, and *in vivo*

cancer models. These findings highlight their therapeutic promise as novel agents capable of targeting metastatic dissemination. Further studies aimed at elucidating their mechanisms of action and evaluating clinical translational potential are warranted.

EACR25-1702

Microneedle-mediated delivery of Iron Oxide Nanoparticles & Oxaliplatin with immune checkpoint inhibitors for its induction of ferroptosis and immunomodulation in Head and neck squamous cell carcinoma

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Introduction

Head and neck squamous cell carcinoma (HNSCC) has a poor prognosis with less than 50% mortality rate with the multimodal therapies. Although surgery, chemotherapy and radiation therapy are most important treatment modalities used, there are associated long term side effects with poor life expectancy which bids for new emerging treatment strategies. In this study, MN mediated delivery of iron oxide nanoparticles & oxaliplatin with immune checkpoint inhibitors (anti PD-L1, anti-CTLA-4) was evaluated for its induction of ferroptosis and immunomodulation in Head and neck squamous cell carcinoma.

Material and method

HNSCC cell line MOC-1 was treated with IONPs, Oxaliplatin, and ICI which were assessed in both In-vitro & In-vivo systems. For In-vitro studies, Cytotoxicity (CCK-8), Ferroptosis induction ROS (DCF-DA) and LPO (BODIPY C11), Apoptosis (Annexin-PI), Cell Cycle (PI) analyzed through spectrophotometry & Flow Cytometry and Ferroptosis/Apoptosis markers (GPX4, COX2, c-Caspase 3) through Western blot to compare treated/untreated groups. For In-vivo studies, MOC1-lucigtag cells injected in C57BL/6 mice subcutaneously and tumor sizes assessed in dissolvable MN NP treated groups by bioluminescence signals. IHC-P was performed with treated/untreated tissue samples.

Differential Expressed Genes (DEGs) and PCA deduced through RNA-Seq and data was analyzed through EBSeq.

Result and discussion

IONP DLIN showed the most effective cytotoxicity with least IC50 (0.20 mM) compared to unconjugated IONP (0.90 mM) and Oxaliplatin (0.45 mM) in 24h MOC-1 cells In-vitro. Significant ferroptosis induction seen in DLIN (0.30 mM) treated cells with high LPO+ (52%) and high ROS+ (64%) compared to untreated cells. Oxaliplatin (0.30 mM) showed higher ROS/LPO induction among all treatment groups In-vitro. Apoptosis induction was higher in Oxaliplatin with Annexin V + cells (30%) than DLIN (14%) or Erastin (9%) as compared to untreated (5%). Significant cell death with

high sub G1 % in DLIN (86%) than apoptotic cells and changes in protein markers such reduced GPX4 and increased COX2 expression in Erastin, DLIN and mix treated cells is implicative of ferroptosis induction. In-vivo MN-treatment showed decreased tumor with highest reduction in mixed (IONP/Oxali/ICI) as compared to untreated group. Immunomodulation was corroborated with high CD8 than CD4 and lower PD-L1, Ki67 expression in DLIN, Oxaliplatin & Mix IHC-P groups. RNA-Seq data revealed ~ 4600-7500 differential expressed genes (DEGs) (FDR < 0.05) in DLIN samples as compared to untreated both in-vitro/in-vivo groups.

Conclusion

IONP NPs has shown significant cytotoxicity with ferroptosis induction in cancer cells which was comparable to Oxaliplatin. The synergistic effect of the MN-mediated delivery of IONP/Oxali with the immune checkpoint inhibitors had enhanced cytotoxicity in tumor cells which could be targeted in cancer therapeutics strategies.

EACR25-1710

Targeting RRM2: A Dual Approach to Apoptosis and Cell Cycle Control in Atypical Teratoid Rhabdoid Tumors

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Introduction

Atypical teratoid rhabdoid tumor (ATRT) is a highly aggressive central nervous system (CNS) embryonal tumor that predominantly affects young children and remains incurable. Current treatment strategies, including upfront high-dose chemotherapy (HDC) and radiation therapy (RT), have improved survival rates; however, they often result in significant long-term neurocognitive deficits. Despite these aggressive interventions, the overall prognosis for ATRT patients remains poor. This highlights the urgent need for novel, targeted therapeutic approaches that can improve patient outcomes while minimizing detrimental side effects.

Material and method

To identify potential therapeutic targets, we analyzed multiple independent ATRT clinical cohorts and identified ribonucleotide reductase subunit M2 (RRM2) as significantly overexpressed, with elevated expression correlating with poorer patient survival. Functional studies were conducted to assess the impact of RRM2 depletion on ATRT cell proliferation, colony formation, and migration. Additionally, we evaluated the therapeutic efficacy of COH29, a pharmacological inhibitor of RRM2, in both in vitro ATRT cell models and in vivo orthotopic mouse model. Mechanistic studies were performed to elucidate the effects of RRM2 inhibition on genomic stability, homologous recombination (HR) DNA repair, and apoptotic signaling pathways. Furthermore, we investigated the involvement of the ATM/Rb/E2F1 signaling axis in mediating the cellular effects of RRM2 inhibition.

Result and discussion

Our study demonstrated that RRM2 depletion significantly reduced ATRT cell proliferation, migration, and colony formation. Treatment with COH29 effectively suppressed ATRT tumor growth both in vitro and in vivo, underscoring the potential of RRM2 as a therapeutic target. Mechanistic analyses revealed that RRM2 inhibition led to increased genomic instability, impaired HR DNA repair, and enhanced DNA damage-induced apoptosis. Further investigation into the underlying signaling pathways showed that COH29-mediated RRM2 inhibition activated the ATM/Rb/E2F1 pathway. Specifically, this resulted in reduced phosphorylation of Rb, decreased E2F1 transcriptional activity, and subsequent inhibition of cell cycle progression. These effects culminated in G1-phase cell cycle arrest, impaired S-phase entry, and compromised DNA repair capacity, ultimately driving ATRT cells toward apoptosis.

Conclusion

Our findings establish RRM2 as a critical oncogenic factor in ATRT and highlight its potential as a therapeutic target. By disrupting both DNA damage response and cell cycle regulation, RRM2 inhibition presents a promising strategy for ATRT treatment. These insights provide a strong foundation for the development of novel therapeutic approaches aimed at improving outcomes for patients with this devastating pediatric tumor.

EACR25-1725

Rhenium Complexes as Promising Selective Anticancer Agents

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Introduction

Despite the intensive development of targeted therapies, chemotherapy still plays a central role in cancer treatment. One group of classical therapies is platinum(Pt)-based drugs. However, their side effects, lack of selectivity, high systemic toxicity, and drug resistance limit their clinical use. Consequently, due to these limitations and the efficacy in cancer cells of Pt-based drugs, there is an ongoing intensive search for new, more promising metal-based anticancer drugs. Consequently, due to these limitations and the efficacy of Pt-based drugs in cancer cells, there is an ongoing intensive search for new, more promising metal-based anticancer drugs. One of the potential therapeutic anticancer metals is rhenium (Re). This study presents several new Re complexes and their cytotoxicity on cancer and non-cancerous cell lines.

Material and method

All the chemicals were purchased from Sigma-Aldrich. The neutral complexes $[Re(CO)_3((R/S)-LNO)Cl]$ and $[Re(CO)_3(LNS)X]$ ($X=Cl, Br$) were obtained in the

reaction of $Re(CO)_5X$ with the respective bidentate N, O- or N, S-donor ligands. In turn, during the reaction of $Re(I)$ precursor and (R/S)-LNO or LNS with a heterocyclic monodentate N-donor ligand (3-methylpyrazole (Hmpz), 3,5-dimethylpyrazole (Hdmpz) or imidazole (Him)), after earlier precipitation of Cl^- ions using the $AgPF_6$ salt, cationic complexes $[Re(CO)_3((R/S)-LNO)Him]PF_6$, $[Re(CO)_3(LNS)Hmpz]PF_6$ and $[Re(CO)_3(LNS)Hdmpz]PF_6$ were obtained. The dose-effect curves for the rhenium(i) complexes in the 100–3.125 μM range were plotted to determine the IC₅₀ values in the MTT assay after 24 and 48 hours of treatment. The cytotoxicity of the studied compounds was evaluated against cancer cells, including breast (MDA-MB-231), ovarian (SKOV-3), colon (Colo-205), lung (A549), and prostate (PC-3), as well as non-cancerous cells, including breast (MCF-10A), colon (CRL-1790), and prostate (PNT1A).

Result and discussion

Among the tested compounds, $ReClCl$ and $ReClBr$ exhibited the most promising activity and selectivity, demonstrating high cytotoxicity against prostate and colon cancer cells while remaining significantly less toxic to their non-cancerous counterparts. This selective cytotoxicity suggests their potential as targeted anticancer agents, effectively eliminating malignant cells while sparing healthy tissues. Notably, the $ReRLnoCl$ complex demonstrated potent cytotoxicity against triple-negative breast cancer (TNBC) cells while showing less significant toxicity toward non-cancerous breast cells, further highlighting its therapeutic selectivity.

Conclusion

Three proposed complexes showed selective cytotoxicity, highlighting their potential as selective anticancer agents.

Acknowledgements: This research was funded by the National Science Centre, 2022/47/B/NZ7/02765.

EACR25-1733

Mechanism of Action of DNA Polymerase Inhibitors in Triple-negative Breast Cancer Cells and Anti-tumor Effects in an Intraductal Breast Cancer Mouse Model

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Introduction

Breast cancer is the most prevalent malignancy in women and the second leading cause of cancer-related mortality worldwide. Triple-negative breast cancer (TNBC) is the most aggressive subtype of breast cancer and is characterized by high metastatic potential, severe side effects, and lack of targeted therapies. Therefore, novel and targeted TNBC treatment strategies are warranted. DNA polymerase 1 (POLA1) and histone deacetylase 11

(HDAC11) are critical regulators of cell proliferation, DNA replication, and epigenetic mechanisms, making them promising therapeutic targets in TNBC. We have previously shown that the adamantyl retinoid ST1926 is a POLA1 inhibitor with potent anti-tumor activities in breast cancer cells. We have synthesized two ST1926 analogues, MIR002 and GEM144, which exhibit dual inhibitory activity against POLA1 and HDAC11. We investigated the anti-tumor properties of these adamantyl compounds in human TNBC *in vitro* and *in vivo* models, focusing on GEM144 due to its superior pharmacokinetic properties.

Material and method

In silico analysis was conducted to evaluate the expression of POLA1 and HDAC11 in human TNBC versus normal breast tissues. POLA1 and HDAC11 levels were determined by immunoblotting techniques in different subtypes of human TNBC cell lines (basal-like HCC1806, luminal androgen receptor (apocrine) MDA-MD-453, and the mesenchymal stem-like MDA-MB-231) as well as the normal-like breast cell line, MCF10A. The mechanism of action of GEM144 was studied by assessing TNBC cell viability, cell death, DNA damage, and apoptosis induction. For *in vivo* studies, the mouse intraductal breast cancer model using female NSG mice was explored as a true orthotopic model for breast cancer studies. Tumor progression and treatment response were monitored using the *in vivo* imaging system (IVIS).

Result and discussion

In silico analysis did not indicate any statistically significant differences in the transcript levels of POLA1 and HDAC11 between human TNBC tumors and normal breast tissues. However, basal protein levels of POLA1 and HDAC11 were elevated in several TNBC cell lines compared to normal-like breast cells. ST1926, MIR002, and GEM144 reduced viability and increased cell death in the different tested TNBC cell lines at sub- μ M concentrations while sparing normal-like breast cells. GEM144 induced DNA damage in treated TNBC cells, as shown by increased gH2AX levels, and resulted in apoptosis, confirmed by TUNEL positivity and PARP cleavage. *In vivo* administration of GEM144 at 20 mg/kg reduced tumor burden in HCC1806 intraductal xenografts, without any major signs of toxicity.

Conclusion

Our findings highlight the therapeutic potential of DNA polymerase inhibitors in TNBC. Next, we will evaluate the effectiveness of GEM144 in combination with standard chemotherapies in TNBC cell lines and patient-derived xenografts.

EACR25-1745

Targeting RSK1 in Colon Cancer: Discovery and Evaluation of a New Small Molecule Inhibitor

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Introduction

Ribosomal Protein S6 Kinase 1 (RSK1) is a member of the RSK family. The expression levels and activities of

RSK family members have been reported to be dysregulated in various cancer types. RSK inhibition represents a promising therapeutic strategy, as it serves as a common target for multiple signaling pathways in cancer therapy. Currently, several small molecule inhibitors, including SL0101, BID1870, and APIO-EE-07 have been documented for use in the treatment of colon and breast cancers. However, there is a need for the discovery of more potent small molecules as RSK inhibitors. In this study, a novel inhibitor candidate targeting RSK1 was determined through *in silico* analyses and the activity and cytotoxic properties of the compound were evaluated on two different types of colon cancer cells.

Material and method

In a scope of *in silico* analyses, a pharmacophore-based virtual screening study was carried out. For this purpose, a pharmacophore model of known inhibitor was generated by using Auto Pharmacophore Generation module. Screening of various small molecule libraries through Screen library module of DS using generated model was performed. Then, molecular docking and molecular dynamics (MD) simulation analysis were performed by Glide/SP and Desmond of Maestro, respectively. Finally, binding free energy (MM/GBSA) was calculated using Prime of Maestro. Cell studies were conducted using HT-29 and SW480 cell lines. In our previous study, the cytotoxic effect of this compound (IC₅₀ value) was determined using MTT assay. The determined IC₅₀ dose of compound (IC₅₀: 78.37 μ M) was applied to the cells, and colony formation and scratch assays were performed. Additionally, the mechanism of cell death induced by the compound was identified through flow cytometry analysis.

Result and discussion

As a result, among the screened compounds, a potential inhibitor candidate of RSK1 (6190-0389) showed a strong binding ability with a docking score of -7.849 kcal/mol against RSK1 compared to known RSK1 inhibitor (-6.698 kcal/mol). Interactions were observed with key amino acid residues including Asp142, Asp148, Asp205, and Leu144 during 100 ns MD simulations. Binding free energy (MM/GBSA) was calculated to be -53.52 ± 4.82 kcal/mol. The compound inhibited colony numbers approximately 1000 fold compared with control group. In the groups treated with the compound, an increase in the percentage of wound area was observed compared to the control group. Additionally, flow cytometry analyses revealed an increase in the number of early and late apoptotic cells in the treated groups compared to the control group.

Conclusion

This compound was determined to be a potential RSK1 inhibitor candidate. The initial findings from detailed analyses of this inhibitor provide a basis for evaluating its potential applicability as a therapeutic molecule for colon cancer.

The project was supported by TÜSEB, 27777.

EACR25-1751

Mitochondrial Transplantation from Oxidatively Stressed Adipocyte-Derived Mitochondria Induces Ferroptosis in

Glioblastoma

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Introduction

Glioblastoma (GBM) is a lethal brain tumor with a median survival of 15 months, resisting standard therapies like surgery, radiation, and chemotherapy due to its ability to withstand oxidative stress and ferroptosis, an iron-dependent cell death pathway. GBM cells scavenge normal mitochondria from surrounding cells to meet energy needs and rely on low-ROS energy systems, suppressing their own oxidative phosphorylation to reduce oxidative stress. We hypothesized that transplanting dysfunctional mitochondria from oxidatively stressed adipocytes, differentiated from human preadipocyte or 3T3-L1 cell line, could disrupt these adaptations and induce ferroptosis in GBM.

Material and method

Adipocytes were differentiated from human preadipocyte or 3T3-L1 cell line (ATCC) using differentiation medium (DMEM, 10% FBS, 1 µM dexamethasone, 0.5 mM IBMX, 10 µg/mL insulin) for 48 h, then maintenance medium for 7–10 days, confirmed by Oil Red O staining. Adipocytes were stressed with 200 µM H₂O₂ for 24 h, and mitochondria were isolated, standardized to 50 µg protein equivalent as a mitochondrial quantity scale. These were transplanted into U87 and U251 GBM cells (1×10^5 cells/well) and orthotopic mouse models: U87 in BALB/c nude mice (n = 10/group) and SB28 or GL261 in C57BL/6 mice (n = 10/group/cell line), injected intratumorally (50 µg protein in 5 µL PBS) on day 7. Ferroptosis was assessed via ROS, lipid peroxidation, mitochondrial potential, GPX4, iron levels, and IHC (4-HNE, GPX4). Tumor growth was tracked by bioluminescence.

Result and discussion

In vitro, mitochondrial transplantation into U87 and U251 increased ROS 3-fold, lipid peroxidation 2.5-fold (p < 0.01), reduced mitochondrial membrane potential by 60% (p < 0.05), lowered GPX4 by 70% (p < 0.01), and raised iron 1.8-fold (p < 0.05), confirming ferroptosis. In vivo, tumor radiance dropped 50% in U87, 45% in SB28, and 48% in GL261 (p < 0.01) by day 28, with IHC showing higher 4-HNE and lower GPX4, indicating ferroptosis. This approach exploits GBM's mitochondrial scavenging and low-ROS metabolism, with consistent effects across models suggesting broad therapeutic potential.

Conclusion

Mitochondrial transplantation from stressed adipocytes induces ferroptosis in GBM, targeting its metabolic vulnerabilities and offering a novel therapeutic avenue.

EACR25-1764

ALDH1 Isoform Landscape in Breast Cancer: Advancing Precision Therapies with a Potent ALDH1A3-Selective Inhibitor

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Introduction

Triple-negative breast cancer (TNBC) is among the most aggressive and clinically challenging breast cancer subtypes, marked by high metastatic potential, poor prognosis and limited treatment options. This underscores the urgent need to identify novel molecular targets for effective therapies. Aldehyde dehydrogenase 1 (ALDH1) enzymatic activity is a hallmark of cancer stem cells (CSCs), strongly associated with drug resistance, tumor aggressiveness, and metastasis, making it an attractive therapeutic target. However, the distinct roles of ALDH1 isoforms hindering the rational design of selective inhibitors.

Material and method

This study integrates bulk RNA-seq data from 1,103 primary breast tumors, 50 breast cancer cell lines, and single-cell RNA-seq data from 26 patients to characterize ALDH1 isoform expression across molecular subtypes and the tumor microenvironment. Functional in vitro studies, including cell signaling and evaluation of metastatic potential, along with preclinical in vivo efficacy tests were performed to demonstrate the therapeutic potential of a novel ALDH1 inhibitor, ABD0171.

Result and discussion

ALDH1A3 was predominantly expressed in basal-like TNBC tumors, primarily by epithelial tumor cells, while ALDH1A1 was enriched in stromal components, including myeloid cells and cancer-associated fibroblasts, implicating this isoform in immune suppression and metastasis. Building on this insight, we developed a novel ALDH1A3-selective inhibitor derived from DIMATE, an ALDH1A1 inhibitor currently in clinical trials for AML. Structure-activity relationship studies identified a lead compound with enhanced selectivity for ALDH1A3, sparing the critical ALDH2 and ALDH3. Functional assays revealed that this inhibitor selectively induced apoptosis in ALDH1A3-expressing basal-like TNBC cell lines. Furthermore, it suppressed metastasis by inhibiting IL-6/JAK2/STAT3 and tPA/Src/FAK signaling pathways, potentially via a regulatory mechanism involving modulation of retinoic acid production mediated by ALDH1 in both tumor cells and the microenvironment. Preclinical TNBC models demonstrated its efficacy in reducing tumor growth and preventing distant metastases.

Conclusion

This work establishes ALDH1A3 as a promising biomarker and therapeutic target in basal-like TNBC, offering a promising approach to address unmet clinical needs in aggressive TNBC.

EACR25-1772**AI and physics based methods to design biopharmaceuticals to fight triple-negative breast cancer**

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Introduction

Breast cancer is among the most prevalent cancers globally, with triple-negative breast cancer (TNBC) being the most aggressive subtype. TNBC is marked by rapid metastasis, treatment resistance, and high recurrence, often linked to the Wnt/β-catenin signalling pathway, where Frizzled Class Receptor 7 (FZD7) plays a key role in carcinogenesis. The current standard of care for TNBC involves nonspecific chemotherapy, radiation, or surgery. Antibodies against FZD7 have reached clinical trials, but failed due to off-target toxicity. Small antibody-like protein scaffolds are emerging as alternatives to antibody therapeutics. These scaffolds offer higher tissue penetration, can be easily modified to have increased specificity to the target, and are more cost-effective to produce, making them attractive candidates for tackling TNBC.

Material and method

In this work, we devised a robust deep-learning based computational pipeline to design and evaluate small antibody-like protein scaffolds to target FZD7. Molecular dynamics (MD) simulations were used to better characterize the interaction of the receptor with known antibodies and with its natural effector ligand WNT3a. Binding site analyses, conducted using PyMOL, identified critical residues within FZD7 essential for disrupting Wnt/β-catenin signalling. Guided by these insights, an exhaustive protein design campaign was implemented and different protocols resorting to deep-learning methods, including RFdiffusion, ProteinMPNN, MaSIF and BindCraft were evaluated.

Result and discussion

Thousands of candidate molecules were obtained and extensive filtering was conducted using Rosetta and AlphaFold3. Rosetta scoring functions evaluated their predicted stability, binding free energy, and structural complementarity to the FZD7 binding site. Further refinement of top candidates was conducted using AlphaFold3 to predict binding interactions and structural confidence. Metrics such as predicted template modeling scores, alignment error, and folding stability were used to select the most promising designs. These were subjected to detailed analyses through MD simulations, with simulation times of at least 1 μs performed using the GROMACS package. Key properties such as root mean square deviation (RMSD), secondary structure stability, and analysis of the interaction between the designed protein and the target revealed stable interactions

between selected designs and FZD7 under physiologically relevant conditions. Experimental validation is currently being implemented to validate the computational design strategy and generate data that will be used to refine future design pipelines.

Conclusion

This work highlights how the integration of advanced computational tools can accelerate the development of innovative therapies, offering new hope for patients with aggressive cancers like TNBC.

EACR25-1779**Patient-Derived 3D metastatic melanoma models for target discovery and personalized therapy**

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Introduction

Advanced-stage melanoma remains a critical clinical challenge, with high mortality rates (~90%) and limited therapeutic success due to its potential for rapid progression and resistance to standard treatments. While immunotherapies have revolutionized cancer care, their success is often limited by resistance mechanisms driven by genetic mutations and the tumor microenvironment (TME). Traditional 2D cell culture models lack the complexity to accurately mimic the TME, reducing their predictive power for therapeutic outcomes. To address this, we developed 3D tumoroids and 3D-bioprinted melanoma models that integrate patient-derived tumor cells and TME cells. The patient cells are obtained from an ongoing clinical trial involving 80 patients across seven cancer types. These models provide a robust platform for studying cancer progression and evaluating personalized treatment strategies.

Material and method

Tumor tissues and blood samples collected from clinical trial patients at Sheba Medical Center were processed to generate single-cell suspensions and isolate peripheral blood mononuclear cells (PBMCs), respectively. These cells were incorporated into our 3D tumoroids and 3D-bioprinted tumor models. The 3D-bioprinted model utilized two distinct bio-inks: one containing the tumor and TME cells, and the other one for endothelial cells and pericytes to construct perfusable vascular structures. Patient-specific H&E-stained slides were analyzed using AI to generate tailored treatment recommendations. These findings were discussed in consultation with the on-call oncologist and the research team to explore potential therapeutic options. To validate the models, their structural integrity and functionality were assessed through confocal microscopy, flow cytometry, and viability assays.

Result and discussion

The 3D tumoroid models demonstrated robust capability in predicting patient-specific therapeutic responses. For instance, in a mucosal melanoma case, standard treatments failed both clinically and in the tumoroid model. Through an AI-guided approach, regorafenib (Stivarga®) was recommended, tested in the model, and administered as compassionate treatment, resulting in a durable partial response lasting almost a year. The 3D-tumoroid models from different cancer indications have consistently mirrored clinical outcomes, demonstrating their potential to accurately predict responses and reduce the translational gap between preclinical findings and real-world therapeutic applications.

Conclusion

Our 3D patient-derived models provide a transformative approach to studying tumor biology and therapy resistance. By incorporating melanoma patient-derived cells and mimicking the clinical scenario, our 3D platform offers a powerful tool for drug discovery and personalized medicine, advancing precision oncology and translational research.

EACR25-1780

GSK-3 and BCL-XL inhibition mitigates the competitive advantage of APC-mutant colorectal cancer cells

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Introduction

Colorectal cancer (CRC) development is primarily driven by mutations in the gene APC, which disrupt WNT signaling and provide APC-mutant intestinal stem cells (ISCs) with a competitive advantage over their wild-type counterparts. This advantage is mediated by the secretion of WNT antagonists, allowing APC-mutant cells to out-compete the wild-types in the crypts and initiate tumor growth. In this study, we explored whether GSK-3 inhibition, which leads to upregulation of WNT signaling could enhance the effects of BCL-XL – a key regulator of cell survival – inhibition by BH3 mimetics and potentially restore the competitive fitness of normal ISCs over APC-mutants.

Material and method

CRC spheroids and intestinal organoid models were treated with the GSK-3 inhibitors, alone or in combination with the BCL-XL inhibitor A-1155463. WNT pathway activity was assessed using the TOP-GFP reporter assay, quantitative PCR for WNT target gene expression, and RNA sequencing analysis to capture transcriptional changes following treatment. Apoptotic responses were assessed via caspase-3 activation, mitochondrial BAX aggregation, and propidium iodide exclusion assays. Competitive co-culture experiments between wild-type and APC-mutant ISCs were performed to evaluate the impact of combination therapy on cell fitness.

Result and discussion

We found that GSK-3 inhibition significantly sensitized CRC cells to BCL-XL inhibition, leading to a strong synergistic induction of apoptosis. Mechanistically, GSK-3 inhibition amplified WNT signaling, potentially pushing APC-mutant cells beyond an oncogenic stress threshold. This was reflected in increased mitochondrial BAX aggregation and caspase-3 activation, indicating enhanced apoptotic priming. Gene expression analysis confirmed upregulation of WNT target genes upon GSK-3 inhibition, consistent with the observed increase in WNT pathway activity measured by the TOP-GFP reporter. Importantly, while APC-mutant cells underwent apoptosis, wild-type ISCs exhibited enhanced fitness, reversing the competitive imbalance in organoid models. This suggests that the combination therapy not only eliminates mutant cells but also restores wild-type ISC dominance, counteracting early tumor progression.

Conclusion

Our findings highlight the therapeutic potential of combining GSK-3 and BCL-XL inhibitors in CRC treatment. By simultaneously exploiting oncogenic stress in APC-mutant cells and restoring wild-type ISC fitness, this strategy may provide a targeted approach for early-stage CRC, particularly in patients with familial adenomatous polyposis (FAP).

EACR25-1788

Mechanistic Insights into the Uptake, Trafficking, Drug Release, and Cytotoxic Efficacy of 5-FU/Irinotecan-Loaded core@shell Nanoparticles in Colorectal Cancer

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Introduction

Colorectal cancer (CRC) remains a leading cause of cancer-related mortality, and improving outcomes upon chemotherapy is critical for patient survival. Nanoparticle (NP)-based drug delivery offers a strategy for controlled chemotherapeutic drug release. Since their cell uptake

and intracellular trafficking play key roles in therapeutic efficacy, this study investigates the uptake mechanisms, endosomal trafficking, and drug release kinetics of core@shell NPs co-loaded with 5-fluorouracil (5-FU) and irinotecan (ITC), along with their cytotoxic effects in cell models of CRC.

Material and method

Core@shell NPs were synthesized with high chemotherapy drug loads (28 wt.-% ITC, 29 wt.-% 5-FU monophosphate) and an optimized ITC:5-FU ratio of 1:1.8. Cellular uptake and trafficking were analyzed using confocal microscopy imaging and pathway inhibition assays in CRC cell lines. NP degradation and drug release were assessed in late endolysosomal compartments. Cytotoxicity was evaluated using CellTiter-Glo (CTGlo) assays and Incucyte live-cell imaging in different CRC cell lines and rectal cancer patient-derived organoids (PDOs).

Result and discussion

Core@shell NPs were internalized in CRC cells primarily via micropinocytosis and CAV1-dependent pathways. Confocal imaging confirmed core@shell NP trafficking through the endosomal pathway, accumulating in late endolysosomes where NPs are degraded to facilitate drug release. Despite slow uptake kinetics, NPs effectively released both active chemotherapeutic drugs over time. Cytotoxicity assays in CRC cells revealed that core@shell NPs exhibited a delayed onset of cell death compared to free drugs. Still, sustained drug release ultimately reduced cell viability, leading to prolonged cytotoxic effects.

Conclusion

Core@shell NPs exhibited cytotoxic effects comparable to free chemotherapeutic drugs in CRC cells. However, unlike free drugs, core@shell NPs showed distinct cytotoxicity kinetics, characterized by a delayed onset and prolonged time to induce cell death. This aligns with slow NP uptake, intracellular trafficking, and sustained drug release. These findings highlight that understanding NP uptake and trafficking pathways provides a foundation for refining NP design to enhance drug delivery efficacy in NP based CRC treatment.

EACR25-1795

Pan-cancer therapy using oncofetal-chondroitin sulfate targeting antibody-drug-conjugates

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Introduction

Cancer remains the 2nd leading global cause of death despite therapeutic advancements, as novel targeted therapies face limitations due to tumor heterogeneity and low tumor specificity of antigens. The novel tumor-agnostic target, oncofetal chondroitin sulfate (ofCS), originally discovered through malaria research, was

shown to reappear in cancer. This secondary carb-hydrate modification is up to 1000x more abundant in malignant tissues (primary tumors and distant metastases) compared to healthy tissues (excluding placenta), with little intra- or inter-tumor heterogeneity, and minimal expression in benign and inflammatory tissues. Similar to its function in placenta, ofCS ensures cell growth, migration and tumor immune evasion. Its abundance and expression across various cell types, including cancer cells and stromal cells within the tumor microenvironment, makes it an ideal target for antibody-based therapies.

Material and method

Using phage display technology, we developed two high-affinity antibody fragments, Vartumabs, binding ofCS. Their specificity was validated in vitro on human cancer cells and tissues, and biodistribution and tumor uptake were assessed in mouse models upon intravenous (i.v) injection. We functionalized them into antibody drug conjugates (ADC) by conjugating cytotoxic payloads (MMAE, DXd) and evaluated their safety and efficacy across allo- and xenografted mice models of hematological and solid cancers. Non-GLP toxicity study was performed in rats in a single dose escalation and repeat dose study with ADC-MMAE, including blood profile, and kidney and liver biochemistry.

Result and discussion

Vartumabs exhibited high affinity towards ofCS, identified as a highly sulfated epitope along an extended CS. Their broad cancer specificity was confirmed, with strong reactivity to both primary and metastatic tumors across dozens of cancer types, while demonstrating minimal interaction with healthy, benign and inflammatory tissues. These results showcased their potential for tumor-agnostic cancer targeting. Biodistribution studies confirmed specific tumor accumulation upon i.v. injection, with limited uptake in normal tissues, indicating their potential for delivery of therapeutics. Vartumab ADCs eradicated tumors in multiple cancer models with no recurrence, evident adverse effect or development of treatment resistance. Lastly, pre-clinical toxicity assessment demonstrated well tolerated treatments in rats with reversible toxic effects upon recovery after the 4th dose.

Conclusion

Our strong pre-clinical package highlights these antibody fragments as promising candidates for broad cancer treatment and support further clinical development. Vartumabs are currently being tested in a Phase 0 clinical trial in PET/SCAN imaging studies across multiple cancers as the first-in-human study of ofCS target validation.

EACR25-1803

EGFR targeting restores primary cilia loss upon ETS-driven oncogenic transformation in prostate cells

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Introduction

Primary cilia (PMC) are non-motile microtubule-based organelles presumably found on most human cells, acting as key sensory and signalling hubs. PMC loss is a common early event in carcinogenesis, including of the

prostate. Despite the well-known molecular heterogeneity that characterizes prostate cancer (PCa), genetic rearrangements leading to overexpression of ΔERG or ETV1 are the earliest known genetic drivers of oncogenic transformation, and represent ~50% and ~10%, respectively, of all PCa cases. Recently, we demonstrated that ETV1 overexpression induces EGFR activation in prostate cells. Given that EGFR inhibitors, as Erlotinib, were described to restore ciliogenesis in cancer cells, we aimed to understand whether ETS-mediated oncogenic transformation is sufficient to induce PMC loss in prostate cells and whether Erlotinib could reverse this phenotype in an ETS-specific cell context.

Material and method

We used clonal tumorigenic cell populations with de novo ΔERG or ETV1 overexpression, alongside with the respective Neo control, established from the non-tumorigenic PNT2 prostate cells. The percentage and length of PMC were evaluated in the presence or absence of FBS or EGF, followed by treatment with sub-cytotoxic Erlotinib concentrations (2- and 3-fold below IC₅₀), using immunofluorescence to Arl13B and Centrin-1.

Result and discussion

We observed that de novo ΔERG or ETV1 overexpression leads to significant PMC loss. Additionally, cells overexpressing ΔERG exhibited PMC characteristics of ciliogenesis instability, namely, shorter PMC, weakened Arl13B signal in the ciliary membrane, and frequent loss of Centrin-1 expression in the basal body. Treatment with Erlotinib partially restored primary cilia in all prostate cells, with a more pronounced effect in cells with oncogenic ΔERG or ETV1 overexpression, even in the presence of PMC disassembly-promoting growth factors (FBS or EGF). Additionally, ETV1-overexpressing cells showed enhanced sensitivity to EGF, exhibiting a faster decrease in ciliated cells upon EGF stimulation compared to control cells.

Conclusion

Ciliogenesis is strongly affected and differentially regulated across distinct ETS-driven oncogenic backgrounds, with either ΔERG or ETV1 overexpression being sufficient to drive PMC loss in prostate cells. Furthermore, we confirm a link between EGFR signalling and PMC loss and unveil a sub-cytotoxic mechanism of Erlotinib-mediated PMC restoration in ETS-positive PCa cell models of early-carcinogenesis. These findings strengthen the potential of EGFR inhibition as a therapeutic strategy for the treatment of ETS-overexpressing PCa.

EACR25-1814

A novel gene therapy combination targeting non-small cell lung cancer

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Introduction

The PI3K/Akt/mTOR pathway is highly deregulated in several cancers including lung cancer. AZD2014 (vistusertib), a dual mTOR inhibitor, targets both mTORC1 and mTORC2 complexes within this pathway. Translationally controlled tumour protein (TCTP) and heat shock protein 27 (Hsp27), commonly overexpressed in cancers, interact with the PI3K/Akt/mTOR pathway, leading to eIF4E hyperactivity, causing increased cell growth, proliferation and drug resistance. We rationalise that by targeting several proteins on a common pathway, treatment dosages can be reduced, while potentially enhancing efficacy and minimising adverse effects in prospective *in vivo* studies. Therefore, we aimed to study the therapeutic potential of combining the small-molecule AZD2014 with antisense oligonucleotides (ASO) designed to target TCTP and HSP27 expression.

Material and method

Cell viability analysis was carried out using AZD2014 in a dose-dependent manner on A549 (adenocarcinoma), H460 (large cell carcinoma) and H520 (squamous cell carcinoma) non-small cell lung cancer (NSCLC) cell lines at 24, 48 and 72 h post-treatment. Cytotoxicity of AZD2014 was studied via lactate dehydrogenase (LDH) release together with molecular and phenotypic markers of cell death mechanisms related to apoptosis and autophagy. Magnetofection-mediated cellular uptake of different concentrations of these ASOs into NSCLC cells was investigated at several time-points using flow cytometry. Changes in TCTP and HSP27 gene expression were investigated, while Western blots confirmed protein knockdown at selected ASO concentrations. 3D spheroid models of each of the three NSCLC cell lines were also developed and characterised to better study pharmacodynamics *in vitro*.

Result and discussion

AZD2014 monotherapy exhibited cytostatic activity in a time- and concentration-dependent manner. This cytostatic action of AZD2014 was also observed in NSCLC 3D models, with significant spheroid growth reduction over six days of treatment. Magnetofection induced a high cellular transfection efficiency causing TCTP and Hsp27 protein knockdown to occur between two and three days post-transfection. Combination treatment resulted in synergistic effects on NSCLC cell viability.

Conclusion

These results elucidate the validity and effectiveness of this tripartite combination which is currently being further studied in both 2D and 3D models to effectively target NSCLC and to establish safety profiles of this novel NSCLC treatment.

EACR25-1836

Oncofetal Chondroitin Sulfate represents a Target for Antibody Drug-Conjugate therapy of Acute Myeloid Leukemia

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Introduction

Acute Myeloid Leukemia (AML) is a highly aggressive hematological malignancy with poor clinical outcome due to resistance or early relapse after treatment with current standard therapies. Despite recent therapeutic advancements, there remains an unmet need for novel, targeted strategies. Oncofetal chondroitin sulfate (ofCS) is a promising new cancer target due to its high expression on many cancer entities and low expression on healthy tissues. Our previous studies have demonstrated the potent anti-tumor efficacy of anti-ofCS antibody drug-conjugates (ADC) in solid tumor models. Here, we explore the therapeutic potential of an anti-ofCS ADC in AML.

Material and method

Cell surface expressed ofCS was analyzed in bone marrow (BM) samples from AML patients and healthy donors using flow cytometry. The cytotoxic effect of anti-ofCS ADC was assessed in vitro drug-killing assays utilizing four AML cell lines. Additionally, in vivo efficacy was evaluated in two AML patient-derived xenograft (PDX) models, established from bio-banked AML patient BM samples.

Result and discussion

Flow cytometry analyses showed significantly higher ofCS levels on BM cells from AML patients and PDX compared to BM cells from healthy donors, which exhibited low or undetectable ofCS levels. Consistently, anti-ofCS ADC demonstrated effective in vitro killing of four ofCS+ AML cell lines. Furthermore, anti-ofCS ADC treatment led to significantly prolonged survival in two AML PDX models compared to a protein drug-conjugate control, with minimal adverse effects. Notably, endpoint analysis of AML PDXs demonstrated ofCS expression on human CD45+ AML cells following the anti-ofCS ADC treatment, indicating intrinsic maintenance of ofCS expression by AML cells independently of treatment. Collectively, our findings highlight the ability of anti-ofCS ADC to selectively target and eliminate AML cells both in vitro and in vivo in preclinical trials.

Conclusion

This study provides the first evidence supporting ofCS as a specific and therapeutically relevant target for AML. Our results underscore the potential of anti-ofCS ADC as a promising new strategy for AML therapy, warranting future exploration in clinical trials.

EACR25-1843

Combination of cerium oxide nanoparticles and antimalarial drug chloroquine: characterization and anti-cancer potential for triple negative breast cancer

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Introduction

The most common type of cancer in women is breast cancer including triple negative breast cancer (TNBC). Among other subtypes, TNBC shows high aggressiveness, a poor prognosis and standard therapy is often associated with harmful effects. In this regard, drug repurposing and nanomedicine have gained huge interest in cancer therapy. Therefore, we tested the anti-cancer effect of the antimalarial drug chloroquine (CQ) in combination with cerium oxide nanoparticles (CNP, nanoceria) as a novel and possibly promising tool to treat TNBC.

Material and method

Initially, material characterization was conducted via UV-Vis spectroscopy, X-Ray photoelectron spectroscopy (XPS), high-resolution transmission electron microscopy (HR-TEM), dynamic light scattering (DLS), and superoxide dismutase (SOD) activity measurements of CNP in absence and presence of CQ. Furthermore, cellular uptake of CNP, treatment efficacy and selectivity of CNP and CQ was tested in a 2D in vitro model using MDA-MB-231 and MDA-MB-468 TNBC cells as well as normal (healthy) MCF-12A breast epithelial cells. To refine the translation to a more in vivo situation, studies were also done with a 3D in vitro spheroid model.

Result and discussion

Material characterization revealed good compatibility of the novel drug combination. The presence of CQ did not affect the physical and biological nature of the nanoparticles. Thereafter, cellular uptake of CNP was verified both in the 2D and 3D in vitro spheroid model. Again, the cellular uptake of CNP was not notably affected by the presence of the antimalarial drug. Subsequently, treatment efficacy of CNP and CQ was shown in MDA-MB-231 and MDA-MB-468 TNBC cells. The novel combination did not decrease the viability of normal (healthy) MCF-12A indicating a strong selectivity. Finally, the selective anti-cancer effect of CNP and/or CQ was reproduced in a 3D in vitro spheroid model. Likewise, CNP and CQ selectively decreased the

viability of MDA-MB-231 and MDA-MB-468 spheroids without harming MCF-12A spheroids.

Conclusion

In summary, combining the antimalarial drug CQ with bioactive CNP effectively lowered the individual doses of CQ needed to kill TNBC cells and, therefore, may also lower ‘harmful’ effects of CQ-treated patients in the future. As a consequence, this study revealed that a combination of CNP and CQ may be an alternative treatment option for TNBC.

EACR25-1851

The role of drug efflux and uptake transporters in the plasma and tissue disposition of KRASG12D inhibitor MRTX1133

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Introduction

Due to the high frequency of oncogenic KRAS driver mutations in human tumors and the relevance of KRAS aberrant signaling pathways, intensive therapeutic development efforts have been directed towards KRAS inhibitors. To date, there are no FDA-approved KRAS inhibitors other than Sotorasib and Adagrasib, which target uniquely the KRASG12C mutation. Targeting KRASG12D is of great importance, as it is the dominant KRAS driver mutation in pancreatic cancer (37%), colorectal cancer (12.5%), bile duct carcinoma (10.9%) and the second most common KRAS alteration in NSCLC (16.4%). The first KRASG12D inhibitor to enter clinical trials was MRTX113, currently in phase 1/2, directed towards solid tumors harboring the KRASG12D mutation (NCT05737706). Pharmacokinetic (PK) studies of newly developed drugs are essential to determine their ADME properties, effective dosing regimens, toxicity and clinical drug–drug interactions. The aim of this PK study is to define which drug transporters play a role in the modulation of plasma pharmacokinetics and tissue disposition of MRTX1133.

Material and method

We tested the transport of MRTX1133 across a monolayer of MDCK-II cells overexpressing the ABC transporters human P-gp(ABCB1), human BCRP (ABCG2) and mouse Bcrp(Abcg2) in an in vitro transwell assay. In vivo, we used different knockout mouse strains lacking the drug transporters P-gp, Bcrp, and Oatp1.

Result and discussion

The transwell assay suggested that MRTX1133 is actively transported by P-gp and Bcrp. The plasma pharmacokinetics of intraperitoneally administered MRTX1133 were not substantially affected by the absence of Abcb1 and Abcg2. The brain, liver and kidney concentrations and organ-to-plasma ratios of MRTX1133 in Abcb1a/b;Abcg2^{−/−} mice were significantly increased compared to those in wild-type mice. The mouse Oatp1a/b transporters did not appear to affect the pharmacokinetics of MRTX1133. Unexpectedly, we observed

significant toxicity of MRTX1133 in some of our mice. We are currently looking further into the background and causes of this toxicity.

Conclusion

This study highlights the relevance of ABCB1/ABCG2 in the transporter-mediated restriction of brain penetration and reduction of kidney and liver accumulation of MRTX1133. We hypothesize that co-administration of the dual ABCB1/ABCG2 inhibitor elacridar could promote brain penetration and increase kidney and liver exposure for MRTX1133. The insights gained may contribute to enhancing the safety and efficacy of MRTX1133 in the clinic.

EACR25-1858

Engineering Subtype-Specific Breast Cancer Models: Decellularized ECM-Based Bioinks for Organoid Culture and Precision Oncology

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Introduction

Breast cancer (BC) is the most frequently diagnosed cancer in women and ranks as the second most prevalent malignancy worldwide. Tumor progression is strongly influenced by the tumor microenvironment (TME), which consists of a complex network of stromal cells – such as fibroblasts, mesenchymal stem cells, immune cells, and vascular components – embedded within the extracellular matrix (ECM). This dynamic environment plays a crucial role in regulating tumor growth, invasion, and metastasis. Recent studies emphasize the importance of ECM composition and mechanical properties in shaping BC behavior. The development of decellularized ECM (dECM) technology has provided a powerful tool for reconstructing key tumor features in vitro, allowing for more physiologically relevant modeling of the TME.

Material and method

This study focuses on designing a bioink derived from dECM obtained from BC cell lines representing different molecular subtypes. The goal is to enhance the development of advanced 3D tumor models. To achieve this, we cultured MCF-7, MDA-MB-231, SKBR3, and BT474 cells and applied a carefully optimized decellularization protocol to extract ECM with maximum integrity and bioactivity. The dECMs were extensively characterized in structural components by proteomic analysis, biochemical composition, and biomechanical properties. The resulting bioinks were then evaluated for their ability to sustain the growth of key TME cell populations – including stromal, endothelial, and epithelial cells – as well as patient-derived xenograft organoids (PDXOs), which were subsequently used for drug screening applications.

Result and discussion

The dECMs obtained from different BC subtypes exhibited distinct structural and biochemical profiles, reflecting the inherent heterogeneity of BC. Stromal cells embedded within these bioengineered matrices displayed notable differences in morphology and behavior, highlighting the influence of ECM composition on cellular dynamics. Moreover, the bioinks successfully supported the culture of BC PDXOs, enabling the study of ECM-driven chemoresistance mechanisms in response to various anticancer therapies.

Conclusion

The variability observed in ECM composition across normal, tumorigenic, and metastatic states underscores the need for models that faithfully replicate the full spectrum of BC ECM characteristics rather than focusing on isolated matrix components. Our findings demonstrate that tumor-derived dECM provides a promising foundation for the development of bioinks capable of capturing BC heterogeneity. These bioinks hold significant potential for advancing preclinical research by offering a more physiologically relevant platform for studying BC progression and therapy response across different molecular subtypes.

EACR25-1863

Identification of a new ERK5 kinase inhibitor

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Introduction

The Mitogen-Activated Protein Kinase (MAPK) Extracellular-signal Regulated Kinase 5 (ERK5) pathway is involved in almost all the biological features of cancer cells. Indeed, its role in the development and progression of several types of tumours has been demonstrated. Moreover, ERK5 pathway activation is among the resistance mechanisms to RAS/RAF/MEK1/2-ERK1/2 targeted therapy. Hence, several ERK5 kinase activity inhibitors have been developed. Despite their efficacy in reducing cancer cell proliferation, these compounds only work at relatively high concentrations, making them unsuitable for clinical research. In addition, some of these drugs resulted in a paradoxical activation of ERK5, by inducing its nuclear translocation. In sight of this, we aimed at developing new ERK5 kinase inhibitors.

Material and method

To test the efficacy of the compounds on the kinase activity of ERK5 we conducted *in vitro* kinase assays. To evaluate the effects of these new potential inhibitors on

cancer cell viability we performed cell counting using both wild type and ERK5-knockout (KO) cells.

Result and discussion

In silico screening of the commercial database MolPort, that contains 5M compounds, allowed to identify several candidates among which we have tested 12 compounds. With *in vitro* kinase assay we have individuated one (compound 14) that completely inhibits ERK5 kinase activity up until 1 μM and partially inhibits it until 100 nM. Interestingly, comparing the effect of compound 14 with the effects of the commercially available ERK5 kinase inhibitor JWG-071, we were able to highlight that the new inhibitor works at lower concentration with respect to JWG-071. Moreover, we studied the effect of these compounds on cell proliferation. In HeLa cells, compound 14 completely inhibits cell proliferation up until 2.5 μM, showing efficacy already at 100 nM. To deepen whether this effect relied, at least partly, on ERK5, we performed the same experiment in control and ERK5-knockout cells. We used two clones of ERK5-KO ovarian cancer cells (OV8) and one ERK5-KO hepatocellular carcinoma cell line (HUH7). Interestingly, in all three models ERK5-KO cells resulted to be less sensitive to compound 14 with respect to the control cells, starting from 100 nM up until 10 μM. Comparing these results with the ones obtained for JWG-071, we pointed out a higher specificity of compound 14 in inhibiting cell proliferation

Conclusion

In conclusion, we discovered a new potential ERK5 kinase inhibitor that has an effect at nanomolar concentrations, both in inhibiting ERK5 kinase activity and cell proliferation in an ERK5-dependent manner. Even if further evaluation regarding the capability of this inhibitor not to induce a paradoxical activation of ERK5 will be needed, these results pave the way to a new class of small molecules inhibiting ERK5 activity, that may be able to reach clinical relevance after additional preclinical evaluation.

EACR25-1887

Dual-Active Peptides: A Promising Therapy for Brain Metastases

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Introduction

Therapeutic peptides are among the most promising biopharmaceutical agents for the treatment of various malignancies, including infectious, metabolic, and cancer. Their appeal lies in their low toxicity, target specificity, high potency, and minimal drug-drug interactions. In this study, we conjugated an anticancer peptide (ACp) with a blood-brain barrier peptide shuttle (BBBpS) to develop a novel dual-active peptide conjugate. This strategy aims to facilitate BBB crossing while effectively targeting and eliminating brain metastases derived from triple-negative breast cancer (TNBC) - the leading cause of brain metastases in breast cancer patients.

Material and method

We assessed the antiproliferative effect of PepH3-vCPP2319 on a panel of cancer and healthy cell lines, the translocation across an *in vitro* BBB model, and the *in vivo* biodistribution in healthy mice.

Result and discussion

ACp exhibits strong activity against TNBC cell lines, demonstrating high selectivity, low toxicity, and a half-life of 10 hours in human serum. Meanwhile, BBBpS efficiently crosses the blood-brain barrier (BBB) via adsorptive-mediated transcytosis (AMT), a receptor-independent mechanism that allows it to return to circulation for excretion. Therefore, BBBpS preserves BBB integrity, prevents receptor saturation, and minimizes off-target interactions. The BBBpS-ACp conjugate maintains selectivity, with an IC₅₀ below 5.5 μM in various TNBC cell lines, while exhibiting minimal toxicity in non-cancer cell lines (IC₅₀ > 10.0 μM) and red blood cells (IC₅₀ > 50.0 μM). Importantly, the conjugate retains the translocation capacity of BBBpS, showing a fourfold increase in BBB penetration compared to unconjugated ACp in an *in vitro* BBB model. BBBpS-ACp is internalized via clathrin-mediated endocytosis without compromising cell membrane integrity. Furthermore, evidence suggests it induces mitochondrial disruption and reactive oxygen species (ROS) production, ultimately triggering apoptosis and cell death.

Conclusion

We have designed a dual-acting peptide capable of brain penetration and TNBC cell elimination, thus expanding the drug arsenal to fight this BC subtype and its BM.

EACR25-1899

Optimization of Bivalent Immunoliposome Preparation for Selective Cytotoxic Activity Against Triple-Negative Breast Cancer Cells

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Introduction

Triple-negative breast cancer (TNBC) is one of the most aggressive subtypes of breast cancer and is often associated with poor patient prognosis. The studies on

targeted therapy have highlighted the use of antibody-based drugs (ABD) as a promising strategy. In recent years, antibody-drug conjugates have been approved for the treatment of TNBC. Another group of ABD extensively researched is bivalent immunoliposomes (BIM). The surface of these liposomes is functionalized with two monoclonal antibodies against antigens that are specific to the tumor cell or characterized by over-expression on the tumor cell surface. This functionalization increases the treatment efficacy and reduces the side effects of the drug-loaded on the liposomes by increasing the treatment selectivity and internalization efficacy into the cancer cell. This study aims to prepare doxorubicin (DOX) – loaded BIM with selective cytotoxic activity against TNBC cells

Material and method

A lipid mixture of DOPC and DSPE-PEG-COOH was dissolved in chloroform and evaporated to form a thin lipid film. This lipid film was then dissolved in a mixture of DMSO and ethanol and subsequently added to a sodium sulfate solution in PBS under vigorous agitation. The solution underwent sonication, followed by freeze-thaw cycles in liquid nitrogen. The resulting liposomal solution was dialyzed. The mixture was incubated with DOX to facilitate active drug loading. After incubation, the doxorubicin-loaded liposomal solution underwent another round of dialysis. The surface of the liposomes was modified with antibodies by forming an amide bond between the amino group of the antibody and the carboxyl group of DSPE-PEG-COOH. To achieve this, EDC (1-ethyl-3-(3-dimethylaminopropyl) carbodiimide) and NHS (N-hydroxysuccinimide) were added to the liposomal solution and incubated. Unreacted EDC and NHS were then removed through dialysis. In the next step, antibodies (one or two types, in different molar ratios) were added to the activated liposomes and incubated to allow conjugation. Unbound antibodies were subsequently removed via dialysis to obtain the final antibody-modified liposomes. The efficiency of DOX encapsulation into liposomes was evaluated by absorbance measurements at 485nm. The cytotoxic activity of bivalent liposomes was determined by the MTT assay after 72 hours of treatment on cell lines. The quality of the liposomes was assessed by dynamic light scattering (DLS).

Result and discussion

The proportions of lipids used and the timeframes of the various preparation steps were optimized. The parameters determined by the DLS method (zeta potential, polydispersity index and size) and the drug encapsulation efficiency test showed the good quality of the obtained bivalent immunoliposomes. Tests on TNBC cells confirmed the anti-tumor activity of BIM.

Conclusion

The process of optimizing the BIM proved to be successful and resulted in a BIM characterized by cytotoxic activity against TNBC.

EACR25-1913

The development of a USP7 inhibitor for cancer immunotherapy

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Introduction

Ubiquitin-specific protease 7 (USP7) regulates the stability and fate of many proteins, thereby influencing cellular processes such as cell cycle, chromatin remodeling, and protein synthesis. Elevated level of USP7 in cancer contributes to tumor progression by modulation of tumor microenvironment. USP7 has a variety of substrates, including MDM2 (Murine Double Minute 2) and the tumor suppressor p53 – well-established targets in cancer drug development. Here, we explored the USP7-MDM2-p53 pathway in the context of immunomodulation, rather than more studied direct cytotoxic effects on cancer cells.

Material and method

The activity and selectivity of OAT-4828, an inhibitor we designed, was assessed in enzymatic assays. After pharmacokinetic studies, the efficacy of OAT-4828 was evaluated in mouse *in vivo* models of melanoma (B16F10), colon cancer (CT-26), lymphoma (A20) and chronic lymphocytic leukemia (Eμ-TCL1). Tumor microenvironment was analyzed by flow cytometry. In *ex vivo* studies the influence of OAT-4828 was estimated in mouse and human T cells, as well as mouse bone marrow-derived macrophages by Western blotting, qPCR, ELISA tests and by a functional cell killing assay.

Result and discussion

Oral administration of USP7i resulted in a significant reduction in tumor volume in syngeneic mouse models of colon, melanoma cancer, and lymphoma and leukemia. This reduction was associated with a substantial increase in T cell activation, as evidenced by higher levels of CD69 and CD44, as well as increased production of Granzyme B and IFN-γ. Antitumor activity of USP7i was improved when combined with anti-PD-1 antibodies, while the T cell depletion completely abrogated the therapy outcome. OAT-4828 caused increased production of IL-2 and upregulation of CD25 and CD69 in human T cells, which was associated with MDM2 downregulation and p53 stabilization. Similarly, USP7 inhibition led to MDM2 downregulation in macrophages, inhibiting their M2-like functions. These results indicate that the main mechanism of action of USP7i is based on the activation and improved cytotoxic functions of T cells and promoting anti-cancer activity relevant to the tumor microenvironment.

Conclusion

We conclude that USP7-MDM2-p53 is implicated in the antitumor function of immune cells and shapes the tumor microenvironment. Altogether, we confirm USP7 as an attractive target for cancer immunotherapy and present a strong rationale for the development of USP7 inhibitors for clinical use.

EACR25-1954

Extracellular Vesicles Loaded with miRNAs Target LCP-1 to Inhibit Tumor Growth and Metastasis in Triple-Negative Breast Cancer

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Introduction

Extracellular vesicles (EVs) have emerged as a promising next-generation drug delivery platform for cancer therapeutics due to their intrinsic tumor-targeting capabilities. This study presents an EV-based patented platform technology, derived from mesenchymal stem cells of the Wharton's Jelly (WJCs), designed to encapsulate and deliver microRNA_a. This miRNA directly targets LCP-1, an actin-bundling protein implicated in metastatic progression. Given the highly aggressive and metastatic nature of triple-negative breast cancer (TNBC), we explored the therapeutic potential of microRNA_a-loaded EVs in modulating tumor progression and metastasis.

Material and method

MicroRNA_a was overexpressed in WJCs using transfection, followed by EV isolation via differential centrifugation. Characterization of microRNA_a-loaded EVs was performed in accordance with ISEV guidelines, utilizing TRPS technology for concentration and size distribution analysis and western blotting for EV-specific marker validation. To evaluate efficacy, MDA-MB-231 cells were treated with microRNA_a-loaded EVs, and LCP-1 expression was assessed via RT-PCR, western blot, and fluorescence microscopy. Functional assays, including MTT cytotoxicity, transmigration, invasion, and cell cycle analysis, were conducted to examine effects on proliferation, migration, apoptosis, and ECM invasion. For *in vivo* validation, an orthotopic TNBC model in BALB/c mice was developed to assess biodistribution, tumor growth inhibition, metastasis suppression, and survival outcomes following microRNA_a-loaded EV treatment.

Result and discussion

Treatment of MDA-MB-231 cells with microRNA_a-loaded EVs significantly downregulated LCP-1 expression, confirming direct targeting. Functional assays demonstrated reduced proliferation, migration, and invasion, hallmarks of tumor progression and metastatic potential. Furthermore, treatment induced cell cycle arrest, apoptosis, and mesenchymal-to-epithelial phenotypic reversion, as evidenced by actin reorganization and fluorescence microscopy analysis. *In vivo*, microRNA_a-loaded EVs selectively homed to

tumor sites, leading to tumor growth stabilization and metastatic suppression, ultimately enhancing overall survival in treated mice.

Conclusion

This innovative EV-based platform presents a novel and efficient strategy for delivering miRNA therapeutics directly to tumor sites, targeting metastatic pathways in aggressive cancers such as TNBC. By modulating tumor progression, invasion, and survival pathways, this approach holds promise for advancing precision oncology and targeted miRNA-based therapies.

EACR25-1971

Isolation of anti-proliferative bioactive compounds from *Gypsophila erocalyx* plant extracts by activity-guided approach and its activity in prostate cancer

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Introduction

The endemic *Gypsophila erocalyx* species has been traditionally used for several purposes. However, its potential as a source of anti-cancer compound has not been studied. The aim of this study was to isolate anti-cancer compounds from *Gypsophila erocalyx* with an activity lead method.

Material and method

The aerial parts of *G. erocalyx* were dried in the laboratory and hexane, ethyl acetate, methanol, and methanol:water (70:30) extracts were obtained by maceration method. Anti-proliferative activities of the obtained extracts were examined in prostate cancer cells. As a result of the anti-proliferative activity test, it was determined that ethyl acetate extract had higher anti-proliferative activity compared to other extracts and it was decided to work with this extract. Ethyl acetate extract was separated into subfractions by chromatographic methods such as column chromatography (CC), molecular sieve chromatography (Sephadex LH-20), and preparative thin layer chromatography (PTLC). The chemical structure of the bioactive compound was elucidated by spectral methods such as nuclear magnetic resonance (NMR) and Gas Chromatography-Mass Spectroscopy (GC/MS). In vitro assays such as colony formation 3D spheroid culture were applied to assess anti-cancer potential of 3.2 subfraction and isolated bioactive compound 2,4-di-tert-butylphenol (DTBP) in prostate cancer cells.

Result and discussion

As the subfractionation continued from ethanol extract to 3 and 3.2, the IC₅₀ values were decreased. The subfraction 3.2 showed significant anti-proliferative effect in

both 2D cells and in spheroid cultures of PC3 cells. Additionally, the colony formation ability of PC3 cells were inhibited by 3.2 subfraction. As a result of spectral methods, it was determined that the active compound obtained from step-wise fractionation of *G. erocalyx* plant was 2,4-di-tert-butylphenol (DTBP). DTBP showed anti-proliferative effect in 2D and 3D culture of prostate cancer cells and it decreased colony forming potential. These results suggested that plant-based DTBP was a candidate anti-cancer agent in prostate cancer.

Conclusion

G. erocalyx is a source for anti-cancer agents. 2,4-di-tert-butylphenol was identified as an anti-cancer active compound from this plant for the first time in this study.

EACR25-1972

Defining Docetaxel's Antitumor Effects on Calu3 Lung Cancer Cells Using High-Throughput Live-Cell Imaging

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Introduction

Oncology research aims to understand the cellular mechanisms underlying cancer and to develop effective treatments, with a particular focus on evaluating drug toxicity. To assess drug efficacy, a variety of assays, such as cell viability, migration, and clonogenic assays, are used to evaluate cancer cell sensitivity and resistance. These tests help determine a drug's potential by examining its effects on cell proliferation, survival, and migration. Docetaxel, a chemotherapeutic agent that inhibits microtubule depolymerization, is often studied using these assays due to its impact on cell migration and proliferation. Its effects on cancer progression are commonly analyzed in various in vitro models. The results of toxicity studies can vary significantly depending on the assay used, and combining multiple assays can offer a more complete profile of a drug. In this study, we utilized the Omni Pro 12 high-throughput live-cell imaging platform to simultaneously conduct several assays on the Calu3 lung cancer cell line.

Material and method

The Omni Pro 12 platform minimized variability by conducting all assays under consistent experimental conditions, reducing the influence of external factors on the results. The primary objective was to evaluate the impact of Docetaxel on three critical aspects of cancer progression: cell viability, migration, and colony-forming ability. To achieve this, we performed cytotoxicity, scratch, and colony assays using Docetaxel concentrations ranging from 0 to 1000 nM.

Result and discussion

The results demonstrated that higher concentrations of Docetaxel affected cell proliferation, migration, and colony formation in a dose-dependent manner. However, the assays indicated different effective concentrations for each outcome. Docetaxel inhibited Calu3 cell proliferation in a dose-dependent manner, with peripheral cells in clusters being more sensitive than central cells. Higher concentrations of Docetaxel also led to reduced proliferation recovery after 24 hours. In the cell migration assay, Docetaxel slowed Calu3 cell migration

in a dose-dependent manner, though this effect occurred more gradually compared to the inhibition of proliferation. Additionally, Docetaxel decreased the long-term colony-forming ability of single Calu3 cells in a dose-dependent manner, with 250 nM nearly eliminating colony formation after 324 hours. Long-term monitoring revealed that Calu3 cells require at least 180 hours to form detectable colonies.

Conclusion

By incorporating multiple assays and utilizing advanced technologies like the Omni Pro 12, this study provides valuable insights into the therapeutic potential of Docetaxel in lung cancer treatment. It also underscores the importance of reducing assay variability to more accurately predict drug responses and determine effective concentrations.

EACR25-1992

Combination of PARP Inhibitor Olaparib and a Vascular Disrupting Agent Reduces Tumor Growth in a Xenograft Mouse Model of Prostate Cancer

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Introduction

Metastatic castration-resistant prostate cancer (mCRPC) is associated with poor clinical outcomes and acquired resistance to various therapies. Targeted therapies, such as poly (ADP-ribose) polymerase inhibitors (PARPi), has expanded the treatment options by specifically targeting homologous recombination repair (HRR) deficiencies however, questions remain regarding their broader applicability beyond HRR-mutated populations and there is ongoing debate about whether an HRR mutation is a prerequisite for the response to PARPi or if HR proficient patients can also benefit from their use. Research is currently focused on exploring novel anticancer agents that could enhance the therapeutic efficacy of PARPi and improve clinical outcomes for patients. NOV202 is a novel microtubule destabilizer and a vascular disrupting agent (VDA) that has demonstrated significant anti-proliferative effects in ovarian cancer xenograft models and a large panel of cancer cells. This study investigates the combinational efficacy of the PARPi olaparib with the novel agent NOV202, evaluating whether NOV202 can enhance the *in vivo* efficacy of olaparib in prostate cancer xenografts. Additionally, we explore the anti-proliferative effects of olaparib and NOV202 *in vitro* to elucidate the underlying mechanism of action, aiming to provide deeper insights into their therapeutic potential.

Material and method

DU145 and PC-3 prostate cancer xenografts were established in NMRI nude mice and treated with vehicle, olaparib (100 mg/kg), NOV202 (30 mg/kg), or their combination. Tumor growth was monitored by non-invasive bioluminescence optical imaging (BLI) and calipers. Xenograft tissue sections were analyzed by immunohistochemistry (IHC) to detect γH2AX, RAD51,

HIF-1α, Ki67, and CD31 expression. *In vitro*, cell viability and molecular responses to treatments were studied to evaluate antiproliferative effects and investigate the mechanism of action.

Result and discussion

Both olaparib and NOV202 independently inhibited tumor growth in prostate cancer xenografts, but the combination exhibited additional efficacy only in BRCA2-mutated DU145 xenografts, suggesting that BRCA2 mutations may sensitize tumors to the combined effects of NOV202 and olaparib. Mechanistically, our study shows that DNA damage is the primary cell death mechanism followed by apoptosis in response to treatments. *In vitro*, the combination of NOV202 and olaparib exerts synergistic antiproliferative effects at low NOV202 concentrations suggesting that NOV202 in combination with olaparib could achieve enhanced therapeutic efficacy while minimizing toxicity.

Conclusion

NOV202 enhances olaparib efficacy in BRCA2-mutated prostate cancer models by inducing DNA damage and reducing vascularization. Combining NOV202 with olaparib is a promising strategy for mCRPC.

EACR25-2000

Cellular response triggered by anticancer agent, C-2028 in breast cancer cells with different expression of key receptors under normoxia and hypoxia conditions

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Introduction

Breast cancer (BC) is one of the most common cancers in the world and the leading cause of death among women. Comparing different types of breast cancer, the treatment of triple-negative breast cancer (TNBC), which does not pose estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor-2 (HER2) remains the most difficult to cure due to its aggressiveness and limited therapies. Unsymmetrical bisacridines (UAs) are anticancer compounds synthesized at the Gdańsk University of Technology. They have shown high cytotoxicity against many human cancer cells and anticancer activity against several xenografts in nude mice. This study aimed to evaluate the impact of C-2028, one of the UA compounds, on several breast cancer cell lines with different statuses of ER, PR, and HER2 in the condition of normoxia and hypoxia.

Material and method

The studies were conducted on four BC cell lines: MDA-MB-231 (ER-, PR-, HER2-, TNBC), MDA-MB-453 (ER-, PR-, HER2+), MCF-7 (ER+, PR+, HER2-), and BT-474 (ER+, PR+, HER2+). MTT assay was used to assess the cytotoxicity of C-2028 and reference compounds: cyclophosphamide, doxorubicin, tamoxifen, paclitaxel, and cisplatin. The cellular response caused by C-2028 was evaluated via cell cycle analysis, phosphatidylserine externalization, cell membrane integrity, cell nucleus morphology, and caspase-3/7 activity. The protein level of ER, PR, HER2, and VEGF was checked

by Western blot. The hypoxic condition was achieved in a hypoxia chamber filled with proper gas containing 1% oxygen.

Result and discussion

Western blot analysis confirmed the status of ER, PR, and HER2 in each studied BC cell line. C-2028 exhibited high cytotoxicity against all four breast cancer cell lines and its IC₅₀ varied from 0.012 to 0.1 μM wherein cells lacking HER2 were more sensitive to C-2028. Reference compounds showed various activity against studied cell lines. The most active was paclitaxel with a similar to C-2028 IC₅₀ values, however, 30% of cells stayed alive even at high concentrations. Doxorubicin exhibited cytotoxicity at the level of 0.25–0.4 μM (IC₅₀).

Tamoxifen and cisplatin showed moderate activity with IC₅₀ 2.0–11.0 μM. Cyclophosphamide was the weakest drug (IC₅₀ = 400 μM). C-2028 induced apoptosis in all breast cancer cell lines. Around 40 to 55% of MDA-MB-231, MDA-MB-453, and MCF-7 cells showed features of apoptosis. In the triple-positive cell line, BT-474 cell death was observed in only 15% of cells. In MDA-MB-231 and MDA-MB-453 cells C-2028 under hypoxia condition slightly lost its activity and the IC₅₀ value was up to 2 times higher. What's more C-2028 under hypoxic conditions led to decrease in the level of VEGF in these cells.

Conclusion

C-2028 was highly active in different BC cell lines, including those lacking HER2 receptor and caused apoptotic cell death in them, which is a promising result for future therapy. C-2028 remained active under hypoxia conditions with the contribution to VEGF protein level.

EACR25-2017

Synergistic effect of 5-fluorouracil and the small molecule Wnt/beta-catenin inhibitor iCRT3 on Caco-2 colorectal cancer cells in vitro

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Introduction

Although 5-fluorouracil (5-FU) is a cornerstone of colorectal cancer (CRC) treatment, its efficacy is often limited by resistance. Wnt/β-catenin signalling plays a crucial role in CRC carcinogenesis and resistance, as Wnt expression is upregulated in 5-FU-resistant cells, protecting them from cell cycle arrest and apoptosis, thereby contributing to drug resistance. The small molecule inhibitor β-catenin responsive transcription inhibitor 3 (iCRT3) disrupts Wnt/β-catenin signalling and may enhance CRC sensitivity to 5-FU, overcoming resistance.

Material and method

In this study, the cytotoxic effects of 5-FU and iCRT3 were investigated using the Caco-2 colon adenocarcinoma cell line, marking the first investigation of their combined effects. To this end, the half-maximal inhibitory concentration (IC₅₀) values were determined using the MTT assay. Subsequently, the drugs were combined in different ways, and drug combination index

(DCI) calculations were performed to evaluate their interaction.

Result and discussion

iCRT3 was found to be 2.45-fold more potent than 5-FU ($p = 0.1982$). Drug combination significantly increased the IC₅₀ compared to 5-FU, with a 40.95-fold increase ($p = 0.0022$) when 5-FU was fixed (2.56 μM) and a 43.5-fold increase ($p = 0.0023$) when iCRT3 was fixed (2.41 μM). Two-way ANOVA showed significant impacts from both drug concentration (50.93 %) and treatment condition (25.31 %) on cell viability ($p < 0.0001$). DCI analysis confirmed strong synergism with fixed 5-FU (DCI = 0.154) and synergism with fixed iCRT3 (DCI = 0.618).

Conclusion

Our results indicate that combining 5-FU and iCRT3 could be a promising strategy for CRC treatment, warranting further investigation.

EACR25-2028

Polyoxovanadates with Anti-breast Cancer Activity and Ca²⁺-ATPase Inhibition Potential

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Introduction

Female breast cancer is the second most commonly occurring and the most diagnosed and deadliest worldwide. Triple-negative breast cancer is more aggressive and harder to treat than hormone-receptor-positive cancers. The intracellular calcium ions (Ca²⁺) act as a second messenger and regulate cellular migration and proliferation, two mechanisms linked to cancer [1]. Regarding breast cancers, it is known that drugs may target calcium signaling to reduce tumoral growth or cause breast cancer cell death. Decavanadate (V10), the most studied member of the class of polyoxo-vanadates (POVs), is a promising anticancer drug (Bijelic et al. Angew. Chem. 2019) and acts as an uncompetitive inhibitor of the Ca²⁺-ATPase (Aureliano et al. J. Inorg. Biochem. 2022). In this work, the mixed valence polyoxovanadates, [V15O36Cl]₆₋V15, and two [V18O42I]_{n-} polyoxidoanions with tetramethylammonium (MeV18) and tetrabutylammonium cations (ButV18) [Missina et al., Inorg. Chem. 2018; Kita et al., FEBS Lett., 2022], were evaluated in their potential anti-breast cancer activity, as well as inhibitors of the Ca²⁺-ATPase activity.

Material and method

The cytotoxicity towards MDA-MB-231 (human triple negative breast cancer cells) and HB4a (human mammary epithelial normal cells) cell lines was assessed

by the MTT assay, 24 h after treatment. Ca²⁺-ATPase activities were measured using the coupled enzyme pyruvate kinase/lactate dehydrogenase assay, using sarcoplasmic reticulum (SR) vesicles as model.

Result and discussion

MeV18 and ButV18 ($IC_{50} = 4.85$ and $9.89 \mu\text{M}$) were observed to be approximately three and two times more active against the MDA-MB-231 cells, respectively, than V15 ($IC_{50} = 17.2 \mu\text{M}$). In addition, they were also cytotoxic to the non-tumor cell line, following the order V15 > MeV18 > ButV18. The POV, V15 and MeV18 and ButV18, inhibited Ca²⁺-ATPase activity in vitro, with IC_{50} values of 14.0, 5.16 and $5.65 \mu\text{M}$, respectively. All compounds exhibited mixed-type inhibition. The most potent Ca²⁺-ATPase inhibitor, MeV18, is also the most cytotoxic POV to breast cancer cells. Moreover, stability studies performed by EPR and ⁵¹V NMR spectroscopic techniques showed that V18 presents higher stability in water than V15, which also suffers a partial breakage in the enzymatic medium.

Conclusion

POVs are promising candidates to develop effective therapeutic agents for dealing with breast cancer, using a combined strategy based on targeting calcium signaling pathways and cytotoxicity therapy.

Acknowledgments: Fundação para a Ciência e a Tecnologia (FCT-Porugal), UIDB/04326/2020, UIDP/04326/2020, LA/P/0101/2020 (M.A.), CNPq-Brasil, 406946/2021-3; 309691/2021-4 e CAPES-Brasil (CAPES/PrInt/UFPR, pela bolsa cód. 001).

EACR25-2036

Enhancing Therapeutic Efficacy in Patient-Derived Models of Pancreatic Ductal Adenocarcinoma Using Engineered Placental Mesenchymal Stromal Cells

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Introduction

Pancreatic ductal adenocarcinoma (PDAC) is a highly aggressive cancer exhibiting a dismal five-year survival rate of less than 10%. A major obstacle in the PDAC treatment is a dense stromal environment, primarily regulated by cancer-associated fibroblasts (CAFs), the most abundant cell type in PDAC stroma. Our study aimed to develop engineered extracellular vesicles (EVs) derived from transduced placental mesenchymal stromal cells (PlacMSCs), carrying mRNA encoding the yeast cytosine deaminase::uracil phosphoribosyl transferase (yCD::UPRT) fusion gene, converting the non-toxic prodrug 5-fluorocytosine (5-FC) into the cytotoxic agent 5-fluorouracil. Furthermore, the influence of CAFs on therapeutic response was evaluated to improve the reliability of the organoid model.

Material and method

Conditioned media from PlacMSCs transduced with yCD::UPRT was concentrated (yCD::UPRT-PlacMSC-cCM) using a tangential flow filtration system (TFF-Easy, HansaBioMed Life Sciences) and EVs characterized by Nanoparticle Tracking Analysis (NTA) using NanoSight NS500 Instrument (Malvern Instruments Ltd.). The organoids (PDXOs) were generated from subcutaneous patient-derived xenografts (PDXs). Donor patient tumors and corresponding PDXOs were characterized by immunohistochemical analysis (IHC). Besides, the therapeutic effect of yCD::UPRT-PlacMSC-cCM + 5-FC was determined on patient-derived CAF primocultures (CAF31, CAF78, CAF79) and PDXOs (PDXO71 and PDXO113) by CellTiter-Glo® Luminescent/3D Cell Viability Assay (Promega Corporation). Finally, the biological activity of yCD::UPRT-PlacMSC-cCM was assessed on the cocultivation model of CAF31 and both PDXO samples.

Result and discussion

NTA detected particles in concentrations between $7.8 \times 10^9/\text{mL}$ and $9.6 \times 10^9/\text{mL}$ and the mean size ranging from 67 to 95 nm. IHC analysis revealed substantial interindividual heterogeneity in the expression of Ki-67, CEA, and CK7 markers across patient tumors and corresponding PDXOs, providing valuable insights into the proliferation, differentiation, and aggressiveness. The treatment with yCD::UPRT-PlacMSC-cCM + 5-FC induced uniform cytotoxic effect across CAF samples and PDXOs. Besides, there were no apparent differences in cell viability after the 5-day treatment with yCD::UPRT-PlacMSC-cCM + 5-FC between monocultures and coculture models.

Conclusion

Our study demonstrates the potential of engineered PlacMSC-derived EVs as a new therapeutic approach for PDAC, inducing cytotoxic effects across patient-derived models. Given the limited translation of preclinical findings into clinical practice, our findings emphasize the need for more advanced preclinical models that better recapitulate the PDAC tumor microenvironment.

EACR25-2066

Investigation of Novel Duocarmycin Payloads Antibody-Drug Conjugates (ADCs) targeting HER2 for Therapeutic Intervention

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Introduction

Antibody-Drug Conjugates (ADCs) have emerged as a revolutionary class of targeted therapy for cancer, with 15 ADCs clinically approved for different haematological and solid malignancies and hundreds of candidates currently undergoing clinical evaluation. By combining the selectivity of monoclonal antibodies with the potency of cytotoxic payloads, ADCs enhance the therapeutic index and efficacy while minimizing systemic toxicity. However, the poor penetration of these conjugates in solid tumours, tumour heterogeneity and acquired drug

resistance to payloads remain therapeutic limitations. Duocarmycins are ultrapotent DNA-alkylating agents with strong anticancer properties, but their clinical use as standalone therapeutics has been hindered by a narrow therapeutic window. ADCs provide an ideal platform for incorporating duocarmycin derivatives, improving their therapeutic index and broadening their clinical potential.

Material and method

In this study, we characterized a novel library of duocarmycin analogues for use as ADC payloads, conjugated to the anti-HER2 antibody Trastuzumab.

Result and discussion

Biochemical assays revealed that these analogues exhibit a slower spirocyclization (activation) rate (>2 hours) compared to the control compounds CPI-MI and CBI-MI. Transwell experiments provided insights into their ability to penetrate multilayered cancer cells. The library of analogues was tested across multiple cancer cell line types, including breast, rhabdomyosarcoma, colon or prostate lines, displaying a range of IC₅₀ values (0.1 - 100 nM). Notably, no significant differences in cytotoxicity were observed between wild-type and different drug-resistant cell lines or between p53^{+/+} and p53^{-/-} cell lines, suggesting these payloads are not greatly affected by common resistance mechanisms associated with ABC transporter expression, DNA repair or p53 status. Finally, lead duocarmycin analogues were selected for a cysteine residue bioconjugation to Trastuzumab enabling the generation of novel anti-HER2 ADCs. These conjugates are currently undergoing *in vitro* testing in HER2⁺ and HER2⁻ cancer cell lines, and new data will be disclosed at the event.

Conclusion

In conclusion, our work builds on our previous duocarmycin-based research and focuses on implementing their cancer cell-killing power in ADC designs with clinical potential.

EACR25-2084

Triptonide induces pro-apoptotic effects against medulloblastoma subtypes

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Introduction

Medulloblastoma is a major cause of paediatric brain tumours worldwide. Whilst effective, current treatments are often associated with lifelong off-target effects resulting in lower quality of life and early mortality in paediatric patients. This necessitates the need to develop new therapies. One source of novel therapeutics are those derived from plants, as they are associated with potent effects along with lesser off-target effects (Jones et al., 2023). One species, *Tripterygium wilfordii*, has gained interest over the last decade for the treatment of cancers. Whilst many compounds from this species have shown anti-cancer efficacy, triptonide, has garnered interest due to its diverse biological activity, including the induction of apoptosis and cell cycle arrest in

multiple cancer cell types (Song et al., 2023). However, the efficacy of triptonide against medulloblastoma subtypes is limited. To address this, we sought to elucidate the anti-medulloblastoma efficacy of triptonide.

Material and method

The effect of triptonide on medulloblastoma (HD-MB-03, & DAOY) viability was determined using MTT and washout assays. Time-lapse live-cell microscopy was utilised to examine the effect of triptonide on cell morphology and cell fate profile. Flow cytometry was used to examine the cell death mechanism (Annexin V/ Propidium Iodide), and mitochondrial membrane potential (Rhodamine 123) [1].

Result and discussion

Triptonide produced time and concentration-dependent irreversible decreases in viability against both HD-MB-03 and DAOY cell lines, with IC₅₀ values of 11 nM and 0.64 μM respectively after exposure for 72 hrs. HD-MB-03 cell fate profiling found triptonide (5 nM) significantly increased total cell cycle time by 118.4 ± 22.6 % (P < 0.0001) compared to control. All concentrations examined (5-20nM) resulted in a significant concentration-dependent increase in cellular death in interphase (P < 0.0001) and produced morphological changes associated with apoptosis. Flow cytometry revealed treatment with triptolide (20 nM) significantly increased apoptosis 5.2-fold (P = 0.046) compared to control. Triptonide also significantly decreased mitochondrial membrane potential (42.8 ± 6.7 %, P = 0.006), indicative of intrinsic apoptosis.

Conclusion

These findings suggest that triptonide shows promise as a treatment for group 3 medulloblastoma subtypes through the induction of apoptosis. Further research is now required to determine the molecular mechanisms of action underpinning this observed activity.

[1] The study was ethically approved by the University of Salford Ethical Review Board (ID: 10513).

EACR25-2087

Identification of HMGA protein inhibitors as potential cancer therapy

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Introduction

The overexpression of HMGA proteins plays a pivotal role in neoplastic transformation and is responsible for aggressive tumor phenotypes, characterized by enhanced metastatic potential and resistance to chemotherapy. Notably, HMGA1 has been identified as a key regulator

of cancer stem cell self-renewal, which is crucial for tumor initiation, maintenance, therapeutic resistance, and recurrence. Several studies, including our own, have demonstrated that HMGA1 overexpression contribute to resistance against multiple chemotherapeutic agents, including cetuximab. Consequently, HMGA proteins represent promising targets for novel cancer therapies.

Material and method

To identify potential inhibitors of HMGA protein function, we conducted high-throughput screening analyses using two libraries of molecules: the Sigma LOPAC 1280 and the Prestwick Chemical Library 1280. Electrophoretic mobility shift assays (EMSA) were subsequently performed to assess the ability of the selected molecules to displace HMGA proteins from their target DNA sequences and to inhibit HMGA binding to target proteins *in vitro*. Functional assays were conducted on breast and colon cancer cell lines, with or without HMGA1 expression, to evaluate the specificity and efficacy of the identified inhibitors.

Result and discussion

Our screening identified seven molecules capable of interacting with HMGA proteins. EMSA experiments revealed that some of these compounds effectively disrupted HMGA-DNA interactions and inhibited HMGA binding to its target proteins *in vitro*. Functional assay further demonstrated that two of these molecules, tannic acid and benserazide, selectively inhibited HMGA function in breast and colon cancer cells, and reduced their aggressive phenotype, including high proliferation rate and stem cell potential.

Conclusion

These findings highlight the possibility of targeting HMGA proteins as a novel strategy to overcome chemo-resistance in aggressive cancers.

EACR25-2098

Doxorubicin and cGAMP-loaded hypoxia-sensitive polymersomes: A strategy for enhanced tumor growth inhibition and reduced systemic toxicity

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Introduction

Hypoxia plays a crucial role in shaping tumor progression and influencing treatment outcomes. This unique microenvironment presents both obstacles and opportunities for developing more precise therapeutic strategies. In this regard, nanomedicine has gained significant attention, utilizing hypoxia-sensitive polymersomes to enable targeted drug delivery to tumors while minimizing off-target effects. Hypoxia-sensitive polymersomes provide a versatile approach to addressing the challenges associated with the tumor microenvironment. These specialized carriers are designed to

selectively degrade in hypoxic tumor regions, ensuring more precise drug release. As a result, hypoxia-sensitive polymersomes represent a promising platform for advancing next-generation cancer therapies. In the present study, we focused on the development and characterization of hypoxia-sensitive doxorubicin or cGAMP encapsulating polymersomes for enhanced anticancer efficacy with reduced systemic toxicity.

Material and method

The experimental approach involved the synthesis of hypoxia-sensitive polymersomes comprising a poly(lactic acid)-azobenzene-poly(ethylene glycol) diblock copolymer, loaded with DOX or cGAMP. Further evaluations were conducted to assess the internalization and degradation of these synthesized vesicles in murine cancer cell lines: 4T1 (breast cancer) and B16-F10 (melanoma), at various time points and concentrations. *In vivo* studies were conducted on BALB/c mice with 4T1 tumors and C57BL/6 mice with B16-F10 [1].

Result and discussion

Hypoxia-triggered drug release from the synthesized polymersomes was confirmed, with increased cytotoxicity of DOX-loaded carriers under hypoxic conditions. IVIS imaging enabled visualization of polymersomes localization and drug release within tumors, revealing enhanced accumulation in tumor tissues while minimizing off-target distribution to healthy organs. Therapeutic efficacy was assessed by measuring tumor growth inhibition and monitoring body weight changes in treated mice. While both DOX- and cGAMP-loaded polymersomes individually suppressed tumor growth, their combination exhibited the strongest inhibitory effect. Additionally, immunohistochemical analysis of post-treatment tissues provided insights into tumor architecture and microenvironmental changes.

Conclusion

Our findings demonstrate that hypoxia-sensitive polymersomes exhibit preferential accumulation and efficient release of drugs within hypoxic tumor microenvironment, resulting in enhanced therapeutic outcomes. Additionally, the reduced systemic toxicity observed in treated mice highlight the clinical potential of these engineered nanocarriers for safer and more effective cancer treatment.

*The work is a result of the research projects no. UMO-2020/39/B/NZ5/00745 financed by National Science Center, Poland.
[1] Local ethics committee approval No. 6/2024.*

EACR25-2115

Idasanutlin in combination with PD-1 blockade - synergy or antagonism?

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Introduction

The blockade of the PD-1/PD-L1 immune checkpoint has become a well-recognized strategy in the treatment of cancer. Due to the limited response in a considerable proportion of patients, diverse drug combinations have

been proposed, including small-molecule antagonists of MDM2, such as idasanutlin. In p53 wild-type cancer cells, MDM2 antagonists lead to forced activation of the p53 protein, causing potent cell cycle arrest but only limited cancer cell apoptosis. A combination of PD-1 blockade and p53 activation has been proposed to be beneficial for both treatments. This study aims at a verification of this hypothesis *in vitro* and *in vivo*.

Material and method

MDM2 antagonist idasanutlin was tested against mouse and human cancer cell lines in terms of p53-induced protein expression, cell cycle blockade, cell survival decrease, activation of apoptosis, and induction of PD-L1 expression. A syngeneic CT26/BALB/c mouse model was used to assess the therapeutic effects of the combination of PD-1 blockade and p53 activation *in vivo*.

Result and discussion

Idasanutlin effectively reactivates p53 in p53-wt human cancer cell lines, leading to potent cell cycle arrest but only limited apoptosis. Similar results are observed for mouse cell lines but at considerably higher concentrations. p53-wt/p53-mut selectivity observed for human cell lines is retained in mouse cell lines.

Idasanutlin seemingly increases the expression of PD-L1, but the introduction of a new *in vitro* model questions this notion. The combination of PD-1 blockade with low doses of idasanutlin *in vivo* leads to a positive interaction of both treatments, while high doses of MDM2 antagonist cause deleterious effects to this immunotherapy approach.

Conclusion

The results confirm the limited ability of MDM2 antagonists alone to eliminate cancer cells. The *in vivo* study underscores important aspects of combining the two therapeutic approaches, which can bring both completing and opposing effects.

This research was supported by the Preludium grant 2019/35/N/NZ5/03991 and Sonata Bis grant 2021/42/E/NZ7/00422 from the National Science Centre, Poland.

EACR25-2119

Multiparametric Analysis of High-Throughput 3D Imaging-Based Drug Screening Reveals Cancer Drug-Induced Vulnerabilities in Patient-Derived Metastatic Colorectal Cancer Organoids

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Introduction

Metastatic colorectal cancer (mCRC) treatment outcomes are significantly hindered by inter- and intra-tumor heterogeneity in patient response. Several studies have shown that three-dimensional patient-derived organoids (PDOs) can preserve specific tumor characteristics. Considering the high heterogeneity of colorectal cancer among patients and the ability of PDOs to replicate their parental tumor characteristics, they function as valuable models for drug profiling via high-throughput screening in the context of personalized therapy. The present study aimed to establish a living PDO biobank from 20 heavily pretreated mCRC patients encompassing the morphological, genomic, and transcriptomic characteristics and drug sensitivities via multi-parameter analysis.

Material and method

Hematoxylin-eosin staining was performed to evaluate phenotypic similarities between PDOs and their parental tumors. Genomic and transcriptomic profiles were analyzed using whole-exome sequencing (WES) and RNA sequencing (RNA-Seq). We then conducted drug screening using a 3D image-based approach with a library of 50 compounds to assess anti-cancer drug sensitivities by measuring organoid volume, nuclei count, and proliferation index via the EdU incorporation assay, along with three distinct fluorescent stainings. The drug responses across these three separate parameters were assessed, identifying both common and patient-specific hits.

Result and discussion

The histologic features of PDOs were validated by an independent pathologist. WES and RNA-Seq analyses of PDOs and matched tumors demonstrated a high concordance in mutations, copy number variations, and transcriptomic signatures. Multiparametric drug sensitivity analyses, including organoid volume, nuclei count, and proliferation, demonstrated greater sensitivity in identifying both common and patient-specific hits across all patients, indicating the platform's potential use and expansion into clinical decision-making.

Conclusion

In conclusion, we established a living biobank of PDOs derived from 20 patients with mCRC, incorporating genomic and drug sensitivity analyses while maintaining intratumor heterogeneity and preserving key molecular features relative to their parental tumors. Additionally, we established a protocol for image-based 3D high-content screening on PDOs and successfully applied this pipeline to evaluate 50 drugs. These results suggest that our PDO biobank, in combination with image-based screening, are effective tools for translational research and eventually for the development of personalized medicine.

EACR25-2123**Celastrol induces pro-apoptotic and anti-migratory effects against medulloblastoma subtypes**

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Introduction

Medulloblastoma accounts for 20–25% of all paediatric brain tumours diagnosed worldwide (de Medeiros et al., 2019). Current treatment regimens are effective.

However, most options lead to long-term off-target effects. Therefore, the continued development of new, safer treatments for children is paramount. One source of novel therapeutics are those derived from plants, due to decreased side effects compared to synthetic alternatives (Jones et al. 2023). One species which has emerged in recent years is that of *Tripterygium wilfordii* and the bioactive compounds it contains. One compound of significant interest is that of celastrol, which has been shown to possess anti-cancer properties (Shi et al., 2020). However, there is limited evidence to showcase its efficacy against medulloblastoma. Therefore, this study sought to determine the cytotoxic and anti-migratory effects of celastrol against medulloblastoma subtypes.

Material and method

The effect of celastrol on medulloblastoma (HD-MB-03, & DAOY) viability was determined using MTT assays. Time-lapse live-cell microscopy was utilised to examine the effect of celastrol on cell morphology, and cell fate profile, in addition to bulk and single-cell migration. Fluorescence microscopy was used to measure caspase 3/7 activation [1].

Result and discussion

Celastrol produced concentration and time-dependent decreases in medulloblastoma (DAOY & HD-MB-03) viability with IC₅₀ values after 72 hours found to be 151 and 232 nM respectively. Cell fate profiling showed significant increases in death in mitosis ($P < 0.0001$) but did not significantly increase cell cycle time ($P = 0.132$). Morphological analysis highlighted changes indicative of apoptosis (cell shrinkage & blebbing). Fluorescence microscopy revealed significant increases in caspase 3/7 activation ($P < 0.001$) indicative of apoptotic cell death. Exposure to celastrol significantly decreased bulk cell migration by $50.4 \pm 3.8\%$ ($P = 0.0002$). Medulloblastoma single-cell migration analysis also revealed significant decreases in cell migratory distance and speed ($30.4 \pm 4.1\%$, $P < 0.0001$).

Conclusion

These data highlight the potential of celastrol as a potential treatment for medulloblastoma through the induction of apoptosis and inhibition of cellular migration, warranting the need for further investigation into the underpinning molecular mechanisms of action.

[1] The study was ethically approved by the University of Salford Ethical Review Board (ID: 10513)

EACR25-2140**The effects of a dual GIP/GLP-1 receptor agonist, Tirzepatide, on the response of prostate cancer cells to therapy**

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Introduction

Worldwide, 10.3 million new cases of prostate cancer (PCa) have been diagnosed, making it the second most common cancer in 2022. Repurposing drugs can provide a new way to identify different approaches to optimising cancer treatment. We aimed to investigate the effects of combining tirzepatide (Mounjaro®), approved for the treatment of type 2 diabetes, with the antiandrogen drug enzalutamide.

Material and method

Cell culture; Prostate cancer cells- LNCaP; Western blot; Tritiated Thymidine (3H-thymidine) Incorporation Assay; Muse cell count

Result and discussion

With LNCaP prostate cancer cells, enzalutamide alone inhibited proliferation, while tirzepatide had no significant effects. However, there was an additive reduction in cell growth when tirzepatide and enzalutamide were added in combination. In contrast, no significant interactions were observed between tirzepatide and the chemotherapy drug, Docetaxel. Analysis of data from the cancer genome atlas revealed that in normal prostate tissue there was an inverse correlation between the gastric inhibitory polypeptide receptor (GIPR) and androgen receptor (AR) ($P < 0.05$), but no association was found with glucagon-like peptide-1 receptor (GLP-1R). In tumors, GLP-1R was weakly positively correlated with AR, while the GIPR remained inversely correlated.

Conclusion

The results suggest that tirzepatide appears to have no effect on the sensitivity to docetaxel but may have a role in optimizing the inhibitory effects of enzalutamide. And future work aims to understand the underlying mechanism of action and translate the mechanistic findings in prostate tissue.

EACR25-2146**Generation of Knock-In RAS Oncogenic Mutations in a KP Lung Cancer Cell Line Using CRISPR to Test the Effects of SHOC2 Inhibition**

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Introduction

RAS is the most frequently mutated oncogene across all human cancers, yet its effective clinical targeted inhibition is limited due to resistance and toxicity. The SHOC2-MRAS-PP1 complex has been identified as a potential therapeutic target for RAS-driven cancers, as it has been reported that SHOC2 genetic knockout can reduce cell growth in KRAS mutant breast carcinoma and

increase the sensitivity to MEK inhibitors, widening their therapeutic index. Increasing evidence suggests functional differences between different codon and amino acid substitutions, and the Cancer Dependency Map project (DepMap) has indicated that the Q61x and G13D mutations may be more sensitive to inhibition of the SMP complex than other RAS oncogenic mutations.

Material and method

We used a CRISPR knock-in approach to generate an isogenic mouse model of the most prevalent RAS oncogenic mutations and explore the effects of SMP pharmacological inhibition, protein degradation or genetic ablation of SHOC2, alone or in combination with MAPK pathway inhibitors in CellTiter-Glo viability assays.

Result and discussion

Our results showed that the robust sensitization to MEK inhibitors seen upon SHOC2 absence is reproducible with genetic ablation, protein degradation and pharmacological inhibition of the SMP complex. Data also suggests that this sensitization is particularly enhanced in cells harbouring KRASQ61 and G13 mutations, correlating with the findings from the DepMap project.

Conclusion

These results provide evidence for further development of SMP inhibitors to potentially use in a clinical setting in combination with MEK inhibitors to treat RAS-driven cancers, which could be of significant benefit especially in patients positive for KRAS G13D/Q61H mutations.

EACR25-2150

Exploring Polyoxotungstates as Novel Antitumor Agents for Pancreatic Cancer Therapy

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Introduction

Pancreatic cancer is one of the most lethal malignancies, with a 5-year survival rate of just 5%. This scenario is attributed to the aggressive nature of the disease, the late onset of symptoms and the limited diagnostic and therapeutic options, which reinforces the urgent need for innovative treatments. Polyoxometalates (POMs), compounds formed by transition metal oxyanions such as tungsten, vanadium and molybdenum, have attracted increasing interest due to their structural diversity and biomedical potential (Bijelic et al, 2019, *Angew. Chem.; Carvalho et al, 2023, IJMS*). Among the POMs, those containing tungstate (POTs) stand out for their stability and effectiveness as anti-tumor agents. They act on essential cellular processes, such as inducing apoptosis, stopping the cell cycle, interfering with ion transport and inhibiting key enzymes, affecting calcium homeostasis and intercalate DNA, compromising cellular repair mechanisms. The aim of this study was to evaluate the potential of polyoxotungstates (POTs) as antitumor therapy for pancreatic cancer.

Material and method

Three human pancreatic cancer cell lines - PANC-1, Hs766T and MIAPaCa-2 were used to evaluate the cytotoxic effects of five different POTs. Cytotoxicity was measured over a range of concentrations (0.1–100 µM) using the SRB assay, at 24h, 48h and 72h after treatment to quantify protein content.

Result and discussion

The results revealed that higher concentrations of POTs were associated with a significant decrease in protein content levels, suggesting a direct inhibition of cell proliferation. The P5W17 and P5W30, were found to be the most active, with P5W30 being the most potent one. P5W30 differs from the other compounds in its chemical formula, containing 30 tungsten and 5 phosphorus atoms, and consequently, it has a distinct three-dimensional structure, forming a Preyssler type, while the others are Keggin type (P2W12), lacunary Dawsons (P2W15; P2W17), and Dawson types (P5W18). In fact, Preyssler type have been shown to present higher levels of efficacy in several biochemical processes, in comparison with other POTs (Poejo et al, 2024, *J. Inorg Biochem.*).

Conclusion

In summary, the results obtained in this study demonstrate the promising efficacy of POTs as anti-tumor agents against pancreatic cancer, with P5W30 proving to be particularly potent. Given the therapeutic potential observed, it is essential to continue with more in-depth research, particularly into toxicity, specific molecular mechanisms and clinical application of POTs.

Funding: CIBB strategic projects 10.54499/UIDB/04539/2020, 10.54499/UIDP/04539/2020, and Associated Laboratory funding 10.54499/LA/P/0058/2020, as well as projects UIDB/04326/2020, UIDP/04326/2020, LA/P/0101/2020 (M.A.) from FCT. Scholarship grant from FCT and European Social Funding 2021.05543.BD. University of Vienna and Austrian Science Fund [DOI 10.55776/P33089, DOI 10.55776/P33927].

EACR25-2164

Investigating the repurposing potential of cardiometabolic and hormonal medications for breast and ovarian cancer

treatment using Mendelian randomisation

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Introduction

Approved cardiometabolic and hormonal medications targeting key hallmarks of cancer represent potential repurposing opportunities for cancer treatment. We aimed to investigate the repurposing potential of approved anti-hypertensive, lipid-lowering, anti-adiposity and anti-oestrogenic medications for breast and ovarian cancer treatment using the causal inference method Mendelian randomisation (MR).

Material and method

We obtained or constructed germline genetic instruments from GWAS to proxy cardiometabolic traits (body mass index (BMI), low density lipoprotein cholesterol (LDL-c), systolic blood pressure (SBP)), and the following medication targets: anti-hypertensive (angiotensin-converting enzyme (ACE), beta-1 adrenergic receptor (ADRB1), sodium-chloride symporter (NCC)), lipid-lowering (3-hydroxy-3-methylglutaryl coenzyme A reductase (HMGCR), Niemann-Pick C1-Like 1 (NPC1L1), proprotein convertase subtilisin/kexin type 9 (PCSK9)), anti-adiposity (gastric inhibitory polypeptide receptor (GIPR)) or anti-oestrogenic (cytochrome P450 Family 19 Subfamily A Member 1 (CYP19A1)). The genetically proxied effects of these traits or targets on breast and ovarian cancer mortality were investigated using summary-level MR. Current indications for these medications, for example risk of coronary artery disease, stroke, type 2 diabetes and elevated SBP, were employed as positive control outcomes to assess validity of the genetic instruments. To assess risk of collider bias, summary-level MR was performed with cancer incidence outcomes and where required, adjustments for collider bias are ongoing.

Result and discussion

Genetically proxied BMI lowering reduced breast cancer mortality (HR per SD decrease BMI: 0.89; 95% CI: 0.82–0.97), whilst genetically proxied HMGCR inhibition (HR per SD decrease LDL-c: 1.81, 95% CI: 1.15–2.83) and PCSK9 inhibition (HR per SD decrease LDL-c: 1.51, 95% CI: 1.05–2.18) increased risk of breast and ovarian cancer mortality, respectively. There was no strong evidence to support effects of any other traits or targets investigated. In general, there was evidence supporting effects of genetically proxied targets on their respective current indications, for example evidence supporting

genetically proxied HMGCR inhibition decreasing risk of coronary artery disease (OR per SD decrease LDL-c: 0.67, 95% CI: 0.56–0.80).

Conclusion

Aside from a protective effect of lower BMI on breast cancer survival, we did not observe strong evidence to support repurposing of anti-hypertensive, lipid-lowering, anti-adiposity or anti-oestrogenic medications for breast or ovarian cancer treatment. General limitations of applying MR to cancer prognosis outcomes including limited power of analyses, low heritability of cancer survival and treatment effects, in addition to collider bias may have affected analyses.

EACR25-2172

Overcoming Gemcitabine Chemoresistance in PDAC with Nanoparticle-Delivered Active Phosphorylated Gemcitabine

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Introduction

Pancreatic Ductal Adenocarcinoma (PDAC) is a highly aggressive and complex disease, with nearly 80% of cases being inoperable at diagnosis and reliant on systemic chemotherapy. Gemcitabine, the standard treatment for patients with poor survival (PS), has limited efficacy due to chemoresistance, with only a 30% response rate. Enhancing drug delivery via nanoparticles (NP) loaded with active gemcitabine forms, gemcitabine monophosphate (GMP), or gemcitabine triphosphate (GTP) may overcome resistance and improve gemcitabine efficacy.

Material and method

We developed high-load (around 76% drug content) inorganic-organic hybrid nanoparticles (GMP-IOH-NPs and GTP-IOH-NPs) and tested their efficacy in advanced patient-derived PDAC organoids. Gemcitabine-resistant murine and human PDAC cells were established through gradual gemcitabine exposure. The anti-tumor efficacy of GMP-IOH-NPs and GTP-IOH-NPs on these resistant cells was assessed using live imaging (Incucyte S3) and CellTiter-Glo assays.

Result and discussion

Live imaging of PDAC organoids from three patients treated with GMP-IOH-NP demonstrated a similar reduction in organoid size and number compared to free gemcitabine, indicating comparable efficacy. However, GTP-IOH-NP exhibited superior cytotoxicity against both human and murine gemcitabine-resistant PDAC cells in comparison to free gemcitabine or GMP-IOH-NP, suggesting that the triphosphate form enhances drug potency.

Conclusion

Encapsulating the active forms of gemcitabine in nanoparticles resulted in a comparable therapeutic effect to the free drug in advanced 3D PDAC cell models. Furthermore, GMP-IOH-NPs and GTP-IOH-NPs demonstrate potent efficacy in treating gemcitabine-resistant PDAC cells, as monotherapies. This optimized

drug delivery approach holds significant promise for effectively targeting PDAC tumors that have developed resistance to gemcitabine.

EACR25-2208

Combinations of HDAC and KRAS inhibitors in lung cancer

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Introduction

Lung cancer is one of the problematic cancers due to the low 5-year survival rate (< 15%). Mutations and dysregulated proteins contribute to chemoresistance in non-small (85%) and in small cell lung cancers (15%). Increased RTK signaling (e.g. EGFR family, AKT, mTOR) and overexpressed epigenetic erasers such as histone deacetylases (HDACs) lead to dysregulation of metabolism, cell cycle, cell death and signal pathways. Whereas inhibitors of RTK signaling are established therapies based on the mutation status of a patient, HDAC inhibitors are experimental in solid cancers so far. HDAC inhibitors have the potential to increase chemosensitivity of cancers against classical cytostatics and targeted therapies as e.g. kinase or KRAS inhibitors. This project investigates drug combinations of HDAC and KRAS inhibitors for synergy in drug-resistant and KRAS wildtype or mutated non-small and small cell lung cancers.

Material and method

Non-small cell lung cancers A427/97 and DV90 and small cell lung cancers DMS114 and SHP77 were investigated. Cytotoxic effect of compounds were analysed by MTT assay. Caspase activation was determined by CellEvent Caspase-3/7 Green Detection Reagent from Thermo Scientific. Drug synergy analysis was performed by Chou-Talalay using Combenefit. HDACs, kinases, KRAS and phosphorylated proteins were analyzed by western blotting.

Result and discussion

HDAC1/2/3, EGFR, pEGFR, HER2, pHER2, and KRAS are highly expressed in lung cancer cells. KRAS mutations G12D, G13D and G12V were identified in A427/97, DV90 and SHP77, respectively. Incubation with 0.5 μM of the class I HDAC inhibitor entinostat for 48h prior to addition of the pan-KRAS inhibitor BI2865 decreased the IC50 of BI2865 by a factor of 5- to 50-fold in A427/97, DV90, and SHP77. This chemosensitization of lung cancer cells against the KRAS inhibitor BI2865 by entinostat was shown to be synergistic according to Chou-Talalay. Furthermore, the combination of entinostat and BI2865 increased caspase 3/7 mediated apoptosis in a synergistic manner. Observed synergistic effects between entinostat and BI2865 are likely mediated by increased p21 expression and changes in the expression of pro- and antiapoptotic proteins.

Conclusion

Pretreatment with the HDAC inhibitor entinostat and subsequent treatment with the pan-KRAS inhibitor BI2865 is better than treatment with entinostat or BI2865

alone. The combination not only led to an increase in cytotoxicity, but also to an increased activation of caspase 3/7 with subsequent apoptosis. A proteome analysis before and after treatment provides mechanistic evidence. p21 was upregulated and various pro- and antiapoptotic proteins were differentially regulated. This combination of epigenetic and targeted therapy represents a new clinical option for the treatment of chemoresistant lung cancers.

EACR25-2245

Nanocarriers for Targeted Boron Delivery to Tumor Cells in BNCT Applications

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Introduction

Cancer represents a significant global health challenge due to its uncontrolled cell growth and resistance to conventional therapies. Among innovative treatments, boron neutron capture therapy (BNCT) has emerged as a promising approach. BNCT relies on the selective accumulation of boron-containing agents in tumor cells, which, after neutron irradiation, produce cytotoxic α particles via a nuclear reaction, allowing the destruction of malignant cells while minimizing damage to healthy tissues. However, the clinical success of BNCT faces challenges such as nonspecific biodistribution and rapid metabolism of boron agents and the necessity for a significant number of boron atoms to be localized on or within neoplastic cells. In this study, we explore the use of carriers, specifically apoferritin and β-cyclodextrins, to accumulate boron clusters inside tumor cells. A boron cluster, cobaltabis(dicarbollide) (Cosan) containing 18 boron atoms per molecule was used, and its nanoformulations were tested for the treatment of different types of cancer, including breast cancer, lung cancer, and mesothelioma.

Material and method

Apo ferritin nanohybrids were synthesized by encapsulating Cosan through pH-mediated disassembly at pH = 2 and reassembly at physiological pH. Encapsulation efficiency was assessed using Bradford assay and ICP-MS. Uptake studies were performed on different cell lines, with boron content analyzed by ICP-MS. Cell viability and uptake efficiency were evaluated. AB22 mesothelioma cells incubated with free Cosan or Apoferritin nanohybrids were subjected to neutron irradiation, and post-treatment effects were assessed via clonogenic assay. Cyclodextrin adducts were synthesized by incubating at pH 7.4 and 37°C β-cyclodextrin derivatives with Cosan or TLP, a curcumin derivative that includes a Cosan moiety.

Result and discussion

A high cargo loading capacity of Apoferritin was achieved, specifically 6.1 ± 1.7 Cosan molecules were effectively encapsulated within the inner cavity of apoferritin. Uptake experiments with AB22 mesothelioma cells showed a significant boron uptake, sufficient for neutron irradiation. For BNCT, AB22 cells incubated with only Cosan or apoferritin nanohybrid were neutron irradiated. Clonogenic assays showed a 50% colony reduction compared to irradiated and untreated controls, highlighting more efficacy of apoferritin for boron delivery for BNCT. Cyclodextrin adducts with Cosan and TLP showed a high affinity of both cyclodextrins, suggesting their potential use as carriers. Additionally, preliminary results indicated a TLP higher toxicity compared to Cosan in lung cancer A549 and LLC cell lines, further supporting its potential as an effective boron delivery system.

Conclusion

We successfully developed an apoferritin nanohybrid and cyclodextrin adducts, which can act as delivery systems that efficiently encapsulates boron-containing molecules for potential use in BNCT.

EACR25-2248

Subcutaneous controlled release of antibodies and antibody-drug-conjugates with zwitterionic chitosan-based hydrogels

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Introduction

Subcutaneous (SC) administration of monoclonal antibodies (mAbs) enhances patient compliance and therapeutic efficacy but remains challenging for antibody-drug conjugates (ADCs) due to formulation instability, delivery constraints, and immunogenicity risks. Conventional SC formulations require cold-chain storage and often display variable bioavailability. Here, we introduce a zwitterionic chitosan-based hydrogel that enables controlled release and prolonged stability of mAbs and ADCs at ambient temperature, offering a scalable alternative for clinical translation.

Material and method

Zwitterionic chitosan-based hydrogels were designed for the sustained release of trastuzumab, rituximab, trastuzumab/pertuzumab, and trastuzumab-emtansine (T-DM1). Comprehensive characterization was performed, including rheology, injectability, stability, and in vitro release kinetics. Two lead formulations were developed: chitosan@DOTAGA:chitosan for mAbs and a zwitterionic chitosan-only hydrogel optimized for ADCs to ensure cGMP compliance. Pharmacokinetic (PK) studies in murine and nonhuman primate (NHP) models compared these formulations to standard-of-care therapies (Herceptin® SC and Kadcyla® IV, respectively). Tolerability was assessed using ex vivo human skin explants, while therapeutic efficacy was evaluated in HER2+ xenograft models with multidose (trastuzumab) and monodose (T-DM1) administration.

Result and discussion

The chitosan@DOTAGA:chitosan-mAb hydrogel preserved the structural integrity of trastuzumab for up to

15 days at ambient temperature. Functional stability was confirmed via SECnMS and peptide mapping. In vivo, the hydrogel underwent complete biodegradation in both murine and NHP models, with no detectable immune activation. PK studies in NHPs revealed comparable systemic exposure profiles to SC formulations utilizing rHuPH20. For the zwitterionic chitosan-only hydrogel encapsulating T-DM1, ADC integrity was preserved for over one month at ambient temperature, with no inflammatory response detected in patient skin explants. In murine models, SC administration of the hydrogel resulted in a prolonged systemic exposure duration (9.5 vs. 5.6 mg·day/mL) compared to IV administration. In a HER2+ xenograft tumor model, therapeutic efficacy studies confirmed that tumor growth inhibition following hydrogel-mediated ADC delivery was comparable to clinically approved IV T-DM1, underscoring the potential for sustained SC drug delivery.

Conclusion

Our zwitterionic chitosan-based hydrogel formulations overcome key barriers in SC biologic delivery, providing extended stability and precise drug release while eliminating cold-chain constraints. By ensuring prolonged bioavailability and therapeutic efficacy, this platform offers a clinically viable strategy for next-generation SC biosimilars and ADC therapies.

EACR25-2253

Antitumor immune mechanisms elicited by NLRC5 expression in cancer cells

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Introduction

Downmodulation of MHC class-I and antigen processing and presentation machinery (APM) genes is a key mechanism of cancer immune evasion. MHC-I defects in cancers occur mainly via the loss of expression of NLRC5 (NOD-Like Receptor CARD domain containing 5). NLRC5 is the key transcriptional activator of MHC-I and APM genes. We have shown that stable expression of NLRC5 in B16-F10 (B16-NLRC5) melanoma cells upregulates MHC-I, promotes tumor immunogenicity and elicits protective antitumor immunity against parental B16-F10 cells. Here, we elucidated the cellular and molecular mechanisms underlying the ability of B16-NLRC5 cells to elicit protective antitumor immunity towards exploiting NLRC5 for cancer immunotherapy.

Material and method

We carried out a proteomic analysis of B16-NLRC5 cells and B16-F10-vector controls to understand the molecular pathways modulated by NLRC5. We implanted B16-NLRC5 and B16-F10-vector cells in syngeneic C57BL/6 mice and monitored tumor growth and histological features. We examined the immune cell composition within tumors and tumor draining lymph nodes by flow cytometry. We evaluated the impact of depleting CD8+ T or NK cells on tumor growth.

Result and discussion

Proteomic analysis of B16-NLRC5 cells showed enrichment of proteasome, ubiquitin-mediated proteolysis, protein processing in the ER, spliceosome, ribosome biogenesis and peptidase pathways, which can impact the generation of canonical and non-canonical

MHC-I peptides. Notably, B16-NLRC5 cells showed downmodulation of PMEL17/gp100, DCT, TYR and TYRP1, which harbor dominant CTL epitopes. These data suggest that NLRC5 could increase tumor immunogenicity by expanding the diversity of the MHC-I peptidome. Tumors formed by B16-NLRC5 cells showed increased vascularization and abundant infiltration by CD45+ cells. Flow cytometry analysis revealed that B16-NLRC5 tumors harbored elevated numbers of CD8+ CD69+ activated/effector T cells in tumor draining lymph nodes (tdLN) and within tumor infiltrating lymphocytes (TILs). Notably, TILs from B16-NLRC5 tumors contained 10-fold more NK1.1+ cells. Depletion of either CD8+ T cells or NK1.1+ cells in B16-NLRC5 tumor bearing mice inhibited NLRC5-mediated tumor growth control.

Conclusion

Our findings indicate that (i) NLRC5 expression in tumor cells upregulates MHC-I, promotes tumor antigen presentation, broadens the MHC-I peptidome and increases tumor vascularity, and (ii) collectively, these events facilitate efficient priming of antitumor CD8+ T and NK cells, their infiltration into the tumor microenvironment and killing of tumor cells. Thus, NLRC5-based approaches can be developed for molecular immunotherapy of diverse immune escape cancers.

EACR25-2268

Effect of doxorubicin and quercetin combined treatment on osteosarcoma model systems

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Introduction

Osteosarcoma (OS) is a highly aggressive bone tumor primarily affecting pediatric patients. Standard treatments include surgical resection, chemotherapy, and radiation for tumors that cannot be surgically removed. Although the 5-year survival rate is 65.5%, patients with metastases and recurrence have a significantly lower survival rate of ~30%. Despite this concerning statistic, the treatment for OS has remained largely unchanged over the past three decades. This stagnation in treatment innovation highlights the urgent need for further research and development in therapies tailored specifically for OS.

Material and method

We identified the DEGs between bone (7 samples) and OS (27 samples). To conduct in-depth study of the obtained upregulated DEGs, we constructed a PPI network and identified the most significant gene cluster. We investigated the effects of the combined treatment with doxorubicin and quercetin on SAOS-2 osteosarcoma cells in 2D condition and immobilized in alginate microbeads in 3D condition. We assessed the effects of treatments on cell viability using MTT and the expression of genes using qPCR.

Result and discussion

We have analysed DEGs between bone and human osteosarcoma samples and identified 630 upregulated genes. We extended the networks with information from

DrugBank to identify potential therapeutics for osteosarcoma focusing on the top 10% of interconnected genes in cluster due to their important biological functions. The identified cluster had enrichment in biological processes connected to oxidative phosphorylation and we found quercetin as a promising candidate for treating OS. We analysed quercetin's effect utilizing the Saos-2 in 2D and 3D on viability and gene expression, alone or in combination with doxorubicin. Following treatment, we assessed cell viability and the expression of genes. Our results have shown that the combined treatment statistically significantly decreased the viability of SAOS-2 cells cultured in 2D and 3D conditions compared to cells treated with doxorubicin. We analyzed the expression of genes associated with poor prognosis in patients such as pluripotency genes, an OS marker, and a resistance-related gene. Collectively our results show different responses to the combined treatment depending on the model system used.

Conclusion

The combined treatment substantially reduced cell viability in 2D and 3D models and decreased expression of genes associated with poor prognosis compared to doxorubicin alone in 3D models. We can hypothesize that microenvironment-based mechanisms modulate cell sensitivity to therapy and increase resistance to treatment of osteosarcoma cells cultured in 3D condition. Understanding the molecular mechanisms will significantly contribute to the development and enhancement of existing therapies, thereby facilitating advancements in the treatment of osteosarcoma. Acknowledgement: This study was supported by the Ministry of Science, Technological Development and Innovation of the Republic of Serbia, grant no. 451-03-136/2025-03/200042 and the Science Fund of the Republic of Serbia, grant no. 7503

EACR25-2280

Unlocking the Dual Power of miR-302b in BRCA1 Wild-Type Triple-Negative Breast Cancer: PARP Inhibitor Sensitivity Enhancer and Tumor Microenvironment Shaper

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Introduction

miRNAs are small non-coding RNAs, strongly regulated at epigenetic level, which play an important role in both tumor-intrinsic mechanisms and tumor microenvironment (TME) shaping. They can be released and transferred through microvesicles, contributing to the crosstalk between neoplastic and TME cells and influencing cancer progression and response to therapies. Recently, we demonstrated that miR-302b enhances chemotherapy response both in *in vitro* and *in vivo* triple negative breast cancer (TNBC) models by regulating cell cycle genes and DNA repair pathways. Additionally, we proved that the miR-302b/cisplatin combination treatment down-

modulates double-strand break repair pathways in neoplastic cells and reduces pro-tumoral M2 macrophages number, identified as CD68/Arginase-1 positive stromal elements. Starting from these findings, we aimed to further investigate miR-302b's potential as a therapeutic tool when combined with PARP inhibitors, a class of DNA repair-based drugs, and its involvement in pro-tumoral M2 macrophages phenotypic switch.

Material and method

We used MDA-MB-468 and MDA-MB-231 as BRCA1 wild-type cell models. MDA-MB-468 were treated for 5 days with 500 nM cisplatin and/or 250 nM olaparib, while MDA-MB-231 with 2 μ M cisplatin and/or 2 μ M olaparib. MiR-302b overexpression was achieved by transfection with miR-302b mimics or negative control, delivered by commercial lipid nanoparticles. Panobinostat was administered for 24 h at 1 μ M in MDA-MB-468 and 10 μ M in MDA-MB-231 cell lines, and bone marrow derived macrophages. IL-4 and/or supernatant of 4T1, treated or not with cisplatin and transfected with miR-302b or control, have been used to condition macrophages.

Result and discussion

Analyzing a proprietary TNBC cohort (GSE86948), we demonstrated that higher miR-302b expression levels correlate with BRCAneSS phenotype and PARP inhibitor sensitivity signatures. Notably, we discovered and validated BRCA1 as direct target of miR-302b, which enhances response to PARP inhibitors in combination with cisplatin in BRCA1 wild-type TNBC models, by mimicking a BRCAneSS phenotype. We also observed that miR-302b downregulation in breast cancer can occur through histone deacetylation at miRNA promoter region. Indeed, treatment with the histone deacetylase inhibitor panobinostat induces miR-302b expression, likely contributing to BRCA1 downmodulation. Lastly, we proved that miR-302b impairs M2 phenotypic switch in macrophages by directly targeting the M2 marker Irf-4, thus counteracting an immunosuppressive microenvironment.

Conclusion

These results strongly support the idea that miR-302b might be a promising adjuvant therapeutic tool in combination with PARP inhibitors/chemotherapy in BRCA1 wild-type TNBC, exerting a dual role on both neoplastic cells and the TME.

EACR25-2293

Preclinical validation of a promising protein-protein interaction peptide inhibitor towards metastatic breast cancer

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Introduction

Triple-negative breast cancer (TNBC) accounts for approximately 10–15% of all breast cancer (BC) cases. Historically, TNBC is characterized by the absence of targetable receptors, which makes chemotherapy the primary treatment option. However, recent evidence suggests that the chemoresistance and high rates of metastasis associated with TNBC may be linked to cancer cells that overexpress the frizzled-7 receptor (FZD7). While monoclonal antibodies targeting FZD7 have reached clinical trials, they have shown off-target effects. Consequently, peptide-based inhibitors are emerging as promising alternatives due to their specificity, lower toxicity, and ease of modification.

Material and method

In this study, we employed a multi-step approach to identify effective anti-FZD7 peptides. First, we used patient samples to confirm the presence of FZD7 in primary and brain metastatic TNBC biopsies. Next, we applied computational methods to identify peptide sequences found within the binding domains of published anti-FZD7 antibodies and to assess in silico binding affinity and serum stability for these peptides. Third, we synthesized ten peptides that exhibited low binding affinity ($K_D < 10^{-6}$) and high stability ($t_{1/2} > 120$ min) and evaluated their activity in vitro. Fourth, we assessed the in vivo biodistribution of the top three peptides and used this information to enhance their pharmacokinetics. Finally, the peptide with the most suitable properties was conjugated to gold nanoparticles for further in vivo studies on tumor accumulation.

Result and discussion

The data obtained demonstrated the therapeutic potential of the peptides. First, histological characterization of tumor biopsies confirmed the overexpression of FZD7 in TNBC. Notably, brain metastases exhibited significantly higher levels of the receptor, which may be linked to FZD7's role in cancer metastasis. Second, among the ten anti-FZD7 peptides identified in silico, three showed high binding affinity (nM), strong anticancer activity ($IC_{50} < \mu$ M), low toxicity ($IC_{50} > mM$), and high stability ($t_{1/2} > 120$ min). Third, the in vivo biodistribution studies indicated that all tested peptides had a short circulation time due to high renal clearance. However, one anti-FZD7 peptide exhibited better stability and tumor accumulation than the others. Based on these data, this peptide was further conjugated to gold nanoparticles to enhance circulation time and tumor accumulation. We observed significantly improved circulation time and tumor accumulation compared to the native peptide, while not increasing accumulation in other organs, thus validating the advantages of the nanoparticle-peptide conjugate.

Conclusion

In conclusion, we successfully developed a peptide-based inhibitor targeting FZD7 that is both effective and safe,

with the potential for further optimization to explore its use as a therapeutic and/or diagnostic tool.

EACR25-2303

Development of an optogenetic lytic system in *E.coli* based on phage-derived lytic proteins as a light-controlled and safe drug delivery platform

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Introduction

Synthetic biology, a multidisciplinary field that integrates principles from engineering, biology and mathematics to create biological systems with new “synthetic” functions, plays a key role in immunotherapies due to its capacity to engineer biological systems with precision and customization. Indeed, synthetic biology has recently emerged as a game changer in engineered cell-based immunotherapies based on T cell (CAR-T), or bacteria engineering. The latter leverage on bacterial ability to infiltrate tumors, due to unique features of the TME including necrosis, hypoxia, and reduced immuno-surveillance. The ease genetic modification of bacteria contributes to their appeal as therapeutics. Particularly, probiotic *E. coli* Nissle 1997 gained attention as smart-microbe platform to deliver therapeutic cargos in the tumor, particularly due to its safety in humans. Synthetic bacterial therapy is based on the design of intracellular sensor-actuators that specifically recognize the conditions of tumor microenvironment (e.g. hypoxia, acidosis or stiffness) and trigger a specific output in response (e.g. cytokines, cAMP, ICIs nanobodies). On the other hand, optogenetics is a powerful tool for studying new genetic circuits in bacteria, particularly for precise spatio-temporal control and multiplexing applications. It allows researchers to control gene expression and cellular behavior using light, enabling dynamic and reversible modulation.

Material and method

The project aims to engineer *E. coli* to undergo lysis upon light exposure using optogenetic tools. To achieve this, we engineered *E. coli* with synthetic DNA circuits carrying the CcaS-CcaR system, which regulates the expression of phage-based lytic proteins in response to light: they are activated under green light and repressed under red light. We also tested the potential of this platform for cargo delivery, specifically for the controlled release of immune-stimulating nanobodies and cytokines.

Result and discussion

We successfully engineered an optogenetically controlled lytic system, characterizing its behavior and identifying its limitations. To counteract the emergence of resistant strains, we took a rational design approach to improve the system, incorporating a third light-inducible module for effective biocontainment. Furthermore, we developed a comprehensive mathematical framework to describe the critical genetic elements, complementing theoretical insights with experimental validation. Our engineered

bacterial platform also demonstrated precise, light-controlled delivery of immune-stimulating nanobodies and cytokines.

Conclusion

Our platform enables precise biocontainment and targeted therapeutic delivery, with applications in cancer immunotherapy. It also holds potential for control by synthetic promoters responsive to the tumor microenvironment, expanding its therapeutic possibilities.

EACR25-2325

Triptolide induces pro-apoptotic effects against medulloblastoma subtypes

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Introduction

Medulloblastoma is a common cause of cancer in children, accounting for 20–25% of all paediatric brain tumours worldwide (de Medeiros et al., 2019). Whilst current treatments are effective, they are associated with substantial off-target effects, which impact patients’ quality of life for decades after remission is achieved (Jones et al., 2023). This necessitates the need for the development of new treatments, with phytochemicals offering a novel source of new compounds. Extracts of *Tripterygium wilfordii* have been used in Traditional Chinese Medicine for centuries (Shan et al., 2023), with recent evidence suggesting that compounds derived from this species, such as Triptolide, exert anti-cancer effects (Noel et al., 2019). However, there is no evidence of if this effect translates to medulloblastoma. To address this, we sought to elucidate the fundamental anti-medulloblastoma effect of triptolide.

Material and method

The effect of triptolide on medulloblastoma (HD-MB-03, & DAOY) viability was determined using MTT, long-term proliferation and washout assays. Time-lapse live-cell microscopy was utilised to examine the effect of triptolide on cell morphology, cell fate profile and cellular migration. Flow cytometry was used to examine the cell death mechanism (Annexin V/Propidium Iodide) [1].

Result and discussion

Triptolide produced irreversible concentration and time-dependent decreases in medulloblastoma viability in both HD-MB-03 and DAOY cells, with IC₅₀ values after exposure for 72 hours found to be 18 nM and 169 nM respectively. HD-MB-03 cell fate profiling revealed that exposure to triptolide (10 nM) produced significant concentration-dependent increases in cellular death within interphase ($P < 0.0001$) and a significant $38.5 \pm 6.9\%$ increase in the time take to progress through the cell cycle compared to control ($P < 0.0001$). Morphological analysis showed triptolide to induce blebbing and cellular shrinkage, indicative of apoptosis. Flow cytometric analysis confirmed this, with a significant 5.2-fold increase in apoptotic cells ($P = 0.0002$) following treatment with triptolide (20 nM) for 72 hours. Triptolide

produced no significant impact on cellular migration at all concentrations (5–20 nM) examined.

Conclusion

These data highlight the potential of triptolide as a novel potent treatment for medulloblastoma. Further work is now required to elucidate the molecular mechanism of action associated with the observed pro-apoptotic effect.

[1] The study was ethically approved by the University of Salford Ethical Review Board (ID: 3582).

EACR25-2329

Identifying on-target and off-target effects of hundreds of oncology drugs in PRISM large-scale cell line profiling with machine learning analysis of baseline and functional genomic features

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Introduction

Oncology clinical trials fail frequently due to not fully characterizing the clinical candidates to identify the true target, off-target effects, or mechanism of action. Clinical candidates generally are characterized with limited tools based on the target and the known target indications. These biases have contributed to failed clinical trials. Using cutting-edge novel technologies, like large-scale PRISM profiling of 900 cell lines that have been genetically profiled in the Dependency Map, with machine learning analysis will provide a more thorough and agnostic understanding of oncology drugs. We show that systematic characterization of our Oncology Reference (OncRef) library of over 200 well-known oncology drugs, of which 64% have never been tested before with large-scale cell line profiling, enables better understanding of on-target and off-target effects.

Material and method

Over 200 drugs, including encorafenib, dabrafenib, pevonedistat, palbociclib, ribociclib, abemaciclib, docetaxel and paclitaxel were screened across ~900 PRISM cell lines at 8 point dose in triplicate. Mixtures of 20–25 cell lines were plated in 384 well plates in RPMI 1640 with 10%–20% FBS. After 5 days, cells were lysed and mRNA was amplified by RT-PCR prior to detection with Luminex FlexMap scanners.

Result and discussion

Of the over 200 drugs that were screened, 41% of 192 inhibitory molecules had their target as the top CRISPR or shRNA correlation. This confirmed that PRISM can be used to generate high-quality data in a short amount of time across a large panel of cell lines. Two interesting findings include the reported target of pevonedistat, of NEDD8 activating enzyme (NAE) inhibitor, and the specificity of the CDK4/6 inhibitors palbociclib, ribociclib and abemaciclib. Pevonedistat, a drug that failed in phase III clinical trials in AML was found to be broadly toxic and had no gene expression or functional genomic correlations with the target. If pevonedistat had been tested with a large-scale cell line panel, they would have further optimized the compound prior to clinical trials. For the CDK4/6 inhibitors that are approved in

HR+/HER2- breast cancer, palbociclib only had CRISPR and shRNA correlations with CDK6 and ribociclib and abemaciclib only had CRISPR and shRNA correlations with CDK4.

Conclusion

Our findings establish the OncRef dataset and PRISM as a benchmark for evaluating current and emerging cancer therapies. Using large-scale systematic PRISM cell line profiling, genomic characterization and machine learning analysis enables better characterization of oncology drugs and will potentially reduce clinical trials failures.

EACR25-2331

Assessment of novel therapeutic treatments in KRAS mutant Colorectal Cancer using Patient-Derived Organoids

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Introduction

Colorectal Cancer (CRC) comprises a complex family of tumors different for morphology, immune infiltration, drug response and genetic alterations, including APC/KRAS/TP53/SMAD4. Mutations in KRAS oncogene, mostly at codons 12 and 13, are associated with CRC initiation and progression, but they are missing in clinics of target inhibitors. Patient-Derived Organoids (PDOs) have been demonstrated a cutting-edge 3D in vitro model suitable for drug testing since they resemble the patient specifics.

Material and method

We performed a selective KRAS/MAPK pathway targeted drug screening of seven compounds on NCM460D normal colon mucosa cells and Difi (KRASWT), HCT116 (KRASG13D), LS-174T (KRASG12D) CRC cells and on 14 PDOs, including two normal colon PDOs and 12 CRC PDOs presenting KRASG12*/G13*, KRASA**, KRASWT and BRAFV600E. We tested ten concentrations of each drug (0.1 nM–30 uM) and after 72hrs of treatment we evaluated the cell viability to calculate each drug-response curve. Next, we performed western blot and immunofluorescence (IF) on treated CRC PDOs followed by selective phosphoproteomic analysis in Trametinib (MEKi) and MRTX1133 (KRASG12Di) treated CRC PDOs.

Result and discussion

We identified Trametinib as the most effective inhibitor of the MAPK pathway (low pM EC50), while MRTX1133 had an on-target effect at 20pM–1uM range exclusively in KRASG12D CRC PDOs. Western blot and IF pointed out that Trametinib and MRTX1133 were able to downregulate pErk expression in all PDOS and in

KRASG12D, respectively. Phosphoproteomic data showed downregulation of MAPK linked pathways, like mTOR and PI3K/Akt, in both Trametinib and MRTX1133 treated PDOs, while highlighted a possible selective effect of MRTX1133 in KRASG12D CRC PDOs on Casein kinase (CSNK) and Ribosomal Proteins (RPs) activity. These results might possibly show a preferential pattern of differentially expressed proteins linked to KRASG12D.

Conclusion

Our CRC PDOs model greatly strengthens the use of patient-derived 3D *in vitro* cultures as a more reliable platform to explore new therapeutic strategies in CRC precision medicine, and pointed out mutation-driven perturbed systems, as for KRASG12D. We aimed to understand the biological landscape of KRASG12D in CRC, and we are currently investigating in depth the effect of MRTX1133 in KRASG12D CRC PDOs to highlight the drug specific mechanism and the KRASG12D linked landscape of altered pathways.

EACR25-2338

Targeted Metabolomics for Early-Onset Colorectal Cancer: Identification of Novel Therapeutic Targets and Biomarker-Driven Treatment Strategies

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Introduction

Colorectal cancer (CRC) is one of the most prevalent malignancies worldwide, and its increasing incidence in individuals under 50 years old is a growing concern. Early-onset CRC (EOCRC) often presents at more advanced stages and with aggressive clinical features, making early detection and targeted therapies a major unmet need. Emerging evidence suggests that EOCRC exhibits unique metabolic alterations compared to late-onset CRC, which could serve as potential biomarkers or therapeutic targets. The METABOAGE study applies targeted metabolomics to uncover metabolic vulnerabilities in EOCRC, aiming to develop biomarker-driven therapeutic strategies that could improve patient outcomes.

Material and method

Peripheral blood samples were collected from CRC patients and healthy individuals in two age groups (<50 and >70 years old; n = 10). A targeted metabolomic analysis was conducted using the Shimadzu LCMS-8050 triple quadrupole system with the LCMS/MS Method Package for Primary Metabolites. Statistically significant metabolic alterations were identified and evaluated in colorectal cancer cell lines (HCT-116, HGUE-C1, HT-

29, Caco-2, SW480). Functional assays assessed cell viability, cell cycle progression, migration, and transcriptomic changes, providing insights into their therapeutic potential.

Result and discussion

Targeted metabolomic profiling revealed distinct metabolic signatures in EOCRC compared to late-onset CRC and healthy controls, with key metabolic pathways showing significant alterations. Several metabolites were identified as potential biomarkers or therapeutic targets, as they appeared to correlate with disease progression and cellular phenotypes associated with tumor aggressiveness. Functional assays suggested that some of these metabolic changes may play a role in tumor cell proliferation, survival, and adaptation to stress, highlighting their potential as novel therapeutic targets. Pathway analysis indicated an association between these metabolic alterations and oncogenic signaling networks relevant to CRC progression, supporting further investigation into their therapeutic modulation.

Conclusion

The METABOAGE study provides evidence that EOCRC is characterized by metabolic alterations distinct from late-onset CRC, identifying potential biomarkers and novel metabolic dependencies that could serve as therapeutic targets. These findings highlight the potential of targeted metabolomics in precision oncology, guiding the development of biomarker-driven therapies tailored to younger CRC patients. Future research will focus on validating selected metabolic targets and exploring their modulation through small molecules, metabolic inhibitors, or alternative therapeutic strategies. Integrating metabolomics into personalized CRC treatment could contribute to innovative therapeutic approaches addressing the specific challenges of EOCRC.

EACR25-2358

MASLiN as hydrophobic anticancer drug carrier: *in vitro* activity and *in vivo* biodistribution

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Introduction

Maslinic acid (MA) is a natural pentacyclic triterpenoid that has gained increasing attention due to its broad therapeutic potential and lack of harmful effects. In particular, MA exhibits significant antitumor activity both in vitro and in vivo, making it a promising candidate for cancer therapy. However, its clinical application is limited by its poor solubility in aqueous solutions. To overcome this challenge, we developed Maslinic Acid Solid Lipid Nanoparticles (MASLiN), which significantly enhance MA solubility, enabling its biomedical application. In this study, we evaluated the potential of MASLiN as a nanoplatform for cancer therapy, assessing their ability to encapsulate hydrophobic drugs, their stability under physiological conditions, cytotoxicity, cellular uptake in pancreatic and breast cancer cells, and their in vivo toxicity and biodistribution.

Material and method

MASLiN were synthesized using the solvent displacement method with Poloxamer 407 and Dicarboxylic acid-Poloxamer 407 as surfactants. The formulation was optimized, and the colloidal stability of MASLiN was assessed under varying pH and ionic strength conditions. Curcumin encapsulation efficiency was also evaluated. The cytotoxic effects of free MA and MASLiN were assessed in BxPC3 pancreatic cancer cells and MCF7 human breast cancer cells, with comparisons to primary human fibroblasts. Cellular uptake studies were conducted to determine nanoparticle internalization. Additionally, the toxicity and biodistribution of orally and intravenously administered MASLiN were investigated in mice.

Result and discussion

MASLiN significantly improved the solubility of both MA and curcumin, addressing a key limitation of these compounds. Cytotoxicity assays demonstrated that MASLiN exhibited higher cytotoxic effects against BxPC3 and MCF7 cancer cells compared to human primary fibroblasts, highlighting their selective antitumor activity. Nile Red-loaded MASLiN were rapidly internalized by BxPC3 and MCF7 cells, with distinct cytoplasmic distribution patterns depending on the cell line. In vivo studies revealed no signs of toxicity following oral or intravenous administration of MASLiN in mice. Furthermore, fluorescence imaging of intravenously administered MASLiN demonstrated homogeneous biodistribution, supporting their potential as nanocarriers.

Conclusion

Our findings highlight MASLiN as a promising nanoplatform for cancer therapy, demonstrating their potential as efficient nanocarriers for hydrophobic anticancer drugs. Additionally, their stability and inherent cytotoxicity support their use as targeted nanodevices for improved cancer treatment strategies.

EACR25-2360

Evaluation of citrate and acetyl-l-carnitine in combination with docetaxel as a potential chemotherapeutic strategy in

cellular models of advanced prostate cancer

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Introduction

Prostate cancer (PC) is a pressing public health issue, ranking first in incidence and second in mortality in Colombia. Approximately 15–20% of patients with advanced PC develop castration resistance (CRPC), a phenotype highly refractory to Docetaxel (DCT), the most cost-effective approved treatment according to clinical practice guidelines. Consequently, the search for therapeutic alternatives, such as drug repurposing for CRPC treatment, is imperative. Considering this, we propose using two compounds, citrate (CIT) and acetyl-L-carnitine (ALCAR), as a promising combination therapy with DCT for treating advanced PC and CRPC.

Material and method

Two cell lines were used: PC3, a cellular model of the CRPC phenotype, and LNCaP, a model of advanced metastatic PC. The lethal concentration 50 (LC50) of DCT, CIT, and ALCAR was determined using crystal violet and MTT assays. Additionally, pharmacological combinations of DCT:CIT and DCT:CIT:ALCAR were tested at different treatment ratios (5:1, 3:1, and 1:1 of the LC50). Combination indices (CI) were evaluated using isobolograms with the Chou-Talalay method. For the most promising combinations, apoptosis and glucose/lactate metabolic profile.

Result and discussion

The LC50 values of DCT and CIT were lower than the reported maximum plasma concentrations. No cytotoxic effect was observed with ALCAR in either cell line; therefore, its incorporation was adjusted to form a new active compound with CIT at a concentration of 6 μM ALCAR, following pharmacokinetic parameters. Isobologram analysis highlighted a strong synergistic effect in most interactions, which was more pronounced at 3:1 and 5:1 ratios for both DCT:CIT and DCT:CIT:ALCAR. Differences were observed in apoptosis induction and glucose/lactate metabolism among the most promising combinations.

Conclusion

We found strong synergistic effects in the combinations of DCT and CIT, as well as CIT and ALCAR, in the LNCaP and PC3 cell models. By using pharmacological concentrations lower than the maximum reported plasma levels for each drug in treatment, we demonstrated that these combinations exhibit a promising effect as a potential therapy for CRPC.

EACR25-2362

Photodynamic therapy with ring-fused chlorins: a vision-preserving approach for retinoblastoma treatment

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Introduction

Retinoblastoma (Rb) is the most common intraocular malignancy in children, with high metastatic potential. Local therapies like cryotherapy and thermotherapy target small tumors, while advanced cases require systemic chemotherapy, radiation, or enucleation. PDT offers tumor-selective cytotoxicity without inducing DNA mutations, reducing secondary malignancy risks. Among 4,5,6,7-tetrahydropyrazolo[1,5-a]pyridine-fused tetraphenylchlorins, the dihydroxymethyl derivative (PX1) shows promise. PX1 was selected for its superior *in vitro* performance, including a significantly lower IC50. This study evaluates PX1-based PDT efficacy and safety in Rb treatment.

Material and method

In vivo studies were conducted on RNU rats injected intravitreally with Y79-GFP-luc cells in the left eye.

After PX1-based PDT, treated and control animals were examined using optical coherence tomography (OCT), electroretinography (ERG), bioluminescence imaging (BI) and immunohistochemistry (IMH) to assess PDT outcomes.

Result and discussion

Initially, OCT identified tumors in the vitreous cavity alongside retinal structures. In untreated eyes, tumors expanded, obstructing OCT imaging. PDT-treated eyes were categorized as large (LT) or small tumors (ST). Post-PDT, LT remained opaque to OCT, whereas ST allowed light passage, showing no major retinal alterations. PDT had no significant impact on retinal layer thickness. Scotopic threshold response, scotopic and photopic ERG, and the Flicker test indicated stable retinal function post-PDT in the ST group. BI confirmed PDT's efficacy in reducing ST, though LT showed a modest response. Regrowth was observed one week post-treatment, suggesting that multiple PDT sessions may be necessary. IHC revealed decreased Ki67 expression in tumor cells in PDT-treated eyes. Rb induced microglial and astrocyte activation, while PDT per se induced minor effects and preserved retinal ganglion cells.

Conclusion

PX1 is a promising photosensitizer for Rb treatment. IMH, OCT and ERG confirmed retinal structure and function preservation, supporting its safety profile. PDT effectively targets ST, warranting further investigation into optimized protocols to prevent recurrence and disease progression.

FCT supports CIBB (10.54499/UIDB/04539/2020; 10.54499/UIDP/04539/2020; 10.54499/LA/P/0058/2020); CQC (10.54499/UIDB/00313/2020; 10.54499/UIDP/00313/2020; TEMA (10.54499/UIDB/00481/2020; UIDP/00481/2020 (10.54499/UIDP/00481/2020); Projects CarboNCT (10.54499/2022.03596.PTDC) and Chem4LungCare (10.54499/PTDC/QUI-QOR/0103/2021).

EACR25-2370

Exploring the impact of photodynamic therapy conditioning on endometrial cancer cells

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Introduction

Endometrial cancer (EC), which primarily affects post-menopausal women, also impacts young women (5% under 40), posing challenges regarding treatment options. Photodynamic therapy (PDT), which induces cytotoxicity in tumour cells using a photosensitiser (PS), light and molecular oxygen, has become a promising conservative treatment for EC, especially in women with a reproductive desire or high surgical risk. PDT is minimally invasive, maintains anatomy and organ function, and has few adverse reactions. Even so, PDT can present some challenges, such as insufficient light penetration into deeper tissues, insufficient accumulation of PS and hypoxia in certain tumour areas. Therefore, this study aimed to evaluate how the remaining EC cells after PDT behave, focusing on their division, migration and invasion ability.

Material and method

HEC-1-A and ECC-1 cell lines were treated with two PS, the dihydroxymethyl derivative of tetraphenylchlorin (PX1) and temoporfin, at an inhibitory concentration of 25% of cell proliferation and submitted to an irradiation for activation after 24 hours. Doubling time (DT), migration and invasion studies were performed after 72 hours of this conditioning protocol.

Result and discussion

The cell line DT was not significantly affected by the PDT conditioning with PX1 and temoporfin. While untreated and PX1-conditioned HEC-1-A cell migration significantly reduced the gap from 72 hours ($p < 0.04$), temoporfin-conditioned cells showed a significant gap decrease immediately after 48 hours ($p = 0.005$). ECC-1 cells showed limited migration without significant differences between controls and conditioned cells until 72 hours. Concerning the invasion capacity, conditioning with PX1 and temoporfin also did not induce changes both in HEC-1-A (1.09 and 0.91) and ECC-1 (0.99 and 1.02) cells, respectively.

Conclusion

The results indicate that the remaining EC cells exhibited similar DT and invasion profiles after PDT. Nevertheless, temoporfin appears to potentiate the migration ability of HEC-1-A cells.

FCT supports CIBB (doi:10.54499/UIDB/04539/2020; doi:10.54499/UIDP/04539/2020; doi:10.54499/LA/P/0058/2020); CQC (doi:10.54499/UIDB/00313/2020; doi:10.54499/UIDP/00313/2020; TEMA (doi:10.54499/UIDB/00481/2020), UIDP/00481/2020 (doi:10.54499/UIDP/00481/2020); Projects CarboNCT (doi:10.54499/2022.03596. PTDC) and Chem4LungCare (doi:10.54499/PTDC/QUI-QOR/0103/2021); PhD Scholarship from FCT and European Social Fund to BS (10.54499/2020.07672.BD).

EACR25-2371

Evaluation in vitro and in vivo of the therapeutic effect of the melatonin-furanochalcone hybrid in colorectal cancer

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Introduction

Colorectal cancer (CRC) is one of the most prevalent types of cancer worldwide, ranking among the main causes of morbidity and mortality. Current therapeutic strategies for CRC are often associated with significant adverse effects, driving the search of alternative treatments based on natural, synthetic, or biological compounds that offer greater efficacy and specificity. In recent years, melatonin (N-acetyl-5-methoxytryptamine) and chalcone have garnered considerable attention due to their anti-inflammatory and antioxidant properties, making them as promising candidates for the treatment of various diseases, including cancer. Therefore, this study aimed to evaluate the therapeutic potential of a novel hybrid compound combining melatonin and furanochalcone (MFC) in colorectal cancer.

Material and method

First, in vitro assays were performed to evaluate the cytotoxicity, selectivity and antiproliferative activity of the MFC hybrid using SW480 (human colon adenocarcinoma), CT26 (murine colon adenocarcinoma) and NCM460 (non-tumorigenic human colon) cell lines. Then, lethal dose 50 (LD50) of hybrid compound was determined in BALB/c mice via oral administration, following the procedure described in the OCDE guideline 425 for testing of chemicals. Subsequently, 50% of the LD50 dose was orally administered to mice to evaluate genotoxic effect in liver and colon tissues using the comet assay, and mutagenicity in bone marrow through the micronucleus assay. Finally, a first pilot study was conducted to assess the antitumor effect of the MFC hybrid by administering 25% of the LD50 to mice with colitis-associated colorectal cancer, induced via azoxymethane and dextran sodium sulfate.

Result and discussion

In vitro assays showed selectivity, cytotoxic effect and antiproliferative activity of the MFC hybrid on the malignant cells, mainly in the SW480 cell line. In vivo, the hybrid compound exhibited an LD50 greater than 2000 mg/kg, suggesting low acute toxicity. Furthermore, no DNA damage or chromosomal alterations were observed under the evaluated conditions. In the pilot study, administration of the MFC hybrid between four and eight weeks after CRC induction, resulted in delayed tumor progression. This effect was evidenced by a reduced tumor burden and slower progression of preneoplastic lesions in treated mice compared to untreated controls.

Conclusion

These findings suggest that the melatonin-furanochalcone hybrid could be a promising therapeutic candidate for CRC, as demonstrated by its antiproliferative activity and capacity to delay tumor progression while exhibiting low acute toxicity and genotoxicity. However, further studies are required to validate its efficacy and clarify its mechanisms of action including its potential roles in immunomodulation, apoptosis induction, cell cycle arrest, inflammatory regulation, among others.

EACR25-2381

Anagrelide induced remodeling of the PDE3-SLFN12 interactome provides insights into translation initiation dynamics and molecular glue mechanisms

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Introduction

The molecular glue anagrelide induces proximity between phosphodiesterase 3 A (PDE3A) and Schlafend-12 (SLFN12) in cancers with high PDE3A expression, triggering SLFN12 tRNase activity, tRNA-Leu-TAA degradation, and ultimately, translation inhibition and cell death. However, other mechanisms, such as the GCN2-eIF2α-mediated stress response, also contribute to the anticancer effects of molecular glues, suggesting multiple parallel mechanisms. Here, we aimed to elucidate these mechanisms by using an interactomics approach to identify the altered protein-protein interactions (PPIs) in the PDE3-SLFN12 complex.

Material and method

We used proximity dependent biotin labeling (Bio-ID) to discover the PDE3A-SLFN12 complex PPIs. SLFN12, PDE3A, and GFP-containing MAC-Tag-N expression plasmids were generated and transfected into SA-4 and HeLa cells. After biotin and either DMSO or anagrelide treatment, cells were lysed and analyzed via LC-MS. High-confidence interactors were filtered with Significance Analysis of INTERactome (SAINT) and Contaminant Repository for Affinity Purification (CRAPome). The results were validated with immunofluorescence, and new complexes were modeled with AlphaFold.

Result and discussion

Peripheral eIF3 subunit PPIs were induced, while core eIF3 subunit PPIs were suppressed. As the peripheral eIF3 complex localizes to the opposite ribosomal face relative to the core eIF3 complex, these results suggest a shift in PDE3-SLFN12 localization to the ribosomal mRNA entry channel upon complex formation. Immunofluorescence confirmed increased colocalization of PDE3 and SLFN12 with ribosomes and each other following anagrelide treatment. AlphaFold modeling of the PDE3-SLFN12-eIF3 PPIs further supported these findings. Functional enrichment analysis via the STRING database revealed significant enrichments in ribonucleoprotein biogenesis, translation initiation and aminoacyl tRNA biosynthesis, reinforcing the complex's role in translation control. Conversely, suppressed PPIs linked to protein folding, antiviral defence, and proteasome function indicated impaired nascent polypeptide folding, loss of

SLFN12's innate immune role, and disrupted PDE3A/SLFN12 degradation, respectively.

Conclusion

Anagrelide-induced remodeling of the PDE3-SLFN12 interactome localizes the complex to the ribosomal mRNA entry channel, inducing ribosomal and peripheral eIF3 PPIs while suppressing proteasomal, chaperonal, and core eIF3 PPIs. This provides insights into complex stabilization, altered translation dynamics and affected signaling pathways. Suppressed PPIs suggest impaired protein folding and disrupted PDE3A/SLFN12 turnover, contributing to translational stress and cell death. Together, these findings enhance our understanding of molecular glue mechanisms and support the design of improved therapeutics.

EACR25-2388

The m6A RNA modification sustains neuroblastoma tumor aggressiveness

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Introduction

Neuroblastoma (NB) is the most frequent extracranial solid tumor in children, accounting for 15% of all childhood cancer deaths. Although the 5-year survival rate of patients with high-risk disease has increased in recent decades, NB remains a challenge in pediatric oncology, and identifying novel therapeutic targets and agents is an urgent clinical need. N6-methyladenosine (m6A), the most abundant post-transcriptional mRNA modification in mammals, is tightly regulated by 'writers' and 'erasers', which deposit and remove the modification, respectively, and by 'readers', which can detect changes in mRNA modification status and influence downstream cellular processes. m6A and related key proteins have been found frequently altered in many different tumors and may represent a good therapeutic target also in NB.

Material and method

By modulating the expression of the writer complex component METTL14 or the demethylase ALKBH5, we evaluated the impact of m6A mRNA modification on NB tumor aggressiveness both *in vitro* and *in vivo*. We also investigated the effect of inhibiting m6A recognition by knocking out YTHDF reader proteins. Next, to identify a molecule capable of interfering with YTHDF m6A mRNA recognition, we screened *in silico* a library of 1.5 million non-redundant compounds against the YTHDF1 protein (PDB entry: 4RCJ) and subsequently evaluated the 113 best-scoring compounds by homogeneous time-resolved fluorescence. We selected compound A as a low micromolar pan-YTHDF inhibitor and synthesized and tested more than 30 analogs in a qualitative structure-activity relationship (SAR) study.

Result and discussion

METTL14 overexpression promotes cell proliferation and invasion *in vitro* and tumor progression *in vivo*,

leading to faster tumor growth and larger tumor masses. Conversely, ALKBH5 overexpression or METTL14 knockout leads to opposite results, with a significant decrease in cell proliferation, an increase in apoptosis, and reduced invasion ability *in vitro*, while dramatically slowing tumor growth *in vivo*. Consistent with these observations, knockout of YTHDF paralogs or YTHDF inhibition with compound A also results in decreased NB cell proliferation and reduced clonogenic potential. We are now finalizing our SAR study.

Conclusion

Our results show that high levels of m6A correlate with higher NB aggressiveness and that targeting m6A deposition or m6A recognition on mRNAs could be an effective therapeutic strategy for NB treatment.

EACR25-2418

Cold Atmospheric Plasma as a Potential Strategy to Target the Invasion and Metastasis Hallmark in Breast Cancer

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Introduction

Breast cancer (BC) is the most prevalent cancer among women of all ages, with high incidence and mortality worldwide. It is a heterogeneous disease, and current therapies have limitations, including recurrence and adverse effects. In this regard, new therapies are

emerging, and cold atmospheric plasma (CAP) has been investigated as a potential anti-tumor approach, however, the mechanisms of action remain unclear. As we have been investigating how CAP influences the hallmarks of cancer, the main goal of this work was to evaluate its effect on BC cell invasion and metastasis potential regarding cell migration and E-cadherin expression. Cell membrane permeability was also evaluated.

Material and method

The migration assay was performed in five distinct cell lines: two triple-negative (HCC1395 and HCC1806), two luminal A subtype (HCC1428 and MCF7), and one representative of the normal breast tissue phenotype (MCF12A). These cells were submitted to CAP treatment for 60 and 120 seconds after a scratch. To assess E-cadherin expression and cell membrane permeability, flow cytometry was used.

Result and discussion

CAP treatment inhibited cellular migration in BC cell lines over time. In HCC1395, HCC1806 and MCF7 cells, migration was significantly reduced at 48 and 72 hours. The effect was stronger with longer exposure: (83.72 ± 5.17)% to (30.53 ± 7.29)%, p < 0.0001; (96.96 ± 1.96)% to (60.68 ± 3.86)%, p = 0.0023; and (83.85 ± 3.05)% to (33.59 ± 3.23)%, p < 0.0001, for each cell line respectively. However, MCF12A showed no significant migratory changes. Previously, we observed that CAP significantly reduced cell invasion in a time-dependent manner on triple-negative BC, supporting its potential to modulate this hallmark. Moreover, E-cadherin expression and cell permeability seemed to be dependent on BC cell type. HCC1395 showed a significant decrease after 120 s (73.96 ± 3.96)%, p = 0.0012. Our preliminary results for MCF7 and HCC1428 suggest a tendency to increase the membrane permeability after 120 s, (125.88 ± 1.00)% and (115.74 ± 3.37)%, respectively. Additional studies will be performed to confirm these findings.

Conclusion

These findings suggest that CAP impairs breast cancer cell migration and invasion while modulating E-cadherin expression and membrane permeability in a cell-type-dependent manner. These results encourage further studies on CAP to pave the way for novel therapeutic approaches in BC treatment.

FCT supports CIBB (10.54499/UIDB/04539/2020, 10.54499/UIDP/04539/2020, 10.54499/LA/P/0058/2020); TEMA (10.54499/UIDB/00481/2020, UIDP/00481/2020 (10.54499/UIDP/00481/2020); Projects CarboNCT (10.54499/2022.03596.PTDC); PhD Scholarship from FCT and European Social Fund to CA-F (10.54499/2022.12228.BD) and BS (10.54499/2020.07672.BD).

EACR25-2428

Harnessing DNA repair to enhance therapy efficacy in myeloid leukemia: The impact of chronotherapy

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Introduction

Circadian rhythms are natural, internal biological cycles that regulate various physiological and behavioral processes over a roughly 24-hour period, including metabolism and DNA repair. Disruption of these rhythms can profoundly affect tumor development and therapeutic response. Leukemic cells often exhibit altered expression patterns of circadian rhythm genes, underscoring the need to better understand their role in disease progression and treatment efficacy. In this context, this study aimed to evaluate, *in vitro*, the influence of circadian rhythms on DNA damage repair capacity and the impact of chrono-therapy on the treatment of myeloid leukemia.

Material and method

For this purpose, two myeloid leukemia cell lines, HL-60 and K562 cells, and a normal lymphocyte cell line, IMC, were used. The expression levels of circadian rhythm genes (CLOCK, BMAL1, BMAL2, PER1, PER2, PER3, CRY1, CRY2, and ROR α) and BCR::ABL1 were quantified by qPCR. DNA repair activity was evaluated by measuring chromosomal damage levels (micronucleus assay; microscopy) and double-strand breaks (γ H2AX; flow cytometry) in synchronized cells every 4h for 24h after circadian synchronization. The trypan blue exclusion test assessed the sensitivity to imatinib (IMA) and cytarabine (ARA-C) at the time points of highest and lowest DNA repair. Statistical analyses were performed with statistical significance defined as $p < 0.05$.

Result and discussion

Our results showed that HL-60 and K562 cells exhibit circadian rhythm gene alterations compared to normal cells, such as ultradian expression of CLOCK gene and arrhythmic expression of PER2. DNA repair capacity oscillated throughout the day in both leukemia cell lines. At the highest DNA repair activity (4h after circadian synchronization), HL-60 cells repaired 35% of induced DNA damage, while at the lowest (12h after circadian synchronization), only 21% was repaired. In the K562 cells, repair was more effective at 4h (29%) and less effective at 16h (12%). Additionally, treatment with IMA in K562 cells and ARA-C in HL-60 cells was more effective when administered at the time of lowest DNA repair activity (IMA = 30 ± 1%; ARA-C = 35 ± 2%; $p < 0.05$) and less effective when administered at the time of highest DNA repair capacity (IMA = 49 ± 2%; ARA-C = 76 ± 3%; $p < 0.05$), compared to conventional administration without circadian synchronization (IMA = 40 ± 3%; ARA-C = 62 ± 2%). No rhythmicity was observed in the expression of the BCR::ABL1 gene, the molecular target of IMA.

Conclusion

In conclusion, *in vitro* myeloid leukemia cells exhibit circadian dysregulation and rhythmicity in DNA repair activity. Chronotherapy demonstrated promising results, improving therapeutic response when drugs were administered during the phase of the day with lower DNA repair activity.

EACR25-2443

Antitumoral compounds: a nutraceutical approach in cancer therapy

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Introduction

Hepatocellular carcinoma (HCC) is the primary tumor of the liver and the 5th most prevalent cancer in the world. The incidence of HCC has been rising worldwide over the last 20 years, despite the progress in vaccination strategies and management of hepatitis virus infections. Currently, first-line therapies remain poorly effective, highlighting the urgent need for novel treatment options. Nutraceuticals provide a vast array of bioactive molecules with potential pharmacological exploitation. The Mediterranean diet (MD), known for its health benefits, is a rich source of natural compounds, including secoiridoids found in extra-virgin olive oil (EVOO). Among them, Oleacein has demonstrated antioxidants, anti-inflammatory and anti-proliferative properties contributing to its potential anti-cancer effects. In this study, we explore the anti-tumoral potential of Oleacein and investigate the molecular mechanisms underlying its anti-proliferative capacity in HCC cell lines.

Material and method

Human HCC cell lines, HepG2 and Huh7, were treated with Oleacein and cell viability was determined by MTS assay. Cell cycle profiles of Oleacein-treated cells were analyzed by flow cytometry following propidium iodide staining. Senescent cells induced by Oleacein were identified by an SA- β -gal assay, while senescence-associated gene expression related to the SASP was examined via quantitative real-time PCR. Additionally, Western blot analysis was conducted to investigate the molecular mechanism underlying the anti-proliferative effects of Oleacein in HCC cells.

Result and discussion

Oleacein inhibits the viability of HCC cells with an IC₅₀ >100 μ M in Huh7 cells and approximately 30 μ M in HepG2 after 72 hours. Our findings demonstrate that Oleacein reduces cell viability of HCC cells by inducing cell cycle arrest and promoting senescence. After 24 hours of incubation with 50 μ M Oleacein, HepG2 cells exhibited a slight increase in the G0/G1 phase cell populations with a dose-dependent increase of p21 and p16 mRNA. In contrast, Huh7 cells showed an accumulation of cells in G2/M phase of the cell cycle along with increased p21 mRNA levels, when compared with untreated control cells. In both cell lines, Oleacein treatment drove cells into a senescent state, as evidenced by senescence-associated β -galactosidase (SA- β -GAL) activity. This was further supported by increased expression levels of interleukin 8, interleukin 1, interleukin 6 and matrix metalloproteinase 3 (MMP3) genes,

factors secreted by senescent cells. Additionally, a reduction in Lamin B levels, a senescence-associated biomarker, further confirmed the induction of senescence.

Conclusion

Overall, our findings suggest that oleacein exhibits anti-cancer effects inducing senescence in HCC cells, highlighting its potential as a promising therapeutic candidate for HCC.

EACR25-2462

Expression profiles of target genes for approved antibody-drug conjugates in the context of high-grade serous carcinoma

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Introduction

High-grade serous carcinoma (HGSC), the most common subtype of ovarian cancer, is characterised by a five-year survival of only <40%. While patients initially respond well to the standard platinum-based chemotherapy, most will experience disease recurrence and eventually treatment resistance. Platinum-resistant HGSC lacks alternative treatment options, and the molecular heterogeneity makes it challenging to find precision approaches via clinical trials. Last year the first antibody-drug conjugate (ADC), Mirvetuximab soravtansine-gynx (Elahere), targeting FOLR1, was approved for platinum resistant ovarian cancer. This brought HGSC to the frontline of ADC-based cancer therapy. Here, we have analysed the expression profiles of the approved ADCs' target genes to map the potential for drug repurposing.

Material and method

For discovery analysis, we used bulk RNA (910 samples, 313 patients) and single-cell RNA (95 samples, 57 patients) sequencing data from the DECIDER clinical trial (NCT04846933). The bulk RNA data was decomposed with PRISM to correct for varying cancer fractions, and TPM normalised. To identify patterns of inter- and intra-patient variation in the expression of the ADC target genes, we fitted linear mixed effects model on the bulk RNA data, with tumor site as a fixed co-factor, the patient and patient-tumor site interaction as random effects.

Result and discussion

Seven ADC target genes (FOLR1, ERBB2, TACSTD2, EGFR, NECTIN4, F3, and CD22) had detectable baseline expression in the cohort. FOLR1 and TACSTD2 demonstrated several patients with high expression, while in contrast, high expression of the other genes was limited to a few patients. On the cohort level, we observed wide variance in the expression for all genes, suggesting high inter-patient heterogeneity. Based on our linear model, only a small part of the observed variance

was explained by tumor site, while the patient-effect explained most of the variation. We used single-cell data to show that the high FOLR1 or TACSTD2 expression was cancer cell-specific. Furthermore, in cancers with high overall expression level of these genes, the expression was highly stable across all cancer cells. For the other ADC target genes, we detected notable heterogeneity with an overall high expression in a cancer sample accompanied by heterogeneity in expression across individual cancer cells.

Conclusion

Our analysis suggests that TACSTD2-targeting ADC could potentially be repurposed to HGSC. TACSTD2 demonstrated patterns of expression comparable to FOLR1 with potentially even wider patient group suitable for the treatment. The other genes studied here might be targetable in a few patients, but the decreased stability of their expression might pose a challenge for the clinical practice. Since our analysis enables the investigation of both intra- and inter-sample heterogeneity, we could use this setup as a discovery platform in the future to identify novel ADC targets.

EACR25-2475

Comparative analysis of proteomics and transcriptomics in patient-derived tumoroids for precision oncology and drug discovery

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Introduction

Traditional 2D cancer cell lines often fail to accurately model primary cancer cells, hindering the translation of in vitro research to the clinic. An emerging solution is the use of patient tissue-derived cells expanded in 3D, known as cancer organoids or tumoroids. In this study, we performed a comprehensive proteomic analysis of patient-derived tumoroids using the Thermo Scientific™ Orbitrap™ Astral™ Mass Spectrometer.

Material and method

Tumoroids were first established from dissociated cells from patient tumor resections using Gibco™ OncoPro™ Tumoroid Culture Medium, and have previously been shown to maintain strong concordance in gene expression with primary tumor material. However, little has been done to investigate protein-level expression patterns in these patient-derived models. The proteomic profiles, generated from over 10,000 unique proteins identified in each sample, revealed significant insights into the signaling pathways associated with breast cancer, specifically MAPK, ALK, and ERBB2 pathways, as well as protein and RNA metabolism.

Result and discussion

In comparing transcriptomic and proteomic datasets, particularly where expression varied across tumoroid

models, there was significant overlap in terms of ECM and adhesion-related proteins, as well as lipid metabolism. This overlap underscores the importance of these proteins in the biological processes of cancer and emphasizes the value of integrating proteomic and transcriptomic data to achieve a more comprehensive understanding of tumoroid biology. While transcriptomics provides a snapshot of gene expression, proteomics offers a deeper understanding of the functional state of the cell by identifying post-translational modifications and protein interactions. This multi-omics approach enhances our ability to model the complex heterogeneity of cancer and supports the development of personalized treatment strategies.

Conclusion

Future work will focus on validating these proteomic findings, including making protein-level comparisons between established tumoroids and primary tissue, and exploring post-translational modifications. Taken together, these new insights are expected to further improve the efficacy of tumoroid models in precision oncology and drug discovery.

EACR25-2477

Coffee Exosomes Have Distinct Effects on Melanoma and Glioblastoma Cancer Cells via PTEN Tumor Suppressor Protein

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Introduction

Exosomes composed of bilayer lipids release nucleic acids, proteins, lipids, and other biomolecules, playing a function in delivering biological signals to surrounding cells. Plant-derived exosomes have the capacity to penetrate mammalian cells and regulate biological activities. Plant exosomes are being investigated for cancer treatment as a possible approach for conveying therapeutic cargo to mammalian cells due to their low immunogenicity and high intracellular intake rate. While cohort studies related to coffee consumption indicate an adverse association between coffee drinking and risk of many cancers there is no study investigating mechanistic effects of coffee exosomes in cancer cells. We previously examined the effects of coffee exosomes on melanoma skin cancer and glioblastoma multiforme (GBM) brain tumor cells. While coffee exosomes had apoptotic and anti-proliferative effects on SK-MEL-28 melanoma cells, they did not induce apoptosis and caused an increase in proliferation of T98G GBM cells.

Material and method

In order to investigate these distinct effects of coffee exosomes on SK-MEL-28 and T98G cells, we conducted transcriptome analysis, and the alterations in the expression levels of target signaling pathway-related proteins were then evaluated in both SK-MEL-28 and T98G cells by western blot analysis. Moreover, we also investigated the effects of coffee exosomes on migration ability and tumor growth in these cells by using wound-healing and spheroid formation assays, respectively.

Result and discussion

Our results showed that coffee exosome treatment significantly decreased the SERPINA1 mRNA levels in SK-MEL-28 cells. Since SERPINA1 could decrease the expression level of PTEN, which is mutant in T98G cells, we investigated the expression levels of proteins involved in PI3K/Akt and MAPK signaling pathways that are regulated by PTEN activity. We revealed that the coffee exosome reduced the expression of MAPK and PI3K/Akt signaling pathway-related proteins in SK-MEL-28 cells, whereas the levels of these proteins were increased in T98G cells. These findings suggest that coffee exosomes decrease proliferation of SK-MEL-28 cells by increasing the activity of PTEN and thereby suppressing PI3K/Akt and MAPK signaling pathways. However, since PTEN is inactive in T98G cells due to mutations, the decrease in SERPINA1 expression could not affect PI3K/Akt or MAPK signaling pathways. Supporting these findings, we observed that exosome administration reduced the size of spheroids formed by SK-MEL-28 cells while increasing the size of those formed by T98G cells. Finally, wound-healing analysis showed that coffee exosomes decreased migration of SK-MEL-28 cells, whereas they prevented migration of T98G cells.

Conclusion

These results suggest that coffee exosomes could potentially be used in treatment of both melanoma and glioblastoma cancer cells by targeting proliferation and migration properties, respectively.

EACR25-2497

Highly-targeted personalised siRNAs for BRAF-fusions inhibit melanocytic proliferation

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Introduction

Congenital melanocytic naevus (CMN) syndrome is a mosaic disorder caused by classical melanoma oncogenic variants arising during in utero development, and is the highest risk factor for melanoma development in children worldwide. BRAF-fusions are a recently described cause of this disease, with a particularly proliferative and symptomatic phenotype. Oral trametinib reduces cutaneous proliferation, however the naevi themselves persist and the melanoma risk therefore remains. We have recently reported induction of apoptosis of NRAS-variant naevus cells using allele-specific siRNA in vitro, and successful delivery into mouse skin. Given the specificity of the sequence of gene fusions and the highly targeted nature of siRNA designed against the fusion breakpoint, as well as the accessibility of skin for delivery of siRNA, we sought to explore the potential of BRAF-fusion siRNA therapy in primary cultures.

Material and method

Primary melanocytic cells were derived from affected skin biopsies from two patients with BRAF-fusion driven CMN syndrome, one from benign naevus tissue and one from a primary melanoma. Patients each had a different 5' partner gene fused to the BRAF kinase domain: PHIP:BRAF and GOLGA4:BRAF, respectively. siRNAs were designed across the fusion breakpoint to target

specifically the fusion transcript, transfected alone and in combination with MEKi trametinib, and compared to non-targeting siRNA and vehicle controls. Proliferation and apoptosis were assessed over five days using IncuCyte® high-throughput phase contrast imaging alongside a DNA-binding fluorescent caspase 3/7 dye. Gene expression was evaluated through qRT-PCR and MAPK activation by Western Blot, both three days post-treatment.

Result and discussion

Fusion-specific siRNAs in all cases specifically and effectively reduced the expression of the fusion transcript compared to non-targeting controls, leaving wild-type transcripts unaffected. Treatment with a single dose of siRNA alone markedly decreased proliferation compared to controls, and significantly more effectively than current standard of care trametinib. Combination of siRNA with trametinib was not more effective than siRNA alone. Induction of apoptosis was seen in both patient cell lines. Mechanistically, siRNA therapy significantly reduced MAPK activation in all lines and decreased the expression of survival genes BIRC5 and ARL6IP1.

Conclusion

Taken together, these findings suggest that siRNA therapy might serve as a more effective treatment for CMN patients with BRAF fusions than trametinib, and induction of apoptosis supports potential resolution of lesions rather than only stabilisation. The accessibility of skin for delivery of siRNA and the specificity of fusion-targeted siRNA would be expected to keep off-target effects to a minimum.

EACR25-2499

Evaluation of pleural mesothelioma sensitivity to targeted DNA damage response inhibitors

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Introduction

Malignant pleural mesothelioma (MPM) remains a lethal cancer due to difficulties in early diagnosis, a scarcity of targets for innovative treatments, inherent resistance to chemotherapy, and an immune-suppressive tumor microenvironment. Previous research has demonstrated that asbestos fibers induce DNA damage in cells, thereby contributing to the molecular diversity seen in MPM. It has been reported that 10–15% of MPM samples contain alterations in genes associated with homologous recombination (HR) or other DNA repair mechanisms. We proposed that these genetic alterations might create a susceptibility in certain MPMs to treatments that target the DNA damage response (DDR) pathways.

Material and method

We assembled a panel consisting of 22 MPM human cell lines and 3 murine cell lines sourced from commercial suppliers or developed from patients at Azienda Ospedaliera SS. Antonio e Biagio in Alessandria and San Luigi Hospital in Orbassano, Italy. Molecular characterization involved whole-genome sequencing and comprehensive methylome profiling of the cell lines. Long-term cell proliferation assays lasting 7 to 15 days were conducted using various concentrations of the following DDR inhibitors: berzosertib and ceralasertib (ATR inhibitors), AZD0156 (an ATM inhibitor), olaparib (a PARP inhibitor), adavosertib (a WEE1 inhibitor), and rabusertib (a CHEK1 inhibitor). We quantified RAD51 nuclear foci after treatment with DDR inhibitors or exposure to ionizing radiation to evaluate DNA damage and HR activation.

Result and discussion

The paucity of preclinical MPM models has hindered advancements in identifying targets and effective therapies for this uncommon tumor type. By gathering a substantial collection of MPM models, we discovered that 10 out of 22 human and 2 out of 3 studied murine MPM lines exhibited remarkable sensitivity to all DDR inhibitors. We identified genomic alterations of unclear significance impacting 112 HR and 132 DDR genes; however, these did not correlate with sensitivity to the drugs. Additionally, the deficiency of BAP1 protein did not serve as a predictor for drug response. MPMs that failed to form RAD51 foci after exposure to ionizing radiation, which are known to induce double strand breaks, were among the most DDR-sensitive cell lines. Intriguingly, drug refractory cell lines showed either sarcomatoid or biphasic histology, whereas drug sensitive lines showed epithelioid features, suggesting a previously unrecognized role of tumor histology in modulating DDR in MPM.

Conclusion

These results represent a preclinical rationale for designing clinical trials with DDR for MPM patients.

EACR25-2559

Preclinical Evaluation of Novel Phosphoinositide 3-Kinase Inhibitors: Antitumor Activity and Toxicity Assessment

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Introduction

Dysregulation of the phosphatidylinositol-3-kinase (PI3K) pathway, via mutations and amplifications, is common in cancers, including colorectal cancer (CRC). This study evaluated 10 novel class I-selective PI3K inhibitors for targeted therapy in CRC models.

Material and method

The efficacy of the PI3K inhibitors was assessed in a two-step process using HCT-116, HCT-15, and DIFI

CRC cell lines, with HaCat cells as a normal control. In the first step, cells were exposed to the inhibitors and Alpelisib (a PI3K inhibitor) at a single dose, followed by a five-dose step to determine IC₅₀ values using the MTS assay. Selectivity indices were calculated by comparing IC₅₀ values between tumor and healthy cell lines. Proliferation pathways were analyzed via Western blot, and apoptosis was assessed by flow cytometry. A 3D cell culture model of HCT-116 was used to evaluate cell viability using CellTiter-Glo, Calcein AM, and Propidium Iodide staining. Six patient-derived CRC organoids were also treated with the most effective PI3K inhibitors. To correlate treatment response with molecular characteristics, the mutational status of 48 genes was assessed by next-generation sequencing (NGS), and the PI3K expression profile was evaluated by immunohistochemistry [1]. In silico analyses using ADMETlab software was conducted. Molecular docking studies were performed to assess binding interactions between the inhibitors and class I PI3K isoforms.

Result and discussion

The compounds VL336 and VL311 interacted with the PI3K α isoform, particularly binding to valine and lysine residues within the active site. Both molecules significantly inhibited tumor cell growth (<50%), outperforming Alpelisib. These compounds also demonstrated high selectivity for tumor cell lines, with low IC₅₀ values of 1.51 ± 0.35 μM and 1.59 ± 0.44 μM , respectively, in HCT-116 cells. Among the CRC cell lines, HCT-116 was the most sensitive to VL336 and VL311, showing greater responsiveness than to Alpelisib. Further analysis revealed that VL311 significantly reduced the phosphorylation of AKT and ribosomal protein S6 expression, which demonstrates disruption of PI3K-mediated signaling and induced late apoptosis. In 3D spheroid models, VL311 reduced cell viability more effectively than Alpelisib. Finally, 3 out of 6 patient-derived samples were sensitive to VL311 and VL336. PIK3CA mutations were absent, but high PI3K expression correlated with greater inhibitor sensitivity.

Conclusion

VL336 and VL311 showed significant in vitro efficacy in 2D, 3D, and patient-derived CRC models, highlighting their therapeutic potential, warranting further investigation of their therapeutic potential, clinical applicability and toxicity.

[1] ethics approval: 71447723.9.0000.5437

EACR25-2568

Alpha-aminobutyric acid attenuates DEN and diet-induced MASLD/MASH mice model by suppression of oncogenic signalling pathways KRAS, STAT3 via gut microbiome modulation

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Introduction

As obesity continues to rise and metabolic dysfunction-associated steatotic liver disease (MASLD) becomes more prevalent, the occurrence of hepatocellular carcinoma (HCC) linked to MASLD and/or metabolic dysfunction-associated steatohepatitis (MASH) is expected to grow, emphasizing the critical need for effective treatment options. Alpha-aminobutyric acid (ABA) is a non-proteinogenic amino acid, a metabolite which could be generated from the metabolism of methionine, threonine, serine and glycine or as a gut-microbiome-derived metabolite. It had previously been shown to ameliorate the progression of MASLD, showing anti-inflammatory and antioxidative properties.

Material and method

This present study aimed to investigate the effect of oral ABA supplementation on the progression of diethylnitrosamine (DEN)-high-fat/high-cholesterol diet (HFD)-induced MASLD/MASH-associated hepatocellular carcinoma (HCC) mice model.

Result and discussion

The administration of ABA was found to mitigate several parameters associated with MASLD/MASH-related HCC, including liver weight, tumor count, and size, along with decreased levels of serum ALT, AST, triglycerides, and both free and total cholesterol. Additionally, ABA markedly diminished hepatic steatosis and the expression of inflammatory markers while enhancing antioxidative and anti-inflammatory capacities. Mechanistically, ABA was shown to suppress the oncogenic KRAS/RAF/ERK and STAT3/SOCS3 signaling pathways, as well as inhibit the activity of the mTOR/PI3K/AKT pathway. Notwithstanding, ABA was shown to remodelled the gut microbiome composition, decreasing the abundance of 12 bacterial species, and enriching 7 bacterial species. Additionally, the glucuronate pathway was seen to be both enriched in liver RNAseq and the KEGG microbial function analysis, which was previously shown to be linked to promoting the synthesis of the antioxidant glutathione and could contribute to the overall improved outlook of HCC progression.

Conclusion

Overall, our findings demonstrated the anti-tumor effects of ABA and its possible mechanism and suggest that ABA could be used as a promising therapeutic approach for the intervention of MASLD/MASH-associated HCC.

EACR25-2601

Isothiocyanate-derived mercapturic acids potentiate tubulin polymerization inhibitors anticancer activity in urinary bladder cancer models (in vitro and in vivo studies)

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Introduction

Urinary bladder cancer is currently the fourth most common cancer in men in Poland, with a continuously increasing incidence and mortality rate. Over the last 30 years, numerous studies have demonstrated that naturally occurring isothiocyanates possess significant chemo-

preventive and antitumor potential, combined with low toxicity. Their multi-targeted mode of action, including effects on mitotic division, along with their natural origin, makes them promising adjuvants for currently used oncological therapies. The metabolism of isothiocyanates via the mercapturic acid pathway leads to the formation of biologically active isothiocyanate-derived mercapturic acids, which accumulate at high concentrations in urine and urinary bladder tissue, making them particularly relevant for bladder cancer treatment.

Material and method

A set of tubulin polymerization modulators (e.g., vinorelbine, colchicine, paclitaxel, combretastatin A4) was tested in vitro with structurally different mercapturic acids. Experiments included assessments of anti-proliferative activity, apoptosis rate, cell cycle analysis, and glutathione levels. Antiproliferative effects were analyzed using synergy models to determine interactions. The most promising combinations were further evaluated in vivo using orthotopic cancer models established by ultrasound-guided cancer cell inoculation.

Result and discussion

Our in vitro studies demonstrated for the first time that mercapturic acids synergistically enhance the anti-proliferative activity of microtubule polymerization inhibitors (such as colchicine, vinflunine, and combretastatin A4), but not microtubule stabilizers (such as paclitaxel). This leads to reduced proliferation and increased apoptosis. We also discovered that the chemical structure of mercapturic acids influences their potency; for instance, the benzyl isothiocyanate derivative was significantly more potent than the sulforaphane derivative. Follow-up studies revealed that the observed synergistic effect resulted from an increased apoptosis rate during the G2/M phase (without substantial changes in the overall cell cycle profile), decreased glutathione and βIII-tubulin levels in combination-treated cells, and reduced tubulin polymerization in cell-free experiments. In vivo studies using orthotopic models indicated a moderate enhancement of the anticancer activity of combretastatin A4 and vinflunine by benzyl isothiocyanate-derived mercapturic acid. This effect was associated with reduced angiogenesis and tumor perfusion, without signs of increased toxicity.

Conclusion

These findings suggest that mercapturic acids enhance the anticancer efficacy of tubulin polymerization inhibitors (but not stabilizers), synergistically increasing mitotic catastrophe rates.

This work was supported by the National Science Centre, Poland (grant no. 2017/26/D/NZ7/01152).

Immunotherapy

EACR25-0072

Novel Antibody Therapeutics: Anti-BCMA.ECD for Personalised Cell Therapy in Multiple Myeloma

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Introduction

Multiple myeloma is characterized by the expansion of malignant plasma cells in the bone marrow (BM), which is associated with excessive production of monoclonal immunoglobulins in blood and urine in patients. The addition of monoclonal antibodies as immunotherapies in MM has further improved patient outcome. However, MM remains incurable for most patients, since drug-resistant clones constantly emerge and evolve. BCMA has been found to be overexpressed in MM and is being explored as a target for both antibody based and live cell therapy.

Material and method

BCMA expression in MM patients was quantified using RQ PCR, and soluble BCMA was quantified using ELISA. The consensus BCMA.ECD sequence was cloned into a vector, sequenced, and protein expression was carried out using the Expi293 system, characterized by SDS-PAGE. Mice were immunized, and hybridoma cells were generated for the production of Anti-BCMA.ECD antibodies, which were further purified and characterized using western-blotting and other assays. The best binders were identified, and the ScFv sequence was incorporated into a novel second-generation CAR (Chimeric Antigen Receptor) T-cell, which was screened using flow-cytometry.

Result and discussion

We characterized BCMA expression in a cohort of MM patients and found it to be elevated at the mRNA level. Serum or soluble BCMA levels were also elevated, predicting inferior therapeutic outcomes in MM patients. BCMA was validated as a candidate for Anti-BCMA therapy. BCMA.ECD protein was expressed in a eukaryotic system and used to generate hybridomas, resulting in twenty different clones. These clones were screened for the best binders, characterized by their binding affinity to BCMA.ECD protein using ELISA. The best binder (9C4) was used for deciphering the ScFv sequence for the development of a novel second-generation CAR.

Conclusion

This study led to the characterization of BCMA expression and variation in Indian MM patients and the development of an Anti-BCMA.ECD antibody for a second-generation CAR.

EACR25-0102

DLL3-targeting CAR-T cells enhanced by co-deletion of PD1 exhibit better efficacy and persistence in a pre-clinical animal model for small cell lung cancer

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Introduction

Small cell lung cancer (SCLC) is an aggressive and high-grade neuroendocrine disease with few treatment options. Delta-like ligand 3 (DLL3) is highly expressed on SCLC and several other types of neuroendocrine cancers with minimal or absent expression in normal adult tissues, making it a promising CAR-T target for SCLC and other solid tumor indications.

Material and method

A VHH antibody (D4) for human DLL3 was selected and generated from a humanized Camelid synthetic library. Subsequently, it was made into a second-generation CAR format and the one with additional PD1 intracellular retention (a non-gene editing technique to remove membrane proteins), both of which were compared for in vitro activities and an in vivo subcutaneous SHP77 tumor animal model for target-dependent T cell cytotoxicity and persistence.

Result and discussion

The DLL3 CAR with PD1 intracellular retention demonstrated a much better long-term cytotoxicity against SHP77 tumor cells than the unmodified CAR in vitro by repeatedly adding two more rounds of the tumor cells in an Incucyte's real-time killing assay at an E:T ratio of 1:2. To further evaluate the activities of both DLL3 CAR T cells in vivo, 5×10^6 /mouse of SHP77 cells were implanted subcutaneously in nonobese diabetic SCIDgamma (NSG) mice and allowed to engraft for 7 days, reaching a mean size of approximately $40\text{--}60\text{mm}^3$ before treatment with a single tail vein injection of 2×10^6 CAR+ T cells/mouse. For this well-established in vivo solid tumor model, we found that the PD1-deleted CAR-T group started to show the tumor growth slowing down D13, soon reaching the peak volume D20 at a mean size of 1500 mm^3 , and then steadily declining close to the baseline D30. But the unmodified CAR-T group was observed to have a much delayed and gradual decline of the tumor volume starting D20 instead at a mean size of 2500 mm^3 , with shrinkage down to a mean tumor volume of 600 mm^3 D30 and further down to the 300 mm^3 D34. In addition, both groups showed the CAR-T cell expansion during the tumor shrinkage and no signs of GVHD in the study.

Conclusion

Although both CAR-T groups exhibited good tumor suppression capabilities, the PD1-deleted DLL3 CAR-T showed a quicker and stronger response in this adoptive T cell therapy against solid tumors in a pre-clinical animal model. The findings of Improved preclinical efficacy and persistence warrant further evaluation of PD1-deleted DLL3 CAR-T cells as a potential clinical candidate for the treatment of SCLC.

EACR25-0109

Nanobodies against PSMA as the foundation for new tracers for prostate cancer imaging and treatment

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Introduction

Nanobodies (VHHs) are the variable domains derived from heavy-chain-only antibodies (HcAbs) found in camelids or sharks. Their small size, stability, and ease of engineering make them valuable for research and clinical applications. Prostate-specific membrane antigen (PSMA) is a transmembrane glycoprotein highly expressed in prostate cancer, serving as a key biomarker for imaging and therapy. To enhance the specificity and affinity of new tracers, we identified and validated PSMA-binding nanobodies.

Material and method

Three llamas were immunized with PSMA-expressing prostate cancer cell lines or patient-derived materials to generate nanobody libraries. Phage display technology was used to screen for PSMA-specific nanobodies, followed by Next Generation Sequencing (NGS) of ~ 1 million bound phages per panning. PSMA knockdown in LNCaP cells was achieved using FOLH1 shRNA lentiviral infection, and knockdown efficiency was assessed via Western blot. Nanobody candidates with high PSMA-binding ratios were validated through a four-step workflow, including Cell ELISA, immunocytochemistry, tissue microarray staining, and flow cytometry using LNCaP WT/shPSMA cells. Selected nanobodies were mapped for epitope binding, produced as His-tagged proteins, and tested in competitive binding assays using Alexa-labeled phage nanobodies. In silico docking analyses with AlphaFold3, Robetta, and HADDOCK2.4 identified binding epitopes.

Result and discussion

Fourteen PSMA nanobodies (PSMANbs) were selected for in vitro analysis. ELISA confirmed their high affinity for PSMA-positive cell lines (LNCaP, B16-PSMA) with minimal binding to PSMA-negative lines (DU145, B16-WT). Six PSMANbs demonstrated strong specificity, with A7 and PSMANb9 exhibiting the highest selectivity in immunocytochemistry and tissue histology across 20 cell lines and 20 normal human tissues. Flow cytometry confirmed reduced binding of AF488-labeled A7 and PSMANb9 in PSMA-knockdown cells. Competitive binding assays showed that A7 and PSMANb9 target distinct, non-overlapping PSMA epitopes, which was further supported by in silico structural modeling. Importantly, their binding does not interfere with the clinically used PSMA-617 tracer.

Conclusion

Six PSMA-specific nanobodies were identified, with A7 and PSMANb9 showing the highest affinity and specificity. Both bind distinct PSMA epitopes without interfering with PSMA-617, making them promising candidates for diagnostic and therapeutic applications in prostate cancer.

EACR25-0177

Efficacy and toxicity of half dose BCG vaccine in bladder cancer

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Introduction

Bacillus Calmette–Guérin (BCG) has been successfully used as immunotherapy to treat non-muscle invasive bladder cancer (NMIBC) for more than four decades. BCG is the only intravesical agent shown to reduce the risk of progression of NMIBC to muscle-invasive disease. Unfortunately, BCG therapy is not a universal panacea and it still fails in up to 40% of patients. This prospective cohort study was designed to document efficacy and toxicity of half dose (40 mg) BCG.

Material and method

Eligibility criteria include intermediate and high-grade NMIBC and carcinoma in situ after 3 weeks of TURBT. Weekly BCG therapy (40 mg, half dose) was given for 6 weeks as induction and a weekly dose for 3 weeks at 3, 6, 9 and 12 months was given as maintenance therapy. The entire procedure was done as an outdoor procedure.

Result and discussion

21 patients were included in the study from 2019 to February 2023. All patients had T1 disease, 7 had low grade (Intermediate risk) and 14 had high grade (High risk without very high-risk features) cancer. Cystitis is the most common symptom experienced by all patients to varied extent but fortunately all are self-limiting. 3 patients had fever which subsided with paracetamol. No serious adverse effect observed in any of the 18 patients, and all were discharged on the same day of admission. After 36 months of mean follow up period, 8 patients had recurrence.

Conclusion

BCG therapy is an effective treatment in intermediate and high-grade NMIBC and carcinoma in situ after TURBT. With half dose of BCG the toxicity is low and the cost of treatment is just over 20\$ for each session.

EACR25-0203

Scoparone attenuates PD-L1 expression in human breast cancer cells by MKP-3 upregulation

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Introduction

Breast cancer is a frequently occurring malignant tumor that is one of the leading causes of cancer-related deaths in women worldwide. Monoclonal antibodies that block programmed cell death 1 (PD-1)/programmed cell death ligand 1 (PD-L1) – a typical immune checkpoint – are currently the recommended standard therapies for many advanced and metastatic tumors such as triple-negative breast cancer. However, some patients develop drug resistance, leading to unfavorable treatment outcomes. Therefore, other approaches are required for anticancer treatments, such as downregulation of PD-L1 expression and promotion of degradation of PD-L1. Scoparone (SCO) is a bioactive compound isolated from *Artemisia capillaris* that exhibits antitumor activity. However, the effect of SCO on PD-L1 expression in cancer has not been confirmed yet. This study aimed to evaluate the role of SCO in PD-L1 expression in breast cancer cells *in vitro*.

Material and method

SCO was obtained from MedChem Express, and various antibodies, including anti-PD-L1 and phospho-protein antibodies, were sourced from Cell Signaling Technology, Abcam, and Santa Cruz Biotechnology. MCF7 and MDA-MB-231 cells were cultured in RPMI 1640 medium with 10% FBS and 1% antibiotics at 37°C in a 5% CO₂ incubator. Cell viability was assessed using a CCK-8 assay after 24-hour SCO treatment (0–500 μM), with absorbance measured at 450 nm. Western blot analysis was performed using SDS-PAGE and chemiluminescence detection. Total RNA was extracted using the AccuPrep kit, and RT-qPCR was conducted with 18S rRNA as the reference gene. MKP-3-targeting and control siRNAs were transfected into MDA-MB-231 cells using Lipofectamine RNAiMAX, and knockdown efficiency was verified by western blotting. Statistical analyses were performed using ANOVA, with data expressed as mean ± SD, and significance set at *p* < 0.05.

Result and discussion

Our results show that SCO downregulated PD-L1 expression in a dose-dependent manner, via AKT inhibition. Interestingly, SCO treatment did not alter PTEN expression, but increased the expression of mitogen-activated protein kinase phosphatase-3 (MKP-3). In addition, the SCO-induced decrease in PD-L1 expression was reversed by siRNA-mediated MKP-3 knockdown.

Conclusion

Collectively, these findings suggest that SCO inhibited the expression of PD-L1 in breast cancer cells by upregulating MKP-3 expression. Therefore, SCO may serve as an innovative combinatorial agent for cancer immunotherapy.

EACR25-0279

Exercise to boost the immune system and optimise immunotherapy responsiveness in pancreatic cancer

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Introduction

Evidence suggests exercise boosts the immune system supporting anti-cancer activity, potentially enhancing cancer immunotherapy. The pancreatic tumour micro-environment (TME) lacks immune cells inhibiting immunotherapy responsiveness. Treatments are limited contributing to its poor prognosis. We hypothesise that exercise will improve immunity in pancreatic cancer and enhance the response to immunotherapy.

Material and method

20 C57BL/6 mice underwent orthotopic implantation of Panc02 (murine pancreatic adenocarcinoma) cells and were divided into 4 groups (*n* = 5); exercise with anti-Programmed Cell Death Protein 1 (PD1), exercise with isotype, no exercise with anti-PD1 and no exercise with isotype. Treadmill running was performed 20 minutes/day, 4 days/week at 12 metres/minute. Resistance training consisted of hanging upside down on a wire mesh screen for 1 minute 2 days/week. Flow cytometry

determined TME immune populations. Tumour and liver samples were harvested, paraffin wax embedded/sectioned and analysed using SlideViewer®. Alongside this, immune profiling of bloods from healthy volunteers was carried out pre and post high intensity interval exercise and the effects of exercise conditioned serum on pancreatic cells (PANC1) in vitro were explored.

Result and discussion

Exercise/anti-PD1 was feasible from Day 2 following surgery throughout the study. A 73.5% reduction in Myeloid Derived Suppressor Cells (MDSCs) occurred in the TME of exercising mice ($p = 0.0426$). Liver metastatic burden was significantly reduced with treadmill running (92.9%, $p = 0.0087$) with a 66.8% reduction seen in the exercise/anti-PD1 group ($p = 0.0286$). Tumour necrosis was significantly higher in the exercise/anti-PD1 group ($p = 0.0182$). Similarly, in healthy volunteers an increase in NK cells (32.6%, $p < 0.0001$) and a reduction in regulatory T cells by 31.0% ($p < 0.0001$) was seen post exercise. Exercise conditioned serum significantly reduced PANC1 cell migration ($p = 0.03$) and increased caspase 3/7 (apoptotic) activity ($p < 0.0001$).

Conclusion

Exercise/anti-PD1 improves tumour response and limits metastatic potential in this aggressive pancreatic cancer model. MDSCs in the TME promote immune tolerance allowing tumour propagation. Exercise significantly reduces MDSCs and trends towards regulatory T cell reduction. These TME modulations promote anti-cancer immune responses demonstrating exercise as an effective immunotherapy enhancer. High intensity interval aerobic exercise in healthy volunteers significantly increases NK cells and reduces Regulatory T cells, promoting cytotoxicity and reducing immune tolerance. Exercise conditioned serum increases apoptosis and reduces migration of pancreatic cancer cells in vitro. Exercise could be a promising immunotherapy adjunct in pancreatic cancer. Clinical studies are warranted to explore these benefits in pancreatic cancer patients.

EACR25-0289

Epigenetic Modulation of CAR-T cells against Group 3 Medulloblastoma

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Introduction

Medulloblastoma (MB) is the most common malignant brain tumour in children. Group 3 MB represents the most aggressive subgroup, with high recurrence rates and poor survival outcomes. Current treatments, including surgery and chemotherapy, are associated with significant morbidity, underscoring the need for innovative therapies. Chimeric antigen receptor T-cell (CAR-T) therapy has shown promise in hematologic malignancies and preclinical solid tumour models, but its efficacy against solid tumours is often hindered by T-cell exhaustion and limited persistence. Epigenetic regulation, particularly DNA methylation, plays a crucial role in T-cell exhaustion. Azacytidine (AZA), a DNA methyltransferase inhibitor approved for clinical use, has been

proposed to enhance CAR-T function by preventing exhaustion-associated epigenetic modifications.

Material and method

We investigated the effects of AZA priming on CAR-T cells targeting B7-H3 and EphA2, antigens highly expressed in Group 3 MB but absent from normal paediatric brain tissue. CAR-T cells were expanded in the presence of AZA, and their cytotoxicity, cytokine secretion, proliferation, and memory/exhaustion phenotypes were assessed via flow cytometry, ELISA and luciferase assays.

Result and discussion

While previous studies have suggested that hypomethylating agents, including decitabine and AZA, may enhance CAR-T function in certain contexts, our findings indicate that AZA priming of B7-H3 and EphA2 CAR-T cells did not significantly alter cytotoxicity, cytokine production, or exhaustion/memory marker expression in vitro. However, AZA treatment led to a notable decrease in CAR-T proliferation, as demonstrated by Cell Trace Violet dilution assays, suggesting that DNA methylation inhibition may influence CAR-T expansion kinetics. Given the promising role of epigenetic modulation in T-cell function reported in other CAR-T models, further investigations are warranted to clarify context-dependent effects of AZA on different CAR constructs and tumour types. To explore an alternative strategy, we are now evaluating whether treating Group 3 MB cells with AZA can enhance tumour immunogenicity and synergize with CAR-T therapy.

Conclusion

This study highlights the complex effects of epigenetic modulation in CAR-T therapy and suggests that AZA may differentially affect CAR-T cell function depending on target antigen, tumour type, and in vitro expansion conditions. By shifting our focus to tumour-directed AZA priming, we aim to assess whether epigenetic modification of Group 3 MB cells can enhance CAR-T efficacy. These findings contribute to the growing understanding of how hypomethylating agents interact with immunotherapy and may help refine epigenetic combination strategies for rare paediatric CNS tumours.

EACR25-0347

Unveiling interactions between senescent tumor cells and the host immune system - Implications for Senolytic Immunotherapy

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Introduction

Therapy-induced senescence (TIS) halts tumor growth and enhances treatment outcomes in numerous preclinical cancer models. However, it also alters tumor biology and reshapes the tumor environment, thereby contributing to treatment resistance and tumor relapse. Several studies have described the complex and dynamic relationship between senescent tumors and the host immune system, yet the molecular mechanisms underlying this interaction remain unclear, and its impact on treatment efficacy contingent upon cancer type. Consequently, elucidating the interplay between senescent tumor cells and the host immune system is a critical priority for improving cancer therapy, including the development of novel senolytic immunotherapies.

Material and method

We employed RNA and proteome analyses, along with immunophenotyping by flow cytometry, in various pediatric tumor mouse models and human tumor cell lines before and after DNA-damaging therapy to characterize TIS-associated changes in tumor biology. To further investigate the interaction between TIS tumor cells and different cells of the host immune system (e.g., macrophages, T cells, and NK cells) *in vivo*, we utilized immune mass cytometry (IMC) and spatial multiplex immunofluorescence imaging (MACSima). Additionally, we applied genetic and pharmacological approaches to modulate T-cell interactions with TIS tumor cells and alter T-cell cytotoxicity in senolytic therapies.

Result and discussion

Upon TIS, various mouse and human cancer cell lines exhibited increased expression of immunologic gene sets and surface markers associated with immune system activation. Investigation of *in vivo* tumor-host interactions by spatial imaging revealed that TIS promotes the infiltration of both CD4⁺ and CD8⁺ T cells into senescent tumor sites, facilitating their direct interaction with TIS cells. Immunophenotyping of these T cells identified Fas ligand (FasL) overexpression as a key actionable moiety that induces apoptosis in Fas receptor (FasR)-positive TIS cells. To further elucidate the significance of this interaction in senolytic therapies and treatment outcomes, we employed *in vivo* and *in vitro* genetic and pharmacological approaches to manipulate FasL–FasR binding, demonstrating its crucial role in TIS-targeted therapeutic strategies and treatment outcome.

Conclusion

This study elucidates the impact of TIS on tumor cell immunogenicity, demonstrating enhanced immune system activation and increased susceptibility to T cell-mediated apoptosis through direct interaction of both CD4⁺ and CD8⁺ T cells via the FasL–FasR pathway. These findings highlight novel actionable moieties to enhance the efficacy of senescence-based immunotherapies across different cancer types.

EACR25-0435

Investigating the repurposing potential of immune checkpoint inhibitors to additional cancer patient populations using Mendelian randomisation

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Introduction

Inhibiting programmed-cell-death protein 1 (PD-1) and programmed-death ligand 1 (PD-L1) by immune checkpoint inhibitors (ICIs) has proven efficacy in the treatment of multiple cancers through targeting evasion of host immune responses, a key hallmark of cancer. We aimed to assess the repurposing potential of these ICIs to additional cancer indications using the causal inference method Mendelian randomisation (MR).

Material and method

Germline genetic instruments were constructed to proxy serum PD-1 and PD-L1 protein levels in normalised protein expression (NPX) units with summary statistics from a GWAS of general population participants.

Summary-level MR was performed to investigate the genetically proxied effects of PD-1 and PD-L1 lowering on breast, colorectal, lung, melanoma, ovarian or prostate cancer mortality. Where data were available, HRs were estimated for participants stratified by cancer stage or subtype. Cancer survival at sites with current PD-1 or PD-L1 inhibitor indications (breast, colorectal, lung, melanoma) were positive control outcomes. Instrument validity was evaluated in cancer patient cohorts. Risk of collider bias was assessed through investigating the effects of PD-1 and PD-L1 on cancer incidence using summary-level MR.

Result and discussion

We observed evidence to support effects of genetically proxied PD-1 lowering on risk of death in colorectal (HR

per NPX unit decrease PD-1: 0.84, 95% CI: 0.71-1.00), lung (HR per NPX unit decrease PD-1: 0.88, 95% CI: 0.78-1.00) and ovarian cancer (HR per NPX unit decrease PD-1: 0.88, 95% CI: 0.78-0.99). Little evidence was observed for effects of genetically proxied PD-L1 lowering, aside from on risk of colorectal cancer mortality (HR per NPX unit decrease PD-L1: 0.90, 95% CI: 0.81-0.99). There was no strong evidence to suggest that effects of PD-1 and PD-L1 on colorectal cancer mortality were restricted to any specific stage or site. Generally, the lead PD-1 and PD-L1 instruments demonstrated consistent direction of effect with their respective protein levels in the cancer patient compared to general population cohorts. There was no strong evidence to support effects of PD-1 or PD-L1 lowering on cancer incidence at any included site.

Conclusion

We found evidence to support PD-1 inhibitor repurposing for ovarian cancer treatment, a possible new indication. We additionally found evidence to support potential PD-1 and PD-L1 inhibitor repurposing to broader colorectal cancer patient populations than current indications. Although there was evidence to support the validity of the instruments in cancer patient populations and we observed a low risk of collider bias, MR evidence was not consistent across cancers with currently approved indications. These findings should thus be interpreted with caution and highlight the remaining challenges of applying MR in cancer survival settings, for example low heritability of cancer prognosis or treatment effects.

EACR25-0448

POSTER IN THE SPOTLIGHT

Fishing for new immunotherapy compounds to boost innate-tumor rejection

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Introduction

Despite significant advances in cancer immunotherapy, tackling immunosuppression remains critical for more effective responses. While developing a zebrafish xenograft model, we found that some tumors engraft very efficiently (“progressors”), while others are cleared by the host (“regressors”). We showed this to be dependent on innate immunity, which recognizes and eliminates certain cancer cells, while others are able to evade/suppress it. This opened an opportunity to perform an *in vivo* phenotypic drug screen to find compounds that induce clearance by boosting innate-tumor rejection.

Material and method

Zebrafish xenografts of human colorectal (CRC) and breast cancer (BC) cell lines that show a high engraftment/low clearance phenotype (progressors) were used to screen an FDA-approved small compound library. Tumor cells were injected into 2 days post-fertilization embryos and subjected to compound testing. At 3-5 days post-treatment, clearance rates were quantified, and hits defined as compounds that increase tumor clearance.

Result and discussion

We screened 500+ drugs and obtained a total of 23 confirmed hits, with multiple of them showing effect in both CRC and BC xenografts. We characterized how the hits modulate the innate tumor microenvironment (TME) using transgenic zebrafish (myeloid reporters and mutants) and have observed phenotypes such as increasing macrophage/neutrophil recruitment and/or infiltration, as well as a macrophage reversion from a pro to an anti-tumoral polarization. We are currently characterizing the molecular mechanism behind the top hit, a muscarinic receptor inhibitor (MRI). The MRI was also tested in a CRC xenograft mouse model, to assess its efficacy in a fully immune-competent host, as well as their synergy with the αPD-1 immune checkpoint blocker. Treatment led to a significant decrease in tumor volume as monotherapy and showed a synergistic effect with αPD-1, with an additional increase in survival. We are currently performing immune profiling of the tumors, to better understand how the compound modulates immunity.

Conclusion

We are confident that we have found an FDA-approved compound that can be repurposed to be used in combination with immune checkpoint blockers, engaging both the innate and adaptive arms of the immune system to overcome the suppressive TME, and consequentially increase treatment efficacy rates.

EACR25-0481

Assessing the Potential of Epigenetic Inhibitors to Improve Immunotherapy Response via In Vitro Models

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Introduction

Immunotherapy based on immune-checkpoint inhibitors (ICIs) is the first-line treatment for advanced cancers like non-small cell lung cancer (NSCLC) and clear cell renal cell cancer (ccRCC). However, only a small percentage of patients benefit from it, becoming crucial to find therapies that enhance ICIs effectiveness. Epigenetic therapies, involving DNA methyltransferase (DNMT) and histone deacetylase (HDAC) inhibitors, could modify tumor cells and the tumor microenvironment, potentially improving ICIs responses. Recent studies have shown

that epigenetic drugs can activate transposable elements (TEs) like LINE-1 and human endogenous retroviruses (HERVs), suggesting a link between epigenetic drug-induced antiviral responses and increased immunogenicity. An abnormal expression of these elements can induce a state of viral mimicry, in which the cell triggers an immune response upon detecting foreign nucleic acids in the form of double-stranded RNA (dsRNA). This study aimed to evaluate the effect of two epigenetic drugs on NSCLC and ccRCC cell lines to improve ICIs responses.

Material and method

We used two NSCLC cell lines (H-2009 and H-2087) and two ccRCC lines (786-O and Caki-2). Epigenetic treatments included azacitidine (DNMT inhibitor) and vorinostat (HDAC inhibitor). Cell viability was assessed using the XTT assay, and gene expression was analyzed via qPCR. The cell cycle was examined by flow cytometry, and dsRNA production was assessed using immunofluorescence.

Result and discussion

The IC₅₀ values were calculated for each cell line and drug after 48 hours of treatment. IC₅₀ is the concentration of a drug required to cause 50% cell viability. All cell lines were sensitive, with IC₅₀ values ranging from 12.83–29.19 μM for azacitidine and 4.62–8.00 μM for vorinostat. Azacitidine induced morphological changes, especially in H-2009, and increased mesenchymal markers (ZEB1, ZEB2 and SNAIL) in all cell lines. CDH1 expression slightly decreased in lung cell lines. S-phase arrest was observed in H-2087 and Caki-2. No significant changes in LINE-1 expression, except for a slight increase in 786-O. Both renal lines showed an increase in HERVs, while lung cell lines remained unchanged. dsRNA production increased in 786-O and H-2087. Regarding vorinostat, no notable morphological changes were observed, but mesenchymal markers (VIM, ZEB1, ZEB2 and SNAIL) increased. G1 and G2 arrest were seen in H2009 and 786-O, respectively. Both LINE-1 and HERVs expression rose, and dsRNA production was enhanced in all lines.

Conclusion

Both treatments induced mesenchymal traits in all cell lines, with vorinostat causing more prominent TEs activation and dsRNA production. Therefore, vorinostat may induce a state of viral mimicry, triggering an immune response. These findings suggest HDAC inhibition could be a promising therapy to enhance ICIs efficacy in lung and renal cancers.

EACR25-0518

Preclinical double humanized CD47/SIRPα mouse model and MC38 cell line expressing human CD47 for efficacy and safety assessment of anti-CD47/SIRPα-targeting therapies

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Introduction

CD47 is overexpressed on many human tumor cells and interacts with its ligand, the signal-regulating protein alpha (SIRPα), a protein expressed on macrophages and dendritic cells, to induce a “don’t eat me” signal. CD47 and its interaction with phagocytes is therefore a promising therapeutic target to treat cancer (S.B. Willingham et al., Proc Natl Acad Sci U S A. 2012). Here, we describe a double humanized knock-in model, the genO-hCD47/hSIRPα model, designed for the efficacy and safety assessment of human CD47/SIRPα-targeting therapies in immunocompetent mice.

Material and method

This model was generated by intercrossing genO-hCD47 and genO-hSIRPα mice, with both models designed to enable the expression of all human isoforms of hCD47 and hSIRPα.

Result and discussion

hCD47 and hSIRPα are expressed at physiological levels, which enabled the assessment of biodistribution of anti-SIRPα nanobodies, used as tracers in non-invasive in vivo tumor imaging (T.R. Wagner et al., Front Immunol. 2023). Activity of anti-CD47-targeting compounds were investigated in the model and a murine MC38 colon adenocarcinoma cell line expressing hCD47 was developed to reproduce as much as possible the interactions between hCD47 and hSIRPα upon tumor engraftment in this syngeneic mouse model. Magrolimab analog (Hu5F9-G4) induced tumor growth inhibition studies in the genO-hCD47/hSIRPα model inoculated with MC38 expressing hCD47. Furthermore, safety assessment of Magrolimab analog in naïve genO-hCD47/hSIRPα mice showed a drop on the number of red blood cells (RBCs) and hemoglobin 1 day post-treatment, with a recovery by 8 days post-treatment. Additionally, MC38-hCD47-bearing genO-hCD47/hSIRPα mice treated with Magrolimab also recapitulates the clinical findings, with a decline in RBCs and hemoglobin post treatment (C.K. Brierley et al., Transfusion. 2019).

Conclusion

Altogether, these data suggest that the genO-hCD47/hSIRPα model enables assessment of efficacy and safety of CD47 and/or SIRPα-targeting agents. The model is currently being upgraded to express humanized Fcγ receptors (FcγR), which will facilitate the assessment of Fc-mediated functions of antibodies targeting human CD47 and/or SIRPα.

EACR25-0586

Development of the in vitro 3D Spheroid High-Throughput Screening Platform for the Development of Immune Checkpoint Inhibitors

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Introduction

Immune checkpoint inhibitors (ICI) have revolutionized cancer immunotherapy, leading to significant clinical success in multiple malignancies. Despite their effectiveness, the high costs associated with ICI development and treatment pose a substantial economic burden. Additionally, the reliance on animal models in preclinical

research raises ethical concerns and often presents translational limitations due to the difficulties to accurately predict human immune responses. There is a pressing need for more physiologically relevant and cost-effective *in vitro* pre-clinical models to evaluate novel ICI candidates.

Material and method

To address this challenge, we developed a high-throughput 3D spheroid culture platform in our proprietary AkuraTM384-well format for the evaluation of immuno-modulatory and anti-tumor efficacy of ICIs. Our system utilizes spheroids composed of tumor cell lines and primary cancer-associated fibroblasts, to effectively recapitulating the tumor microenvironment. HLA-matched aCD3/CD28 pre-activated peripheral blood mononuclear cells (PBMCs) were added to 3D human tumor spheroids to assess immune-mediated tumor killing. Treatment conditions included ICIs and isotype control antibodies. Tumor viability and growth were monitored via bright field images and fluorescence measurements, while cleaved caspase-3/7 activity served as a marker of T-cell-induced apoptosis. Additionally, cytokine levels were quantified in the supernatant. Flow cytometry analysis of single spheroid was performed to assess immune cell phenotype and expression of tumor markers such as PD-L1 and EpCAM.

Result and discussion

Our results demonstrate that CD3/CD28/ICI treatment significantly enhances T-cell-mediated tumor cell apoptosis, as evidenced by increased cleaved caspase-3/7 activity compared to controls. Fluorescence-based viability assays confirmed a reduction in tumor spheroid growth upon ICI treatment. Moreover, cytokine profiling revealed an upregulation of immune-activating cytokines, supporting the immunostimulatory effect of ICI within the spheroid model. Flow cytometry analysis indicated an induction of an activated phenotype in T cells, further validating the model's relevance for immune response evaluation. The platform successfully captures key aspects of the tumor-immune microenvironment, providing a robust and scalable assay for drug screening.

Conclusion

We have developed a physiologically relevant, high-throughput 3D spheroid model for the evaluation of novel ICI candidates. This platform closely reflects clinical scenarios, allowing for efficient screening of immunotherapies while reducing reliance on animal models. The system's scalability and reproducibility make it a powerful tool for accelerating the development of next-generation immune checkpoint inhibitors.

EACR25-0656

Integrating the Fasting-Mimicking Diet to Augment Anti-tumor Immunity following Adoptive Cell Transfer in Breast Cancer

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Introduction

Cancer arises within a complex cellular milieu, where the extent and composition of tumor-infiltrating immune cells strongly influence tumor defense and patient prognosis. Additionally, tumors do not exist in isolation, but rather, engage in constant crosstalk with the immune system including the bone marrow and the spleen. Emerging research highlights the therapeutic promise of fasting-mimicking diets (FMDs) in delaying cancer onset, providing cellular protection, and regulating immunity. Adoptive cell transfer (ACT) is a treatment approach that isolates and amplifies potent anti-tumor immune cells before re-infusing them into cancer patients for therapeutic purposes. My project investigates immune profiles resulting from FMD exposure, either independently or with therapeutic drugs, before and after ACT. It aims to elucidate the distinct roles of immune cell subsets and their impact on tumor advancement through promoting anti-tumor immunity.

Material and method

Using the 4T1 TNBC syngeneic model in BALB/c mice, the impact of FMD and drug treatments on tumor growth and immune competence is assessed. The project includes two rounds of 4T1 cell transplantation in hosts exposed to either a normal diet or FMD, with some groups receiving chemotherapeutic drugs. The first round focuses on the characterisation of favourable immune cell subsets recruited at tumor sites, spleens, and bone marrows using FACs and IHC. In the second round, infiltrated tumor masses and splenocytes are transplanted into recipient hosts with naïve 4T1 cells to assess tumor progression, immunity acquisition, and cytotoxicity.

Result and discussion

Preliminary data show reduced tumor volumes and delayed progression following exposure to FMD and doxorubicin, confirming synergistic effects. The combinatorial treatment also led to a reduction in splenomegaly while preserving the morphology and structure of a healthy spleen. Additionally, it induced phenotypic and functional changes regarding total and specific subpopulations of leukocytes in both, spleens and bone marrows, underlying the enrichment of immune signatures previously, associated with improved clinical prognosis. Thorough characterisation remains ongoing as we continue to define the most relevant immune cells in modulating anti-tumor responses in this setting, prior to the initiation of the transplantations.

Conclusion

Combining FMD with ACT may enhance anti-tumor immunity, improve treatment outcomes, and expand efficacy across cancer types. Exploring FMD mechanisms could uncover ways to stimulate beneficial immune cell patterns, opening new avenues in cancer treatment.

EACR25-0710

ROR1-Specific CART-Cells: Advancing Targeted Immunotherapy for Cancer

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Introduction

The receptor tyrosine kinase-like orphan receptor 1 (ROR1) is critical during embryogenesis regulating cell migration, differentiation and growth. It is demonstrated that ROR1 expression is low in adult tissues, and unfortunately the expression increases in various malignancies such as Mantle Cell Lymphoma (MCL), Multiple Myeloma (MM), Triple Negative Breast Cancer (TNBC), Non-Small Cell Lung Carcinoma (NSCLC), colorectal cancer (CRC) or Melanoma (MEL), all being highly aggressive and their management is challenging. ROR1 targeted therapies are continuously developing, with remarkable achievements in terms of novel mAbs, ADCs or small molecules. Our study aims to highlight that the use of CAR T-cells could improve the outcomes and offer new perspectives by targeting ROR1.

Material and method

The full length of CAR was synthesized and subcloned into lentivirus vector by Creative Biolabs. The insert was confirmed by Sanger seq. The iCasp9-pMD2.G-psPAX2 virus was generated by transfecting HEK293FT packaging cells. The target pH-R-iCasp9 plasmid, the packaging vector, psPAX2 and the envelope, pMD2.G were acquired from AddGene. FuGENE® was used as transfection reagent. Lentiviral supernatants were harvested at 24h and 48h. CAR ROR1 Jurkat cells were spinoculated in 8 µg/mL polybrene and viral supernatant. On day 3 after transduction, fluorescence microscopy confirmed the presence of mCherry+ cells. CAR ROR1 Jurkat cells were expanded for 14 days, followed by mCherry/GFP-highly positive cells sorting, on a FACS ARIA III platform. The co-culture efficacy of different effector-target (E:T) ratios was evaluated by flow cytometry. Target cells were identified by staining with an APC anti-human ROR1 antibody. The supernatant was stored and further used for LDH titration by an ELISA assay.

Result and discussion

The presence of eGFP and mCherry red signal confirmed the successful generation of anti-ROR1 CAR T-cells. The analysis at different E:T ratios showed that the CAR T cells successfully inhibited the targets, with a significant cytokine release measured by ELISA assays, all tests being done in comparison with Mock - Jurkat cells. The highest inhibitory rate was observed in the MCL cell line, accompanied by a significantly higher cytokine release compared to the controls. As the CAR T-cells are immortalized due to the use of Jurkat cells, we evaluated the suicidal capacity using various concentrations of AP1903. AP1903 induced iCasp9 dimerization, and the flow cytometry analysis revealed that apoptosis was initiated in most of the CAR T-cells. Apoptotic rate was determined by a 7-AAD/PO-PRO1 flow cytometry assay on an LSRII cytometer. This remained evident at a very

low concentration (6.25 nM), which is still well below the clinical concentration for AP1903.

Conclusion

Anti-ROR1 CAR T-cells showed a significant inhibitory rate against multiple targets, indicating that the new approach could be considered for further in vivo evaluations.

EACR25-0726

Improved Adoptive T Cell Therapies for Colorectal Liver Metastases by Stabilization of IFNAR1 on Tumor-Specific T-Cells

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Introduction

Diagnosis for Colorectal cancer (CRC) are surging, and distant metastases, particularly to the liver, plays a crucial role in determining poor prognosis, despite improvements in surgical, chemotherapeutic and targeted therapies. Adoptive T-cell therapies (ACT) show promise for cancer treatment but often struggle in solid tumors due to the immunosuppressive tumor microenvironment (TME) and one factor to this failure is the down-regulation of the type I Interferon receptor (IFNAR) on T cells, which abolished their ability to respond to anti-tumor immune signals. To overcome these limitations, we improved tumor-specific CD8+ T cells or CAR T cells by the stabilization of IFNAR1 on cell surface, through genetic and pharmacologic approaches, blocking its endocytosis, induced by tumor-released type I IFNs, thus avoiding cells exhaustion and death, and promoting regression of CRC liver metastasis.

Material and method

We dissected the TME of human CRC synchronous liver metastases ($n = 3$) with AKOYA spatial multiplex-immunofluorescence staining for IFNAR1 and immune cells markers. Then, with mouse established CRC cell lines we investigated the molecular mechanisms. MC38 cells have been chosen, also by RNASeq data, as model of high releasing type I IFNs, opposed to mouse established CRC-derived organoids MTO-140, which are low releasing. We utilized both in vitro differentiated OT-I and OT-I-SA CD8+ T cells for ACT targeting MC38OVA liver metastases, and Chimeric Antigen Receptor T cells (CAR-T) genetically engineered to express IFNAR1-SA and that recognize hCEA, and MC38hCEA as target.

Result and discussion

Here, we observed that IFNAR1 is downregulated in liver metastasis in a cohort of human CRC synchronous liver metastasis ($n = 250$) and this correlates with a high production of type I IFNs by the same tumors. Type I IFNs chronically released by tumor contribute to reducing the anti-tumor immune response exhibited by the sustained internalization of IFNAR1 on CD8+ T cell that cause their death, and a decreased frequency of intra-

tumor effector CD8+ T cells (Teff). Thus, exploiting advantage for genetics and pharmacological compounds to block receptor phosphorylation or internalization by endocytosis, we highlighted that the stabilization of IFNAR1 on CD8+ T cells, is necessary and sufficient to leverage tumor-released type I IFNs to control the growth of liver metastasis by preventing T cell death. Indeed, MC38-OVA-derived liver metastasis escape OT-IWT CD8+ T immune response and continued to grow, but not with the OT-ISA T cells that are highly infiltrated in TME and control tumor growth, and this occurs even with engineering CAR-T with both IFNAR1-SA and hCEA receptor.

Conclusion

Overall, these results indicate that genetic stabilization of IFNAR1 on ACT cellular products enhances their viability and persistence within CRC metastatic lesions, significantly improving the efficacy of ACT and hold promise for future development.

EACR25-0738

A Pro-Tumorigenic Cytokine with an Unexpected Anti-Cancer Role: TGF β Enhances NK cell Immunotherapy for pancreatic cancer

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Introduction

TGF β has been recognised for decades as a driver of tumour progression and resistance to therapy. It induces exhaustion or inhibition in immune cells within the tumour microenvironment (TME), such as cytotoxic CD8+ T cells and NK cells, thus representing a major barrier to cancer immunotherapy. Recent studies have found tissue-resident NK cells (trNK) to be enriched in solid tumours, highlighting their anti-cancer potential. Specifically, TGF β in the TME promotes their anchoring by inducing CD103 expression. While these cells are positioned to attack tumours, their cytotoxicity is suppressed by the immunosuppressive TME. We hypothesised that combining TGF β exposure with cytokine stimulation could generate tissue-resident NK cells with preserved cytotoxic ability for solid cancer immunotherapy.

Material and method

PBMCs from healthy donors were expanded in vitro using IL-2, IL-15, and IL-21, with or without TGF β , to generate cytotoxic NK cells (cNK) or cytotoxic tissue-resident NK cells (c-trNK). Their phenotype was characterized using spectral flow cytometry, and their cytotoxicity was assessed against K562 leukemia and MiaPaCa2 pancreatic cancer cells. To evaluate their in vivo localization and tumor control, mCherry-expressing MiaPaCa2 cells were orthotopically injected into Rag2^{-/-} Il2rg^{-/-} mice, followed by intravenous NK cell

administration. NK cell distribution was tracked using IVIS fluorescence imaging.

Result and discussion

The optimised expansion protocol generated viable cells with minimal T cell contamination (CD56+CD3-fraction: 98.1 ± 1.1), critical for reducing the risk of GvHD in translational applications. The addition of TGF β was essential for the optimal expansion of NK cells expressing tissue residency markers, with over 90% of c-trNK cells expressing both CD49a and CD103. TGF β did not significantly alter the expression of most activating or inhibitory receptors but led to decreased CD16 expression, impairing c-trNK cells' ability to perform antibody-dependent cell cytotoxicity. Chemokine receptor expression varied, with TGF β promoting higher CXCR6 expression, a key factor for tumour site migration. At high effector-to-target ratios, TGF β did not significantly impair cytotoxicity, and c-trNK cells successfully eliminated K562 cells and nearly all MiaPaCa2 cells in vitro. In vivo, c-trNK cells mediated partial tumour control and were detected in high numbers in the livers, lungs and guts of tumour-bearing mice, suggesting successful migration to tumour and non-tumour epithelial sites. Interestingly, c-trNK were also detected in the blood and spleen.

Conclusion

These findings demonstrate that TGF β -exposed NK cells, when combined with cytokine stimulation, can generate tissue-resident NK cells with preserved cytotoxic function. This approach holds promise for enhancing the effectiveness of cancer immunotherapy, with potential for improved tumour control and tissue-specific dissemination.

EACR25-0752

Development of a humanized anti-ANXA3 monoclonal antibody for potential treatment of hepatocellular carcinoma

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Introduction

Hepatocellular carcinoma (HCC) is the predominant form of primary liver cancer. It ranks the third leading cause of cancer-related death globally, with high incidence and mortality in Southeast Asia. Unfortunately, majority of HCC patients are diagnosed at intermediate to advanced stages when treatment options are limited and the chance of tumor recurrence is high. Our previous studies demonstrate that endogenous and circulating annexin A3 (ANXA3) promotes self-renewal, tumor growth, and resistance to standard chemotherapy and targeted therapy in HCC, thus potentially offering a new target for HCC treatment. The use of humanized antibodies is more clinically relevant than non-humanized counterparts because of their reduced risk of triggering immune responses in human patients. In the era of precision medicine, there is a growing emphasis on tailor-made treatment considers personal genetic differences or environmental conditions. With advances in technology enabling the rapid detection of ANXA3 expression in an individual, the potential use of a humanized anti-ANXA3 antibody alone or in combination with other approved therapeutics represents a meaningful step forward to improving therapeutic outcomes of HCC patients with ANXA3 upregulation.

Material and method

Herein, a humanized anti-ANXA3 monoclonal antibody was developed and extensively characterized for its therapeutic efficacy utilizing both *in vitro* and *in vivo* HCC models.

Result and discussion

In vitro, our humanized anti-ANXA3 mAb in combination use with standard chemotherapy cisplatin or targeted therapy sorafenib or lenvatinib demonstrated synergistic effects in suppressing cell proliferation, inducing apoptosis, reducing tumor-initiating cell frequency, and re-sensitizing therapy-resistant cells to their respective treatments. *In vivo*, the combinational treatments similarly showed maximal tumor shrinkage, tumor-initiating frequency reduction, and therapy-resistant tumor re-sensitizing effects. Importantly, the humanized anti-ANXA3 mAb administration did not elicit any toxicity on a humanized peripheral blood lymphocyte (Hu-PBL) mouse model with respect to animal body weight and tissue weight changes and plasma biochemistry panel readouts. Animal behavioural tests also showed no significant difference in animal muscle strength, locomotor ability, and anxiety level.

Conclusion

Findings of this study also explored the potential of expanding the use of the humanized anti-ANXA3 mAb to multiple malignancies with ANXA3 overexpression.

EACR25-0825

Next Generation Protein Characterization of CD19-CAR Signaling Cascades

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Introduction

Chimeric antigen receptor (CAR)-T cell therapy is a revolutionary new pillar in cancer treatment with success in treating specific subsets of B cell leukemia, lymphoma, and multiple myeloma.¹ Much attention is focused on expanding CAR-T cell therapy to other hematologic malignancies and solid tumors. Central to engineering effective CAR-T cell therapies is a detailed understanding of the signaling cascades that regulate CAR-T cells, including trafficking and tumor infiltration, preventing antigen escape, resisting immunosuppressive responses, and ameliorating potentially fatal toxicity.¹ Detailed characterization of extracellular and intracellular signaling molecules is required, often with limited and complex sample types.

Material and method

To study CAR-T signaling, we leveraged a non-viral gene editing platform (TcBuster) to introduce a CD19-CAR into T-cells. Chemically selected cells were isolated and CAR surface expression was verified by flow cytometry analysis using an antibody raised against the peptide linker within the CAR scFv. We then stimulated the CAR-T cells with immobilized CD19 and implemented automated solutions to characterize IFN- γ secretion using automated ELISA (Simple Plex) and intracellular signaling events using a capillary immunoassay platform (Simple Western). The capillary immunoassay provided multiplex protein expression measurements of multiple signaling molecules, including phosphorylated and total protein isoforms, with specific molecular weight characterization, and required only 3 μ L of lysate for analysis.

Result and discussion

Here, we leveraged non-viral genome editing and advanced protein analytical solutions to shine new light on CAR-T cell activation, CAR trafficking, and signal transduction. We validated an antibody targeting the Whitlow/218 linker (CST) using Simple Western technology, enabling the measurement of CAR expression in whole-CAR-T cell lysates in addition to flow cytometry. The flow cytometry and Simple Western results showed that CAR-T cells downregulate CAR expression in less than 10 minutes following antigen engagement. However, CAR presence at the cell surface may linger for 15 minutes or longer. Furthermore, the Simple Western results indicate CAR expression increases again at 4 hours, suggesting a ‘recycling’ of the CAR in T cells. Simple Western analysis revealed detailed kinetics of intracellular CAR-T cell signaling events with reproducible quantification for both CAR expression and intracellular signaling molecules. Finally, the Simple Plex assay provided quantitative results of 4 secreted cytokine concentrations ranging in ng/mL levels at 4 hours post-activation in a single hands-free run in less than 90 minutes.

Conclusion

Together, these data show the importance of Fit-For-Purpose Analytical Tools to enable the measurement and understanding of the delicate interplay between signaling molecules that regulate CAR-T function.

EACR25-0955

The effect of STING agonist on lung metastases and immune landscape in murine model of spontaneous breast cancer metastasis

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Introduction

Immunotherapy emerged as the most promising tool in cancer treatment. Among its modalities the activation of the cGAS-STING pathway, which boosts anti-tumor immune response, is considered as encouraging therapeutic approach. STING activation enables production of type I interferons and pro-inflammatory cytokines leading to the activation of multiple immune cells. The process of metastasis represents one of the hallmarks of cancer. The appearance of metastases constitutes the major poor prognosis factor which limits the possibilities and opportunities of cancer therapies and is responsible for the greatest number of cancer-related deaths. Thus, the aim of current research was to assess the impact of STING agonist administration on the number and size of lung metastases and immune landscape in murine model of spontaneous breast cancer metastasis.

Material and method

The experiments were conducted on murine model of 4T1 breast cancer. cGAMP was injected intratumorally in a dose of 5 µg/mice. To estimate cGAMP-induced oxidative burst, the assessment of luminol chemiluminescence have been conducted. In the metastasis assessment experiments four days post cGAMP administration mice were deeply anaesthetized and the primary tumors were surgically excised. After next 16 days lung metastases were visualized by intratracheal injection of 15% India Ink solution. To assess STING agonist-induced reprogramming of lung immune cells, twelve hours post cGAMP administration lungs were collected and digested. The isolated cells were stained with antibodies against: neutrophils, monocytes macrophages, NK cells, T lymphocytes. The analyzes were performed with flow cytometry, gates dividing negative from positive cells were based on fluorescence minus one (FMO) control.

Result and discussion

Intratumoral administration of STING agonist resulted in the appearance of luminescence signal around the tumors twelve hours post treatment. Both cGAMP-treated and control mice exhibited luminescence signal in the area of lungs. Local - intratumor administration of STING agonist decreased the number and size of spontaneous 4T1 lung metastases. STING agonist induced decreased in the number of lung neutrophils with simultaneous switch of their phenotype toward anti-tumor one.

Increase of pro-inflammatory monocytes (CD11b+ Ly6Chi) and polarization of pro-tumor (MHC-II- CD206+; CD86+CD206-) macrophages toward anti-

tumor one (MHC-II+CD206-; CD86+CD206-) was noted. STING agonist increased infiltration and activation of NK cells (CD49b+NKP46+; CD49b+ CD69+) and decreased anergic (CD8+PD-1+; CD8+ CD69-) T lymphocytes number.

Conclusion

Local treatment with STING agonist induces systemic immune response, enabling reprogramming of lung immune cells phenotype that leads to decrease of metastasis spread in murine breast carcinoma model.

The work is a result of the research project no. UMO-2019/35/N/NZ5/02506, financed by National Science Centre

EACR25-0974

Tumor-infiltrating immune cell types predicting recurrence-free survival in melanoma patients receiving adjuvant PD-1 inhibitor therapy

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Introduction

Adjuvant treatment of melanoma patients with PD-1-based immunotherapy has improved recurrence rate, and became a standard treatment. However, a considerable proportion of patients still recur within 1-2 years, necessitating the identification of predictive markers. Our study aimed to examine the association of intratumoral infiltration by specific immune cell subsets with the recurrence-free survival of melanoma patients receiving adjuvant PD-1 inhibitor therapy.

Material and method

Archived paraffin blocks of pretreatment surgical samples (71 lymph node and 12 skin/subcutaneous metastases) from melanoma patients receiving adjuvant PD-1 inhibitor therapy at the National Institute of Oncology between 2019 and 2024 (nivolumab n = 20, pembrolizumab n = 28) were selected. The intratumoral density of immune cells expressing the following markers: CD8, FOXP3, CD20, CD103, OX40, PD-1, and PD-L1 were determined by immunohistochemistry, and the associations with recurrence-free survival were analyzed. The follow-up time was median 46 months (14–72). The Fisher's exact test was used for statistical analysis comparing the proportions of cases in different groups, and Kaplan-Meier analysis with log-rank test for calculating survival differences.

Result and discussion

Eighteen of the 48 patients developed recurrence during the follow-up period. In this group the ratio of patients showing strong immune cell infiltration was significantly lower compared to recurrence-free patients in the case of CD8+ T cells (2/18 vs. 16/30, p = 0.0050), CD103+ tissue resident T cells (2/18 vs. 13/30, p = 0.0259), and the activation/checkpoint markers OX40 (4/17

vs. 19/30, p = 0.0145) and PD-1 (2/18 vs. 14/30, p = 0.0134). High intratumoral density of the above cell types was accompanied with significantly longer recurrence-free survival.

Conclusion

Our findings indicate the importance of immune cell infiltration in the efficacy of adjuvant PD-1 inhibitor treatment in a “real world” patient cohort.

The work was supported by the National Research, Development and Innovation Office, National Laboratories Program (project: National Tumor Biology Laboratory, 2022-2.1.1-NL-2022-00010).

EACR25-1001

Pioneer Platform: A Novel Biotherapeutic Antibody Discovery Platform

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Introduction

The Pioneer™ Antibody Discovery Platform by Bio-Rad is designed for biotherapeutic lead generation. Central to this platform is the Pioneer Antibody Library, which has 2.25×10^{11} unique antibodies and is optimized for therapeutic candidate selection. The platform uses SpyDisplay, a novel selection system based on SpyTag technology, enabling rapid selection of high-affinity antibodies. These candidates can be integrated with Bio-Rad’s TrailBlazer™ modular antibody assembly platform for versatile screening. This poster presents data on antibody selection against several antigens, showing the platform’s capability to deliver diverse, high-affinity leads. Using anti-TIGIT antibodies as an example, the performance is comparable to clinical trial antibodies.

Material and method

Library Design: The Pioneer Antibody Library comprises 2.25×10^{11} unique antibodies, with 96% functionality confirmed by next-generation sequencing (NGS).

Selection Technology: The SpyDisplay system uses a SpyTag/SpyCatcher technology to display Fab fragments on *E. coli*, enabling a rapid one-day panning protocol without subcloning. **Screening and Characterization:** After phage selections, unique antibodies were expressed and purified in Fab format. Monovalent binding affinities were determined using bio-layer interferometry (BLI). Functional assays, including the TIGIT/CD155 blockade bioassay, evaluated antibody efficacy

Result and discussion

Library Quality: The Pioneer Library showed high sequence diversity and quality, with reduced post-translational modification sites in CDRs. The design focused on germlines with favorable properties, increasing the probability of selecting useful hits.

Selection Efficiency: The SpyDisplay system was efficient, yielding high-affinity antibodies. The absence of a Fab-pIII fusion resulted in correctly folded and displayed Fabs. **Anti-TIGIT Antibody Selection:** The platform selected high-affinity, potent antibodies against TIGIT. Of the identified antibodies, 25% had affinities below 1 nM. The selected antibodies exhibited high diversity and covered nine distinct epitope bins.

Functional Assays: The anti-TIGIT antibodies showed comparable or superior performance to benchmark

antibodies in a TIGIT/CD155 blockade bioassay. They demonstrated good developability parameters, including stability and low polyreactivity.

Conclusion

In summary, the Pioneer Antibody Discovery Platform, with its large and diverse library, efficient selection technology, and robust screening capabilities, is a powerful tool for generating high-affinity therapeutic antibodies. The platform’s ability to deliver lead candidates comparable to clinical-stage antibodies highlights its potential in biotherapeutic development.

EACR25-1026

Novel Harnessing of Innate and Adaptive Anti-Cancer Immunity: NLRP3-Inflammasome Activation Facilitates Immune Checkpoint Blockade in the Treatment of Mesenchymal Stage IV Colorectal Cancer

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Introduction

The role of the NLRP3 inflammasome in colorectal cancer (CRC) progression remains unclear (1). While some studies link high NLRP3 activity to cancer progression, others suggest protective roles, depending on the clinical context (2–4). Clinical trials mainly focus on inhibition of NLRP3 activity (5). This highlights the urgent medical need for the evaluation and development of targeted NLRP3-activating therapies in tumors where activation could provide therapeutic benefit. Hence, we evaluated the effect of NLRP3 activation in the treatment of mesenchymal stage IV CRC in an organoid-based metastatic CRC mouse model *in vivo* and in organoid-immune-cell-cocultures *in vitro*.

Material and method

In vitro experiments involved coculturing of peritoneal mouse immune cells with murine CRC organoids.

Within, NLRP3 inflammasome activation was induced using EMT-244, a novel and potent NLRP3 activator. Results were validated utilizing immune cells from mice harboring a CRE-inducible active NLRP3 mutant.

Therapeutic effects on the coculture were quantified using viability assays. **In vivo**, we employed an orthotopic, organoid-driven mesenchymal stage IV CRC mouse model, which mimics an aggressive and treatment-resistant human CRC subtype (6). To evaluate the impact of EMT-244 in combination with immune checkpoint blockade (ICB: anti-PD-1 antibody) on the metastatic process, tumor samples were analyzed using macroscopic and microscopic approaches, including Ki67 staining to assess proliferation and TUNEL staining to detect apoptosis.

Result and discussion

In vitro, we demonstrated an EMT-244-dependent reduction of CRC organoid viability during coculture with peritoneal murine immune cells ($p = 0.0498$). Importantly, this effect was not abundant when treating

CRC organoids alone, highlighting the necessity of an immunological tumor microenvironment. We identified a concomitant upregulation of pro-apoptosis-related proteins, e.g. ‘cleaved Caspase-8 and -3’ in respective coculture. In vivo experiments using our organoid-driven stage IV CRC mouse model revealed that EMT-244, in combination with ICB, significantly reduced liver and even more drastically peritoneal metastatic burden compared to ICB monotherapy ($p = 0.0114$ and $p = 0.0026$), which was also confirmed on histological level ($p = 0.0006$). Furthermore, liver metastases exhibited a significant decrease in Ki67 positivity ($p = 0.0157$) and an increase in TUNEL positivity ($p = 0.0388$) in the EMT-244 + ICB group, indicating reduced proliferation and increased apoptosis.

Conclusion

NLRP3 inflammasome activation by EMT-244 facilitates ICB in mesenchymal stage IV CRC, presenting a promising new treatment approach, particularly as a first-line immunotherapy for metastases. This study highlights the critical need for a paradigm shift towards investigating NLRP3 inflammasome-activating therapies in the context of CRC disease progression.

EACR25-1028

Immunopeptidomics approach for the identification of neoantigens in multiple myeloma

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Introduction

Multiple myeloma (MM) is a clonal plasma cell malignancy characterised by abnormal production of monoclonal proteins (M proteins). It accounts for approximately 10% of all haematological malignancies and remains incurable despite therapeutic advances. Immunotherapeutic strategies, including chimeric antigen receptor (CAR) T-cell therapy, monoclonal antibodies, antibody-drug conjugates and bispecific antibodies, have demonstrated the potential to harness the immune system to target MM cells. Adoptive T-cell receptor (TCR)-T cell therapy, which targets tumour-specific neoantigens presented via the human leukocyte antigen (HLA) complex, is not limited to extracellular proteins, thereby broadening the spectrum of targetable antigens. Early

studies show promising results and a favourable safety profile.

Material and method

We used an immunopeptidomics approach to identify neoantigens in MM. Whole exome sequencing (WES) and RNA sequencing (RNAseq) of MM cell lines, primary MM tissue from osteolytic lesions and autologous PBMCs (as healthy controls) were combined with immunoprecipitation of HLA peptide complexes (pHLA) and liquid chromatography-tandem mass spectrometry (LC-MS/MS) analysis of myeloma samples. Candidate neoantigens (NeoAgs) were rigorously validated based on MS spectra and a database search was performed to exclude single nucleotide polymorphisms (SNPs) and canonical RNA editing events. To assess immuno-genicity, HLA-matched allogeneic and autologous PBMCs were stimulated in an accelerated co-cultured dendritic cell (acDC) assay followed by IFN γ ELISpot readout. Neoantigen-specific TCRs were analyzed by comparing the clonal frequencies of unstimulated and stimulated cells using single-cell RNA sequencing (scRNAseq).

Result and discussion

A total of twelve neoantigens were identified in three MM cell lines and seven in eight primary tumour samples. In MM cell lines, neoantigens were derived equally from DNA and RNA variants, whereas in primary tumour tissue, most were derived from RNA variants. Four neoantigens from MM cell lines showed immunogenicity. Three were derived from somatic mutations in PRPF8, FBXL6 and IQGAP2, while the fourth immunogenic candidate arose from an RNA variant in an IgV pseudogene. One neoantigen from MM cell lines and three from primary tumour tissue were used in an scRNAseq approach to identify neoantigen-specific TCRs. The 20 clones with the highest fold change in clonal frequency are currently being tested for reactivity.

Conclusion

Mass spectrometry-based immunopeptidomics allows the identification of neoantigens in multiple myeloma with potential for personalised TCR-based therapies. However, given the low mutational burden of MM, direct neoantigen identification remains challenging, highlighting the need to enhance MS sensitivity for improved detection.

EACR25-1044

Bridging the Gap Between In Vitro and In Vivo: STC-1010 Cancer Vaccine Efficacy in 3D Tumor Spheroids

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Introduction

Immunotherapy has revolutionized cancer treatment, but low-immunogenicity tumors often escape immune detection. To address this challenge, Brenus Pharma developed the Stimulated Ghost Cells (SGC) platform, an allogeneic cancer vaccine technology that enhances immunogenicity by expanding antigen coverage. It

overcomes also previous cancer vaccines challenges thanks to a standardized manufacturing process increasing access for patient. Here, we present STC-1010, a therapeutic colorectal cancer (CRC) vaccine, developed by exposing HCT116, HT-29, and LoVo cell lines to stress conditions – serum deprivation, irradiation, heat shock, and chemotherapy – mimicking standard CRC treatments and relapse conditions. The cells were then haptenated to boost immune response induction.

Material and method

Initial validation in a 2D ex vivo model demonstrated STC-1010's anti-tumor efficacy: Functional validation via ex vivo immune activation assays using monocyte-derived dendritic cells (mDCs) from several donors demonstrated STC-1010's immunostimulatory capacity.

Result and discussion

Vaccine-exposed mDCs showed increased IL-8 secretion, enhanced antigen processing and presentation, and activation of IL-6/JAK/STAT3 and phagosome pathways, confirmed by GSVA using KEGG/Hallmark databases. STC-1010 successfully primed mDCs, with haptenated epitopes detected. Primed CD8+ T cells induced higher apoptosis in HCT116, HT-29, and SW620 cells compared to controls. However, conventional 2D models inadequately replicate tumor complexity, contributing to high failure rates in clinical translation. To better mimic the in vivo tumor microenvironment, we developed 3D CRC spheroids by co-aggregating HCT116, HT29, SW620, and LoVo cells with colorectal fibroblasts (CCD-18Co). Tumor cells were fluorescently labeled to monitor viability in co-culture with stromal and immune cells. Spheroids were characterized by bright-field microscopy, fluorescence analysis, and histology. Quality control (QC) and release criteria were established, including tumor size evaluation and chemotherapy response. Additionally, the 3D CRC spheroid models were co-cultured with T-cells, and their activation was assessed by monitoring tumor killing and cytokine secretion. Furthermore, we developed a process to enable preservation of the 3D spheroids – an essential step toward generating off-the-shelf preserved spheroid panels for STC-1010 potency testing.

Conclusion

This innovation overcomes ethical, financial, and time limitations of in vivo models while preserving key tumor resistance mechanisms. Our results support STC-1010 as a promising immunotherapy and demonstrate the value of 3D spheroids as reliable preclinical tools for evaluating new treatments. Overall, this work enhances strategies to overcome immunotherapy resistance and accelerates translational research with scalable, physiologically relevant models.

EACR25-1120

CAM Assay: An Alternative, 3Rs-compliant, In Vivo Model for the Development of Anticancer Immunotherapies

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Introduction

Recently, immunotherapy has rapidly become the "fifth pillar" of cancer treatment, along with surgery, radiotherapy, chemotherapy, and other targeted treatments. Various categories of immuno-oncology (IO) treatments have been developed, including monoclonal antibodies, Antibody Drug Conjugates (ADCs), Immune Checkpoint Inhibitors (ICIs), vaccines, adoptive cell therapies, immune system modulators. During pre-clinical phase, rodents still represent the most commonly used in vivo model. However, these models have several drawbacks, such as the immunodeficiency of humanized models, ethical constraints, time requirements and a high cost. Therefore, an alternative, pertinent, 3Rs-compliant in vivo model is strongly needed for accelerating cancer immunotherapy research. In this work, the chicken ChorioAllantoic Membrane (CAM) assay is used to investigate different types of IO treatments.

Material and method

On embryonic development day (EDD) 9, different human tumor cell lines are grafted on the upper CAM. A treatment with different immunotherapy reagents is performed between EDD10 and EDD18: monoclonal Abs (bevacizumab, trastuzumab, rituximab) in 4 doses; ADC (trastuzumab emtansine) in 1 dose; ICIs (pembrolizumab, nivolumab, atezolizumab, avelumab) in 5 doses; tumor cell based vaccine (STC1010) in 3 doses; CAR-T cells & CAR-NK cells in 1 dose. IO reagents are evaluated in monotherapy or in combination with other drugs. The in ovo part ends at EDD18. Anti-cancer efficacy is evaluated on tumor growth through tumor mass weight, on metastatic invasion through Alu sequence detection in the lower CAM based on the qPCR, on angiogenesis development by counting the vessels surrounding the tumor, on immune response activation through tumor infiltrating immune biomarker quantification based on the RT-qPCR.

Result and discussion

The efficacy of different IO reagents is revealed in ovo: all treatments lead to a significant tumor growth regression (24.79%-49.53%), on different tumor models. Additional analyses show more conclusive evidence, including metastasis regression, the inhibition of angiogenesis development, as well as immune activation and immune cell infiltration in the tumor. Furthermore, the combination regimens show increased anti-tumoral potential when compared to the monotherapy.

Conclusion

Our work demonstrates that the CAM assay is suitable for testing different IO anticancer treatments. The presence of an active immune system allows a suitable characterization of the effects of immunotherapy reagents on tumor cells, the microenvironment, and the entire organism. As one part of New Approach Methodologies (NAMs), the CAM assay provides a relevant alternative, 3Rs-compliant, in vivo model for testing novel immunotherapies on a large spectrum of cancer types, as a monotherapy or as an in combination approach.

EACR25-1140

Targeting UMG1, a highly glycosylated cancer specific CD43 epitope, by a

Bispecific T-Cell Engager (BTCE) in Malignant Melanoma

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Introduction

Malignant Melanoma (MM) is an aggressive disease of melanocytes, characterized by high metastatic potential and mortality rate. Although recent immunotherapies and targeted agents have improved outcome, there remains a pressing need for additional therapeutic strategies. Bispecific T-cell Engagers (BTCEs) have recently emerged as a promising approach to harness the immune system by directing T cells specifically against tumor cells, gaining increasing relevance in the evolving therapeutic landscape. UMG1 is a unique epitope of CD43 that differs from the molecule's canonical expression profile. While CD43 is broadly expressed across many cell types, thus limiting its suitability for targeted interventions, the UMG1 epitope is notably restricted to cortical thymocytes and a small subset of peripheral T lymphocytes, becoming prominent only when CD43 is aberrantly expressed in malignancies. This distinctive pattern makes UMG1 an appealing immuno-therapeutic target. Previous studies have demonstrated potent antitumor activity of UMG1/CD3ε-BTCE in hematologic malignancies, prompting further investigation of UMG1 as a tumor-specific target in MM.

Material and method

UMG1 reactivity was assessed by immunohistochemistry on Tissue Microarrays (TMAs) and pathology slides from MM specimens. Immunofluorescence was performed on primary and metastatic MM cell lines to confirm UMG1 expression. UMG1-positive MM cells were co-cultured with healthy donor-derived Peripheral Blood Mononuclear Cells (PBMCs) in the presence of escalating concentrations of UMG1/CD3ε-BTCE for 72 hours. Cytotoxicity was measured via tumor cell viability, and T-cell activation was evaluated using markers CD69, CD25, and CD107a.

Result and discussion

Approximately 50% of MM samples showed variable membrane staining for UMG1, while normal skin tissues were negative. Immunofluorescence confirmed these findings in both primary and metastatic MM cell lines, underscoring UMG1's role as a MM-specific marker. UMG1/CD3ε-BTCE induced a robust, dose-dependent T cell-mediated cytotoxic response against UMG1-positive MM cells. This response occurred together with significant upregulation of CD69 and CD25, and enhanced CD107a expression on CD8+ T cells, reflecting effective T-cell activation and degranulation. These results indicate that targeting the UMG1 epitope effectively engages T cells to eliminate MM cells.

Conclusion

UMG1 is a novel promising therapeutic target in MM due to its distinct, tumor-specific expression. UMG1/CD3ε-BTCE elicits potent T cell responses and significant cytotoxicity, offering a novel immunotherapy approach. These findings support *in vivo* investigations to validate the translational potential of UMG1-targeted therapy against MM.

EACR25-1142

Exploiting human ALK.CAR-T cell therapy for the treatment of ALK+ small cell lung cancer

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Introduction

Small cell lung cancer (SCLC), representing 15% of all lung cancers, is an aggressive and highly metastatic neuroendocrine carcinoma of the lung with an extremely poor prognosis. SCLC is classified into four molecular subtypes based on the expression of key transcriptional factors, with ASCL1 or NEUROD1 subgroups accounting for the vast majority of cases. Nevertheless, SCLC biology remains largely unexplored, highlighting the urgent need for preclinical studies to investigate more effective therapeutic strategies. Anaplastic lymphoma kinase (ALK) is a receptor tyrosine kinase expressed in specific regions of the nervous systems. Given the neuroendocrine nature of SCLC, we evaluated ALK expression in a subset of SCLC cell lines.

Material and method

Correlation studies between ALK and NEUROD1 expression were conducted *in silico* using the SCLC CellMiner Cross Database. ALK, NEUROD1 and ASCL1 expression were evaluated by Western blot, while ALK extracellular expression was confirmed by flow cytometry. NEUROD1 knock-out was performed by CRISPR-Cas9 genome editing. Human ALK.CAR-T cells were generated using CD3+ cells from healthy donors. Luciferase-GFP-expressing (Luc-GFP) COR-L279 and H82 ALK+ SCLC cell lines were exposed to hALK.CAR-T and killing activity was measured by luciferin addition 72h post-coculture. INFγ, TNFα, Perforin and Granzyme B levels were assessed by intracellular flow cytometry.

Result and discussion

Bioinformatic analysis revealed an overall positive correlation between NEUROD1 and ALK expression in SCLC cell lines, which we verified *in vitro* in NEUROD1+ SCLC cells by Western Blot. Upon NEUROD1 knock out, ALK expression was lost both at the transcriptional and protein level. Remarkably,

NEUROD1 knock out in SCLC cells was also associated with the downregulation of neuroendocrine differentiation markers such as Synaptophysin and Chromogranin A, along with the upregulation of the epithelial marker E-cadherin, indicating a transcriptional rewiring towards a more epithelial phenotype.

Susceptibility of COR-L279 and H82 ALK⁺ SCLC cells to hALK.CAR-T cells was then evaluated. Both cell lines were significantly and specifically killed in vitro by hALK.CAR-T cells in a dose-response manner. Large amounts of Perforin, Granzyme B, and pro-inflammatory cytokines (i.e., IFNg and TNFa) were released when hALK.CAR-T cells were challenged with ALK⁺ tumor cells. In vivo studies to confirm these findings are currently ongoing and will be presented at the meeting.

Conclusion

Although further investigation and clinical validation of ALK-targeted immunotherapy are required, targeting ALK with CAR-T cells could represent a promising strategy to overcome current treatment limitations and improve patient outcome in a subset of ALK⁺ SCLC.

EACR25-1143

Understanding bystander killing by bispecific monoclonal antibodies

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Introduction

Bispecific T cell engaging monoclonal antibodies (BiMAbs) provide an exciting immunotherapy option as they simultaneously bind an antigen on tumour cells and a surface molecule on T cells drawing the T cells to the tumour to induce antibody mediated tumour lysis (Labrijn et al. 2019). This is, so far, the only antibody based immunotherapy that can kill bystander target negative cells (Bodmer and Golubovskaya 2023). This is highly advantageous for successful treatment since cancer is generally heterogeneous with respect to expression of potential targets for immune attack.

BiMAbs can trigger bystander killing by T cells that have been activated by killing target-positive cancer cells and then killing target-negative cancer cells in their vicinity (Ross et al. 2017). However, much is still unknown about the mechanism of action as to how the activated T-cells kill surrounding target negative cancer cells (Meier et al. 2022). This project aims to study the basic biology of bystander effects by different populations of T-cells.

Material and method

By treating cells from a panel of colorectal cancer derived cell lines with BiMAbs and peripheral blood mononuclear cells (PBMCs), we investigated targeted immune responses against target-positive cells. Target-positive cell lines were treated with BiMAbs and PBMCs. Following treatment, activated CD4⁺ and CD8⁺ T cells were sorted to evaluate their cytotoxic roles. A variety of viability assays, including the M30 CytoDeath™ ELISA kit, were used to quantify apoptosis while the xCELLigence Real-Time Cell Analysis provided a direct quantitative readout of cell killing.

Result and discussion

Our data suggests that PBMCs activated by BiMAbs though attaching them to appropriately targeted cancer cells, mediate bystander killing by killing non-target cancer cells through binding to ICAM-1 and other possible non-specific receptors. The expression of these non-specific receptors is induced in the cancer cells by cytokines released by the T cell activation. Preliminary data has shown that, for example, ICAM-1 blockade partially inhibited activated T cell killing of cells negative for the BiMAb target, suggesting that the activated T cell attack of ICAM-1 is contributing to bystander killing.

Conclusion

These findings highlight the potential of bystander killing by BiMAbs of target negative cancer cells and provide insights for optimizing BiMAb-based anti-cancer immunotherapies.

EACR25-1156

Redirecting cytomegalovirus immunity against pancreas cancer for immunotherapy

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Introduction

Immunotherapy has had limited success in pancreatic cancer, largely due to a low mutational burden and immunosuppressive microenvironment. Here we hypothesized that pre-existing antiviral immunity could be redirected to control pancreatic cancer growth. Cytomegalovirus (CMV, a β-herpesvirus) was chosen, as the majority of the population has been previously infected and it induces an extremely large/broad memory T cell response.

Material and method

Mice latently infected with murine CMV (MCMV) were orthotopically implanted with pancreatic cancer cells and treated with systemic injections of MCMV T-cell epitopes.

Result and discussion

The therapy promoted preferential accumulation of MCMV-specific T cells within pancreatic cancer, delaying tumor growth and increasing survival. Immuno-phenotyping and scRNAseq analyses showed these T cells were highly activated and cytotoxic, leading to increased tumor necrosis and caspase-3 activation.

Finally, therapy was enhanced when combined with low dose gemcitabine chemotherapy. Together, these results show that CMV-specific T cells can be repurposed to combat pancreatic cancer.

Conclusion

Our studies reveal that CMV immunity can be re-directed to control a solid tumor normally refractory to immunotherapy via intravenous injection of viral epitopes. This mutation agnostic approach has significant potential for development of “off-the-shelf” therapeutics by

stimulating pre-existing antiviral T cells and is widely applicable due to high prevalence of CMV immunity.

EACR25-1196

Comparative analysis of neoadjuvant PD1+Lenvatinib versus PD1 alone in the tumour immune microenvironment of melanoma: Correlation of immune profiling with pathological response.

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Introduction

Neoadjuvant immunotherapy (NeoIT) has emerged as a transformative approach for resectable stage III melanoma, with a major pathological response (MPR) being a strong predictor of recurrence-free survival. However, the impact of different PD-1 based regimens on the tumour microenvironment (TME), and their association with response, remains unclear. This study examines the immune profiles in the TME from patients (pts) receiving neoadjuvant anti-PD1 alone (PD1 alone) versus PD1+Lenvatinib (NeoIT-L), to identify treatment specific features associated with pathological response.

Material and method

Stage III melanoma pts treated with 6 weeks of NeoIT-L (NCT04207086) or PD1 alone (NCT02858921) were included. Multiplex immunohistochemistry (mIHC) and RNA sequencing (RNAseq) analysis were performed on tumour samples collected at baseline (BL) and at week 6 (wk6) post treatment.

Result and discussion

Of 40 pts included, 19 had PD1 alone (7 [37%] had MPR ($\leq 10\%$ viable tumour cells at wk6) and 21 had NeoIT-L (12 [57%] had MPR). As previously reported by our group, mIHC analysis confirmed that in NeoIT-L MPR pts, mature follicular B cells (CD21+CXCR5+ B cells) ($p = 0.03$) and lymphoid aggregates ($p = 0.02$) significantly increase from BL to wk6. These changes were not observed in non-MPR pts. In contrast, no significant changes, in these cell types, were detected in mIHC, from BL to wk6, in pts treated with PD1 alone irrespective of response. When analysing gene signatures, NeoIT-L induced a significant upregulation of B-cell receptor (BCR) and T-cell receptor (TCR) signalling along with downregulation of cell cycle pathways, including the G2M checkpoint ($p < 0.05$), in MPR pts, but not in non-MPR. PD1 alone led to an upregulation of antigen processing, chemokine and cytokine signalling, including IFN- γ and TNF- α pathways ($p < 0.05$), in MPR pts, but not in non-MPR. When analysing 897 genes that were commonly upregulated from BL to wk6 in MPR pts from both treatment groups, we found these were specifically enriched for chemokine signalling pathways ($p < 0.001$). When the unique differentially expressed genes were directly compared between treatments, NeoIT-L specifically promoted BCR signalling and down-regulation of cell cycle pathways ($p < 0.05$). In contrast,

no distinct pathways were exclusive to PD-1 alone, as some genes related to IFN- γ response for example in fact overlapped between cohorts.

Conclusion

NeoIT-L and PD-1 alone induce distinct immune changes linked to pathological response. NeoIT-L responders exhibited changes in BCR/TCR signalling and suppressed cell cycle pathways suggesting enhanced immune activation and reduced tumour proliferation with the addition of a short duration (6 weeks) of lenvatinib. In contrast, PD-1 alone responders showed changes in antigen processing and cytokine signalling without significant cell cycle changes. Ongoing CyTOF analysis will further characterise the peripheral immune changes.

EACR25-1203

A novel synthetic peptide HS1002 Enhances T Cell-Mediated Antitumor Immunity

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Introduction

T cells play a crucial role in antitumor immunity. However, their function is frequently suppressed within the tumor microenvironment, limiting the efficacy of immunotherapy. Treatment with hTERT-derived peptides has been shown to enhance the production of effector T cells. HS1002, a novel synthetic peptide, was designed using sequences from gonadotropin-releasing hormone (GnRH) and human telomerase reverse transcriptase (hTERT). The present study aimed to evaluate the ability of HS1002 to enhance T cell-mediated immunity and improve the effectiveness of anti-PD-1 therapy.

Material and method

To assess the immunostimulatory effects of HS1002, ELISpot assays were performed to measure IFN- γ secretion in PBMCs. The Incucyte ZOOM system was used to evaluate PBMC-mediated cytotoxicity against LNCaP cells. Cytokine array analysis was conducted to characterize HS1002-induced changes in cytokine secretion. Flow cytometry was performed to analyze immune cell populations in syngeneic tumor models.

Result and discussion

HS1002 treatment increased IFN- γ secretion and enhanced PBMC cytotoxicity against LNCaP cells. Cytokine profiling revealed elevated levels of CXCL1, CXCL10, CCL2, TNF- α , and IL-2, indicating a heightened immune response. To evaluate the antitumor effects of HS1002, tumor growth inhibition was assessed in syngeneic tumor models. HS1002 significantly reduced tumor volume in the MC38 model, whereas no significant effect was observed in the 4T1 model, suggesting that tumor immune environment influences HS1002 responsiveness. Flow cytometry analysis showed increased CD45 $^+$ immune cell infiltration and CD8 $^+$ T cell activation, as evidenced by elevated granzyme B and IFN- γ expression in MC38 tumors. Gene analysis further confirmed enrichment of immune-related pathways in HS1002-treated MC38 tumors. In the poorly immunogenic 4T1 model, co-administration of HS1002 with anti-PD-1 antibody significantly reduced tumor volume and

increased immune cell infiltration and T cell populations, which were diminished by anti-PD-1 monotherapy. While PD-1 expression on T cells remained unchanged, CD8⁺ T cells in the combination group exhibited increased granzyme B and IFN- γ expression, suggesting an enhancement in cytotoxic activity.

Conclusion

HS1002 enhances T cell-mediated antitumor immunity by promoting immune cell infiltration and T cell function. Moreover, its synergy with anti-PD-1 therapy highlights its potential as a promising immunotherapeutic strategy.

EACR25-1288

CD25-Biased IL-2/Anti-IL-2 Complexes Enhance Antigen-Primed CD8⁺ T Cell Expansion, Overcome Treg-Mediated Suppression, and Exhibit Significant Antitumor Activity

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Introduction

Interleukin-2 (IL-2) is essential for T cell proliferation, survival, and effector function. However, its clinical use in cancer immunotherapy is hampered by severe toxicity and the unintended expansion of regulatory T (Treg) cells. Complexes of IL-2 and anti-IL-2 monoclonal antibodies have previously demonstrated high biological activity in vivo. In particular, IL-2/anti-IL-2 mAb JES6-1A12 complexes (IL-2/JES6) are highly selective and potently stimulate the expansion of CD25⁺ cell populations. Our study examines whether these CD25-biased IL-2/JES6 complexes can selectively expand antigen-primed CD8⁺ T cells while mitigating Treg-mediated suppression. The primary objective is to assess and optimize these complexes for further application in cancer immunotherapy.

Material and method

We employed an adoptive transfer model using OT-I CD8⁺ T cells in congenic B6 mice primed with ovalbumin. The expansion of transferred CD8⁺ T cells and the expression of activation markers (CD25, granzyme B, and perforin) were analyzed by flow cytometry. In vitro Treg suppression assays were performed to assess the ability of IL-2/JES6 complexes to counteract Treg-mediated inhibition, and in vivo antitumor efficacy was evaluated in murine models of glioma (GL261) and colon carcinoma (CT26).

Result and discussion

CD25-biased IL-2/JES6 complexes induced robust expansion of antigen-primed OT-I CD8⁺ T cells and enhanced their expression of CD25, granzyme B, and perforin. Although Treg cells were also expanded, in vitro assays confirmed that saturating doses of IL-2/JES6 effectively overcame Treg-mediated suppression. Notably, treatment with IL-2/JES6 led to significant antitumor activity in both GL261 and CT26 models, supporting the potential of this strategy for tumor immunotherapy.

Conclusion

Despite the concurrent expansion of Treg cells, CD25-biased IL-2/JES6 complexes enhance CD8⁺ T cell effector functions and overcome Treg-mediated suppression to achieve marked antitumor responses. Future work will focus on optimizing dosing regimens and advancing clinical translation to maximize therapeutic benefit.

EACR25-1425

Induction of TWIST1-specific antitumor immunity by DNA vaccine encoding a truncated TWIST1 antigen

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Introduction

TWIST1, a basic helix-loop-helix transcription factor, is overexpressed in a variety of tumors such as melanoma, breast, colon cancer and mesothelioma, and associated with tumorigenesis and metastasis. We have previously shown that full-length TWIST1 vaccine elicited cytotoxic T lymphocytes (CTLs) that recognized TWIST1-expressing tumors and conferred protection against tumor challenge in the form of DNA vaccine and adenovirus-associated viral vector system, highlighting TWIST1 as a promising target for cancer immunotherapy. In this study, we sought to apply optimization approach on the TWIST1-based vaccine platform in order to enhance T cell immunity and reduce potential toxicity.

Material and method

We developed a novel soluble PD-1 (sPD1)-based DNA vaccine encoding a truncated version of TWIST1 (sPD1-TruncT1) that includes a conserved region found in both human and mouse proteins. The immunogenicity and antitumor efficacy of the TruncT1 vaccine were evaluated and compared in parallel to full-length TWIST1 vaccine in two murine models of TWIST1-expressing tumors.

Result and discussion

By doing epitope mapping, we identified immunodominant T cell epitopes within the TWIST1 antigen, showing that majority of T cells recognized a C-terminal conserved region. sPD1-based DNA vaccine encoding epitopes of this conserved region, via in vivo electroporation (EP), elicited more robust TWIST1-specific T cell responses compared to full-length TWIST1 vaccine. Furthermore, these TWIST1-specific T cells were polyfunctional because they produced multiple effector cytokines when encountering TWIST1-expressing tumors. In addition, immunotherapy with the sPD1-TruncT1 vaccine showed a notable enhancement in antitumor efficacy in both preventive and therapeutic scenarios.

Conclusion

With the widespread expression of TWIST1 in different cancer types, truncated TWIST1 vaccination has high potential for cancer immunotherapy and warrants investigation in human clinical trials to evaluate its

applications as a vaccine for patients with WT1-expressing cancers.

EACR25-1428

RALA-PRAME DNA Vaccine: A Novel Immunotherapy for High-Grade Serous Ovarian Carcinoma

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Introduction

High grade serous ovarian carcinoma (HGSC), accounting for >75% of ovarian cancer, is a highly aggressive gynaecological cancer with ≤30% 5-year and <15% 10-year overall survival rate. This study addresses the unmet clinical need for effective immunotherapeutic strategies by leveraging the antigens overexpressed in the tumour microenvironment to develop a pDNA vaccine for the treatment of HGSC. DNA vaccines produce higher CD8+ CTL response along with a CD4+ T-cell response in comparison to other vaccination strategies. The vaccine was delivered using nanoparticles (NPs) formulated with a cationic cell penetrating peptide RALA designed to encapsulate anionic nucleic acid cargo and overcome the barriers of naked DNA delivery, facilitating cellular entry.

Material and method

Antigen expression was confirmed using multi-dataset analyses. A plasmid was designed encoding the selected antigen (PRAME), which is overexpressed in all stages of HGSC. pPRAME was complexed with RALA to make NPs. The size, charge and PDI was measured using a DLS zetasizer and encapsulation efficiency was measured by Ion exchange Chromatography. In vitro transfection efficiency of the NPs was measured in NCTC-929 and D.C 2.4 cells using ELISA. The optimal NPs were lyophilised with trehalose and assessed in a murine (C57BL/6) model to analyse the immune response and therapeutic effect of the NPs.

Result and discussion

Elevated PRAME expression was observed throughout the different stages of HGSC in analysed datasets. Physicochemical characterisation revealed optimal RALA/pPRAME NPs with size (< 150 nm), high encapsulation efficiency (>95%) and stability upon lyophilisation with Trehalose. In vitro transfection demonstrated significant overexpression of PRAME in D.C 2.4 and NCTC-929 cells with cell viability >80%. In vivo C57BL/6 mice immunised with RALA/pPRAME NPs elicited robust immune response, evidenced by ELISpot analysis showing higher INF-γ+ splenocytes with both RALA/pPRAME and RALA/pPRAME+GM-CSF vaccine groups ($p < 0.05$) compared to GM-CSF only. Therapeutic efficacy was evaluated in a E0771 PRAME overexpression tumour model, demonstrating

reduced tumour burden in RALA/pPRAME NP treated groups compared to controls.

Conclusion

The findings in this study demonstrate RALA/pPRAME NPs as a promising vaccine platform for therapeutic vaccination against PRAME overexpressing HGSC with strong potential for clinical translation.

EACR25-1486

Establishment of Ex-Vivo Cultures from Pleural Effusions of Metastatic NSCLC Patients as a Model to Study Response to Immune Checkpoint Inhibitors

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Introduction

A significant challenge in studying cancer immunotherapy is the lack of robust, accessible experimental models that can closely represent human tumors and immune responses. We developed an ex-vivo organ culture (EVOC) model using malignant pleural effusions (PEs) to investigate immunotherapy responses in advanced non-small cell lung cancer (NSCLC) patients. PEs, accumulation of abnormal fluid in the pleural space surrounding each lung, occur in approximately 20% of NSCLC patients and are associated with worse prognosis. Our project's main goal was to develop a tool to study response to immune checkpoint inhibitors (ICIs). To achieve this, we established EVOCs from PEs of metastatic NSCLC patients, serving as a valuable model to investigate the mechanisms of ICI response and resistance.

Material and method

PEs were collected from metastatic NSCLC patients at Sheba Medical Center. Cell populations were characterized by flow cytometry and immunohistochemistry. Freshly isolated cells were cultured for 4 days and treated with ICIs: Durvalumab (anti-PD-L1) alone or combined with Tremelimumab (anti-CTLA4) and IL2 as an immune activator. Response was assessed by IFNγ protein secretion (ELISA) and gene induction (RT-PCR). Bulk RNA seq analysis was conducted to find differentially expressed genes between responders and non-responders.

Result and discussion

We analyzed 49 PE samples, primarily containing T cells lymphocytes (mainly CD4+), B cells, macrophages and varying percentages of cancer epithelial cells. Of 15 PE-EVOCs treated with ICIs, 47% responded to ICIs and exhibited significant IFNγ induction and secretion. RNA-seq analysis of sorted CD3+ T cells and B cells (CD19+/CD20+) from 12 PE samples revealed 27 differentially expressed genes in B cells between responders and non-

responders, while no differences were observed in the CD3+ population, suggesting a specific role for B cells in ICI response. Initial analysis of DE genes revealed some interesting candidate that has a role in B cell activation and response to ICIs.

Conclusion

EVOCs from malignant pleural effusions of lung cancer patients provide insights into immune responses to ICIs, potentially improving patient selection and treatment outcomes. Our findings highlight a role for B cells in ICI response. We are currently investigating the identified genes of interest in our laboratory to elucidate their specific functions in B cells and their impact on ICI response, aiming to enhance our understanding and develop more effective immunotherapy strategies.

EACR25-1491

mRNA Cancer Vaccines: Leveraging Sugar-Functionalized LNPs for Targeted Antitumor Immunity

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Introduction

Cancer immunotherapies have emerged as a leading treatment strategy, particularly those that leverage antigen-presenting cells (APCs) to elicit Th1 immune responses and prime cytotoxic T lymphocytes. Cancer vaccination involves delivering tumor antigens directly to APCs to activate antigen-specific immunity against cancer cells. In this study, we explore sugar-functionalized Lipid Nanoparticles (SF-LNPs) as a cancer vaccine platform for the efficient delivery of mRNA-encoded antigens to APCs and subsequent immune activation/maturation.

Material and method

A sugar-modified lipid was synthesized via a coupling reaction, and the chemical structure of the compound was confirmed by ¹H-NMR spectroscopy. SF-LNPs were generated using the Nanoassemblr™ microfluidic platform. Particle size, polydispersity index, and zeta potential (ZP) were measured using dynamic light scattering and electrophoretic light scattering, respectively, with a Zetasizer Ultra. Encapsulation efficiency was quantified using the Quant-iT RiboGreen™ fluorescence assay. Cellular uptake and eGFP-mRNA expression of DiD-labeled SF-LNPs and non-functionalized LNPs (NF-LNPs) were assessed at multiple time points through flow cytometry and confocal microscopy. Experiments were conducted with RAW264.7 and JAWSII cell lines, as well as with human monocytes, monocyte-derived dendritic cells and monocyte-derived macrophages. Cell surface receptor

expression and cytokine production were evaluated using flow cytometry and ELISA, respectively.

Result and discussion

SF-LNPs exhibited a uniform size distribution with an average particle size below 100 nm, and a neutral ZP. Encapsulation efficiency of eGFP-mRNA within SF-LNPs reached values above 85 %. Flow cytometry and confocal microscopy analysis demonstrated higher and faster cellular uptake of DiD-labeled SF-LNPs than NF-LNPs. Additionally, SF-LNPs showed increased eGFP-mRNA expression compared to NF-LNPs. Flow cytometry results revealed upregulation of activation and maturation cell surface markers, while ELISA assays indicated increased secretion of pro-inflammatory cytokines, suggesting effective immune activation by SF-LNPs.

Conclusion

These findings highlight the potential of SF-LNPs as a versatile and effective cancer vaccine platform, offering a promising strategy for mRNA-based immunotherapies.

Funding: CiNTech (PRR-30) C644865576-00000005; Marie-Sklodowska-Curie fellowship (project 101130813); FCT: 2023.11762.PEX, CIBB (FCT UIDB/04539/2020, UIDP/04539/2020 and LA/P/0058/2020).

EACR25-1528

Accelerating the Manufacture of γδ T Cells for Allogeneic Cancer Therapies: Integrated Cell Differentiation and Expansion in Bioreactors

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Introduction

Chimeric antigen receptor T cell (CAR T) therapies are emerging as a promising therapeutic option for cancer treatment. However, their wide application remains challenging due to barriers imposed by HLA-disparities, limiting most therapies to autologous settings. To overcome this, γδ T cells arise as a powerful alternative. γδ T cells have potent immunosurveillance activity and are HLA-independent so they do not cause graft versus host disease (GvHD), enabling their application in allogeneic settings. Due to the low abundance of γδ T cells in the human body, strategies to generate high numbers of γδ T cells while retaining their quality attributes are needed for their application in the clinical setting. To date, protocols available to generate γδ T cells from hematopoietic stem/progenitor cells (HSPC) rely on OP9 mouse stromal cell lines overexpressing Notch ligands, a strategy not easily scalable and compatible with Good Manufacturing Practices (GMP). Herein, we propose to develop a scalable and animal origin-free bioprocess to differentiate HSPC into γδ T cells.

Material and method

To achieve this goal, we cultured CD34+ HSPC, obtained from human umbilical cord blood, in the presence of

recombinant Notch ligands. This process, optimized to be entirely free of animal-derived products, was initially implemented in static conditions (96 well-plates) and scaled-up to a fully controlled, miniaturized bioreactor system (Ambr®15, Sartorius).

Result and discussion

Differentiation was similar between static and bioreactor systems, with 80–90% of the cells reaching the Pro T cell stage by day 21 of differentiation. Although selection of an optimal timeframe for TCR stimulation is expected to further maximize the yields of $\gamma\delta$ T cells, currently $1-2 \times 10^3$ CD3+TCR $\gamma\delta$ + cells were obtained per initial CD34+ HSPC, after 42 days of culture. $\gamma\delta$ T cells obtained through this strategy expressed key cytotoxicity markers (e.g. CD8, NKG2D, NKp30) and displayed cytotoxic activity in vitro against a leukaemia cell line (MOLM-13), when originated both in static and bioreactor conditions.

Conclusion

Overall, we have developed a scalable and GMP-compatible bioprocess to generate $\gamma\delta$ T cells, suitable for off-the-shelf therapies, namely in the context of allogeneic CAR T cell products.

EACR25-1544

Blockade of PD-L1 endocytosis by uPAR antagonist peptides to improve cancer immunotherapy

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Introduction

Immunotherapy has revolutionized the field of tumor immunology. Recent evidences have revealed the clinical efficacy of programmed cell death-1/programmed death ligand-1 (PD-1/PD-L1) antibodies in patients with metastatic breast cancer, melanoma and non-small-cell lung cancer. The therapeutic efficacy of PD-1/PD-L1 inhibitors is high in patients with high PD-L1 expression. Recently, Tseng et collaborators showed that targeting Plasminogen Activator Inhibitor (PAI-1) by its inhibitor tiplaxtinin (TPX) synergizes with anti-PD-L1 checkpoint blockade in a model of murine melanoma. PAI-1 induced the internalization of surface PD-L1, resulting in the reduction of PD-L1 at membrane level. Binding of PAI-1 to uPA/uPAR complex results in the recruitment of low-density lipoprotein receptor protein 1 (LRP1) and triggers the endocytosis process leading to the internalization of PAI-1-uPA-uPAR-LRP1 quaternary complex. The endocytosed PAI-1 and uPA then undergoes lysosomal degradation, whereas uPAR and LRP1 are transported back to the plasma membrane by recycling endosomes. Another limiting factor of ICIs is the PD-L1 packaging within specific membrane-enclosed extracellular vesicles (EVs), the so-called exosomal PD-L1. We propose to inhibit PDL-1 endocytosis by uPAR inhibitors to maintain high-cell-surface levels of PD-L1 and, at the same time, to reduce the expression of exosomal PD-L1. Moreover, we propose to set up 3D co-culture system between non-small cell lung cancer cells and T cells to assess efficacy of uPAR inhibitors on immunotherapy responses.

Material and method

2D and 3D cultures from A549 (non-small cell lung cancer cells) were treated with TPX and uPAR antagonist (IPR803) to evaluate the effect of TPX and IPR803 treatment on PD-L1 modulation. Conditioned media from untreated and treated TPX tumor cells were used for exosomes isolation to evaluate the effect of TPX treatment on exosomal PD-L1. 3D co-cultures were seeded between A549 previously treated with anti-human PD-L1 and IPR803 and T cells CD8+ CD4+ to assess cytotoxic effect of T cells on tumor cells.

Result and discussion

Our result evidenced that in 2D and 3D cultures of A549 TPX and uPAR antagonist peptides are able to block the PD-L1 internalization and, consequently, to increase PD-L1 membrane levels. Moreover, we demonstrated that exosomes from TPX-treated A549 show a decrease of exosomal PD-L1 levels, compared to untreated cancer cells. In parallel, our data highlighted in 3D co-cultures an improvement of cytotoxic effect of T cells on A549 treated with uPAR antagonists and anti-PD-L1 antibodies

Conclusion

Our results evidenced that blockade of PD-L1 endocytosis induced a decrease of exosomal PD-L1 levels. Furthermore, our data demonstrated that uPAR inhibition by uPAR antagonist peptides result in a significant increase in surface PD-L1 levels, opening the way for new combined therapeutic strategies with uPAR inhibitors and anti-PD-1/PD-L1.

EACR25-1577

Characterization of radiolabelled single-domain anti-HER2 antibodies as potential radioimmunotherapeutics for breast cancer

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Introduction

Human Epidermal Growth Factor Receptor 2 (HER2) is a membrane-bound kinase regulating cell's proliferation and viability. Overexpression of HER2 occurs in 25–30% of breast, 10–15% of ovarian, 5–15% of stomach cancers and is associated with metastases, therapy resistance and poor prognosis. However, HER2 is a promising therapeutic target, due to its extracellular accessibility and differential expression in normal and tumour cells. Radioimmunotherapy delivers radiation selectively to the neoplasm, induces DNA damage and thus enhances therapeutic effect. Here, we use a single-domain antibodies derived from Camelidae heavy-chain antibodies, called nanobodies, offering an improved pharmacokinetics and minimizing off-target radiation. In our study, we radiolabelled two anti-HER2 nanobodies - internalising 2Rb17c and non-internalising 2Rs15d [1] - with various radioisotopes to evaluate and compare therapeutic potential of the final radiobioconjugates.

Material and method

Nanobodies were coupled with DOTA chelators. Bioconjugation kinetics was examined by HPLC, products were characterized by ESI mass spectrometry and radiolabelled with ^{177}Lu / ^{161}Tb / ^{225}Ac . Radiolabelling kinetics and chemical purity were measured by iTLC. Stability was determined in PBS, cell culture medium and human serum. Specificity, binding affinity and internalization assays were done on SKOV-3 cells (HER2+) followed by cell toxicity examined by MTS assay.

Result and discussion

Nanobody-chelator coupling reaction was optimized and products were obtained with high efficiency. Bioconjugates were successfully radiolabelled. Cell studies showed that chelator coupling and radiolabelling are not impairing nanobody specificity to HER2. The resulting radiobiocoujugates were applied to cells and MTS results revealed cytotoxic effect on HER2 positive cells.

Conclusion

Performed studies provided promising results and indicate that both radiobiocoujugates may be considered as potential therapeutics for HER2 positive tumour. Moreover, their application may be shifted to diagnostic agents by means of radioisotope with proper emission spectrum. Collectively, these outcomes encourage to investigate these compounds in further aspects.

Project was financed by Narodowe Centrum Nauki (NCN), award ID: 2019/34/E/ST4/00080, principal award recipient: M. Pruszynski.

EACR25-1641

Glycopolymer-Mediated Gene Delivery for Efficient CAR-T Cell Production

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Introduction

Gene-engineered T cell immunotherapies have shown great promise as a treatment for various non-solid cancers, offering significant therapeutic benefits. However, the significant production costs and limited availability associated with virus-based genetic manipulation present notable challenges for cellular immunotherapies. As a result, non-viral gene delivery platforms, particularly those based on cationic polymers, emerged as a possible alternative. This work aims to develop a new generation of non-viral gene delivery nanosystems based on cationic glycopolymers for T cell engineering.

Material and method

A library of well-defined copolymers based on 2-aminoethyl methacrylate (AMA) and 2-lactobio-namidoethyl methacrylate (LAMA) was synthesized using ARGET ATRP. These glycopolymers were complexed with plasmid DNA encoding green fluorescent protein (GFP) at polymer/DNA N/P ratios of 8/1 and 10/1. After 15 min of magnetic stirring at RT, polyplexes were incubated with Jurkat cells, and GFP expression was assessed after 48 h via flow cytometry. Additionally, the size and

surface charge of the polyplexes were characterized using dynamic light scattering and electrophoretic measurements, respectively.

Result and discussion

Glycopolymers with different carbohydrate/cationic ratios and monomers distribution (random/block) were tested: PAMA90-b-PLAMA113, PAMA92-co-PLAMA95 and PAMA114-co-PLAMA20. All glycopolymers demonstrated the ability to complex with nucleic acid, forming polyplexes with suitable physicochemical properties for cellular uptake. Nano-carriers revealed enhanced gene reporter expression at a N/P ratio of 10/1, indicating that the higher tested N/P ratio improved transfection efficiency. Notably, all the developed formulations exhibited higher transfection activity than that obtained with the gold-standard polymer for gene delivery application – the polyethyleneimine (PEI). Among them, polyplexes prepared with PAMA114-co-PLAMA20 demonstrated the best performance, achieving a threefold increase in the percentage of transfected cells while maintaining low cytotoxicity compared to PEI. This superior performance is likely due to the higher cationic content, which plays a key role in both polyplex formation and endosomal escape.

Conclusion

Results demonstrated the transfection ability of the developed glycopolymer-based polyplexes, with PAMA114-co-PLAMA20-based polyplexes standing out as a promising nanosystem for gene delivery.

EACR25-1649

Cellular Composition and Dynamics Upon Immunotherapy Correlate with Clinical Response

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Introduction

Despite the notable success of immune checkpoint inhibitors (ICI) in increasing survival rates across several cancer types, the complexity of the tumor microenvironment still poses significant challenges cancer therapy. The therapy-induced changes in tumor microenvironment (TME) cell composition and their regulation as predictive biomarkers remain incompletely understood.

Material and method

To comprehensively investigate TME dynamics under ICI therapy, we curated a large-scale single-cell RNA sequencing (scRNA-seq) dataset comprising 417 samples from 242 patients across 11 cancer types, including 187 longitudinally site-matched samples. Additionally, we included 18 scRNA-seq samples from 12 patients

collected before and approximately one week after ICI treatment as a validation cohort.

Result and discussion

We retrieved and processed pan-cancer datasets as reference, enabling the comprehensive annotation of non-malignant TME cells into 68 immune subtypes and 15 non-immune subtypes. Using generalized linear mixed models, we identified a consistent increase in Tpex, Tf_h, TREM2⁺ macrophages, and SPP1⁺ macrophages following immune checkpoint inhibitor (ICI) therapy across multiple cancer types. Nonnegative matrix factorization (NMF) revealed a distinct cellular program dominated by angiogenic and immune suppressive macrophages, which exhibited significant correlations with both treatment status and response. Additionally, we identified a co-regulatory community centered around M2-like C1QC⁺ macrophages, which showed a negative correlation with the naive CD4⁺ T cell community. This polarized trend was further confirmed through unsupervised clustering, which highlighted a clear distinction between non-responder-enriched groups and other patient groups.

Conclusion

The consistency of these dynamics across diverse cancer types, coupled with response-dependent variations, underscores the necessity of systematic approaches to unravel the intricate interactions within the TiME, paving the way for advancements in immunotherapeutic strategies.

EACR25-1671

Redox-Responsive Self-Immulative Nanogels for Cellular Immunotherapies

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Introduction

Cancer has become a major public health challenge and a significant societal and economic issue in the 21st century. These highly complex diseases are characterized not only by the overgrowth of malignant cells but also by an altered immune response. Current treatment options primarily include chemotherapy, radiation, and surgery; however, these often result in adverse side effects and therapeutic resistance. Immunotherapy presents a groundbreaking alternative, utilizing the body's immune system to selectively target and eliminate tumor cells. Chimeric Antigen Receptor T cell (CAR T cell) therapy is a personalized treatment that harnesses T cells to combat cancer. However, the success of this technique depends on efficient gene delivery systems. In this regard, this study explores the potential of redox-responsive nanogel formulations, based on a cationic glycopolymer, as non-viral platforms for gene delivery.

Material and method

The nanogels were prepared at RT for 18 h using a cationic glycopolymer, previously developed by our

group via ARGET ATRP, a plasmid DNA encoding the green fluorescent protein (GFP), and a crosslinker. Various nanogels were prepared at different polymer/DNA (N/P) ratios in PBS or purified water at pH 8.5. After incubation with Jurkat cells, GFP expression was assessed via flow cytometry after 48 h. Particle size and surface charge were characterized using dynamic light scattering and electrophoresis.

Result and discussion

Nanogels prepared in purified water at pH 8.5 exhibited a smaller hydrodynamic diameter and higher surface charge than those in PBS, indicating enhanced colloidal stability due to electrostatic repulsion. These results highlight the critical influence of solvent choice on the formulations' physicochemical properties. Transfection assays confirmed that all nanogels successfully transfected T cells with minimal cytotoxicity.

Conclusion

We successfully developed nanogels with distinct physicochemical properties able to carry DNA, highlighting the versatility of the formulation approach. Additionally, these nanogels effectively transfected T lymphocytes.

EACR25-1684

Targeting solid tumor cell lines of diverse histological origins with nucleolin-specific CAR-T cells - a proof-of-concept

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Introduction

CAR (chimeric antigen receptor)-T cell therapy, which involves genetically modifying T cells with synthetic receptors that target specific tumor antigens, has shown remarkable success in hematologic malignancies. However, its application in solid tumors remains challenging due to limited tumor infiltration and strong immunosuppression. Nucleolin, a membrane-nucleus shuttling protein, has been demonstrated to be over-expressed on the surface of cancer and endothelial cells from tumor blood vessels, across different solid tumors. Accordingly, anti-nucleolin nanobodies, previously developed by our group, showed strong binding to nucleolin-positive cell lines, suggesting nucleolin as a potential CAR-T cell target. Here, we describe the initial steps in generating anti-nucleolin CAR-T cells and evaluate their in vitro activity against solid tumor-derived cell lines of diverse histological origin.

Material and method

Peripheral blood mononuclear cells were isolated from healthy donors, activated with CD3/CD28 agonists, and transduced with CAR-encoding lentivirus. Transduction efficiency and stability were evaluated by both reporter

gene and CAR surface expression, at multiple timepoints. To assess CAR-T cells anti-tumor activity, specific lysis of different nucleolin expressing cancer cell lines was analyzed by real-time impedance-based co-culture assays, at different E(effector):T(target) ratios.

Untransduced and mock-transduced T cells were used as controls. Proliferation in the presence or absence of target cells and immunophenotyping studies by spectral flow cytometry were also performed.

Result and discussion

CAR expression ranged between 40% and 60% and remained stable for at least six days in culture. Anti-nucleolin CAR-T cells displayed significantly higher cytotoxicity than respective controls, and higher effector cell ratios led to increased target cell death. This effect was more pronounced in tumor cells with superior surface nucleolin expression. Phenotypic analysis revealed a predominance of central memory (CCR7⁺ CD45RO⁺) CAR-T cells, and differences on exhaustion markers expression, namely PD-1 and LAG-3, were observed over 10 days in culture, suggesting dynamic changes in activation and exhaustion profiles.

Conclusion

In summary, this work demonstrates the successful generation and in vitro validation of anti-nucleolin CAR-T cells, against different cancer cell lines, supporting nucleolin as a potential new target in CAR-based immunotherapy for solid tumors.

This work was funded by: Fellowship 2020.04685.BD (FCT). Projects: EXPL/MED-FAR/1512/2021 (FCT); CIBB (FCT UIDB/04539/2020, UIDP/04539/2020 and LA/P/0058/2020), Fundación La Caixa BREAST-BRAIN-N-BBB; CInTech (PRR-30) C644865576-00000005; 2022.07746.PTDC.

EACR25-1699

Development of PD-L1 based cancer vaccine conjugating a novel T cell epitope

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Introduction

Programmed cell death 1 (PD-1)/PD-L1 binding negatively regulates adaptive immune response mainly by inhibiting the activity of tumor-infiltrating cytotoxic T lymphocytes and promotes tumor growth and metastasis. An effective PD-L1 antibody producing vaccine is needed to restore antitumor T cell responses and cause long-term remission in a subset of cancer patients. In this study, we have designed a conjugate vaccine containing T cell epitope and PD-L1 epitope. This PDL1 conjugated vaccine was administered to tumor model mice in a form of peptide or plasmid DNA to evaluate tumor control efficacy and antitumor immune reactions.

Material and method

The PDL1 conjugated recombinant protein vaccine was intradermally administered to mice three times at 2-week intervals by using pyro-drive jet injector (PJI), which shoots out a solution by pressure of gunpowder explosion. On day 7 after the third immunization, the mouse was injected with MC38 (murine colon adenocarcinoma) on back. On day 14 after tumor challenge, the

mice sacrificed and tumor tissues and sera were collected. The tumor tissue sections were histologically stained with anti-CD4, -CD8 and F4/80 antibodies to detect tumor infiltrating T cells and macrophages. Anti-PDL1 antibody titers were measured by ELISA. The PDL1 conjugated plasmid DNA vaccine was intradermally administered to mice three times at 2-week intervals by using PJI. On day 7 after the third immunization, the mouse was injected with CT26 (murine colorectal carcinoma). On day 21 after tumor challenge, the mice sacrificed and splenocytes and sera were collected. IFN- γ and TNF- α secretion from splenocytes were measured by ELISpot assay. Tumor diameters were measured every other day and the tumor volumes were calculated.

Result and discussion

Tumor section staining showed that strong CD4+, CD8+ T cell and macrophage infiltration in PDL1 conjugated vaccine injected group. Anti-PDL1 antibody titer in PDL1 conjugated vaccine injected group was up-regulated. Compared with negative control, PDL1 conjugated pDNA inhibited tumor growth. Both IFN- γ and TNF- α secretion was significantly upregulated only in PDL1 conjugated pDNA group with tumor cell stimulation. These data suggest that PDL1 conjugated pDNA vaccine might have induced a PD-L1-specific immune response and delayed tumor growth.

Conclusion

Our data is suggesting that the pDNA vaccine containing PD-L1 epitope reverses the PD1/PD-L1 axis causing suppressive tumor phenotype. Therefore, this novel conjugate vaccine is a promising strategy for cancer immunotherapy.

EACR25-1700

Immunization against a mutant p53-derived neoantigen by a personalized oncolytic adenovirus

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Introduction

Replication-competent oncolytic adenoviruses (OAd) are promising anticancer therapeutics. Adenoviruses have a lytic life cycle and can be engineered to selectively replicate in tumor cells. An important attribute of OAds is their immunogenicity. When they infect and subsequently kill tumor cells, OAds elicit robust local and systemic immune responses that can counteract an immunosuppressive tumor microenvironment. Neoantigens released during a lytic infection can stimulate T cell activation by professional antigen presenting cells, thereby increasing the immune response to the tumor. Unfortunately, immune responses are primarily directed against the virus itself; cross reactivity against the targeted tumor is frequently limited. To enhance anti-tumor immunity in the context of an active infection, we have developed OAds that can reconstitute pHLA-immunopeptide complexes on the surface of infected cells.

Material and method

We employed a platform technology called AdenoBuilder to create OAds that stimulate robust

immune responses to antigenic peptides incorporated into the viral capsid. With the AdenoBuilder platform, synthetic adenovirus genomes are enzymatically assembled from plasmid components and directly packaged. This modular approach permits the rapid production of recombinant viral vectors with modifications across the viral genome. Our personalized OAds enhance neoantigen presentation by the transgenic expression of tumor-specific neoantigens and compatible HLA alleles that can be matched to the allelotype of individual patients. To further enhance antigen presentation, our current replication-competent OAds also express β2-microglobin and the IL-12 complex.

Result and discussion

We tested a prototype OAd designed to elicit specific T cell responses against the public neoantigen HMTEVVRHC (derived from the p53R175H mutant protein) which was systematically modified to enhance its binding affinity for HLA-A*0201. The optimized peptide sequence was incorporated into the capsid of an OAd that also expressed HLA-A*0201, β2-microglobin and the IL-12 complex. We demonstrated that this neoantigen-expressing OAd could stimulate robust, neoantigen-specific anti-tumor responses in an HLA-A2-transgenic mouse model.

Conclusion

OAds that express specified pHla-immunopeptide complexes are an emerging form of precision immunotherapy. This study demonstrates the feasibility of using the AdenoBuilder system to rapidly generate OAds that are personalized to individual patients and their tumors.

EACR25-1757

Selection and identification of nanobodies against a cancer-testis antigen, placenta-specific protein 1

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Introduction

Placenta-specific protein 1 (PLAC1) is a surface cancer-testis antigen protein mainly expressed in the placenta, ovary and testes. In addition, it is also highly expressed in multiple types of cancer such as breast, ovarian, head and neck, liver, and colorectal cancer. High levels of PLAC1 expression promote cancer proliferation, angiogenesis, invasion and metastasis. Therefore, PLAC1 has been considered as an attractive target for the development of cancer therapeutics. In this work, we aimed to select specific nanobodies targeting the PLAC1 protein.

Material and method

We used a synthetic nanobody library for biopanning with the E. coli-expressed extracellular domain of PLAC1 (PLAC1e). We also performed phage enzyme-linked immunosorbent assays (phage-ELISA) for affinity screening of the candidate clones of nanobody-displayed phages. Nanobody nucleotide sequences were verified by DNA sequencing and translated to protein sequences using the Expasy Translate Tool.

Result and discussion

As a result of phage-ELISA screening, the candidate nanobody selected from the biopanning process

demonstrated binding with high affinity to the target protein. Deduced amino acids of ten candidate clones revealed CDR3 length with 14 and 19 amino acids. Nanobodies with a CDR3 length of 19 amino acids were shown by phage-ELISA to have higher absorbance than those with a CDR3 length of 14 amino acids.

Conclusion

This study provides potential specific nanobodies to PLAC1 isolated from a synthetic nanobody library. From these findings, the synthetic nanobody library offers a useful tool for application in the selection of specific antibody domains. Furthermore, the anti-PLAC1 nanobodies discovered can be used in further studies for the development of nanobody-based T cell engagers or other nanobody-based tumor therapeutic agents.

EACR25-1815

Patient-derived tumor tissue cultures (PDTTCs) as a new platform for assessing T cell engager efficacy in glioblastoma

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Introduction

Glioblastoma (GBM) is the most common brain cancer of the adult and a clear medical unmet need. Although immunotherapy is showing great results in several cancer types, in GBM there are still several barriers for its successful implementation. The tumor microenvironment (TME) has been shown to play a fundamental role in the response to immunotherapies and to T cell engagers therapies. However, the study of human TME has been limited by the lack of good experimental models.

Material and method

For this purpose, we have developed patient-derived tumor tissue cultures (PDTTCs), a powerful preclinical model that preserves tumor tissue architecture and TME and accurately represents the complexity of GBM patient tumors and their immune system.

Result and discussion

We recently characterized a novel EGFRvIII-TCB that showed specificity for EGFRvIII mutation and promoted tumor cell killing as well as T cell activation and cytokine secretion in patient-derived models expressing EGFRvIII. We have shown that the PDTTC model is a powerful platform to study the impact of the TME on T cell engagers therapies in GBM that can be deeply explored through single cell and spatial transcriptomics technologies. We showed that incubating the PDTTCs with leukocytes from the same patient that donated the tumor and EGFRvIII TCBs or EGFRvIII CARTs we can assess the interaction, infiltration and response of the immune cells to the tissue, as well as the efficacy of the therapeutic candidates.

Conclusion

Through our studies, we expect to identify novel therapeutic targets and biomarkers that could facilitate the response to T cell engagers and select the patients that may benefit from them.

EACR25-1817**ENO3PEP vaccine enhances chemotherapy efficacy in pancreatic cancer by boosting anti-tumor immunity**

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Introduction

Pancreatic ductal adenocarcinoma (PDA) is a highly aggressive malignancy with poor prognosis and limited treatment options. Standard chemotherapy, such as gemcitabine plus nab-paclitaxel (GemNab), offers only modest survival benefits. Immunotherapy has emerged as a promising strategy to enhance treatment efficacy. One potential immunogenic target is Alpha-Enolase (ENO1), a glycolytic enzyme overexpressed in PDA. The ability of an ENO1-based vaccination (ENO3PEP) to boost GemNab chemotherapy by inducing a more robust anti-tumor immune response was assessed in this study.

Material and method

The most immunogenic ENO1 sequences were identified based on proliferative response and cytokine release in donors and PDA patients, then cloned into the pVax plasmid to generate the ENO3PEP vaccine. KPC mice, which spontaneously develop PDA, were vaccinated at 8 weeks of age and received booster doses every two weeks for four rounds before being sacrificed at 18 weeks. Mice were treated with GemNab (gemcitabine 1 mg/mouse; Abraxane 0.6 mg/mouse) at 9 and 11 weeks alone or in combination with ENO3PEP. Anti-ENO1 antibody levels were assessed by ELISA, and IFN γ -secreting T cells were measured using ELISpot. Tumor burden and metastases were evaluated through hematoxylin and eosin (H&E) staining, immune infiltration via immunohistochemistry (IHC), and collagen deposition using Masson's trichrome stain. Flow cytometry was used to characterize tumor-infiltrating and lymph node-resident immune cells.

Result and discussion

In KPC mice, ENO3PEP significantly reduced pancreatic tumor burden and metastases. It enhanced the humoral response by increasing anti-ENO1 IgG and IgG2c antibodies, the latter associated with Th1-driven immunity, suggesting a shift toward a stronger anti-tumor response. At the cellular level, ENO3PEP increased IFN γ secretion by ENO1-stimulated T cells and remodeled the tumor microenvironment by reducing collagen deposition and recruiting more activated cytotoxic immune cells through enhanced antigen presentation. ENO3PEP combined with GemNab shown enhanced therapeutic efficacy relative to each treatment individually, markedly decreasing tumor burden and enhancing both humoral and cellular anti-tumor immune responses.

Conclusion

Overall, the findings of this study suggest that ENO3PEP has the potential to serve as a promising boost to standard

chemotherapy, improving immune activation and enhancing treatment outcomes for PDA patients.

EACR25-1822**IL17A depletion remodels pancreatic tumor matrix and boosts ENO1 vaccine efficacy**

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Introduction

Pancreatic ductal adenocarcinoma (PDA) accounts for ~90% of all pancreatic cancers and has a poor prognosis, with a 5-year survival rate below 10%. This is largely due to the huge desmoplastic reaction and its immuno-suppressive tumor microenvironment (TME), dominated by a fibrotic extracellular matrix (ECM) rich in collagen, fibronectin, and structural proteins. The ECM acts both as a physical barrier to therapies and as a biochemical shield, releasing suppressive signals that prevent immune infiltration. Recent studies identified Interleukin 17A (IL17A) as a key driver of PDA progression, fostering immune evasion by promoting myeloid recruitment at the expense of T cells. This evidence highlights IL17A as a promising therapeutic target.

Material and method

To assess IL17A's role in ECM remodeling and immune responses, we bred KPC mice, a spontaneous PDA model, with IL17A knockout mice (KPC/IL17A^{+/+} and KPC/IL17A^{-/-}). Decellularized tumor matrices from both IL17A proficient and deficient KPC were analyzed by mass spectrometry to map ECM composition. Cancer-associated fibroblasts (CAFs) from both genotypes were isolated and used to generate 3D-spheroids (homotypic and heterotypic), to evaluate invasive properties and ECM deposition. Atomic Force Microscopy (AFM) was performed on OCT-embedded tumors to assess stiffness. Lastly, IL17A depletion was combined with ENO1-DNA vaccine, and immune responses were analyzed by flow cytometry and ex vivo functional assays.

Result and discussion

IL17A depletion led to significant ECM remodeling, with increased levels of tumor-restraining Collagen I and decreased pro-metastatic Collagen VI, Fibronectin, and VEGFR. IL17A-depleted cancer cells and spheroids showed reduced invasion and downregulation of EMT transcription factors. AFM revealed that IL17A-deficient tumors exhibited softer matrices, which favored immune cell infiltration. IL17A depletion combined with ENO1-DNA vaccination prolonged survival of KPC mice, by increasing CD8⁺ T cells and NK cells within tumors and enhancing their cytotoxicity. Depleting CD8⁺ or NK cells abolished the vaccine's efficacy, confirming their critical role.

Conclusion

Our data show that IL17A promotes a pro-invasive, immune-excluding ECM in PDA, supporting immune evasion and therapy resistance. IL17A depletion reprograms the TME, reducing stiffness, boosting immune

infiltration, and enhancing anti-cancer vaccine efficacy. Targeting IL17A may thus ameliorate immune-based therapies in PDA.

EACR25-1823

Enhanced natural killer cell anti-tumor activity with Erastin mediated ferroptosis and potential therapeutic application in neuroblastoma

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Introduction

High-risk neuroblastoma (NB) is rare, it often relapses and becomes refractory to treatment, making it a significant contributor to childhood cancer mortality. Addressing the challenges posed by NB's resistance to conventional apoptosis-inducing therapies has become a pressing concern in pediatric oncology research. In recent years, the growing comprehension of alternative cell death modalities distinct from apoptosis has revealed a promising avenue in the endeavor to combat treatment-resistant cancers. One such mechanism, ferroptosis, has attracted increasing attention for its potential role in combating therapy-resistant cancer cells. High-risk NB typically aligns with the immune exclusion phenotype, where substantial immune cell infiltration is notably absent. While the role of immune cell infiltration in NB is of significant interest, the underlying mechanisms remain elusive.

Material and method

Cell viability and proliferation were assessed using MTT and colony formation assays. Lipid peroxidation levels were measured to confirm ferroptosis induction.

Transcriptomic analysis was performed using RNA sequencing to identify differentially expressed genes upon Erastin treatment. ULBP1 expression was validated using qRT-PCR and flow cytometry. Co-culture experiments with NK-92 cells were conducted to assess NK cell-mediated cytotoxicity using a standard cytotoxicity assay. ATF4 involvement in ULBP1 regulation was investigated using siRNA-mediated knockdown and western blot analysis. Statistical analyses were performed using GraphPad Prism.

Result and discussion

Here, we show that induction of ferroptosis using Erastin, a cysteine-glutamate antiporter inhibitor reduced NB cell proliferation and foci formation. Furthermore, our transcriptomics analysis showed upon treatment with Erastin of NB cells increased expression of ULBP1 (NKG2D ligand). Co-culture of NK cells and NB cells upon treatment with Erastin showed a significant increase NK cell cytotoxicity. Additionally, we found transcription factor ATF4 driver ULBP1 expression in NB cells upon ferroptosis induction.

Conclusion

Our results suggest that the NK cell's cytotoxic function can be enhanced with Erastin mediated ferroptosis which might be beneficial for NB patients.

EACR25-1904

Generation of versatile tumor target cell lines using the SpyCatcher/SpyTag system

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Introduction

Immunotherapies, such as therapeutic monoclonal antibodies and engineered chimeric antigen receptor (CAR) T cells, are powerful tools in cancer treatment, particularly effective against hematological malignancies. However, tumor escape mechanisms, such as down-regulation of tumor antigens or tumor heterogeneity, often contribute to the clinical failure of these therapies. To address these challenges, it is essential to develop robust in vitro platforms for assessing effector cell potency during the development of novel immuno-therapies.

Material and method

We have established a versatile tumor target cell line, Spy target, which can be post-translationally decorated with tumor antigens. This platform allows precise control over antigen selection, antigen density and the combination of multiple antigens within the same cell line. The Spy target system is based on the SpyTag/SpyCatcher technology, derived from the fibronectin-binding protein (FbaB) of Streptococcus pyogenes. SpyTag and SpyCatcher spontaneously form a covalent isopeptide bond upon interaction. We engineered two cell lines (HEK293T and MDA-MB-231) expressing SpyCatcher on the cell membrane and performed a clonal selection of the transfected cell lines to further obtain a homogenous population expressing high levels of SpyCatcher. As a proof of concept, we tested HER2, a clinically relevant tumor antigen overexpressed in various solid tumors (breast, ovarian, gastric) and associated with poor prognosis. SpyTag-HER2 fusion protein was produced in HEK293-E6 cells as a soluble recombinant protein and subsequently displayed on the Spy target cells. We then performed a functional characterization of Spy target cells displaying different levels of HER2 antigen.

Result and discussion

Antibody-dependent cell cytotoxicity assay (ADCC) was conducted using a reporter cell line that stably expresses the FcγRIIIa receptor and an NFAT response element driving firefly luciferase expression. Titration of the therapeutic anti-HER2 antibody (trastuzumab) demonstrated specific effector cell dose-dependent activation only in the presence of Spy target cells decorated with SpyTag-HER2.

Conclusion

We are currently expanding this platform by incorporating additional tumor antigens and implementing further functional assays. These include

testing the potency of CAR T cells, CAR macrophages, and different monoclonal antibodies in ADCC assays, further advancing the development of novel immunotherapies.

EACR25-1935

The immune and metabolic profile in metastatic and early-stage triple negative breast cancer patients treated with immune checkpoint inhibitors

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Introduction

Immune checkpoint inhibitors (ICIs) are a promising option for triple negative breast cancer (TNBC) treatment. However not all the patients respond equally and causes are unknown. The objective of the present study is to research immune and metabolic profiles through non-invasive methods that allows to differentiate the stage of the disease and the response to ICIs.

Material and method

51 TNBC patients were enrolled: 13 under first line of treatment with ICIs (MET) and 38 under neoadjuvant chemotherapy with ICIs (NEO). Blood samples were collected before the start of treatment (baseline) in all patients. In NEO patients we collected samples after 3 weeks of treatment (3W) and at the end of treatment (endpoint). NEO patients were classified as responders (R) and non-responders (NR) based on whether they achieved histologically complete pathological response respectively after treatment. Immune profile were characterized by flow cytometry and metabolomics profile was determined by mass spectrometry.

Result and discussion

MET group showed significant higher levels of total Myeloid Derived Suppressor Cells (MDSCs) ($p = 0.0057$) and granulocytic MDSCs (G-MDSCs) ($p = 0.0025$) at baseline point compared to NEO patients.

Metabolomic analysis revealed significant differences in metabolites of the Tyrosine and Purine pathways between both groups ($p < 0.005$). In NEO patients we observe a significant increase during the treatment in the percentage of total T lymphocytes ($p < 0.0001$), Natural Killer Cells (NKTs) ($p = 0.001$) and cytotoxic T lymphocytes (TCD8+) ($p < 0.0001$). When classified into responses, only the R group obtained a significant increase in NKT ($p < 0.001$) and TCD8+ ($p < 0.001$) after therapy. NR group showed higher percentages of MDSCs at all sample points. This increment was statistically significant for total MDSCs at baseline ($p = 0.01$) and G-MDSCs at baseline ($p = 0.0087$), 3W ($p = 0.02$) and

endpoint ($p = 0.027$). In addition, results obtained revealed changes in metabolic pathways in all time points including the Phenylalanine, Tyrosine and Tryptophan metabolism. The analysis of 3W samples showed additionally differences in Purine and Steroid hormones pathways.

Conclusion

Advanced disease (MET) presents higher immuno-suppressive cells (MDSCs) than early stage (NEO) and metabolomic differences at baseline point. In NEO patients immunotherapy is capable to promote an activation of the immune system through the increase of antitumor cells TCD8+ and NKTs especially in responder subjects. Non responders present activated immuno-suppression mechanisms such as MDSCs. The cellular changes, along with the differences in the metabolomic profile of each group, allow us to establish personalized patterns that inform the stage of disease and response to ICIs therapy in TNBC and could help in clinical decision-making. This study is still in the recruitment phase with the expectation that this trend will continue.

EACR25-1965

Exploring CAR-T Cell Killing Dynamics Through Live-Cell Fluorescence Microscopy

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Introduction

Harnessing the potent cytotoxicity of chimeric antigen receptor T (CAR-T) cells has revolutionized cancer immunotherapy. Human epidermal growth factor receptor 2 (HER2) has emerged as a critical target in CAR T-cell therapy. HER2 is notably overexpressed in a significant portion of A549 lung cancer cells and SKOV3 ovarian cancer cells, making these cell lines valuable models for studying HER2-targeted therapies. Traditional cytotoxicity assays provide only static endpoint measurements and often rely on 2D cell culture models, which fail to capture the structural complexity of tumors *in vivo*. Therefore, fluorescence live-cell imaging can play a pivotal role in advancing CAR T-cell research by enabling real-time monitoring of CAR T-cell interactions with fluorescent HER2-positive cancer cells in 3D. This study aims to explore the dynamics of CAR-T cell-mediated cytotoxicity using the Omni platform.

Material and method

A549-GFP cells and SKOV3-GFP cells were detached from flasks and dissociated via trypsinization and counted using the Exact FL (Axion BioSystems). Next, cells were centrifuged and resuspended to a final concentration of 20,000 cells/mL, and 100 μ L cell suspension was added to a 96-well U-bottom plate and centrifuged at 1000 rpm for 4 minutes. After 48 hours of incubation, CAR T-cells were added to the SKOV3-GFP and A549-GFP spheroids with the following effector: target (E:T) ratios: 0:1 (Ctrl), 1:10, 1:5, 1:2, 1:1, 2:1, 5:1 and 10:1. The plate was imaged hourly for 72 hours on the Omni.

Result and discussion

Results showed that the potency of CAR T-cell-mediated cytolysis was dose-dependent, with higher E:T ratios leading to greater target cell killing. SKOV3 spheroids, which overexpress HER2, were more susceptible to HER2 CAR T-cell-mediated killing, while A549 spheroids with low HER2 expression required higher E:T ratios for effective cytolysis. For example, SKOV3 spheroids treated with CAR T-cells showed a dose-dependent decrease in fluorescence intensity, with higher E:T ratios (2:1, 5:1, and 10:1) resulting in approximately 75% reduction in fluorescence intensity by 72 hours. In contrast, A549 spheroids only exhibited significant killing at 5:1 and 10:1 E:T ratios. Non-treated spheroids showed continuous growth over time, indicated by a steady increase in fluorescence intensity.

Conclusion

The Omni platform enables real-time monitoring of CAR T-cell interactions with target cells, providing key insights into the cytotoxic potential of these immune cells. Fluorescent metrics were used to track immune cell-mediated killing, with changes in fluorescence confluence and intensity reflecting the extent of target cell death. Real-time monitoring with live-cell imaging tools like Omni provides valuable insights into the dynamics of immune cell interactions, helping to refine and improve CAR T-cell therapies.

EACR25-2022

Detecting changes in the tumour microenvironment and in CART infiltration following chemotherapeutic priming using intravital imaging

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Introduction

Chimeric antigen receptor (CAR) T cell therapies have shown remarkable efficacy in haematological malignancies but demonstrate limited efficacy against solid tumours including prostate cancer. This is in part due to an immunosuppressive and fibrotic tumour micro-environment (TME) which leads to restricted CAR T cell tumour infiltration, reduced CAR T cell persistence and CAR T cell exhaustion. Accumulating evidence suggests that chemotherapies can modulate the TME to dampen immunosuppression and potentially improve CAR T cell efficacy.

Material and method

Our work aims to optimise and develop an intravital imaging pipeline to both longitudinally image and trace changes in the TME following chemotherapeutic priming, and track CAR T cell infiltration in patient-derived xenograft (PDX) models of prostate cancer. To achieve this, we are using optical imaging windows in prostate cancer PDXs and repeated longitudinal imaging to assess tumour dynamics.

Result and discussion

Using this imaging-based approach, we can

longitudinally detect any changes in fibrosis through second-harmonic generation (SHG) imaging, changes in blood vasculature using quantum dots, and assess CAR T cell dynamics in PDX models of prostate cancer following chemotherapy. This approach can enable us to optimise chemotherapeutic priming to modulate the TME and observe improvements in CAR T efficacy including cell tumour infiltration, persistence, movement and dwell time.

Conclusion

Our work highlights the advantages of optical imaging windows in guiding CAR T cell delivery and potentially improving response rates in solid tumors.

EACR25-2039

POSTER IN THE SPOTLIGHT

Cytotoxic ILC-1 cells determine outcome of PD-(L)1 blockade in microsatellite instable cancers

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Introduction

PD-(L)1 blockade has revolutionized the treatment of mismatch repair deficient (dMMR)/microsatellite instable (MSI) cancers. However, dMMR/MSI tumors frequently lose HLA class I-mediated antigen presentation, rendering them resistant to the dominant effectors of PD-(L)1 blockade: adaptive CD8+ T-cells. Previously it has been shown that innate antitumor immunity is an important determinant of response. However, the role and clinical relevance of innate lymphoid cells (ILCs) in this context remains largely undefined.

Material and method

To study the role of ILCs in dMMR/MSI cancers, we analyzed the transcriptomes and clinical outcomes of 74 metastatic dMMR/MSI tumors treated with anti-PD-(L)1 in the DRUP study. We performed an in-depth phenotypic characterization of ILC1-like cells in dMMR/MSI tumors, using flow cytometry, imaging mass cytometry (IMC) and scRNA-sequencing. Furthermore, we optimized an in vitro expansion protocol and performed functional characterization of ILC1-like cells in co-cultures with dMMR/MSI cancer cells with/without antigen presentation defects.

Result and discussion

Among dMMR/MSI cancers in the DRUP study, expression of the key lymphocyte marker CD7 was strongly associated with clinical benefit of PD-(L)1 blockade, broadly implicating lymphocytes at the core of these responses. Surprisingly, however, infiltration by classical lymphocytic effector populations did not seem to underly this effect, as markers of CD8+ T cells, CD4+ T cells, and NK cells were not associated with response. In contrast, marker gene analysis identified ILC1-like cells as the main lymphocytic subset associated with clinical outcome in our cohort. In-depth phenotypic studies showed that ILC1-like cells infiltrating dMMR/MSI tumors highly expressed markers of activation, proliferation, and cytotoxicity, as well as immune check-

points including PD-1 and a selection of activating innate immune receptors and “missing self” KIR-receptors. In vitro expanded tumor-infiltrating ILC1-like cells showed cytotoxic antitumor responses in co-culture with dMMR/MSI tumor organoids and cell lines. Importantly, the powerful tumor killing capacity of ILC1-like cells was independent of HLA class I expression.

Conclusion

Cytotoxic ILC1-like cells are a clinically relevant tumor-reactive pool of ILCs in dMMR/MSI cancers, predicting outcome of PD-(L)1 blockade in this disease. These data contribute to our understanding of the mechanism of action of immune checkpoint blockade in this largely HLA class I-negative disease and demonstrate the potential of ILC1-like cells in cancer immunotherapy.

EACR25-2051

Visualization and quantification of antibody-dependent cellular phagocytosis (ADCP) using a live-cell imaging high-throughput automation system

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Introduction

Antibody-based treatment strategies have revolutionized the therapy of some cancer entities. Yet, assessing antibody-based immunotherapy approaches in vitro is challenging and heterogeneous. One aspect involves studying Fc-mediated effects like antibody-dependent cellular phagocytosis (ADCP). ADCP targets cancer cells with monoclonal antibodies for clearance by phagocytic immune cells. Standard phagocytosis assays rely on endpoint measurements, providing limited kinetic information and often requiring destructive protocols. In contrast, many live-cell imagers have limited capacity, hindering high-throughput screening. This study uses SYNENTEC’s live-cell imaging automation system (CELLAVISTA 4K®, SYBOT-1000®, CYTOMAT®) to analyze ADCP in a high-throughput format.

Material and method

Donor-derived macrophages, differentiated from peripheral blood mononuclear cells (PBMCs), were co-cultured with pHrodo®-labeled REH cell lines or primary patient-derived cells from acute lymphoblastic leukemia (ALL) patients in 96-well flat-bottom plates.

The plates were automatically imaged over time using the automation system, and ADCP was automatically quantified by determining fluorescent spots using YT-SOFTWARE®.

Result and discussion

At first, glycine-treated REH cells were used as a positive control to determine the optimal exposure time and thresholds for the image analysis. Using these parameters, the treated cells showed a significantly higher number of fluorescent spots than untreated cells. Secondly, we compared whether our assay demonstrated differential responses to various treatments. While DMSO or an isotype control antibody did not increase the fluorescent signals, antibody and chemotherapy treatments showed a moderate effect. The highest effect was observed with a combination treatment. Thirdly, we tested whether the assay also worked with primary ALL cells. Again, we observed treatment-dependent differences in ADCP induction, depicting that the device can also be used to analyze primary samples.

Conclusion

Using the automation system improved the reliability and throughput of our imaging processes, enabling us to measure more than two plates for parallel screening. This increased throughput has the potential to greatly advance the study of phagocytosis assays, by reducing the time, resources, and cost required to identify promising drug candidates or combination treatments. Therefore, high-throughput screening can ultimately contribute to the development of more effective antibody-based therapies.

EACR25-2174

Novel NK Cell Immunotherapy Strategy to Enhance Tumor Killing and Overcome Microenvironment Resistance

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Introduction

The effectiveness of immunotherapeutic mAbs or CAR-T therapies can be hindered by tumor cells losing antigen expression and upregulating neoantigens and immune checkpoint (IC) ligands. In contrast, NK cells are not dependent on specific antigens for cytotoxicity, offering promise in cellular immunotherapy. Allogeneic NK cells have a lower risk of graft-versus-host disease (GvHD) and cytokine release syndrome (CRS), making them a safer, “off-the-shelf” option. However, a hostile tumor microenvironment can lead to resistance and reduce NK cell efficacy. To enhance their therapeutic potential, NK cells can be genetically engineered to target tumor markers common to a broad range of tumors (as IC ligands) and to overcome the hostile TME.

Material and method

NK cells, isolated from peripheral blood of healthy donors, were genetically modified using retroviral vectors. The retroviral tri-cistronic vectors encode chimeric proteins composed of IC extracellular domains (specifically extracellular PD1-exPD1) fused to the cytoplasmic NKG2D domain linked to 4.1BB co-stimulatory in frame with NKG2A single chain variable fragment (scfv) and IL15.

Result and discussion

exPD1-NK cells, obtained upon transfection, increased the cytotoxicity against PD-L1+ tumor cells. Moreover, exPD1-NK cells constitutively release NKG2A-scfv fragment and IL15. The NKG2A-scfv fragments disrupted the NKG2A/HLA-E interaction and blocked the NK inhibitory receptor NKG2A signal in the TME. In addition, the release of IL15 supported the NK cell survival and proliferation in vivo. The ability to release NKG2A scfv and IL15 makes exPD1-NK cells better able to kill even PD-L1 negative cells.

Conclusion

Our findings provide a comprehensive strategy to overcome significant obstacles in NK cell therapy, demonstrating the potential of these innovations to improve the effectiveness of NK cell-based immunotherapies and offer new therapeutic options for patients with solid tumors. This research could contribute to a paradigm shift in cancer treatment.

EACR25-2188

POSTER IN THE SPOTLIGHT

Neutrophil serine proteases alter IL-18 to a superkine

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Introduction

IL-18 is a key cytokine stimulating IFNγ production in NK cells. Clinical trials using IL-18 monotherapy have yielded limited success, as IL-18, despite being safe, failed to generate sufficient therapeutic effects. A possible explanation could be its inability to elicit strong immune responses. Therefore, developing IL-18 variants that are more potent is essential for improving its therapeutic potential. Here, we characterize a neutrophil-altered IL-18 variant with increased capacity to induce IFNγ production in NK cells.

Material and method

IFNγ produced by primary NK cells or KG-1 cells upon stimulation with PMN or neutrophil serine protease (NSP) pre-exposed IL-18 was determined using ELISA or flow-cytometric analysis of intracellular IFNγ. NSP enzyme activity was measured with a FRET substrate.

Result and discussion

Our study demonstrates that neutrophils regulate the bioactivity of IL-18 in a fine-tuned manner. Exposure of IL-18 to low numbers of neutrophils significantly

enhanced its bioactivity and induced augmented IFNγ responses in NK cells and KG-1 cells. With increasing numbers of neutrophils this phenomenon shifted to degradation of the IL-18 molecule and abolished IFNγ release. We found that PMN-processed IL-18 increased the percentage of IFNγ+ CD56bright NK cells and IFNγ production per CD56bright NK cells as determined by the mean fluorescence intensity (MFI) of IFNγ+ NK cells. Using purified NSP, we found that in particular Proteinase 3 was capable of triggering increased IFNγ production in KG-1 cells.

Conclusion

Our results suggest the existence of a fine-tuned system to modulate the bioactivity of IL-18 during an acute inflammatory response, affecting cytokine production in immune cells, such as NK cells. NSP-altered IL-18 variants could be harnessed to boost NK cell-based cancer therapies.

EACR25-2225

Distinct chemotherapy regimens differentially modulate the immune landscape of triple-negative breast cancer, with potential impact on combination with immunotherapy

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Introduction

The standard treatment for triple-negative breast cancer (TNBC) relies on chemotherapy, increasingly combined with immune checkpoint inhibitors (ICIs). Different chemotherapeutic agents may uniquely influence both the tumor and its microenvironment (TME), particularly in terms of immune modulation. While some evidences exist in preclinical models, data from clinical tumours remain limited. This study aimed to compare the early impact of various neoadjuvant chemotherapy regimens on cancer pathways, immune-related features, and single gene expression in TNBC patients.

Material and method

We analysed RNA-seq data from paired core biopsies collected before and after the first cycle of neoadjuvant chemotherapy in four TNBC patient cohorts. Patients were treated with nab-paclitaxel/carboplatin (PNabT, n = 93), doxorubicin/cyclophosphamide (AC, n = 19), nabpaclitaxel (NabT, n = 15), or paclitaxel (T, n = 17). Using the singscore R package, we quantified 80 gene sets related to the TME, immune function and signalling in cancer. Treatment-induced dynamics for single genes and signature scores were identified by ANOVA analysis. P-values were adjusted for multiple testing using the Benjamini-Hochberg method. Gene with cell-

type specific expression were annotated using external single cell data to aid data interpretation.

Result and discussion

On-treatment expression profiles showed an overall up-regulation of immune cell- and immune function-related signatures, alongside a down-regulation of proliferation-related pathways. However, treatment-dependent differences emerged. Tumours treated with AC or NabT exhibited the greatest up-regulation of T cells, macrophages and dendritic cell signatures, whereas over 20% of tumours in the PNabT and T groups showed a decrease, particularly when baseline expression was high. NabT-treated patients displayed the strongest up-regulation of B, plasma, and mast cell signatures, while proliferation-related signatures were most significantly down-regulated in PNabT and NabT groups. Expression patterns of well established cell markers confirmed these patterns. Notably, in tumours with high immune infiltration, PD-L1 expression further increased in over 90% of TNBCs receiving AC, while a decrease was observed in 40-60% of tumours receiving alternative chemotherapies.

Conclusion

These findings highlight the early immuno-modulatory effects of neoadjuvant chemotherapy in TNBC, with anthracyclines and nab-paclitaxel alone eliciting a particularly strong immune response. These insights may have clinical implications for optimising chemotherapy combinations with immune checkpoint inhibitors in TNBC treatment.

EACR25-2299

Inhibition of Furin in CAR macrophages directs them toward a proinflammatory phenotype and enhances their antitumor activities

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Introduction

CAR T-cell therapy has shown efficacy in hematological malignancies, but its application to solid tumors is hindered by poor infiltration and tumor-induced immunosuppression. Macrophages, key players in the tumor microenvironment, can infiltrate solid tumors, interact with stromal components, and phagocytose tumor cells. We hypothesize that genetically engineering macrophages to express a CAR targeting tumor antigens, combined with an activation strategy, could enhance their anti-tumor activity and trigger a robust T cell response

Material and method

In this project, we first developed complex preclinical tumoroid models incorporating macrophages to better mimic the tumor microenvironment and assess the engineered macrophages expressing a CAR receptor targeting the HER2 antigen. This CAR, which includes an intracellular CD3ζ domain similar to the FcεRI-γ protein, enhances macrophage phagocytic activity upon activation by antibody-antigen complexes.

Approximately 30% of the transduced macrophages expressed the CAR.

Result and discussion

CAR-modified macrophages (CAR-M) exhibited

significantly enhanced phagocytosis of HER2-coated beads and HER2+ cancer cell lines compared to wild-type macrophages. Co-culture experiments with patient-derived breast cancer tumoroids confirmed the efficacy of CAR-M in a complex tumor environment. However, within the tumor microenvironment, macrophages often adopt an anti-inflammatory phenotype, reducing their anti-tumor potential. To address this issue, we employed a dual strategy involving the inhibition of proprotein convertases, particularly Furin, in CAR-M. Furin inhibition led to increased expression of pro-inflammatory markers and enhanced macrophage activation in the presence of cancer cells. Additionally, Furin-inhibited CAR-M displayed enhanced phagocytic activity against HER2+ targets. Our findings highlight the critical role of proprotein convertases in regulating macrophage phenotype. Furthermore, CAR-M demonstrated the ability to activate T cell proliferation, an effect further enhanced by Furin inhibition.

Conclusion

Our therapeutic strategy is based on the dual activation of tumor-infiltrating macrophages: first, by enhancing their phagocytic activity through CAR receptor expression targeting tumor antigens, and second, by reprogramming them towards a pro-inflammatory phenotype through Furin inhibition. Additionally, while CAR cell immunotherapy is increasingly attractive, identifying sufficiently specific tumor targets remains a challenge. Recent advances in antigen identification have introduced a new class of proteins: alternative proteins (AltProts) derived from previously non-coding regions of the transcriptome. Incorporating these proteins has allowed us to identify new antigenic targets for CAR-M therapies.

EACR25-2311

A positive crosstalk between chemokine receptor CXCR4 and intrinsic programmed cell death-1 receptor (iPD-1): anti PD-1, nivolumab, impairs CXCL12-induced signaling, proliferation and migration in human colon cancer cells

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Introduction

Colorectal cancer is a significant contributor to cancer-related mortality, with metastasis being a primary cause of death. CXCR4 was previously reported to have a prognostic value in colorectal cancer. PD-1 signaling typically mediates immune exhaustion in immune cells. Recently, PD-1 expression was also described on tumor cells. Interestingly, PD-1 expression promotes tumor survival and chemoresistance in melanoma, while in colon and non-small cell lung cancer may contribute to growth suppression. The heterogeneity in PD-1's role within different cancers makes it an intriguing target for therapeutic interventions. Aim of the work is to investigate and delineate the cross talk between the two receptors to identify targetable interactions potentiating

the efficacy of immunecheckpoint inhibitors in human colon cancer cells

Material and method

PD-1 and CXCR4 expression in human colon cancer cell lines HCT116, HT29, and LoVo was determined through flow cytometry with APC anti-human CD279 (clone MIH4) and PE anti-CXCR4 (FAB170P); immunofluorescence using primary anti-hCXCR4 and mouse monoclonal anti-hPD-1. Co-immunoprecipitation assay was performed in HCT116, HT29, LoVo to assess direct interaction between PD-1 and CXCR4. The protein extracts were immunoprecipitated using A/G-Agarose and incubated with mouse monoclonal anti-hPD1. IPs were run on an SDS-PAGE gel followed by immunoblotting to evaluate CXCR4. CXCL12-induced cell viability and transwell migration assay were conducted in the presence nivolumab, NIVO 1-10 μ M, and CXCR4 inhibitor, R54 0.1,1 μ M). Additionally, intracellular signaling CXCR4-PD-1 was assessed through ERK1/2 and Akt and SHP2

Result and discussion

CXCR4 and PD-1 were co-expressed in HT29 (47% CXCR4; 22% PD1), HCT116 (CXCR4 30% and PD-1 30%), LOVO (45% CXCR4 and 25% PD-1) cells. Through immunofluorescence, CXCR4 and PD-1 co-localized in HT29, HCT116 and LoVo cells. Co-immunoprecipitation confirmed a direct interaction between PD-1 and CXCR4 in HT29, HCT116, LoVo cells. NIVO (1 μ M) inhibits CXCL12 migration in LoVo and HT29 (by 2-fold). Similarly, PD-1 ligand, PD-L1 induced colon cancer migration impaired by the newly developed CXCR4 inhibitor- R54 in a dose dependent manner. Additionally, NIVO (1 μ M) impaired CXCL12-induced cell proliferation and CXCL12 induction of ERK/Akt suggesting, as PD-1 is not a known receptor of CXCL12, that CXCL12/CXCR4 signaling impairment depends on PD-1 blocking. Conversely, CXCL12 activates SHP2, a key downstream effector of PD-1 transduction while NIVO (1 μ M) inhibited it.

Conclusion

These results demonstrate the crosstalk between PD-1/PD-L1 and CXCR4/CXCL12 in human colon cancer cells. This positive crosstalk highlights a potential therapeutic target in cancer immunotherapy. Nivolumab not only counteracts immune suppression but also reduces migration induced by CXCL12, offering a potential therapeutic target for inhibiting metastatic progression in colon cancer

EACR25-2321

Combining NK/CAR-NK-based therapy with autophagy inhibition as therapeutic strategy in high-risk medulloblastoma

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Introduction

Autophagy is intimately involved in the immunological control of malignant transformation, tumor progression and therapy response. Medulloblastoma (MB) is the most common pediatric brain tumor with aggressive Group3 (G3) subtype characterized by poor prognosis, therapy relapse, and high dependence on autophagy. Natural Killer cells (NKs)-based immunotherapy represents a promising therapeutic value in the treatment of G3 MB. Here, we explore the targeting of autophagy as an immunomodulatory approach to promote the homing of effector NK cells into MB tumor sites and sensitize it to engineered-NKs based immunotherapy.

Material and method

Flow cytometry and western blotting were used to investigate the G3 MB NK-related activating ligands and the cytotoxic potential of NK cells.

Result and discussion

We demonstrate that G3 MB cell lines and in G3 MB patients-derived cells show low levels of NKs-related activating ligands (ULBPs, CD155, CD112, MICA/B) in compared to others MB subgroups. Interestingly, genetic and pharmacologic inhibition of autophagy increases NKs-related ligands expression on cell surface of MB G3 cells, and boosts G3 MB sensitiveness to NKs-mediated cytotoxicity. In addition, genetic inhibition of autophagy in G3 MB cell line modulates cytokines and chemokines secretion and improves in vitro NKs migration. Chloroquine treatment improves also the efficacy of NKs to reduce tumor growth in G3 MB xenograft model. In order to optimize our model, we hypothesize that using engineered NK cells could improve their therapeutic efficacy. We found that engineered NK cells are more capable of eliminating G3 MB cells in vitro.

Interestingly, autophagy inhibition is able to enhance the therapeutic efficacy of engineered NK cells against G3 MB cells in vitro, as well in an orthotopic G3 MB in vivo model.

Conclusion

The completion of our project would be helpful to design a novel therapeutic approach for children with high-risk MB, aiming to a higher clinical response rate coupled with less toxicity than conventional therapies.

EACR25-2349

Anti-IL-7R α antibodies: a targeted therapy for T-cell Acute Lymphoblastic Leukemia

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Introduction

T-cell Acute Lymphoblastic Leukemia (T-ALL) is an aggressive sub-type of ALL. Despite high therapeutic efficacy in children, adult cases and refractory/relapse disease remain a significant hurdle. Interleukin-7 receptor (IL-7R) is expressed in around 70% of T-ALL cases and IL-7 promotes T-ALL cell proliferation and viability in

vitro and leukemogenesis in vivo. Moreover, 10% of T-ALL cases display IL-7R α gain-of-function mutations. T-ALL cell dependence on IL-7R α provides a robust rationale to target this pathway using anti-IL-7R α monoclonal Antibodies (mAbs).

Material and method

To identify anti-human IL-7R α mAbs with clinical potential, we evaluated the ability and specificity of a panel of 48 mAbs (generated by FairJourney Biologics) to bind to human IL-7R α , using flow cytometry (FC). mAbs with high binding capacity were further characterized for their:

- 1) ability to block IL-7-mediated signaling in T-ALL cells (western blot);
- 2) impact on the viability and proliferation of T-ALL samples (FC analysis);
- 3) internalization kinetics (FC analysis);
- 4) capacity to induce Antibody-dependent cellular cytotoxicity (ADCC) and/or
- 5) Antibody-dependent cellular phagocytosis (ADCP). The FJB45 mAb was tested in vivo, using a T-ALL patient-derived xenograft (PDX) mouse model in a phase II-like clinical trial setting. NSG mice were transplanted with 10 different T-ALL PDX samples and treated with placebo or the FJB45 mAb.

Result and discussion

Four mAbs with high affinity binding to hIL-7R were selected: FJB26, FJB29, FJB45 and FJB48. None of these mAbs blocked IL-7R α -mediated signaling in T-ALL cell lines and PDX cells, as evidenced by the lack of impact on the phosphorylation levels of downstream effectors of IL-7R and on IL-7-mediated viability and proliferation of T-ALL cells. However, they all showed ADCC potential, with FJB45 displaying the strongest effect in T-ALL PDX cells. Moreover, FJB29, FJB45 and FJB48 exhibited ADCP potential, with the latter inducing the strongest effect. Thus, FJB45 was selected to be tested in vivo. In a phase II-like clinical trial setting, FJB45 clearly extended overall mouse survival in 4 out of 10 PDX samples. On average, FJB45 mAb extended mouse survival for 14 days. Remarkably, there was one PDX that responded exceptionally well to FJB45 mAb, with an increase in overall survival of 150 days.

Conclusion

We determined that FJB45 has ADCC- and ADCP-promoting capacity and is effective in vivo. Our studies contribute to the growing evidence that anti-IL-7R mAbs may be valid therapeutic tools for the treatment of IL-7R-positive T-ALL.

EACR25-2430

Boosting Melanoma Immunotherapy: Synergizing Nanovaccines with PD-L1 Modulation

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Introduction

Immune checkpoint blockade (ICB) targeting programmed cell death protein 1 (PD-1)/PD-ligand (PD-L1) has revolutionized cancer therapy, yet resistance remains a challenge, often due to poor T-cell infiltration, an immunosuppressive tumor microenvironment (TME), and T-cell exhaustion. Overcoming these barriers requires innovative strategies to boost tumor immunogenicity and reshape the TME. Here, we developed a nanovaccine (NV) designed to enhance melanoma-dendritic cells (DC) interactions, promoting antigen-specific T cell activation.

Material and method

A poly(lactic acid) (PLA) and mannose-conjugated poly(lactic-co-glycol) (PLGA) NV was engineered to co-deliver a melanoma neoantigen and toll-like receptor (TLR) ligands. NV physicochemical properties, entrapment efficiency (EE), internalization by DCs, and impact on DC viability were assessed. DC activation in lymph nodes (LN) of NV-immunized mice was evaluated. The therapeutic efficacy of NV in combination with three PD-1/PD-L1 modulators – a monoclonal antibody (α PD-L1), small interference RNA (siPD-L1) incorporated within NV, and an in-house discovered small-molecule inhibitor (SM56) – was evaluated in melanoma mouse models via tumor growth analysis and immune profiling [1].

Result and discussion

NV exhibited a mean size of around 200 nm, low polydispersity index, neutral charge, and spherical morphology. EE was 60–65% for the neoantigen and above 80% for TLR ligands. NV were efficiently internalized by DCs, particularly those with mannose conjugation, without compromising viability. NV treatment promoted DC activation in LNs, increasing CD80/CD86 expression and enhancing cytokine-secreting T-cell responses. In vivo, NV combination therapies showed distinct efficacy profiles: siPD-L1 failed to enhance tumor suppression significantly, while α PD-L1 and SM56 demonstrated potent anti-tumor effects. The strongest tumor regression and CD8+ T-cell infiltration were observed in the NV + α PD-L1 and NV + SM56 groups. Notably, only NV + SM56 reduced immunosuppressive cells in the TME, suggesting superior ability to reshape the immune landscape.

Conclusion

These findings underscore the potential of polymer-based NV to overcome ICB resistance by promoting T-cell responses and modifying the TME. Additionally, SM56 emerged as a promising alternative to monoclonal antibodies, demonstrating a strong synergistic effect with NV. This work supports the rationale for nanotechnology-driven combination strategies to improve melanoma immunotherapy outcomes.

Acknowledgments: This work was supported by UIDB/04138/2020, UIDP/04138/2020, PTDC/BTM-SAL/4350/2021 (FCT-MCTES), and LCF/PR/HR22/52420016, LCF/PR/HR24/00968 (La Caixa Foundation). [1] All animal procedures were approved by the regulatory authority and handled in compliance with the National Institutes of Health guidelines and the Directive 2010/63/EU.

EACR25-2457

Improving Triple-Negative Breast Cancer immunogenicity via viral-mediated epigenetic remodeling

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Introduction

Immunotherapy has emerged as a promising treatment for various cancers; however, the efficacy of such therapies is often hindered by the suppression of immune responses via the tumor microenvironment (TME). The TME is characterized by a complex relationship between cancer and its proximal non-cancerous cells and is heavily based on cell-cell communication via direct interaction, secretion of biomolecules, and cancer-derived extracellular vesicles. A clear example of this cancer-induced alteration is represented by myeloid-derived suppressor cells (MDSCs). Several studies have convergently shown that the expression of DNA-methyltransferase-1 (DNMT1) results in MDSCs protection from necroptosis and enhanced accumulation in vivo. Conversely, targeting DNMT1 through hypo-methylating agents (HMAs) has shown promising therapeutic potential by not only reducing the accumulation and suppressive activities of MDSCs but also synergistically with immune checkpoint inhibitors (ICI) to enhance cancer immunogenicity.

Material and method

Murine 4T1 (triple-negative breast cancer) and CT26 (colon carcinoma) cell lines were used as syngeneic tumor models in BALB/c mice. An oncolytic adenovirus expressing a DNMT1-targeting shRNA (OAd-shDNMT1) was engineered and validated for DNMT1 knockdown and genome-wide methylation reduction. Small extracellular vesicles (sEVs) were isolated from virus-infected cancer cells and characterized for RNA content and uptake by myeloid-derived suppressor cells (MDSCs). MDSC differentiation and suppressive function were assessed in vitro using flow cytometry and co-culture assays with murine splenocytes. In vivo, tumor-bearing mice received intratumoral OAd-shDNMT1, Decitabine (DAC), or immune checkpoint inhibitors (anti-PD-1), and tumor growth, immune infiltration, and systemic MDSC levels were evaluated.

Result and discussion

Collectively, in this study, we report that, by using virally infected TNBC murine cells as a source for shDNMT1-loaded sEV production, OAd-shDNMT1 successfully reduced MDSC levels in vitro and in vivo. Furthermore, the co-administration with ICI resulted in a significant tumor growth reduction in mice bearing poorly immunogenic TNBC 4T1 cells, and, in addition, promoting anti-tumor immunity and prolonged survival in modestly immunogenic colon CT26 cancer cells.

Conclusion

This multifaceted strategy based on OV-mediated immune stimulation and reduction of MDSC levels via sEVs, may improve clinical outcomes and the success of immuno-based regimens for patients facing MDSC-rich and highly aggressive cancer subtypes.

EACR25-2510

Anti-CD19 therapy (Blinatumomab®) as a bridge therapy for hematopoietic stem cell transplantation in pediatric patients

with relapsed/refractory B-type acute lymphoblastic leukemia Ph-negative

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Introduction

The most common pediatric hematologic cancer is B-lineage Acute Lymphoblastic Leukemia (B-ALL). Blinatumomab® destroys CD19+ leukemic cells, this study assessed its use in Brazilian children with Relapsed/Refractory (R/R) Ph-negative B-ALL

Material and method

This is a retrospective, prospective, and longitudinal cohort study that analyzed Blinatumomab® as a bridging therapy for Hematopoietic Stem Cell Transplantation (HSCT) in 16 pediatric patients with Ph-negative R/R B-ALL, during the years 2017 to 2024 (Follow-Up: 7 years), treated at the Children's Hospital of Brasília, Brazil. The study included real-world children and adolescents, aged between 0 and 21 years, with B-ALL in first hematological remission, maintaining a positive Minimal Residual Disease (MRD), with $\geq 0.01\%$ blasts in the Bone Marrow (BM) after first-line treatment. The data were collected from electronic medical records and all BM samples were studied following EuroFlow SOPs, with the acquisition of at least 5×10^6 events per sample. The FACSCanto cytometer and Infinicyt software were used to acquire and analyze MRD monitoring. The local ethics committee approved the study

Result and discussion

The recent emergence of targeted therapies has revolutionized the treatment of B-ALL. In this study, a total of 16 bone marrow samples were obtained from children with Ph-negative R/R B-ALL, with 62.5% being male and 37.5% female, with an average age of 6.81 years (0-21). The samples were studied at different times points during treatment with Blina®, including pre-transplant ($n = 16$) and pre- and post-transplant ($n = 3$), defining negative Minimal Residual Disease (MRD) as $< 0.01\%$ blasts in the BM. Blinatumomab® was administered as a continuous intravenous infusion for four weeks, at a dose of $5 \text{ mcg/m}^2/24\text{H}$ during the first 7 days of treatment (1st cycle), increasing to $15 \text{ mcg/m}^2/24\text{ H}$ on the subsequent days (2nd cycle). We observed a response to Blina® in 7 out of 16 patients (43.75%), with 93.75% ($n = 15$) using only the 1st infusion cycle, while 6.25% ($n = 1$) received both cycles. The predominant

side effect was febrile reactions, and 1 out of 16 patients developed an event associated with neurotoxicity and cytokine release syndrome. So far, 43.75% (n = 7) of the patients responded to treatment with Blina® and achieved remission. Among the non-responders, 37.5% (n = 6) were relapsing, and 18.75% (n = 3) died from causes unrelated to the use of immuno-therapy. This study demonstrates that pediatric patients can benefit from the use of Blinatumomab® from the first infusion cycle, paving the way for the HSCT procedure and resulting in complete remission in Ph-negative R/R B-ALL, marking a significant advancement in pediatric treatment.

Conclusion

Although our study had some limitations regarding its retrospective design and the limited patient population, the response rate in our cohort demonstrated that Blinatumomab® has a tolerable toxicity profile, providing hope for improvements in cure rates.

EACR25-2549

Advancing CAR-T clinical development with high-parameter CyTOF technology

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Introduction

Adoptive immunotherapy using chimeric antigen receptor (CAR)-T cells is considered a recent revolutionary treatment in cancer therapy. CAR-T therapy has achieved great success in hematological B cell malignancies, however it has faced significant challenges in solid tumors due to various factors, such as complex tumor microenvironments, restricted trafficking, impersistent anti-tumor activity, and toxicities. A better understanding of CAR-T biology will accelerate development of CAR-T therapies with improved anti-tumor efficacy, durability, and decreased toxicities.

Material and method

High-parameter flow cytometry has been a powerful tool to functionally characterize CAR-T cells at multiple stages of clinical development, from product characterization during manufacturing to longitudinal evaluation of the infused product in patients. However, fluorescence-based cytometry faces significant challenges with signal overlap and autofluorescence, limiting sensitivity and the number of targets that can be detected in CAR-T cells. Consequently, rare cell populations are poorly resolved and functional read-outs of CAR-T cells are unreliable. CyTOF technology overcomes these limitations with low signal overlap and no autofluorescence. Further, CyTOF enables a streamlined and flexible workflow in clinical research using freezing antibody cocktails and stained cell samples. Here, we present a 40+ parameter CyTOF panel to simultaneously analyze phenotypic and functional protein expression in CAR-T cells from in vitro co-culture with tumor cells.

Result and discussion

CD19 CAR-T cells were expanded in vitro and co-cultured with Nalm6 cells at different E:T ratios for 2-5 days. A high-parameter CyTOF panel including over 40 surface, cytoplasmic and nuclear markers was used to

stain CAR-T cells. Samples co-cultured at different time points were stained, barcoded, frozen and simultaneously acquired on CyTOF XT at a later date. The cytotoxicity, activation, proliferation, differentiation and exhaustion of CAR-T cells were evaluated. Comprehensive profiling revealed that CAR-T cells became activated, proliferated and produced cytokines in in vitro co-culture with tumor cells and showed reduced cytotoxicity and exhaustive phenotype after an extended period of expansion.

Conclusion

Overall, we demonstrate that the high-parameter CyTOF panel enables deep functional characterization of CAR-T cells by simultaneous detection of surface, cytoplasmic and nuclear markers, supporting the continuous clinical development of CAR-T products.

EACR25-2554

NK Cell Exhaustion in Multiple Myeloma: Pathways to Novel Immunotherapies

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Introduction

The tumour microenvironment (TME) in multiple myeloma (MM) is characterized by immune suppression, impairing anti-tumour responses. MM progression is driven by complex interactions between malignant plasma cells and the TME, fostering immune evasion and resistance to therapy. Natural Killer (NK) cells, vital for tumour surveillance, often display dysfunction and exhaustion within the MM TME. Characterizing NK cell states in the TME is critical for identifying therapeutic targets to restore their activity.

Material and method

We constructed an MM single-cell atlas using publicly available single-cell RNA sequencing (scRNA-seq) data from GEO and the clinical trial E-PRISM, comprising 108 samples deriving from BM aspirates across all stages of MM and healthy controls. NK cells were classified into active, tissue-resident (rNK), or exhausted (eNK) states using a gene expression signature-based scoring algorithm. Differential gene expression, Cell-cell interaction analysis, and transcription factor (TF) enrichment analysis were conducted to characterize NK cell regulation and prioritize potential therapeutic targets for NK reactivation.

Result and discussion

The MM atlas included 373,981 cells, and the healthy dataset contained 148,692 cells. We found significant shifts in the cell type compositions along the disease progression with the reduction of progenitors cell types (HSC&MPPs, and B cell progenitors the most afflicted), CD56brightCD16- NK cells and non-classical monocytes showed an increasing trend during the disease

progression, and, as expected higher numbers of plasma cells in all the disease stages. eNK proportions were significantly higher in disease states compared to healthy samples (mean: 19.3%), increasing with progression: MGUS (32.6%, $p = 0.04$), SMM (40.9%, $p < 0.001$), PMM (40.8%, $p < 0.001$), and RRMM (59.2%, $p < 0.001$). eNK showed increased expression and activation of immune checkpoint receptors with the most active being KLRC1 (NKG2A), LAG3, and LAIR1. Ranking the active TF by immune suppression-related genes we identified STATs, ETS1, EOMES, and other key TF involved in NK development and regulation. The highest-ranked TF in the dataset after the exclusion of samples receiving any kind of therapy was a nuclear receptor.

Conclusion

This study showed deep changes in the cell type compositions of BM during the progression to MM. Also, eNK cells during MM progression reveal a continuous accumulation with the activation of known immune checkpoint receptors. Finally, we identified a glucocorticoid receptor, that taking the heavy use of dexamethasone to treatment of MM, unveils valuable information about NK biology, and paves the road to the development of novel engineered NK cell-based therapies. We are proceeding with the development of an exhaustion model to further investigate the role of this nuclear receptor in the NK activity.

EACR25-2558

Accelerating immuno-oncology research with flexible workflows using modular Flex-Fit CyTOF panels

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Introduction

Deep functional profiling of immuno-oncology markers and cytokines in whole blood (WB) reveals immune signatures that inform disease prognoses, immunotherapy selection, and outcomes in cancer patients. WB is best analyzed within 24 hours of collection to capture clinically relevant immune signatures; however, WB collection and cytometric analysis are often performed at different sites, which can lead to significant delays. WB stabilizers can overcome this challenge, yet not all antibody panels are compatible with these reagents. To enable flexible experimental workflows for deep profiling of immuno-oncology markers and cytokines in WB we developed modular Flex-Fit CyTOF® panels compatible with stabilizers.

Material and method

CyTOF-mediated high-parameter flow cytometry enables single-tube staining to identify 50+ markers at the single cell level. CyTOF provides exceptional resolution without spectral unmixing or compensation, while Flex-Fit CyTOF panels allow simple and quick panel design by combining a customizable selection of pre-validated sub-panels. We built two high-parameter antibody panels for analysis of fresh and stabilized WB. The first panel has 21 markers and identifies 12 immuno-oncology markers in 10 major cell subsets. The second panel has

31 markers and identifies 10 cytokines in 32 cell subsets. Stimulated WB samples from two healthy donors were either freshly stained, or they were stabilized with PROT1, RBC lysed, and stained. Liquid antibodies in the panel were pooled together and frozen (-80°C) as single-use aliquots for surface and intracellular staining to reduce technical variability from staining.

Result and discussion

Optimal panel design during penel design ensured that all markers in both panels could be used to accurately identify target cell populations in PROT1 stabilized WB. Following stabilization, non-specific staining was not observed when compared with freshly stained samples. Cell populations maintained the anticipated co-expression of markers in PROT1 stabilized WB. Prominent cytokines such as IL-17A, TNF- α , IL-10, etc. could be detected in stabilized WB.

Conclusion

Overall, we show that PROT1 compatible high-parameter antibody panels enable functional immune profiling immune cells by identifying immuno-oncology markers and cytokines. Compatibility with WB stabilization reagents provides workflow flexibility for carrying out functional profiling in longitudinal and multi-center studies in clinical research and healthcare settings. Furthermore, freezing antibody cocktails is a unique feature of CyTOF flow cytometry, ensuring staining consistency. Together, flexible CyTOF workflows enable fast and convenient panel design for high-parameter cytometric analysis of WB and can facilitate biomarker discovery, immunotherapy selection, and tracking disease prognoses in cancer. For Research Use Only. Not for use in diagnostic procedures.

EACR25-2588

RAS(ON) G12C-selective and multi-selective doublet combination overcomes clinical resistance mechanisms to KRAS G12C(OFF) inhibitors and sensitizes to immune checkpoint blockade in preclinical models

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Introduction

Oncogenic RAS promotes carcinogenesis by sustaining cell proliferation and driving immune evasion. KRAS G12C(OFF) mutant selective inhibitors provide clinical benefit to patients with NSCLC however most patients will eventually develop resistance through reactivation of the RAS pathway, underlining the need for therapeutic approaches that maximize RAS inhibition. Elironrasib (RMC-6291), a RAS(ON) G12C mutant-selective, covalent inhibitor and daraxorrasib (RMC-6236), a

RAS(ON) multi-selective, noncovalent inhibitor have shown profound antitumor activity as monotherapies in preclinical models and, more recently, showed promising clinical activity in patients with RAS-driven tumors at doses that were well tolerated.

Material and method

Here we tested the RAS(ON) doublet combination of elironrasib with daraxonrasib in a panel of KRAS G12C NSCLC xenograft models, including those harboring alterations associated with clinical resistance to KRAS G12C(OFF) inhibitors such as increased RTK signaling, KEAP1 co-mutations and KRAS amplification. In addition, we evaluated the potential for the RAS(ON) doublet to sensitize an immune-refractory syngeneic NSCLC model to anti-PD-1.

Result and discussion

The combination of elironrasib with daraxonrasib significantly improved the depth and durability of response compared to the respective monotherapies. PK/PD/efficacy modeling suggested that the combination of elironrasib and daraxonrasib, even at sub-optimal mono-therapy doses, could drive deep and durable MAPK pathway suppression. In an anti-PD-1 refractory NSCLC syngeneic model elironrasib, daraxonrasib, and the RAS(ON) doublet combination induced rapid, transient tumor regressions, but in all treatment arms some tumors relapsed on treatment. Elironrasib, daraxonrasib and the RAS(ON) doublet favorably modulated the tumor micro-environment, increasing tumor infiltrating lymphocytes, decreasing myeloid cells and inducing immunological memory. Only the RAS(ON) doublet combination upregulated MHC I expression, increasing the possibility of recognition of the tumor by T cells. While the combination of either elironrasib or daraxonrasib alone with anti-PD-1 did not achieve significant combination benefit in this model, the triple combination of the RAS(ON) doublet and anti-PD-1 achieved 100% complete regressions.

Conclusion

These preclinical findings suggest that the RAS(ON) doublet combination can improve the depth and durability of response compared to the respective monotherapies and overcome mechanisms of clinical resistance to KRAS G12C(OFF) inhibitors. Furthermore, in an immuno-refractory model the RAS(ON) doublet combination reversed the immune-evasion mechanisms governed by oncogenic RAS. Collectively, these preclinical data support the clinical evaluation of the elironrasib with daraxonrasib RAS(ON) doublet in combination with anti-PD-1 in patients with KRAS G12C mutant NSCLC cancers.

Molecular and Genetic Epidemiology

EACR25-0040

Clinical Analysis of CNS WHO Grade 4 Gliomas with IDH-mutant versus IDH-wildtype Focused on CDKN2A

Homozygous Deletion

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Introduction

Despite there no definite consideration of CDKN2A homozygous deletion as poor prognosis factor in gliomas, its prognostic role has been widely investigated in CNS tumors, such as malignant meningiomas. CDKN2A is a gene acting as tumor suppressors by suppressing the cell cycle, especially blocking traversal from G1 to S-phase. Herein, we are primarily investigating the prognostic role of CDKN2A in CNS WHO grade 4 gliomas.

Additionally, traditional prognostic factors for grade 4 gliomas will be examined, and our results will be validated.

Material and method

We conducted retrospective analysis of glioma cohorts in our institute. Medical records were reviewed for 142 glioblastoma patients for 15 years, and pathological slides were examined again for the updated diagnosis according to 2021 WHO classification of CNS tumors. The IDH mutation and CDKN2A deletion were examined by NGS analysis using ONCOaccuPanel®. Traditional prognostic factors including age, WHO performance status, extent of resection, and MGMT promoter methylation were examined.

Result and discussion

Mean follow-up duration was 27.5 months (ranged from 4.1 to 43.5 months) and mean overall survival (OS) was 19.4 months (95% CI 16.3-20.9 months). After the exclusion of 4 patients with poor status of pathologic samples, total 109 glioblastoma which were diagnosed by previous WHO criteria were changed into 26 (23.9%) astrocytoma, IDH-mutant, CNS WHO grade 4 and 83 (76.1%) glioblastoma, IDH-wildtype, CNS WHO grade 4. Among them, 61 patients (56.0%) had CDKN2A deletion. Group A was classified for 59 patients with IDH-wildtype and present CDKN2A deletion, group B was classified for 16 patients with IDH-mutant and present CDKN2A deletion, group C was classified for 48 patients with IDH-wildtype and absent CDKN2A deletion, and group D was classified for 13 patients with IDH-mutant and absent CDKN2A deletion. Group A had a mean OS of 15.70 months (95% CI 13.86-17.54 months), group B had a mean OS of 19.37 months (95% CI 13.43-25.30 months), group C had a mean OS of 22.63 months (95% CI 20.10-25.17 months), and group D had a mean OS of 33.38 months (95% CI 29.35-37.40 months). Multifactor analysis showed following factors were independently associated with OS; age (\geq 50 years vs. <50years; HR 4.642), extent of resection (gross total resection vs. others; HR 5.523), WHO performance (0, 1 vs. 2; HR 5.007), MGMT promoter methylation, (methylated vs. unmethylated; HR 5.075), IDH mutation (mutant vs. wildtype; HR 6.358), and CDKN2A deletion (absence vs. presence; HR 13.452).

Conclusion

The presenting study suggest that CDKN2A deletion should play a powerful prognostic role in CNS WHO grade 4 gliomas as well as low grade glioma. Even if CNS WHO grade 4 gliomas had mutant IDH, they can have poor clinical outcome due to CDKN2A deletion.

EACR25-0060

Enhanced antitumor immunity following neoadjuvant chemotherapy in triple-negative breast cancer

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Introduction

Peripheral immune responses are increasingly recognized as occupying an important role in anticancer immunity.

Material and method

We evaluated serial blood samples from patients with triple-negative breast cancer (TNBC) undergoing neoadjuvant docetaxel, doxorubicin and cyclophosphamide chemotherapy (TAC) to characterize the evolution of the peripheral immune cell function and composition across the course of therapy. Serial blood samples from 10 patients with advanced TNBC treated with neoadjuvant chemotherapy (TAC) were collected before the initiation of chemotherapy, after the third and sixth cycles, and approximately 2 months after completion of chemotherapy. T-cell function was evaluated using ex vivo IFN γ ELISpot assays, and the dynamics of T-cell repertoire and immune cell composition were assessed using bulk and single-cell RNA sequencing (RNAseq). T cells exhibited an improved response to viral antigens after TAC, which paralleled the decrease in CEA and CA125 levels. Single-cell analysis revealed increased numbers of memory T-cell receptor (TCR) clonotypes and increased central memory CD8+ and regulatory T cells throughout chemotherapy.

Result and discussion

Finally, administration of TAC was associated with increased monocyte frequency and expression of HLA class II and antigen presentation genes; single-cell RNAseq analyses showed that although driven largely by classical monocytes, increased class II gene expression was a feature observed across monocyte subpopulations after chemotherapy.

Conclusion

TAC may alleviate tumor-associated immunosuppression by reducing tumor burden and may enhance antigen processing and presentation. These findings have implications for the successful combinatorial applications of immune checkpoint blockade and therapeutic vaccine approaches in TNBC.

EACR25-0062

Facilitators and barriers of gastroscopy screening in high-risk populations for gastric cancer: a qualitative study

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Introduction

To investigate the facilitators and barriers of gastroscopy screening in high-risk populations for gastric cancer, so as to provide reference for medical staff to develop intervention measures to enhance the compliance of

gastric cancer high-risk individuals with gastroscopy screening.

Material and method

From January to August 2023, the maximum difference purposive sampling was used to select patients in high-risk of gastric cancer who were recruited at the Southwest Hospital of the Army Medical University. A one-to-one, semi-structured in-depth interview method was used to collect data, and the data collection was terminated when saturation was reached.

Result and discussion

A total of 21 patients participated in the semi-structured interview. Facilitators of gastroscopy screening included three themes and eight subthemes: ability factors (rich disease knowledge, rich screening knowledge), motivation factors (awareness of susceptibility, awareness of warning symptoms, enhancement of screening awareness, perceived benefits), and opportunity factors (family support, social support); Barriers of gastroscopy screening included three themes and nine subthemes: ability factors (lack of disease knowledge, lack of screening knowledge, poor literacy), motivation factors (fear of gastric endoscopy, perception barriers), and opportunity factors (economic barriers, environmental and resource constraints, unpleasant screening experiences, low trust in medical care).

Conclusion

Multiple factors affect the compliance in gastric cancer high-risk patients for gastroscopy screening. Medical staff should develop the intervention measures to promote gastroscopy screening based on facilitators and barriers, so as to enhance the early detection rate and survival rate of gastric cancer.

EACR25-0064

Bioinformatics analysis for the identification of key genes and microRNAs related to bone metastasis in prostate cancer

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Introduction

Prostate adenocarcinoma (PCa) is the leading cause of cancer-related mortality in men, with bone metastasis being the primary contributor to mortality in PCa patients. Therefore, elucidating the etiology and molecular mechanisms underlying bone metastasis is crucial for early detection, accurate diagnosis, and personalized treatment.

Material and method

In this study, differentially expressed mRNA(DEGs) and miRNAs (DE-miRNAs) selected from datasets (GSE32269, GSE77930 and GSE21036) available in the Gene Expression Omnibus (GEO). Enrichment analysis for pathway and process among the DEGs was performed and detect the interactions of the DEGs and construct the PPI network and miRNA-mRNA regulatory network. Diagnostic and survival analyses were performed with logistic regression and Cox regression to evaluate the clinicopathological characteristics associated with overall

survival.

Result and discussion

Five eligible miRNAs(miR-636, miR-491-5p, miR-199b-5p, miR-149-5p, and miR-143-3p) had prognostic significance in biochemical recurrence-free survival. Logistic regression analysis revealed that the ectopic expression of these DE-miRNAs was associated with the histological type, anatomical region, and pathological grade of bone metastasis in PCa. The differentially expressed genes were significantly enriched in Erbb signaling pathway, mTOR signaling pathway, FoxO signaling pathway, Ras signaling pathway. Three hub genes (MMP12, NR4A2 and THY1) with worse biochemical recurrence-free survival and one hub gene (MMP9) with worse overall survival were detected.

Conclusion

These DEGs and DE-miRNAs may serve as potential biomarkers for the early diagnosis of bone metastasis in PCa and we have successfully defined molecular signatures of bone metastasis in PCa.

EACR25-0198

Trends of prostate cancer stage and grade over a 12-year period in Northern Ireland; a population-based analysis

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Introduction

In 2022, prostate cancer (PCa) became the most diagnosed cancer among males in the UK. Its incidence has significantly increased over time in comparison to other cancer types. This rise may be attributed to increased use of PSA blood testing, improvements in diagnostic procedures (MRI, PSMA-PET), and an aging population. Whilst it is important to diagnose high risk PCa in a timely manner, contemporary guidelines are in place to minimize the over-diagnosis of low-risk disease. These guidelines, together with advancements in diagnostic methods, may lead to a shift in the classification of lower-grade PCa. Using data from the Northern Ireland Cancer Registry (NICR), this study examines population-based trends in incident PCa according to tumour characteristics at diagnosis, and with respect to age and deprivation status.

Material and method

The NICR provides comprehensive, population-based data on cancer diagnosis in Northern Ireland (NI). This study analysed 14,273 incident PCa cases diagnosed from 2010–2021 in NI to assess trends in stage and grade at diagnosis. PCa cases were categorized using Grade Group (GG) and registry-derived TNM stage. Yearly trends were analysed to assess shifts toward more/less favourable disease characteristics.

Result and discussion

PCa diagnoses in NI increased 45% (973 in 2010 to 1,413 in 2021), with 65–69 years as the most affected age group (20% of cases). Over this 12-year period, there was a fourfold increase in stage 1 diagnoses (139 in 2010 to 663 in 2021) and a fourfold decrease in stage 2 cases (368 to 86). Stage 3 and unknown cases remained stable,

while stage 4 diagnoses doubled (126 to 258). Gleason grade distribution showed a 2.5-fold increase in GG3 cases. Low-risk (\leq GG1) cancers declined, dropping from 313 in 2012 to 153 in 2020. Deprivation analysis is the examination of how the lack of resources and opportunities can impact cancer incidence, outcomes and survival. This analysis showed a correlation between higher deprivation and more advanced disease. More deprived populations had fewer stage 1 diagnoses and more stage 4, suggesting delayed diagnosis or limited access to early detection. Similarly, high-risk Gleason scores (\geq GG4) were more frequent in more deprived areas, whereas low-risk scores (\leq GG1) were more common in less deprived groups.

Conclusion

PCa diagnoses in NI have risen, in conjunction with global trends, with earlier detection due to diagnostic advances. However, the increase in stage 4 cases highlights persistent late-stage diagnoses. Socioeconomic disparities remain evident, with higher deprivation linked to advanced disease, further emphasising the need for early detection and targeted interventions. Future directions include assessing trend correlations with clinical guideline changes (with clinician oversight) and identifying target populations that could benefit from: improved education, earlier screening, or adjustments in clinical settings.

EACR25-0385

Application of Mendelian randomization to appraise causality of the relationship between the gut microbiome and cancer

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Introduction

The gut microbiome has been implicated in cancer aetiology; however, its causal relevance is not clear. Mendelian randomization (MR) is increasingly being applied to assess the causal role of the gut microbiome on several health outcomes; however, the complexities and limitations of the method are not fully addressed in much of the current literature. Here, we aimed to appropriately apply MR to understand the role of the gut microbiome in cancer aetiology, whilst demonstrating the range of sensitivity analyses required to provide clarity in causal estimates.

Material and method

Two-sample MR was used to examine the causal relationship between microbial traits from two large microbiome genome-wide association studies (GWAS) (Hughes et al.(1) and MiBioGen(2)) and risk for cancers of the lung, breast, colorectum, pancreas, prostate, ovary, endometrium and oesophagus from the largest cancer GWASs, with replication sought in UK Biobank and FinnGen. Genetic instruments were selected using SNPs associated with microbial traits at genome wide significance ($P < 5 \times 10^{-8}$). A range of sensitivity analyses were performed to appraise the robustness of findings to violations of MR assumptions. These include ancestry-

specific analysis to account for differences in population structure, use of lenient p-value thresholds to select directionally consistent genetic instruments which are directionally consistent across the contributing GWAS cohorts, genetic colocalisation analysis to identify shared causal variants, a genome-wide approach to identify potential pleiotropic pathways, and Steiger filtering and reverse MR to identify potential reverse causality.

Result and discussion

Whilst our MR analyses suggested a putative causal role of 16 microbial traits across the eight cancers, few results were replicated in both UK Biobank and FinnGen and sensitivity analyses implied violations of MR assumptions, suggesting that these findings were unlikely to reflect causality. We did find that the relative abundance of bacteria in the class of Actinobacteria was associated with an increased risk of colorectal cancer, which was replicated in FinnGen. Colocalisation analysis suggests there could be a shared causal variant between the relative abundance of bacteria in the class of Actinobacteria and colorectal cancer, and MR results using a lenient P-value threshold were directionally consistent across pleiotropic methods and the main MR analysis. However, there were many additional traits associated with the SNP rs182549 in the genome-wide analysis which suggests that the SNP could influence colorectal cancer through a pleiotropic pathway.

Conclusion

Through testing for violations of core MR assumptions, our work demonstrates the importance of performing sensitivity analyses when assessing the robustness of MR findings, especially in the context of understanding the causal relevance of the gut microbiome on health outcomes.

EACR25-0531

Malignancies of the small intestine: incidence and trends in a nationwide registry.

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Introduction

Regardless the rising global incidence, malignancies of the small intestine remain rare. As it is a clinically heterogeneous group, knowledge about the different malignant entities is of importance to optimize diagnostic approaches and treatment strategies, and to ultimately improve prognosis.

Material and method

This nationwide retrospective cohort study, with data from the Netherlands Cancer Registry, included all patients diagnosed with a malignancy of the small intestine in the Netherlands between 2000 and 2022. Malignancies were divided into seven subgroups; adenocarcinomas, neuroendocrine neoplasms (NENs), gastrointestinal stromal tumors (GISTs), lymphomas, other sarcomas, metastases, and “other”. Age-standardized incidence rates, overall- and relative survival were reported.

Result and discussion

A total of 11,194 patients with a malignancy of the small intestine were included. Age-standardized incidence rates (reported per 100,000 person-years) more than doubled between 2000 and 2020 (1.88 to 3.94, $p < 0.001$), with the largest increase in NENs (0.53 to 1.57, $p < 0.001$), followed by adenocarcinomas (0.78 to 1.22, $p = 0.004$) and GISTs (0.14 to 0.53, $p < 0.001$). The age-standardized incidence of lymphomas remained relatively stable (0.32 to 0.46, $p = 0.088$) and sarcomas showed a decrease in age-standardized incidence (0.10 to 0.02, $p = 0.005$). Survival varied significantly between subgroups, with unchanged survival of patients with adenocarcinomas and NENs, in contrast to improved survival of patients with lymphomas and GISTs.

Conclusion

The incidence of malignancies of the small intestine in the Netherlands has more than doubled over the past two decades, mostly due to an increase in NENs and adenocarcinomas. Survival outcomes for these patient groups have not improved, highlighting the urgent need for further research on these rare cancers.

EACR25-1041

Exploring circulating proteins as intermediates between physical activity and cancer risk

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Introduction

Higher levels of physical activity (PA) are associated with a decrease in risk of several cancers, including breast, colorectal and endometrial cancers. In this study we investigated circulating proteins as potential intermediates between PA levels and risk of breast, prostate, endometrial and colorectal cancers.

Material and method

Data were used from UK Biobank (UKB), a cohort of 500,000 participants aged between 40-69 who were recruited between 2006-2010. Circulating proteins were measured by the Olink Explore 3072 panel at baseline. PA was measured between 2013-2015 using an Axivity AX3 wrist-worn triaxial accelerometer. Three PA measures were included: overall acceleration average, proportion of time doing moderate-vigorous activity and proportion of time sedentary. Cancer registry data were leveraged, and the international classification of disease codes (ICD9 and ICD10) were used to determine cancer case status, deriving a prevalent cancer variable, or incident cancer variable if diagnosed more than two years after enrolment into UK biobank. Linear or logistic regression were used to determine the association between variables, adjusting for potential confounders (including body mass index), accounting for multiple testing. Where there was evidence for an association between PA and protein levels, two sample Mendelian randomization (MR) was additionally applied to explore causal evidence for the relationship between PA ($N = 100,000$ in UKB) and Olink circulating proteins ($N = 34,000$ in UKB), using genetic variants strongly ($p < 5 \times 10^{-8}$) and independently ($R^2 < 0.0001$) associated with overall physical activity.

Result and discussion

Higher levels of PA were associated with a lower risk of breast, colorectal and endometrial cancers. All three PA measures were associated with levels of 114 proteins ($p < 2.7 \times 10^{-5}$), for example, more physical activity was associated with lower levels of osteoglycin. Of the 114 proteins associated with physical activity, 16 were associated with cancer outcomes ($p < 4 \times 10^{-4}$), 9 with incident cancer and 14 with prevalent cancer status. Higher levels of osteoglycin were associated with higher risk of breast cancer. For 16 proteins associated with both PA and cancer risk, MR evidence suggested a potential causal relationship between higher overall PA and lower levels of two proteins, osteoglycin and E3 ubiquitin-protein ligase RNF149, and estimates were concordant in direction with the observational result.

Conclusion

These analyses point towards proteins which associate with both physical activity levels and cancer risk. One of the proteins highlighted, osteoglycin, has previously been shown to have altered expression in breast tumour tissue and warrants further exploration. Sensitivity analyses including multivariable MR and reverse MR are ongoing to further understand the likely direction of effects.

EACR25-1151

Time to diagnosis for ovarian cancer in England: Associations with 'alternative explanation' comorbidities and recent menopause

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Introduction

Ovarian cancer is diagnosed at a late-stage 3/4 in the majority (62%) of patients resulting in poor survival and high morbidity and mortality. Several factors, i.e., biological, patient/healthcare system-related, and socio-economic factors contribute to delayed diagnosis of ovarian cancer. This also include lack of specific "alarm symptoms", and cancer symptoms are often attributed to chronic conditions especially irritable bowel syndrome, recurrent urinary tract infections or menopause-related changes.

Material and method

Patients in England born before 1955 and diagnosed with incident ovarian cancer between 1990 and 2019 were identified in the Clinical Practice Research Datalink (CPRD) Gold and CPRD Aurum databases and linked hospital admission and cancer registry data. 'Diagnostic interval' (or time to diagnosis) was defined as time from first presentation in primary care with a relevant sign/symptom to diagnosis. Associations between presenting sign/symptom, comorbidities offering "alternative explanations" for symptoms, and recent menopause and diagnostic interval were investigated using multivariable linear regression models adjusting for patient characteristics including age, presence of other comorbidities and consultation frequency. Models were run for Gold and Aurum databases separately, and results combined using meta-analysis.

Result and discussion

Complete data were available for 2010 patients in CPRD Gold and 1885 patients in CPRD Aurum. Presenting sign/symptom was associated with substantial variation in diagnostic interval. Compared to patients presenting with abdominal or pelvic pain, diagnostic interval was 125% higher in patients with back pain, and 63% higher for patients with urinary symptoms, but was substantially reduced (i.e., halved) in patients presenting with abdominal mass, distension or ascites. The diagnostic interval was also increased by 27% in women with two or more "alternative explanation" comorbidities versus those with none, and by 32% among women with recent menopause.

Conclusion

Our study provides evidence that ovarian cancer diagnosis may be delayed in women with chronic conditions such as irritable bowel syndrome and/or menopause-related symptoms/changes. Raising awareness of this possibility (i.e., symptom ambiguity) among primary care physicians may reduce the number of late-stage diagnosis, improve survival and reduce morbidity and mortality from ovarian cancer.

EACR25-1227

Characterization of the profile of cancer among vulnerable communities at Sanabel Nour in North Lebanon: an NGO setting

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Introduction

Cancer remains a major global public health concern with low and middle-income countries (LMICs) disproportionately affected. Lebanon, a low-income country (LIC), faces a significant cancer burden, particularly in vulnerable communities. This study aims to characterize the cancer profile of vulnerable communities in North Lebanon by analyzing data from patients receiving support from Sanabel Nour, a local NGO providing medical assistance to underserved communities in Tripoli.

Material and method

This retrospective chart review analyzed data from 440 cancer patients. Socioeconomic status was assessed using the Lebanese Multidimensional Poverty index. Data on demographics, tumor characteristics, and comorbidities were extracted from patient records. Descriptive statistics were employed for data analysis.

Result and discussion

The majority of participants were females (65.5%). Among females ($n = 288$), breast cancer is the most prevalent (48.6%), followed by colorectal cancer (7.6%). For males ($n = 152$), bladder cancer is the most common (18.1%), followed by colorectal cancer (14.6%). A concerning trend is the late-stage diagnosis of many cancer cases. Notably, the median age at diagnosis for female breast cancer patients is lower than the national average, and the mean age at diagnosis for colorectal cancer patients is also lower than the national mean.

Conclusion

Comprehensive cancer profiling across the country is essential for tracking disease trends and developing targeted interventions. Early screening programs are crucial to improve survival rates and patient outcomes

EACR25-1381

Brain Tumors and Neurodegenerative Diseases: What's the link?

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Introduction

Brain tumors represent a complex and heterogeneous group of neoplasms arising from abnormal cell growth within the brain. Previous observational studies have demonstrated a correlation between brain tumors and the risk of neurodegenerative diseases (NDs), but the causality and direction of their associations remain unclear. This relationship is complex and an area of ongoing research. Alzheimer's disease (AD) is the most common neurodegenerative disorder that shows epidemic associations with many types of tumors including brain tumors, sharing genetic characteristics. On the other hand, the antioxidant activity of natural compounds derived from olive oil appears to affect cancer behavior and, at the same time, mitigate neuroinflammation related to NDs. We explored *in vitro* the correlation between AD and neuroblastoma to evaluate the effects of the polyphenol Oleacein in modulating the principal genes associated with AD.

Material and method

Oleacein was prepared as reported by Procopio et al. Cell-Titer Glo assay was used to analyze cell viability of SHSY-5Y, a human neuroblastoma cell line, and 3Tg-I-Astro, an established immortalized astroglial cell line from 3xTg-AD mouse. SHSY-5Y cells treated with Oleacein (5 μ M) were tested using TaqMan Array 96-Well Plate AD by qRT-PCR reaction. Datasets from TCGA database at UALCAN were interrogated to obtain the differential expression of specific human genes. Reactome Pathway Browser was used to visualize common correlated canonical pathways.

Result and discussion

First, we evaluated the effect of Oleacein on cell viability (48 hours), resulting in a significant reduction in SHSY-5Y cell proliferation, with no effect in 3Tg-I-Astro cells. In addition, four AD-related genes were modulated by Oleacein in SHSY-5Y cells. In detail, we found an upregulation of Gap Junction Beta1 (GJB1), Beta-Secretase2 (BACE2) and Acetylcholinesterase (ACHE) genes, while Solute Carrier Family 30 member 3 (SLC30A3) gene was downregulated. In TCGA-UALCAN database, the upregulated ACHE and GJB1 genes were reported to be downregulated in human brain tumor samples compared to normal ones. Network analysis provides interactions of modulated genes in common canonical pathways suggesting novel predictive and therapeutic targets.

Conclusion

AD and neuroblastoma seem to have common genetic implications. Oleacein antioxidant and anti-inflammatory activities in SHSY-5Y cells could modulate the expression levels of AD-related genes, offering new diagnostic and therapeutic opportunities.

EACR25-1543

When clinical meets molecular: CTNNA1 germline alterations are associated with hereditary diffuse gastric cancer development

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Introduction

CTNNA1 germline variants were associated with hereditary diffuse gastric cancer (HDGC), and in 2020, CTNNA1 was recognized as a HDGC-associated gene, despite the scarce supporting research. Despite joint efforts to improve knowledge on CTNNA1-associated disease and cancer lifetime risks, CTNNA1-related disease spectrum, variant-type causality and clinical criteria driving genetic testing remain vastly understudied. This lack of knowledge limits clinical management of CTNNA1 variant carrier families and definition of criteria for variant classification. We aim to explore CTNNA1 genotype-phenotype associations to improve clinical criteria driving genetic testing, surveillance and risk-reduction recommendations for carriers.

Material and method

Using molecular, clinical and population data from 351 CTNNA1 variant carrier families from Europe and America, we analysed genotype–phenotype associations with multivariable logistic regression, and further compared results to a CDH1 variant carrier cohort. We knockout (KO) CTNNA1 using CRISPR/Cas9 in gastric cancer (GC) cells, developed CTNNA1 humanized Drosophila models and analysed diffuse GC (DGC) tumour samples from CTNNA1 carriers, to assess CTNNA1 associated loss-of-function mechanisms. We redefined current HDGC clinical criteria to improve CTNNA1 genetic testing yield.

Result and discussion

We found that Nonsense Mediated mRNA Decay (NMD) degrades CTNNA1 transcripts bearing premature stop codons, and that DGC tumours from CTNNA1 truncating carriers lose α E-catenin. Using our humanized Drosophila model, we demonstrated that truncating transcripts are non-functional, unlike non-truncating ones. Analysis of clinical data showed that the likelihood of DGC development is 8-fold higher in truncating than in non-truncating carriers. Also, risk of GC development is 7-fold higher in CTNNA1 truncating carriers compared to wild-type individuals, but is significantly lower than the 38-fold higher risk of GC development in CDH1 carriers. Lobular breast cancer is recurrent among CTNNA1 truncating carrier families, some lacking HDGC criteria. Refining HDGC criteria increased CTNNA1 carrier families' pick-up rate by 9%.

Conclusion

We provide compelling evidence supporting that CTNNA1 truncating variants are positively associated

with DGC development and that NMD is the pathophysiological mechanism leading to CTNNA1 downregulation in HDGC. We demonstrate that, compared to CDH1, CTNNA1 is a moderate penetrance HDGC gene. This knowledge is new and essential to define surveillance and/or prophylactic surgery measures for CTNNA1 carrier individuals/families.

Acknowledgements: This work was developed under the collaborative environment of the CTNNA1 worldwide working group. FCT PhD fellowship (2020.05773.BD); ERN-GENTURIS (Project No.739547); PREVENTABLE project (Grant Agreement n° 101095483).

EACR25-1665

Genetic Insights into Familial Non-Medullary Thyroid Cancer: Whole Exome Sequencing Reveals Potential Susceptibility Genes in a Representative Family

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Introduction

Thyroid cancer is the most common endocrine neoplasia and about 10% of all cases are of familial origin. Non-syndromic familial non-medullary thyroid carcinoma (FNMTC) is a form of hereditary thyroid cancer characterized by its polygenic nature, autosomal dominant inheritance pattern and incomplete penetrance. Despite its clinical significance, few FNMTC susceptibility genes have been identified, making early diagnosis challenging and hindering the development of targeted therapies. Building on our team's previous contributions to the genetic characterization of FNMTC, this study aims to identify novel candidate genes that may confer susceptibility to the disease in a representative family from our cohort.

Material and method

Five members from a large FNMTC family were selected for analysis, including two with papillary thyroid carcinoma (PTC), two with multinodular goiter (MNG), and one with Hashimoto's thyroiditis. Whole exome sequencing was performed in leucocyte DNAs and significant variants were selected through bioinformatic analyses.

Result and discussion

A pathogenic TGFBI p.R666S missense mutation was found to affect three members of the family, one with PTC and two others with MNG and Hashimoto's thyroiditis. A previously known pathogenic FOXE1 variant (p.A248G) affecting other FNMTCs, as well as a pathogenic TPO p.Q660E missense mutation were found in the same two members with PTC and with MNG. Besides the TGFBI p.R666S missense mutation, the third

member with Hashimoto's thyroiditis also harbored pathogenic missense mutations affecting AR (p.P392S), MEFV (p.A744S) and SLC34A3 (p.G196R). In addition, the fourth member with MNG was found to harbor a pathogenic LRP2 p.D2054N missense mutation. The final member with PTC did not exhibit any identifiable pathogenic variants, suggesting that the PTC in this case is likely sporadic rather than genetically inherited.

Conclusion

A range of pathogenic missense variants was identified in the majority of the FNMTC-affected family members, suggesting a polygenic inheritance pattern. Subjecting additional members of the same family to whole exome sequencing is hence required to ascertain which pathogenic variant is susceptible to promote a FNMTC phenotype.

EACR25-1676

Mapping Genetic Risk Variants for Sporadic Colorectal Cancer in a Brazilian Population: Insights from a Genome-Wide Association Study

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Introduction

Colorectal Cancer (CRC) is the second leading cause of cancer-related deaths worldwide, with most of the cases being sporadic and resulting from the combination of external factors and genetic variations, specifically single nucleotide polymorphisms (SNPs). Over the years, genome-wide association studies (GWAS) have identified numerous risk SNPs associated with sporadic CRC. However, most of these studies have been conducted in European populations, which lack representativeness of admixed populations, such as the Brazilian one. Therefore, this study aims to perform the first GWAS on sporadic CRC in Brazil, using 850k Axiom™ PMDA to discover and validate new risk biomarkers.

Material and method

A total of 1,371 CRC patients and 1,922 cancer-free Brazilians were included, with mean ages of 60 and 54 years, respectively. All Brazilian regions were represented, with the predominance of the Southeast region (53%) for both cases and controls. DNA genotyping was performed using the 850K Axiom™ Precision

Medicine Diversity Array (PMMA), constituted by 788,623 variant regions. Data analysis was conducted using the Applied Biosystems™ Axiom™ Analysis Suite, and SNP quality control (QC) was performed via PLINK. Population stratification was assessed by constructing a Multidimensional Scaling (MDS) plot based on the 1000 Genomes dataset. A genome-wide significant threshold of $-\log_{10}(1.58e-07)$ was applied for association testing.

Result and discussion

MDS analysis confirmed our cohort population is genetically admixed. The Manhattan plot identified 50 significant CRC-associated variants, with the most significant being rs10255340 ($p = 1.64e-10$; RELN \leftrightarrow ORC5; OR = 0.6528). Variants rs62251956 (TRIM71; OR = 1.804; $p = 8.11e-8$) and rs11790363 (NTRK2 \leftrightarrow AGTPBP1; OR = 1.866; $p = 1.50e-7$) exhibited higher odds ratio, indicating a significant association with increased CRC risk. These findings provide novel insights into CRC susceptibility in admixed populations and highlight the importance of genetic studies in diverse groups.

Conclusion

This is the largest GWAS assessing the risk of sporadic CRC in Brazil. It suggests 50 potential risk loci for this admixture population. Further studies will be conducted to calculate a significant polygenic risk score (PRS), and additional validation will be performed in a larger population.

EACR25-1701

Incidence of chronic medical conditions in adolescent and young adult survivors of hematologic malignancies in California, 2006–2020

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Introduction

Survivors of adolescent and young adult (AYA) hematologic malignancies have a high risk of chronic medical conditions. We evaluated the cumulative incidence and

incidence rate ratio (IRR) for several conditions in cancer survivors compared to a matched cohort without cancer.

Material and method

We used data from Kaiser Permanente (KP) Northern and Southern California to identify AYAs diagnosed at age 15–39 years with Hodgkin lymphoma (HL), non-Hodgkin lymphoma (NHL), acute myeloid leukemia (AML), or acute lymphoblastic leukemia (ALL), from 2006–2020, who survived ≥ 2 years after diagnosis. KP members without a history of cancer were matched (10:1) to survivors by age, sex, diagnosis year, and KP site. We calculated the cumulative incidence of each condition, accounting for death as a competing risk. Multivariable-adjusted Poisson regression estimated rate ratios in survivors versus the matched cohort, adjusting for age at diagnosis, sex, and race/ethnicity.

Result and discussion

Among 2,624 AYAs with hematologic malignancies (42% HL, 40% NHL, 9% AML, 9% ALL), with a median follow-up of 5.9 years (interquartile range: 3.7–9.3), the 10-year cumulative incidence was 43.9% for any chronic medical condition (vs. 22.4% in the matched cohort) and 16.1% for ≥ 2 conditions (versus 4.4%).

Overall, after adjusting for demographic factors, cancer survivors had 2.7- and 4.8-times higher rates of developing 1 and ≥ 2 conditions, respectively (IRR = 2.7, 95% CI 2.5–2.9 and 4.8, 95% CI 4.2–5.4), with IRRs ranging from 1.8 (95% CI 1.6, 2.1) for respiratory disease to 56.2 for avascular necrosis (95% CI 29.4, 107.5). In patients with ALL and AML, the highest IRRs were, respectively, for VTE (36.8, 16.4), renal disease (15.4, 14.1), diabetes (8.3, 6.1), and cardiovascular disease (6.3, 6.5). In patients with NHL and HL, the highest IRRs were, respectively, for avascular necrosis (34.2, 7.4), VTE (8.1, 7.0), renal (4.8, 5.8), and cardiovascular disease (2.9, 3.1). In AYAs with lymphomas, risk of most conditions was higher for those diagnosed with regional or distant disease (vs. localized). Elevated IRRs were observed across all demographic groups, insurance types, and neighborhood deprivation levels.

Conclusion

AYA survivors of hematologic malignancies had a higher risk of all chronic conditions than the non-cancer comparison cohort. Long-term surveillance, risk mitigation through lifestyle changes, and effective disease management should be evaluated to improve quality of life and reduce premature mortality.

EACR25-1706

Chamosite in Late Permian C1 coal and the high incidence of lung cancer in Xuan Wei

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Introduction

The Xuan Wei area of Yunnan Province, in the southwest of China, bears the abnormally high incidence and mortality rate of lung cancer in the world.

Epidemiological studies have suggested that this has been associated with the domestic combustion of C1 coal from the high incidence of lung cancer area in Xuan Wei, which contains iron-rich chamosite. However, the underlying molecular mechanism of lung cancer induced by chamosite-containing C1 coal is not clear. Thus, our study provides experimental insights into how particles in C1 coal contribute to the development and progression of lung cancer, which may provide potential systemic treatment options for Xuan Wei lung cancer patients.

Material and method

Scanning Electron Microscopy (SEM), Diffraction of x-rays (XRD), X-ray fluorescence spectrometry (XRF) and Particle Size Distribution (PSD) were utilized to characterize the low-temperature coal ash (LTA).

Transmission Electron Microscope (TEM) was performed to validate that LTA enters the cells and lung tissue. Changes in human bronchial epithelial(B2B) cells after C1 and C9 LTA exposure were investigated by XTT assay, colony formation assay and 3D colony formation assay. Immunoblotting, and immunofluorescence staining were also employed for functional studies.

Result and discussion

Our XRD analysis indicates that chamosite is contained in C1 and C9 LTA, not in C1 and C9 high-temperature ash (HTA). The XRF results showed C1 LTA has higher iron content than C9 LTA. The analysis of BEAS-2B cells with TEM demonstrated that the mitochondria were subcellular targets of LTA. LTA was also found in the lung tissue of mice by TEM. Histopathological analysis of lung sections using H&E staining demonstrated that LTA treatment for 3 months caused more severe injury and fibrosis than the mice in the control group.

Additionally, EGFR and KRAS genes were over-expressed both in vivo and in vitro.

Conclusion

Our study indicates that long-term exposure of C1 and C9 LTA may lead to malignant transformation of normal cells as well as alterations in the lung tissue of mice. The deeper mechanisms will be addressed in further work.

EACR25-1726

Effect of Pathogenic Mismatch Repair

(MMR) Genes on the Systemic Metabolome and Their Link to Cancer Risk in a Lynch Syndrome Cohort

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Introduction

Systemic metabolism affects cellular processes and can modulate the tissue microenvironment in complex ways, potentially impacting cancer initiation processes. In Lynch syndrome, a condition characterized by carriers of pathogenic mutations in DNA mismatch repair genes (path_MMR genes), genetic background significantly increases cancer risk. However, not all carriers develop cancer. Various lifestyle factors can influence Lynch syndrome cancer risk, with lifestyle choices actively shaping systemic metabolism, serving as the mechanistic link between lifestyle and cancer risk. In this study, we explore systemic metabolism at different omics levels, including the circulating metabolome, proteome, and enzymatic profiles of Lynch syndrome carriers.

Material and method

This study comprises a two-group cross-sectional analysis to compare the circulating metabolome, proteome, and enzymatic levels of cancer-free Lynch syndrome carriers with healthy non-carrier controls. We correlate these results with clinical parameters and path_MMR status to discern the metabolic distinctions attributable to genetic differences.

Result and discussion

Our analysis detected elevated levels of circulating cholesterol, lipids, and lipoproteins in Lynch syndrome carriers. Furthermore, significant alterations were observed in circulating amino acid and ketone body profiles when compared to healthy non-carriers, suggesting a distinct systemic energy metabolism in Lynch syndrome carriers. These findings highlight a unique metabolic landscape in cancer-free Lynch syndrome carriers, which may contribute to their varied cancer risk.

Conclusion

This study provides valuable insights into the systemic metabolic landscape of individuals with Lynch syndrome, enhancing our understanding of how energy metabolism is linked to cancer risk. The metabolic signatures identified could serve as potential biomarkers for early detection and risk assessment in Lynch syndrome.

EACR25-1750

Sociodemographic and clinicopathologic features associated with rapid relapse in triple-negative breast cancer: A Swedish nationwide registry-based study

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Introduction

Triple-negative breast cancer (TNBC) is associated with high recurrence rates within the first three years of diagnosis. Here, we identify sociodemographic and clinicopathologic factors associated with rapid relapse or death in TNBC using Swedish population-based registry data.

Material and method

Data were retrospectively collected from national registries for 9262 women diagnosed with primary invasive TNBC in Sweden between 2007 and 2021. Eligible patients ($n = 8338$) were categorized into three relapse groups: rapid relapse or death within 2 years (rrTNBC), late relapse or death after 2 years (lrTNBC), and non-rapid relapse for <5 years follow-up and no survival event (non-rrTNBC). Multivariable logistic regression and nomogram modeling were employed to assess predictive factors for relapse.

Result and discussion

Of the 8338 eligible patients, 15% experienced rapid relapse. Rapid relapse was associated with advanced age (71 years vs. 61 years in lrTNBC), larger tumor size (30 mm vs. 20 mm in lrTNBC), and advanced stage at diagnosis (19% Stage III in rrTNBC vs. 4% in lrTNBC) and after surgery (rrTNBC 29% vs. lrTNBC 7%).

Compared to the lrTNBC group, the rrTNBC group frequently received neoadjuvant chemotherapy (NACT), but were less likely to receive breast-conserving surgery combined with adjuvant radiotherapy. Although pathologic complete response (2.6%) was generally poor for those undergoing NACT, regional residual disease was more common in the rrTNBC group (67% vs. 40% in lrTNBC). Furthermore, rrTNBC patients had parents born outside of Sweden, lower education levels, lower income, and a higher proportion of widowed individuals. Logistic regression identified age, tumor size, tumor grade, nodal status, and Charlson comorbidity index as key predictors of relapse risk. The predictive model achieved an AUC of 70%, indicating moderate accuracy.

Conclusion

Taken together, rapid relapse in TNBC is strongly linked to advanced age, tumor characteristics, and socio-economic status. The predictive model provides a valuable tool for identifying high-risk TNBC patients in need of personalized treatment strategies.

EACR25-1850

Exploring the role of monoallelic pathogenic MUTYH variants in colorectal cancer risk: Clinical implications and genetic findings in Serbian population

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Introduction

MUTYH is a crucial gene in the base excision repair pathway that encodes the A/G-specific adenine DNA glycosylase, also known as hMYH. This enzyme is essential for maintaining genomic integrity by removing adenine residues mispaired with 8-oxoguanine, a product of oxidative DNA damage. Biallelic pathogenic variants

in this gene cause autosomal recessive MUTYH-associated polyposis (MAP). Individuals with MAP have a 70%–90% absolute risk of developing colorectal cancer (CRC) if polyposis is left untreated, with the disease most commonly diagnosed between the ages of 45 and 59. However, the cancer risk associated with germline variants in individuals carrying a single defective MUTYH allele remains a topic of debate.

Material and method

Using Next-Generation Sequencing (NGS), the Institute for Oncology and Radiology of Serbia (IORS) has been performing screening for hereditary CRC since 2018. Patients are selected based on early-onset cancer, personal and/or family history of colorectal cancer. In total, 116 individuals meeting criteria for genetic testing for hereditary colorectal cancer were analyzed using the Illumina TruSight Hereditary Cancer Panel at the Genetic Counseling Department at IORS.

Result and discussion

In 6 of 116 patients (5.17%), three different monoallelic pathogenic germline variants in the MUTYH gene were detected (c.1353_1355delGGA, c.1103G>A, c.650G>A). Five of them were diagnosed with colon polyposis and/or CRC between the ages of 29 and 49, with one of these patients developing three primary colon cancers due to many polyps. Four of these five patients have a family history of colorectal, endometrial, and/or breast cancer. The sixth patient carrying the pathogenic MUTYH variant, aged 57, has a long-established history of gastric polyposis and no family history. While some population-based analyses of monoallelic MUTYH P/LP variant carriers suggest that these variants do not significantly increase CRC risk, emerging evidence indicates that even heterozygous pathogenic variants may contribute to a heightened risk of malignancies, particularly CRC and other types of cancer. One proposed mechanism is that MUTYH deficiency in heterozygosity can promote tumorigenesis through Loss of Heterozygosity (LOH) of the functional allele.

Conclusion

According to the 2024 NCCN Guidelines, individuals with a single defective MUTYH allele do not have an increased risk of developing CRC, but individuals with a personal or family history of polyposis are advised to have increased screening compared to the general population. The results of several studies are contradictory and range from no increased risk to a slight or moderate increased lifetime risk of developing colorectal, endometrial, or breast cancer. These findings highlight the need for further research to better define the role of monoallelic MUTYH variants and improve clinical practice in genetic counseling and cancer prevention.

EACR25-1883

Uncovering Missing Heritability in Prostate Cancer: A Functional Genomics Approach Using Allele-Specific Expression

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Introduction

Prostate cancer (PC) has an estimated heritability of approximately fifty eight percent and less than half of its genetic contribution has been elucidated. Identifying target genes and understanding their functional consequences remain major challenges, requiring novel approaches. Since most identified risk loci lie outside protein-coding regions and often act through cis-regulatory mechanisms, we hypothesize that much of PC's missing heritability is attributable to unidentified cis-regulatory variants. We propose an innovative framework to genetic association studies by leveraging differential allelic expression (AE) as a quantifiable readout of cis-regulatory variant effects. This method effectively controls for trans effects using an internal reference within each sample. A similar strategy successfully identified new breast cancer (BC) risk loci and, given evidence of shared regulatory variants between BC and PC, pleiotropy will also be assessed.

Material and method

We analyzed RNA-seq data from prostate tissue of healthy individuals (GTEx n = 116) and tumor-matched normal prostate from individuals with PC (TCGA n = 51). RNA-seq was mapped to a reference genome using STAR and variant calling was performed using GATK. For TCGA samples, genotype data were imputed from microarrays. Case-control association studies will be conducted using allelic expression fraction. daeQTL and functional analyses will be performed on loci of interest to elucidate regulatory mechanisms and pinpoint causal variants.

Result and discussion

The analysed samples include a diverse ancestry, with a predominant european background. The AE analysis pipeline allowed the standardization and cross-comparison between RNA-seq data from two independent datasets. Sample-wise mean AE fraction at heterozygous sites is around 0.5, indicating an overall balanced allelic expression. Ongoing statistical analysis, including effect size estimation, aim to identify variants whose AE levels are associated with PC risk modulation. Variants exhibiting significant associations after multiple test correction will be compared to those identified in breast cancer using the same methodology, as well as to previously reported GWAS.

Conclusion

By applying this approach that enhances case-control studies, we are able to leverage allele-specific expression as a quantitative phenotype and improve the identification of genetic risk factors in PC. The focus on cis-regulatory variants, allows this approach to refine risk prediction and uncover novel mechanisms underlying PC susceptibility.

Work was supported by Portuguese national funding through FCT—Fundação para a Ciência e Tecnologia (2023.01351.BDANA, DL 57/2016/CP1361/CT0042, and PLEIREG 2022.02380.PTDC) and institutional support (CINTESIS UIDB/04255, RISE LA/P/0053/2020, and CCMAR UIDB/04326/2020)

EACR25-2235**Transcriptome-wide Mendelian randomisation exploring dynamic CD4+ T cell gene expression in colorectal cancer development**

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Introduction

Recent research has identified a potential protective effect of higher numbers of circulating lymphocytes on colorectal cancer (CRC) development. However, the importance of different lymphocyte subtypes and activation states in CRC development remains poorly understood and warrants further investigation. CD4+ T cells, a highly dynamic lymphocyte subtype, undergo remodelling upon activation to induce the expression of genes critical for their effector function. Previous studies investigating their role in CRC risk have used bulk tissue, limiting our current understanding of the role of these cells to static, non-dynamic relationships only.

Material and method

Here, we combined two genetic epidemiological methods – Mendelian randomisation (MR) and genetic colocalisation – to evaluate evidence for causal relationships of gene expression on CRC risk in multiple CD4+ T cell subtypes and stages of activation. Genetic proxies were obtained from single-cell transcriptomic data, allowing us to investigate the causal effect of expression of 1,805 genes across five CD4+ T cell activation states on CRC risk (78,473 cases; 107,143 controls). We repeated analyses stratified by CRC anatomical subsites and sex, and performed a sensitivity analysis to evaluate whether the observed effect estimates were likely to be CD4+ T cell-specific.

Result and discussion

We identified six genes with evidence ($FDR-P < 0.05$ in MR analyses and $H4 > 0.8$ in genetic colocalisation analyses) for a causal role of CD4+ T cell expression in CRC development – FADS2, FHL3, HLA-DRB1, HLA-DRB5, RPL28, and TMEM258. We observed differences

in causal estimates of gene expression on CRC risk across different CD4+ T cell subtypes and activation timepoints, as well as CRC anatomical subsites and sex. However, our sensitivity analysis revealed that the genetic proxies used to instrument gene expression in CD4+ T cells also act as eQTLs in other tissues, highlighting the challenges of using genetic proxies to instrument tissue-specific expression changes.

Conclusion

Our study demonstrates the importance of capturing the dynamic nature of CD4+ T cells in understanding disease risk, and prioritises genes for further investigation in cancer prevention research.

EACR25-2243**Subtyping Xeroderma Pigmentosum in Tanzania through blood whole-exome sequencing**

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Introduction

Xeroderma Pigmentosum (XP) is a rare inherited disease caused by mutations in a group of 9 DNA repair genes (XPA, ERCC3, XPC, ERCC2, DDB2, ERCC4, ERCC5, ERCC1, POLH). XP patients develop an extreme sensitivity to ultraviolet radiation and exhibit a high risk of developing skin cancer and serious skin disease due to sun rays extreme sensitivity. The incidence of XP in Tanzania is much higher than in Europe due to consanguineous marriages and the genetic landscape of XP patients in Tanzania remains unknown. Since 2017 we initiated an XP outreach program aiming at providing awareness of the damaging effects of UV radiation and early cancer detection, treatment and follow-up clinic every 2 months. This study aims to determine the genetic subtypes of XP in Tanzania and its correlation with clinical presentation. As genetic profiling has a high cost and it is not affordable to perform it in a routine manner in Tanzania, this study should allow us to predict the genotype, and the risk of complications based on the early symptoms and thus to improve the management of these patients.

Material and method

We performed a whole-exome sequencing on 156 blood samples from patients and parents. To detect variants across samples, we used HaplotypeCaller, a software specifically designed to call germline single nucleotide polymorphisms and indels. Variants were next annotated for pathogenicity with GeneBe. Additionally, we performed bulk RNA sequencing on keratinocyte samples from 40 patients in our cohort.

Result and discussion

We detected 17 variants classified as pathogenic, likely and predicted pathogenic in XP genes. We detected

homozygous and double-hit heterozygous pathogenic mutations in four XP subtypes: XPC, XPA, XPF and XPG. A known XPC splice-site variant was detected in 130 of the 156 individuals. To correlate the emergence of this XP hotspot with consanguinity, we carried out an inbreeding analysis with Automap to identify runs of homozygosity (ROH), i.e. genomic regions that are identical by descent. As expected, we found an enrichment of ROH in the region harbouring the XPC gene in XPC patients compared to parents. This highlights how consanguinity leads to high frequency of XP in this population where the known XPC hotspot is a low-frequency polymorphism.

Conclusion

In this study, we identified four XP subtypes, with XPC being the most prevalent. XPC is primarily caused by a hotspot mutation, which is a splice site variant and mainly results from consanguinity. These findings highlight the genetic basis of XPC and its underlying mutational mechanisms.

EACR25-2528

Changing epidemiology and trends in incidence of childhood acute lymphoblastic leukaemia in Scotland: An analysis of the national cancer registration data, 1971-2020

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Introduction

Acute lymphoblastic leukaemia (ALL) is the most common haematological malignancy in children (aged 0-14 years), accounting for about 25% of all childhood cancers. The highest incidence is observed in developed countries in Europe, North America and Oceania. The disease is relatively more common in males than females, with a M:F ratio of about 1.3-1.5:1. The aetiology of ALL is not well understood – this includes genetic (e.g. Down's syndrome) and environmental (e.g. exposure to high levels of ionizing radiation) factors and 'delayed infection hypothesis'. We conducted a retrospective population-based cohort study to examine whether there have been changes in the epidemiology of ALL in Scotland during the past five decades.

Material and method

Individual-level, national (population-based) cancer registration data for patients diagnosed with childhood (age 0-14 years) ALL (ICD-10 code, C91.0) in Scotland from 1970-2020 were obtained from the Scottish Cancer Registry, Public Health Scotland. Average annual incidence rates were calculated by gender during the 10 five-year time periods (1971-75 to 2016-20). The percentage (%) change in the incidence rates in each gender was calculated as the change in the average annual incidence rate from the first (1971-75) to the last time period (2016-20). The average annual percentage change (i.e. year-on-year increase in incidence rates during 1971-2020) was estimated using the slope of the linear trend line fitted to the incidence rates by year of diagnosis.

Result and discussion

During the 50-year study period (1971-2020), a total of 2,951 new cases of childhood (age, 0-14 years) ALL were registered in Scotland (56.5% males, 43.5% females). The average annual incidence rates increased by about 56% in males (from 2.7/100,000 children in 1971-75 to 4.2/100,000 in 2016-20), and about 95% in females (from 2.1/100,000 in 1970-75 to 4.1/100,000 in 2016-20). The average annual percentage change in incidence rate was 1.2% in males and 1.6% in females.

Conclusion

It appears that there has been a small but steady increase in the incidence of childhood ALL in Scotland during the past 5 decades. Some of this increase may be due to the improvement in diagnostic procedures during this period. Although generally the disease is more common in males, our study showed that the incidence rates in recent years have become similar in both genders (4.2/100,000 vs. 4.1/100,000). There was also a much higher increase in the incidence rates in females than in males (95% vs. 56%) over the study period. This unexpected relatively greater increase in incidence in female children needs further investigation.

Prevention and Early Detection

EACR25-0059

A 8-miRNA risk score-based prediction model for liver cancer rupture

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Introduction

Approximately 700,000 people die of Hepatocellular Carcinoma (HCC) each year worldwide, making it the third leading cause of cancer related deaths. Rupture is a potentially life-threatening complication of HCC. Spontaneous rupture is the third most common cause of death due to HCC after tumor progression and liver failure.

Material and method

Here, we derived a 8-microRNA risk score (8-miRNA RS)-based model with better performance in the prediction of liver cancer rupture in 755 advanced liver cancer patients (273, 265, and 217 in the training, internal, and external validation sets, respectively). This model, presented as a nomogram, included four parameters: the 8-miRNA RS, TNM stage, primary tumor size, and volume transfer constant (Ktrans).

Result and discussion

Favorable calibration and discrimination of 8-miRNA RS-based model with areas under the curve (AUC) of 0.865, 0.811, and 0.804 were shown in the training, internal, and external validation sets, respectively. Patients who have higher nomogram score (>92.2) would have higher liver rupture rate ($P = 0.004$).

Conclusion

In summary, our data showed the 8-miRNA RS-based model could precisely identify more patients who are likely to develop tumor nodule rupture, which may help clinicians formulate the initial prevention and diagnosis

strategy and consequently achieves better clinical prognosis for patients with liver cancer.

EACR25-0066

Implementing cancer prevention strategies in the occupational setting: the Cancer Prevention at Work (CPW) study protocol

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Introduction

Chronic infections account for 13% of human cancer. Helicobacter pylori (Hp), Hepatitis C Virus (HCV) and Human Papilloma Virus (HPV) are responsible together for 75% of this burden. The adoption of evidence-based preventive interventions is suboptimal. The occupational health surveillance (OHS) would be a suitable framework for implementing health promotion activities, given the structured medical examination program. The aims of Cancer Prevention at Work (CPW) are (i) to conduct pilot protocols for prevention of cancers caused by Hp, HCV and HPV among workers; (ii) to estimate the cost-effectiveness of these interventions and compare them with no intervention scenario; (iii) to develop plans for the large-scale implementation of such interventions and engage stakeholders; (iv) to identify barriers and facilitators of occupational-based interventions; (v) to provide data on prevalence of infection and of adherence to cancer prevention strategies in different types of workers.

Material and method

CPW is held in four European Countries with a high prevalence of cancer-related infections: Italy, Spain, Romania and Slovakia. Population involved include healthcare, retail, finance, metal and manufacture workers. Three interventions - Hp test; HCV test; HPV vaccination - will be implemented during the OHS as a prospective pilot study involving at least 1000 workers in each center. The recruitment started in summer 2024 and will continue for 12-18 months. Anonymized data will be collected electronically and centralized. Workers who tested positive for Hp and HCV and their family members (FMs) will be followed up through confirmatory diagnostic tests and eventual treatment. HPV vaccination will be offered to eligible workers and

their FMs. A 6-month follow-up questionnaire will be administered to the participants.

Result and discussion

The project is expected to identify cost-effectiveness advantages from using OHS to implement interventions that are broader in scope than occupational disease prevention. If such advantages are identified, the project has a significant potential to influence future research efforts and practical initiatives within the OHS that prevent all (including non-occupational) cancer. The project will also describe the determinants of the cost-effectiveness of the interventions, providing a robust evidence base for decision makers to draw conclusions on the potential benefits from replicating the interventions in other sectors and countries.

Conclusion

OHS represents an innovative context for cancer prevention implementation and might offer an effective contribution to cancer control, reaching heterogeneous populations and sensitizing to cancer prevention.

This work is supported by the European Union Horizon Europe Research and Innovation Programme under the CPW Grant Agreement No. 101104716.

EACR25-0067

Helicobacter pylori screening integrated to occupational health surveillance – the HPOS Study

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Introduction

Eradication of Helicobacter pylori (Hp) infection can prevent cancer development¹⁻⁵. However, there are still no standardized screening protocols⁶. We aimed at testing an occupational-based Hp screening in a population of Italian healthcare workers (HCW).

Material and method

The study, approved by the Ethical Committee, started in January 2023 and will be completed in March 2025. The population includes HCW employed at the S.Orsola University Hospital, Bologna, aged 40-65. Detailed occupational, lifestyle and clinical information were collected through a written questionnaire. Hp infection was tested with stool antigen test (SAT). Hp positives were referred to the general practitioner (GP) for treatment; they were followed-up at 3 months by phone interview on health status, management of the infection and feedback around the implemented screening.

Result and discussion

Up to July 2024, 567 HCW were contacted, 379 agreed to participate and 278 returned the SAT for analyses. Of these, 213 (76.9%) were women and 64 (23.1%) were men, mean age 51 years old. Overall, 46 (16.6%) were Hp positive and 232 (83.4%) Hp negative. Family history of Hp infection was reported by 46 HCW (17.1%), while 152 (56.5%) did not report any history and 71 (26.4%) did not know; 34 HCW (12.7%) reported family history of gastric cancer. Most participants were nurses (N =

123, 45.4%), and worked in medical units ($N = 107$, 42.7%); 118 (43.5%) performed invasive practices during their activity; 36 (13.3%) worked in gastroenterology units; 115 HCW (42.9%) reported gastritis and 110 (41.5%) abdominal pain; 91 (34.0%) had undergone upper endoscopy. Surprisingly, 16 HCW (5.9%) reported to have no knowledge on Hp infection. To date, 39 of the 46 Hp positives were followed-up: 25 contacted the GP, 11 referred to another physician, the remaining did not report any contact. Of the 36 referring to a doctor, 30 were prescribed antibiotics (14: 10-day sequential; 12: 10-day concomitant therapy; 3: 14-day triple therapy; 1: triple therapy modified; 2: not prescribed any therapy). Feedback on the study were very good: 27 HCW would suggest this screening to their families; 18 HCW involved their families in Hp screening; 22 HCW reported 9-10 points at the experience quality score.

Conclusion

We implemented an occupational-based Hp screening program for gastric cancer prevention within HCW in Italy obtaining good compliance and feedback.

Prevalence of infection was lower than expected. Replication in other populations of workers is needed.

EACR25-0146

Characterize the spatial biology of HPV infection in tissue with multiplexing

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Introduction

Human papillomavirus (HPV) infection is associated with a variety of clinical conditions that range from innocuous lesions to cancer. Cervical cancer is the most common cancer caused by HPV. Other less common cancers affecting both men and women include anal, vulvar, vaginal, mouth/throat, and penile cancers. More than 200 types of HPV have been identified and classified as high-risk and low-risk. Among the high-risk HPV types, HPV 16 and HPV 18 are responsible for most HPV-related cancers.

Material and method

We developed a multiplex method of nucleic acid and protein detection by combining *in situ* hybridization (ISH) application with AMPIVIEW® RNA probes and immunohistochemistry (IHC) applications. Powered by Enzo's LoopRNATM ISH technology, AMPIVIEW® RNA probes are uniquely designed with the precision of targeted, sequence-specific RNA, to deliver superior sensitivity for the detection and the expression analysis of key biomarkers in cells and tissue including formalin-fixed, paraffin-embedded (FFPE) tissue specimen.

Result and discussion

In the progression of cervical lesions to cancer, expression of key markers of proliferation and check-point control such as Ki-67 and p16 can be dysregulated at the transcriptional and protein levels. In this study, we used IHC to detect the expression pattern of these key markers, as well as RNA ISH with AMPIVIEW® HPV RNA probes to spot integrated HPV DNA as well as HPV RNA.

Conclusion

Changes can be assessed between different infection

levels and the severity and extent of marker gene dysregulation, thereby providing a proof of principle for AMPIVIEW®-based multiplexing as a tool to assist tracking the progression of cancer cells due to HPV infection.

EACR25-0234

Insights into Molecular and Immune Mechanism of Chemo Prevention of Aegle marmelos Fruit Extract: Preclinical and Preventive Strategies in Colitis Associated Colon Cancer Pathogenesis

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Introduction

Inflammatory bowel disease (IBD), such as ulcerative colitis (UC) and Crohn's disease (CD), causes chronic inflammation induced tissue damage and repair, that predisposes colitis-associated colorectal cancer (CACRC), the third most prevalent cancer globally. Recently IBD is more common in India than in other Asian countries. The nucleotide-binding domain leucine-rich repeat containing (NLR) proteins are crucial for innate immunity and intestinal tissue repair/renewal and its dysregulation is prime cause of IBD. Fruits of Aegle marmelos, commonly known as "Bael," is a natural immune modulator to treat gastrointestinal (GI) disorders. This study was aimed to evaluate immunomodulatory, anti-inflammatory and anti-cancer activity of Aegle marmelos fruit extract (AME) using preclinical models of inflammation-associated colon cancer.

Material and method

AME chemo prevention were investigated using *in vitro* IBD model of colon cancer cell line (Colo 205) exposed to dextran sodium sulfate (DSS). AME binding affinity to α -tubulin was determined using surface plasmon resonance (SPR) and confocal imaging techniques. The cyto compatibility of AME on peripheral blood lymphocytes was assessed through the CCk-8 assay. DSS-colitis (BALB/c) mice model and HT-29 xenograft tumor model were used to assess anti-cancer and immunomodulatory effects of the AME. Disease activity index (DAI) and hematoxylin-eosin (H&E) staining and immunohistochemical analysis of NLRP3 inflammasome markers were employed to assess its therapeutic effect.

Result and discussion

AME exhibited dose-dependent affinity for α -tubulin protein, obstructed tubulin polymerization, and impaired microtubule formation. AME altered HT-29 cell morphology by disrupting mitochondrial disintegration and impairing membrane integrity. DSS treatment showed a time/dose-dependent effect on Colo-205 cells. DSS (0.5 to 10 μ g/ml) induced hormesis effect and increased vitality of colo-205 cells, while AME treatment decreased cell viability. AME treatment improved DAI scores and pathological damage due to DSS induced colitis in mice. Delayed tumor growth was observed with AME treated HT29 xenograft transplanted mice. AME treatment suppressed levels of NLRP3 inflammasome pathway molecules such as NLRP3, GSDMD, IL-18, TIM-3, and AIM-2 inflammatory factors both in DSS colitis and xenograft tumor models.

Conclusion

These results validated AME as immune regulatory and chemo prevention in colon cancer under inflammatory conditions by targeting NLRP3 inflammasome mediated immune signal. Also, provides insight into AME's potential therapeutic benefits and supporting the use of natural compounds in cancer prevention and highlights the importance of exploring alternative approaches to traditional therapies in the quest for effective treatments.

EACR25-0251

G-Protein coupled Estrogen Receptor 1 (GPER1): A Novel Target for Chemoprevention of Prostate Cancer

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Introduction

The pathogenesis of Prostate Cancer (PCa), the 2nd most common cancer in men, is preceded by a long latency period. G-Protein coupled Estrogen Receptor 1 (GPER1); a plasma membrane localized receptor, is reported to have cell-context dependent role in progression of tumors of various organs. Existing data suggest that GPER1 has a tumor suppressive role in PCa. However, GPER1 has not been explored for its chemopreventive potential for PCa.

Material and method

Publicly available human datasets were screened for prostatic GPER1 expression. Human PCa and Benign Prostatic Hyperplasia (BPH) tissues and prostatic tissues at different stages during PCa progression in TRAMP (Transgenic Adenocarcinoma of Mouse Prostate) model were assessed for GPER1 expression using qRT-PCR or flow-cytometry. Pyrosequencing was carried out to study GPER1 promoter methylation. TRAMP mice subcutaneously administered G1 (GPER1 agonist) alone or in combination with G15 (GPER1 antagonist) were assessed for tumour progression. Stable GPER1-silenced clones of RWPE-1, LNCaP and PC3 cells were assessed for their proliferative, invasive and migratory abilities in the presence and absence of G1. RT2 profiler, qRT-PCR and zymography assays were carried out to identify GPER1-regulated molecules.

Result and discussion

The frequency of GPER1-positive cells was found to be significantly reduced in high-grade human PCa compared to BPH tissues. A similar observation was concluded by the analysis of public datasets for prostate tumours. In the TRAMP mice, GPER1 expression and GPER1-positive cell frequency was found to be significantly higher at the HGPIN (High-Grade Intraepithelial Neoplasia) stage and decreased at the well differentiated carcinoma (WDC) stage, compared to respective age-matched controls animals. Methylation analysis revealed an increase in the percent methylation at the 5 CpG sites spanning the GPER1 promoter in TRAMP mice displaying HGPIN followed by a decrease in the animals displaying WDC, compared to respective age-matched control mice. Pharmacological activation of GPER1 with G1 alone, not when co-administered with G15, inhibited progression of HGPIN to PCa in TRAMP mice. GPER1-silencing in LNCaP, PC3 and RWPE-1 led to an increase in migration and invasion while G1-treatment led to a decrease in proliferation and invasion in vitro. GPER1 mediated epithelial to mesenchymal transition (EMT) was found to be regulated through miR200a-ZEB2-E-Cadherin loop and other metastasis-associated genes.

Conclusion

GPER1 silencing in prostate epithelial cells favored EMT while GPER1 activation inhibited EMT. GPER1 activation by G1 prevented progression of HGPIN to PCa in TRAMP mice. Overall, these observations highlight the potential of GPER1 as a promising target for chemoprevention and warrant clinical studies to test GPER1 agonists for their role in PCa chemoprevention in humans.

EACR25-0302

Personalized Initial Screening Ages for Colorectal Cancer Based on Detailed Family History in Western Europe

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Introduction

Individuals with a family history of colorectal cancer (CRC) face varying levels of increased risk, influenced

by the number of affected relatives, their relatedness, and age at diagnosis. Early detection through timely screening is crucial. Current guidelines often provide broad age ranges for screening and lack precision to account for these risk variations. This study aims to derive risk-adapted starting ages for CRC screening based on varying family history characteristics in Western European countries, where CRC incidence is rising among younger populations.

Material and method

We determined 5-year cumulative risks of CRC for ages 30 to 50 for selected Western European countries (Germany, France, The Netherlands, and Belgium) using the GLOBOCAN 2022 database. Risk estimates were derived from a comprehensive meta-analysis of 20 cohort studies, where relative risks for CRC ranged from 1.09 (95% CI, 1.03–1.15) for individuals with a second-degree relative (SDR) diagnosed with CRC to 3.26 (95% CI, 2.82–3.77) for those with a first-degree relative (FDR) diagnosed before age 50. We then identified the ages at which individuals with different family history characteristics reach the same CRC risk as the average-risk population at age 50 (aCR50), the most common starting age for screening in Europe.

Result and discussion

Those with at least one FDR diagnosed with CRC at or after age 50 reached the aCR50 threshold at ages 45–46 across the four countries. For those with two or more FDRs diagnosed with CRC, aCR50 was reached at age 41 (95% CI, 39–44) in France and at ages 43–44 in the other countries. When an FDR was diagnosed before age 50, the aCR50 threshold was reached earlier, ranging from age 39 (95% CI, 38–40) in France to age 42 (95% CI, 41–43) in the Netherlands. In cases where individuals had at least one SDR with CRC, the aCR50 threshold was consistently reached at 49 years across all included countries. These findings highlight discrepancies between calculated risk-adapted starting ages and current screening guidelines.

Conclusion

This study underscores the need and potential of a tailored approach to CRC screening for individuals with a family history and provides an empirical basis for refining risk-adapted starting ages of CRC screening for people with a CRC family history in the analysed European countries.

EACR25-0311

Assessment of a Fiber-rich Diet in Post-treatment Breast Cancer Survivors

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Introduction

Breast cancer survivors are living longer post-cancer diagnosis and thus are experiencing continued impacts of cancer treatment. Cancer treatments may alter diet intake, including fiber-rich foods; thus, it is important to understand survivors' dietary patterns to inform targeted interventions that may reduce recurrence and improve outcomes. This study aimed to characterize dietary

patterns among breast cancer survivors and compare intake among those receiving chemotherapy, radiation, and surgery.

Material and method

This was an observational secondary analysis of female breast cancer survivors seen at a Midwestern, USA Cancer Center Survivorship Clinic. The Diet History Questionnaire III was used to collect dietary intake, including total fiber, fiber-rich foods and used to score diet quality (Healthy Eating Index-2015 score; HEI-2015). Participants also self-reported fiber and probiotic supplements and treatment history was collected from the medical record. Mann Whitney U-test, Kruskal-Wallis, and Fisher's Exact test examined differences in dietary intake between treatment groups.

Result and discussion

The population (N = 17) were 88% white women (n = 15), with a median (IQR) age of 62 (16). Most were diagnosed with stage 1 cancer (76.5%, n = 13). All participants received surgical treatment, where 59% (n = 10) received radiation and surgical treatment, and only 29% (n = 5) received chemotherapy as a part of any treatment plan. Median daily fiber intake was 13.0 (18) g, meeting 43% of the American Institute for Cancer Research (AICR) goal of 30 g fiber/day, with a median HEI-2015 of 69.9 (10) out of 100. The median daily intake of whole grains was 0.66 (1) ounces, comprising only 24% of median total grain consumption. Participants ate a median of 1.8 (2.3) cups of fruit, 1.5 (1.4) cups of vegetables, and 0.8 (1.5) ounces of nuts, seeds, and legumes, where 41% (n = 7) used probiotics, and 24% (n = 4) used fiber supplements. No significant differences in total fiber, fiber-rich foods, or use of fiber and probiotic supplements were found between breast cancer survivors by treatment type.

Conclusion

When compared to established recommendations of dietary fiber and fiber-rich foods, no treatment groups met the AICR guidelines. There is a need for practical nutrition interventions and education to increase dietary fiber intake among breast cancer survivors.

EACR25-0346

Lifestyle intervention during colorectal cancer screening: a pilot study in France

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Introduction

This pilot study aimed to test the feasibility and optimize

the fieldwork procedures of a lifestyle intervention in French hospital settings for the implementation of the large-scale randomized control trial LIFE-SCREEN [1] that aims to provide evidence-based diet and lifestyle advice for cancer prevention at colorectal cancer screening.

Material and method

A total of 40 patients were recruited at two hospitals, Centre Léon Bérard and Hôpital Edward Herriot in Lyon, France. The intervention material comprised cancer prevention recommendations including recipes, nutrition and physical activity tips, advice on behavioural change, and a logbook to support goal setting, tracking and achievement. Patients received the intervention material in pre- or post-colonoscopy visits and were asked to complete questionnaires at baseline and after at least one month of using the material. They also received a pedometer. Self-administered biospecimens collections (urine, feces, capillary blood collections) and venous blood (collected at hospital) were optional to enable patients to participate while refusing one or more biospecimens. Focus group meetings were organised with patients and then with hospital staff to obtain clear information and understanding regarding their perspective on the intervention.

Result and discussion

Out of the 40 participants enrolled (20 men & 20 women; mean age of 56.7 ± 8.1 years), 65% and 30% responded to the self-administered questionnaires at one-week and one-month post-recruitment, respectively. The collection of biospecimens was generally rated as acceptable to very acceptable by the patients, with the exception of capillary blood collection. Patients participating in the two-focus group meetings were positive about the quality of the intervention folder and material and found the monitoring sheets useful to help them reach their different goals. Hospital staff suggested to simplify the material while referring to more detailed online information to reduce the time needed to explain the intervention material to the patient during the visit. Venous blood collection and following up the participants to arrange self-administered biospecimens collection and transportation was deemed burdensome by hospital staff.

Conclusion

To optimize the feasibility of the LIFE-SCREEN trial, several modifications were made to the intervention material and protocol to improve efficacy and meet the diversified patients' needs while reducing the burden on hospital staff. More specifically, a simplification of the material was performed and supplementary resources online (including short videos) were developed. In addition, biospecimens collections were removed from the protocol (due to logistical challenges).

[1] clinicaltrials.gov: PP201907-26

EACR25-0860

Predictors of colorectal neoplasia among individuals with a family history of CRC aged 40 to 54 years: Objective Risk vs. Perceived Risk

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Introduction

Individuals with a family history of colorectal cancer (CRC) are at increased risk of CRC and recommended to start screening earlier than the average-risk population. Current recommendations are exclusively based family history and genetic predisposition and do not consider lifestyle-related risk factors. This study aimed to evaluate risk factors for CRC precursors and predictors of risk perception among first-degree relatives (FDRs) of CRC patients aged 40–54 in Germany.

Material and method

Data from 922 participants with a family history of CRC in an FDR were analyzed in a multi-center cross-sectional study. Participants completed questionnaires on sociodemographic factors, lifestyle choices, medical history, and perceived CRC risk. Colonoscopy reports were obtained, and participants were categorized according to the most advanced finding at colonoscopy (advanced neoplasia, non-advanced neoplasia, or no neoplasia). Multivariable logistic regression models were used to assess predictors of both presence of neoplasms and perceived CRC risk.

Result and discussion

Two hundred twenty (23.9%) participants were found to have colorectal neoplasia, including 63 (6.8%) with advanced lesions. Strong associations with advanced neoplasia presence were observed for obesity ($BMI > 30$: aOR 2.44, 95% CI 1.12–5.22), smoking (aOR 1.47, 95% CI 1.14–1.88 per 10-pack-years), and physical inactivity (<45 minutes/day; aOR 2.51, 95% CI 1.11–5.25). However, these factors did not match the lifestyle predictors of perceived risk. Instead, alcohol consumption and fruit and vegetable intake were the lifestyle factors most strongly related to people's sense of their own risk.

Conclusion

This study assessed and compared objective risk factors for advanced neoplasia and key predictors of CRC risk perception among individuals with a family history of CRC. Our results show a significant disconnect between objective risk factors and participants' risk perceptions. Individuals with a family history of CRC may benefit from effective risk communication and personalized CRC screening strategies incorporating lifestyle factors.

EACR25-0998

Participant-related risk factors for false positive faecal occult blood test results in screening for colorectal cancer – a systematic review and meta-analysis

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Introduction

Colorectal cancer (CRC) screening using faecal occult blood tests (FOBT) reduces CRC-related mortality, but both guaiac FOBT (gFOBT) and faecal immunochemical tests (FIT) have undesirably high rates of false positives (FP). There is evidence that the number of FP differs by sex, and multiple studies have investigated the effects of sex-specific FIT cut-offs. However, it remains unclear whether other factors are also relevant. We identified participant-related factors associated with the risk of FP results.

Material and method

We systematically searched MEDLINE, EMBASE, Cochrane Central, MedRxiv.org and BioRxiv.org up to April 2022, screened the reference lists of relevant systematic reviews, and tracked citations of included studies through Web of Science. An updated search primo January 2025 is in progress. Eligible studies included average-risk or symptomatic populations aged 18 years or older who were screened with either of the FOBT. We screened full texts in duplicate and assessed the risk of bias using the Joanna Briggs Institute's critical appraisal tools. We calculated relative risks (RR) of false positive rates (FPR) and RR of positive predictive values (PPV) of available risk factors. For each risk factor reported in at least two studies, we estimated the summary effects and corresponding 95% confidence intervals using random-effects models (Prospero CRD42022315767).

Result and discussion

We included 90 studies with 17,728,741 participants. Males had higher FPR [FPR (95%CI): 1.32 (1.23, 1.41) for gFOBT and 1.32 (1.15, 1.48) for FIT] and PPV [PPV (95%CI): 1.79 (1.56, 2.02) for gFOBT and 1.44 (1.35, 1.53) for FIT] than females. In populations tested with gFOBT, age groups were found to differ in PPVs, e.g., the PPV for 65–69-year-olds was 2.16 (95%CI: 1.47, 2.86) times the PPV for 50–54-year-olds. Populations tested with FIT had significantly different FPRs when comparing age groups, use of antiplatelet agents, use of antithrombotic agents, and presence of hypertension. For instance, the FPR for 65–69-year-olds was 1.69 (95%CI: 1.17, 2.22) times the FPR for 50–54-year-olds. When considering PPVs, the results showed differences across age groups, use of aspirin, use of proton pump inhibitors (PPI), alcohol intake, smoking, diabetes mellitus, metabolic syndrome, and diarrhea, e.g., users of PPI had a lower PPV than non-users [PPV (95%CI): 0.75 (0.68, 0.83)]. Most comparisons showed high heterogeneity.

Conclusion

Several participant-related factors are associated with the risk of FP. These observations could be due to differences in cancer incidence between the groups as well as true associations with FP. Despite this uncertainty, the evidence from this study supports the need to optimize screening across subgroups. We suggest further research to assess whether the identified risk factors can be used to tailor screening programs to their intended populations.

EACR25-1004

Effect of TiO₂, as food additive E171 or nanoparticles, on colorectal carcinogenesis and colibactin-producing *Escherichia coli* virulence

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Introduction

Colorectal cancer (CRC) is the third most common cancer worldwide and the second leading cause of cancer mortality. Genetic, host susceptibility, immunological, and environmental factors contribute to its initiation, development, and/or clinical expression. Among the environmental contaminants, titanium dioxide (TiO₂) particles are of particular concern given their potential carcinogenic effects and ubiquity in everyday consumer products, especially as a food additive (E171). TiO₂ nanoparticles may interact with the intestinal microbiota and facilitate the emergence of pathobionts such as colibactin-producing *Escherichia coli* (CoPEC), which abnormally colonise the colonic mucosa of CRC patients. This project aims to investigate the impact of TiO₂ on colorectal carcinogenesis, with a particular focus on its effects on the interactions between intestinal epithelial cells (IEC) and the gut microbiota, including CoPEC.

Material and method

The growth of five CoPEC strains was monitored after a 24-hour exposure to TiO₂ doses equivalent to those reaching the intestine (0.1 and 1 mg·kg⁻¹). Their ability to interact with human intestinal epithelial HT-29 cells was then measured. Interactions with nanoparticles were observed using scanning electron microscopy (SEM) coupled with energy dispersive spectroscopy (EDS). APCmin/+ mice predisposed to develop CRC were exposed to a human equivalent dose of E171 (10 mg·kg⁻¹ of body weight) twice a week for 27 days. A co-exposure scenario was tested in which mice were infected with the 11G5 CoPEC strain and exposed five times a week for 50 days to E171. Macroscopic, histological, and colonic mucosa-associated microbiota analyses were performed.

Result and discussion

In vitro, the doubling time of each CoPEC strain was reduced after TiO₂ exposure. Adsorption of TiO₂ particles was observed on the surface of the 11G5 strain by SEM-EDS. An increase in the ability of 11G5 to adhere and persist in HT-29 was shown along with structural pili modifications. In vivo, after 27 days, some of the E171-exposed individuals exhibited more advanced tumour development than the control group (number and total volume of polyps) and were defined as E171-sensitive group. Occludin gene expression was significantly reduced in this group. These sensitive mice also showed a greater dysbiosis than the E171 non-sensitive and unexposed ones with several over-

represented taxa that are also more abundant in CRC. Additionally, E171 exposure led to an increased colonisation and persistence of the 11G5 strain in the colon and its translocation to the liver and spleen.

Conclusion

Collectively, these results suggest that, in sensitive individuals, exposure to TiO₂ may contribute to CRC aetiology by altering the structure and function of the intestinal microbial ecosystem and the host intestinal barrier. This could promote the emergence of pathobionts such as CoPEC, in addition to inducing changes that enhance their colonisation.

EACR25-1018

Investigating how Mitochondrial Dysfunction Promotes Breast Carcinogenesis in a Stiff Extracellular Matrix

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Introduction

High mammographic density (HMD) is one of the highest independent risk factors for breast cancer, yet the mechanisms by which HMD promotes breast carcinogenesis are not understood. HMD is associated with increased mechanical stiffness of the extracellular matrix in breast tissue. Metabolic reprogramming is a key hallmark acquired by cancer cells to support their survival and progression in the tumour microenvironment (TME). TME stiffening has been shown to drive metabolic changes in various cancer types. However, less is known about the importance of metabolic rewiring in breast carcinogenesis, particularly with respect to HMD. Here we use a mechanically tuneable 3D model to investigate how metabolic changes in a stiff matrix may drive the oncogenesis of normal mammary epithelial cell lines.

Material and method

MCF10A cells were cultured in Matrigel for 14 days to allow the formation of mature, polarised mammary acini. Live acini were then extracted and transferred into soft (0.25 kPa) or stiff (10 kPa) Matrigel-alginate hydrogels, the latter of which were generated by crosslinking alginate polymers with 24 mM Ca²⁺. After 7 days acini were extracted from hydrogels and processed for downstream analysis.

Result and discussion

Global proteomics revealed that GO pathways associated with oxidative phosphorylation (OXPHOS) are down-regulated in MCF10A acini cultured in a stiff 3D matrix. Mitochondrial morphology is an indicator of mitochondrial function, where highly interconnected mitochondrial networks generate ATP through increased OXPHOS. Consistently, confocal imaging showed that mitochondrial networks are dense and elongated in a soft matrix, while those in stiff are fragmented and sparse. Mitochondrial DNA (mtDNA) copy number is reduced

by 20% in stiff, suggesting that mitochondrial biogenesis and/or clearance change in response to increased matrix stiffness. Subcellular fractionation revealed that mitochondria in a stiff matrix are partially depleted of the mitochondrial transcription factor TFAM, which maintains the stability, replication and transcription of mtDNA. Moreover, TFAM is enriched in the cytoplasmic fraction of acini in stiff. The reduction in mitochondrial TFAM may be responsible for mtDNA depletion and impaired OXPHOS in a stiff matrix. Ongoing work aims to understand if there is defective import of TFAM into mitochondria in stiff, or if TFAM is released into the cytosol through partial mitochondrial permeabilization.

Conclusion

We show that non-cancerous mammary acini cultured in a stiff 3D matrix exhibit mitochondrial dysfunction, characterised by increased mitochondrial fragmentation, reduced mitochondrial TFAM and a decreased OXPHOS signature. Future work will investigate whether this mitochondrial dysfunction promotes breast carcinogenesis in a stiff matrix, with the hope to identify targetable pathways for the early detection and prevention of breast cancer in women with HMD.

EACR25-1208

OUTLIVE-CRC "Improving prognosis and quality of life of young CRC patients: Tertiary prevention through multi-marker models and nutritional interventions"

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Introduction

Colorectal cancer (CRC) is increasing in younger individuals and depicts an overall five-year survival rate reaching 65% with a recurrence rate about 10–40%. It is known that lifestyle factors such as physical activity and nutrition are important risk factors. A divergence between chronological and biological age (BA) constitutes an adverse prognostic factor for young adults, especially for those having a metabolic syndrome. The overriding goal of OUTLIVE-CRC is the early detection and prevention of recurrences to improve prognosis and quality of life of early-onset CRC patients (<50 years) by combining functional omics data and nutritional interventions.

Material and method

We develop a biological age (BA) based multimarker risk panel encompassing clinical parameters as well as multi-omics data (metabolomics, proteomics, genomics, epigenomics, microbiomics) using blood and stool samples. Liquid biopsy techniques will be applied to detect recurrence at early stage and/or to identify patients with a high risk of recurrence, and will be combined with BA markers, using artificial intelligence methods. Patient-derived colonic organoid cultures will be implemented to study the individual risk of malignant transformation and to train liquid biopsy marker panels. Microfluidic automation strategies will be used to translate the identified

multi-marker panel into a point-of-care microfluidic platform. Personalized nutritional interventions will be developed based on identified liquid biopsy markers. Patient representatives are integrated in all parts of the study.

Result and discussion

OUTLIVE-CRC is a multi-center study funded in the course of the The National Decade against Cancer by the German Federal Ministry of Education and Research between the years 2022 and 2030. OUTLIVE-CRC will provide a new tertiary prevention concept for early-onset CRC patients by combining liquid biopsy testing with personalized nutritional interventions. The consortium is supported by active patient integration.

Conclusion

The OUTLIVE-CRC study will implement a novel tertiary prevention strategy to improve prognosis and quality of life of early-onset CRC patients.

EACR25-1559

Effects of Immediate Breast Reconstruction on Psychosocial and Sexual Well-Being in Women with Breast Cancer: The Mediating Role of Breast Satisfaction

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Introduction

Breast cancer is the most common cancer in women, and its treatment can have a significant impact on quality of life, particularly psychosocial and sexual aspects.

Immediate breast reconstruction (IBR) has emerged as a solution to improve body image and overall well-being. This study examines the relationship between breast satisfaction, psychosocial well-being and sexual well-being in patients undergoing IBR.

Material and method

A prospective study involving 36 women undergoing IBR. The BREAST-Q questionnaire assessed the quality of life in psychosocial, physical and sexual dimensions, as well as breast satisfaction, and data were analysed using correlations and mediation models. Additionally, differences in results were explored based on the surgical technique and the reason for surgery.

Result and discussion

Participants reported the highest quality of life in the psychosocial and sexual dimensions. No significant differences in these indicators were found based on surgical technique or reason for surgery. Psychosocial well-being and breast satisfaction explained 56.16% of the variability in sexual well-being, with 44.67% attributable to psychosocial well-being. Breast satisfaction acts as a mediator between psychosocial and sexual well-being, independent of physical well-being. These findings suggest that IBR may improve patients' well-being by preserving body image and reducing psychological morbidity.

Conclusion

IBR is a key strategy for improving the quality of life in breast cancer patients, particularly by positively

influencing psychosocial and sexual well-being.

Satisfaction with breast reconstruction is a crucial factor in this improvement, acting as a mediator in the relationship between psychosocial and sexual well-being. These findings highlight the importance of incorporating psychological support and reconstruction information into breast cancer treatment to help patients make informed decisions and improve their long-term well-being.

EACR25-1682

Overview of ovarian cancer risk prediction models evaluating their performance and utility: a systematic review

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Introduction

Ovarian cancer is the deadliest gynecological malignancy and most cases are diagnosed at an advanced stage. There is no population-wide screening and some women with known genetic risk are offered surveillance or prophylactic strategies. Models for predicting risk of ovarian cancer could be useful in decreasing mortality. We provide the first comprehensive overview of ovarian cancer risk prediction models and assess their performance, quality, and applicability.

Material and method

We conducted a systematic literature search through MEDLINE and Embase for articles on ovarian cancer risk prediction models and validation studies. The Prediction model Risk of Bias Assessment Tool (PROBAST) was used to assess model quality.

Result and discussion

We identified twenty-eight studies representing twenty-one ovarian cancer risk prediction models. Fourteen risk prediction models were developed in the general population, while six were developed for high-risk populations, defined by family history, and one for patients with endometriosis. The number of study participants ranged from 80 to 4,943,765 women aged between 18–89 years old. There were eight internally and four externally validated models. Two models had both internal and external validation. Twenty-three studies that reported discrimination and/or calibration had moderate-to-good performance. Risk prediction models with the best predictive ability, both developed in the primary care setting, reported an area under the receiver-operating characteristic curve (AUC) of 0.86 (95% confidence interval (CI) 0.84–0.87) for 2-year and an AUC of 0.77 (95% CI 0.76–0.78) for 10-year ovarian cancer risk in the general population. For women with higher ovarian cancer risk, the 10-year risk predictive ability of the best-performing risk prediction model had a good discrimination with a C-index of 0.77 (95% CI 0.73–0.82) and a good calibration with an expected to observed ratio of 0.87 (95% CI 0.70–1.08). Overall, predictive ability

was strongest in short-term ovarian cancer risk prediction models.

Conclusion

Several models for predicting short- and long-term risk of ovarian cancer have been published, of which a few have been validated in other populations. Their predictive ability varies but is generally moderate-to-good, and some models may be suitable for use in primary care or specialty clinics; however, assessment of their utility and validation in diverse cohorts is warranted.

EACR25-1690

Prevalence and Clinical Characteristics of High-Risk HPV in Women from Underserved and Remote Regions of Colombia

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Introduction

Human papillomavirus (HPV) is the leading risk factor for cervical cancer, the most common malignancy among Colombian women. Colombia is a genetically diverse country, with Indigenous, Afro-descendant, and mixed-ancestry populations residing in different regions.

Despite advancements in screening and vaccination, disparities in prevention and early detection persist, particularly among women in remote and underserved areas with limited healthcare access. HR-HPV prevalence and clinical impact may vary by ancestry due to genetic, immunological, and environmental factors. This study evaluates HR-HPV prevalence and cytological findings in women from low-resource regions with restricted healthcare coverage with diverse ancestral backgrounds.

Material and method

A co-testing approach (HPV testing and cytology) was conducted in four Colombian regions with limited healthcare access: Soacha, Buenaventura, Ciénaga de Oro, and Mitú. The study included women from diverse ancestral backgrounds, including Indigenous, Afro-descendant, and migrant populations. Liquid-based cytology was performed using the ThinPrep system, while HPV genotyping was conducted with the Cobas HPV test, detecting HPV 16, HPV 18, and other high-risk genotypes.

Result and discussion

A total of 561 women were included in the study, with a mean age of 46 years. HR-HPV was detected in 15% of the participants. Among them, 63 women tested positive for other high-risk genotypes, while HPV 16 and HPV 18 were each identified in 5 cases. The prevalence of HR-HPV varied across different regions: Buenaventura (15.3%), Ciénaga de Oro (13.5%), Mitú (12.9%), and Soacha (10%). Cytological abnormalities among HR-HPV-positive women: ASC-US: 16 cases (30.8%). LSIL (CIN I): 8 cases (15.4%). HSIL (CIN II, III, carcinoma in situ): 2 cases (3.8%). ASC-H: 2 cases (3.8%).

Conclusion

These preliminary results show an HR-HPV prevalence of 15%, with regional variations (10%–15.3%), reflecting

global disparities linked to socioeconomic and ancestral factors. HPV 16 and 18 were detected in 1.8% of cases, while other high-risk genotypes (12%) highlight the need for broad-spectrum screening. Cytological abnormalities were present in 53.8% of HR-HPV-positive women, with ASC-US (30.8%) as the most frequent. These findings underscore the need for expanded vaccination, targeted screening, and improved healthcare access to reduce cervical cancer risk in vulnerable populations.

EACR25-1705

Dietary differences exist among rural and urban cancer survivors

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Introduction

The United States ranks fourth globally in cancer rates, with obesity as a major risk factor affecting survivorship outcomes. The American Institute for Cancer Research and World Cancer Research Fund (AICR/WCRF) emphasize maintaining a healthy weight, physical activity, and a plant-based diet. However, addressing obesity also requires understanding social determinants like socioeconomic and geographic factors. This analysis of a randomized control trial aims to assess health factors of cancer survivors and their relationship to these social determinants.

Material and method

Cancer survivors ≥19 years, following completion of primary treatment were enrolled in a multi-site trial. At baseline, self-reported diet (Diet History Questionnaire III), demographic, clinical, and wearable physical activity data (Fitbit) were collected. Healthy Eating Index-2015 (HEI) was used to evaluate diet quality. Those with complete diet data (>600 calories) were analyzed via Chi-square, t-test, one-way ANOVA (Bonferroni correction), and linear regression to examine the relationship between health factors (diet, BMI, physical activity) and social determinants of rural–urban commuting area (RUCA) codes and Area Deprivation Index (ADI).

Result and discussion

Of 79 evaluable participants at a Midwestern site, the majority were non-Hispanic White (95%, n = 75) females (99%, n = 78), with stage 1 breast cancer (55%, n = 42). At baseline, most were overweight or obese (BMI ≥ 25; 75%, n = 59), 19% (n = 15) live in either a large rural city (“micropolitan”) or rural area and 18% (n = 14) live in an area of socioeconomic disadvantage. The average (SD) age was 54.6 (11.7) years old and mean physical activity was 43.4 (30.9) minutes per week. The average HEI score was 65.5 (11.0) out of 100, with a mean daily intake of 15.7 (8.1) g of fiber, 1.2 (1.1) cups of fruit, 1.6 (0.9) cups of vegetables, 39 (29.9) g of added sugar,

1.5 (1.3) oz red and processed meat, and 81% of grain intake from refined grains. HEI scores were significantly lower among those in more rural areas (66.8 vs. 60.1, $p = 0.03$). Those in the micropolitan areas ate significantly higher red and processed meats compared to metropolitan residents (3.1 vs 1.3 oz/day, $p < 0.001$) and rural residents (3.1 vs 1.6 oz/day, $p = 0.04$). On average, micropolitan residents ate 1.75 oz more red and processed meats compared to metropolitan and rural residents, after adjustment of age and calories (β : 1.75, $p < 0.001$). There was no significant relationship between ADI and diet, obesity or activity.

Conclusion

Cancer survivors' health factors fall short of AICR/WCRF recommendations for physical activity, body weight, added sugar, fiber, fruit, vegetables, and whole grains. Those in more rural areas may be at higher risk of adverse diet intake. Identifying at-risk populations early on enables targeted interventions and personalized strategies to reduce obesity among cancer survivors, supported by inter-professional collaboration.

EACR25-1747

Intestinal plasma cell senescence as a biomarker for risk prediction of early-onset colorectal carcinogenesis

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Introduction

The incidence of early-onset colorectal cancer (EOCRC) is increasing globally, influenced by divergence between chronological (CA) and biological age (BA). Lifestyle choices, such as diet, are the main factors to promote diet-related intestinal inflammation and thereby influence the divergence of CA and BA, resulting in an increased risk of EOCRC. Another sign of advanced BA is immunosenescence, a gradual decline in function of the immune system, which is associated with the development of many age-related diseases, like cancer. Searching for a stable biomarker for EOCRC detection, we focused on the functions of intestinal plasma cells, which play an important role in maintaining tissue homeostasis and protection against invading organisms, mostly via secretion of immunoglobulin A (IgA).

Material and method

Human and murine fecal IgA levels were determined by ELISA. Telomer length was measured in blood leukocytes via qPCR and serum metabolites were quantified via NMR. As experimental mouse models we used (i) acute Dextran Sodium Sulfate (DSS)-induced

colitis, by adding DSS into the drinking water for 7 days and (ii) colitis-associated colorectal cancer (CAC), by administering of two treatment cycles of i.p. azoxy-methane (AOM) and oral DSS, over the course of 5 weeks. As part of a nutritional intervention, these mice were fed a diet high in fat and sugar, that resembles modern western diet (WD), for 20 weeks. Fecal samples were collected at several time points for quantification of IgA and mesenteric lymph nodes (mLN) as well as Peyer's Patches (PP) were analyzed by flow cytometry. Finally, colon tissues were collected and analyzed by mass spectrometry-based proteomics.

Result and discussion

In humans, fecal IgA levels decline with CA and strongly correlate with BA, using telomer length of leukocytes as an indirect measure. Metabolomic analysis revealed increased serum LDL lipoprotein levels with CA, with serum LDL levels negatively correlating with fecal IgA levels. Of note, LDL levels were already enhanced in EOCRC patients, compared to age-matched normal controls. In a mouse model of acute DSS-induced colitis we observed that, in contrast to old mice, young mice respond to DSS with IgA secretion into the lumen. In line with that, proteomic analysis identified old mice to down-regulate colonic IGHα level, while up-regulating IGG2B and IGHM in comparison to young mice.

Conversely, female mice do not alter IGHα expression during aging. Finally, in a CAC model, young male mice fed a WD displayed the lowest survival rate, while the percentage of IgA+ cells in the mLN and PP correlated with survival rate under WD amongst all four groups.

Conclusion

Our data suggest that IgA secretion is reduced in old individuals, especially in those with higher risk to develop CRC, and could potentially be used as a non-invasive biomarker for the detection of divergent BA and hence increased risk to develop EOCRC.

EACR25-1888

Menarche, menopause, and female breast cancer risk in China and the UK: comparative findings from three large cohorts

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Introduction

Breast cancer incidence differs significantly between Western and Eastern populations, exemplified by the UK and China. Age at menarche and menopause, well-established risk factors for breast cancer, also exhibit distinct characteristics across these populations. This study aims to compare their distributions and associations with breast cancer risk in the UK and China.

Material and method

We used data from three large prospective cohort studies: the China Kadoorie Biobank (CKB), which included 300,824 women aged 30–79 recruited between 2004 and 2008 across 10 diverse regions in China; the Million Women Study (MWS), comprising 1.3 million women aged 48–91 recruited between 1996 and 2001 in England and Scotland; and the UK Biobank (UKB), with 273,402

women aged 40–69 recruited between 2006 and 2010 in England, Wales, and Scotland. Together, these cohorts covered over 1.8 million women and ~100,000 breast cancer cases (ICD-10: C50). Reproductive data were collected via questionnaires. Cox proportional hazards models were used to estimate hazard ratios (HRs) for menarche, menopause, menopausal status, and reproductive duration, adjusting for potential confounders.

Result and discussion

Breast cancer incidence was 4–5 times higher in the UK (3.93 per 1,000 person-years in MWS; 3.19 in UKB) than in China (0.82 in CKB). On average, Chinese women had later menarche (mean: 15.44 years [SD 1.97] in CKB) than UK women (12.97 [1.62] in UKB; 13.00 [1.60] in MWS), while menopause timing was similar (48.22 [4.41] in CKB vs. 49.74 [5.09] in UKB and 47.53 [5.80] in MWS). Menarche at age 15 or older, compared to below age 12, was more protective in Chinese women (HR 0.81, 95% CI 0.60–0.81) than in UK women (HR 0.91, 95% CI 0.86–0.98 in UKB; HR 0.94, 95% CI 0.92–0.96 in MWS). Later menopause was associated with higher breast cancer risk, with a greater magnitude in Chinese women (HR 1.56, 95% CI 1.30–1.88) than in UK women (HR 1.18, 95% CI 1.08–1.30 in UKB; HR 1.27, 95% CI 1.22–1.31 in MWS).

Conclusion

Differences in reproductive characteristics and in their associations with breast cancer risk between UK and Chinese women may partially explain the disparities in breast cancer incidence between the two populations.

EACR25-2167

Association between area-based socioeconomic status and colorectal cancer mortality in England

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Introduction

Colorectal cancer (CRC) remains one of the leading causes of cancer-related mortality worldwide. While advances in screening, early detection, and treatment have significantly improved survival rates, disparities in outcomes persist. Socioeconomic disparities play a crucial role in influencing CRC mortality – affecting access to healthcare, engagement with screening programmes, early diagnosis, treatment adherence, and overall survival. We conducted a population-based retrospective cohort study to examine the association between area-based socioeconomic status and CRC mortality, by age and sex in England for the period 2011–20.

Material and method

Individual-level anonymised CRC mortality data (ICD-10 codes: C18–20) for 151,050 patients were obtained from the Office for National Statistics. Each patient was assigned to one of five area-based socioeconomic groups using the Index of Multiple Deprivation (IMD quintile 1, most deprived; IMD quintile 5, least deprived). Age- and sex-specific mortality rates and age-standardised

mortality rates (standardised to the 2013 European Standard Population) were calculated. CRC mortality rates (per 100,000 population) were calculated for each year of death (2011 to 2020) by age, sex and area-based socioeconomic group (IMD quintiles 1–5). Poisson regression models were constructed to examine the differences in CRC mortality rates between the most and least deprived socioeconomic groups/area.

Result and discussion

Overall, colorectal cancer patients from the most deprived socioeconomic group/area (IMD quintile 1) experienced 21–28% higher mortality rates than those in the least deprived socioeconomic group/area (IMD quintile 5). This excess risk of mortality in patients from the most deprived socioeconomic group/area was significantly greater (i.e. more than double) for males (29–46%) compared with females (9–20%).

Conclusion

The association between socioeconomic status and CRC mortality is driven by a complex interplay of higher burden of behavioural risk factors (e.g. unhealthy diet, obesity, smoking, physical inactivity), comorbidities (e.g. diabetes) low uptake of CRC screening (43% vs 57%), delayed diagnosis, and disparities in treatment adherence. The excess CRC mortality in males is attributed to relatively high CRC incidence and enhanced burden of these factors compared to females. By understanding these connections and addressing the social determinants of health, it is possible to reduce this disparity in CRC mortality and improve survival rates in all patients, regardless of their gender and socioeconomic status.

EACR25-2300

Melanoma and health inequity in the UK

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Introduction

Melanoma is the 5th most common form of cancer in the UK, with incidence rates rising significantly by 147% since the 1990s and a projected ongoing rise of 9% by 2040. A global burden of disease study identified incidence rates to be the second highest in Western Europe. Early diagnosis improves outcomes for patients; however, health inequity can significantly impact the journey to receiving appropriate care. In England, socio-economically deprived areas have been shown to have the lowest levels of cancer symptom awareness and greatest barriers to seeking medical review.

Material and method

In accordance with the PRISMA guidelines, a systematic review was performed. Databases Ovid MEDLINE, Embase, CINAHL, Cochrane were searched. Articles were included if participants were aged over 18+ with a diagnosis of cutaneous melanoma and any source of health inequity as defined by WHO: employment status, income level, gender, ethnicity, sexual orientation. Data was exported to Raayan, both reviewers performing

article selection independently.

Result and discussion

[table on article selection, mortality and incidence plus T-test/aNOVA to compare mean time to diagnosis to be inserted here on poster] Studies across Europe and America have shown an increased incidence but reduced mortality of cutaneous melanoma in groups which have a higher economic status. Those from economically deprived areas, skin of colour and male have worse outcomes measured by mortality and stage at diagnosis. This is reflected in the findings of studies across the UK, North America and Europe which identify the greatest incidence of melanoma in socioeconomically advantaged Caucasian populations to a significant degree, yet worse outcomes are recorded for people with skin of colour and those living in areas of higher economic deprivation. Across age groups this trend continues, as although melanoma is a cancer associated predominantly with increasing age, it is also one of the most common cancers in young adults aged 20–39 in very high regions on the Human Development Index (HDI). Despite this, young adults in low HDI settings have a five times greater case fatality rate compared to their high HDI counterparts. Acral lentiginous melanoma commonly impact people of colour; a study in North America determined that most patients present later, with worse outcomes and that black male patients are disproportionately burdened by health inequity in diagnosis. There were no large scale studies in the UK, with regional data echoing global trends, in particular the impact of COVID-19 on melanoma stage 4 diagnosis has been shown to have increased from 7% in 2019 to 21% in 2020 in the most deprived populations.

Conclusion

There are no large scale studies on the impact of health inequity on melanoma diagnosis and survivorship in the UK. This is significant as understanding regional trends in melanoma is vital for informing policy, healthcare service planning and public health interventions.

EACR25-2393

The role of diet and dietary patterns on colorectal cancer risk, progression and outcomes

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Introduction

Colorectal cancer (CRC) accounts for nearly 2 million new cases and 1 million deaths annually. Around 50% of CRC cases are preventable through lifestyle changes, with diet playing a key role. Adherence to dietary patterns such as the Mediterranean Diet (MD) and Plant-Based Diets (PBD) has been associated with lower CRC risk, while the Western Diet (WD) has been associated

with a higher risk. Understanding how dietary patterns and specific nutrients are associated with CRC risk and its outcomes (e.g. disease-free progression and survival) is crucial to guide future prospective research and the development of public health initiatives.

Material and method

This multicenter, cross-sectional observational study is part of the GUTBIOME-PT study (NCT06741293) and aims to recruit 5,000 participants aged 40–74 from partner hospitals. Eligible participants include individuals undergoing screening colonoscopy or newly diagnosed with CRC (pre-treatment). Participants are categorised into four groups based on clinical diagnosis: healthy controls, low-risk polyps (LRP), high-risk polyps (HRP), and CRC. Socio-demographic and anthropometric data (weight and height) are collected via self-administered questionnaires. Dietary intake is assessed using two non-consecutive 24-hour dietary recalls, and data is converted into nutrients using the Portuguese Food Composition Table. Additionally, adherence to the MD and PBD is evaluated using validated tools such as the Mediterranean Diet Adherence Score and the Plant-Based Dietary Index. Principal component analysis identifies and characterises dietary patterns while correlation analyses explores associations between dietary intake and CRC risk, disease-free progression and overall survival. Multi-variable models, including linear or logistic regression, adjust for potential confounders such as age, sex, and BMI to ensure robust associations [1].

Result and discussion

Recruitment began in November 2023 and is expected to be completed by late 2029. Currently, 206 participants have been recruited with dietary data collected from 119 participants. Among these, approximately 57% are female and 43% are male with a mean age of 54 years. Regarding group distribution, 55% are controls, 30% have LRP, and 15% have HRP. Adherence to MD is moderate in 53% of participants, high in 30% and low in 17%. By the time of the poster presentation, we anticipate showcasing analyses based on data from 200 participants, providing insights into the relationship between dietary intake and patterns and CRC.

Conclusion

This study will establish a foundational framework for identifying dietary patterns that are associated with a decreased CRC risk and mortality and an increased disease-free progression, contributing to tailored nutritional recommendations and public health strategies aimed at improving CRC prevention and treatment.

[1] This study is approved by the Ethics Committee of the Academic Medicine Center of Lisbon.

EACR25-2420

Capacity for detection of tumor markers in Sierra Leone: a snapshot assessment

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Introduction

Introduction The International Agency for Research on Cancer (IARC) reported that in 2022, there were 1,918 new cancer cases and 1,334 deaths due to cancer in Sierra Leone. Early diagnosis and treatment can taper down these alarming figures. Tumor markers, when used together with imaging and other examinations, are vital diagnostic tools that are critical for early cancer diagnosis and management. This preliminary snapshot assesses the tumor markers testing capacity and accessibility in Sierra Leone.

Material and method

We performed a snapshot review of the testing capacity of 15 secondary to tertiary laboratories – public and private laboratories across Sierra Leone, purposefully selected to represent the regions. The assessment was done by making phone calls and or reviewing the laboratories' websites and extracting information on their testing capacities. We reviewed the laboratories' capacities for the quantitative measurement of common tumor markers such as Cancer Antigen 15-3 (CA 15-3), CA-125, CA 19-9, and prostate-specific antigen (PSA) for diagnosing breast, ovarian, pancreatic, and prostate cancers respectively, and Alpha-fetoprotein (AFP) – a marker that suggests liver, ovarian, or testicular cancer. The availability of equipment and competent staff for tumor markers tests was also reviewed.

Result and discussion

Of the 15 facilities assessed, only 4 (26.7%) had testing capacity for tumor markers. One (1) of these (25%) was a public tertiary hospital-based laboratory. All 4 laboratories with cancer markers testing capacities were located in Freetown, the capital city. There is a research laboratory located in the Kambia district, which is a study site for the Human Papillomavirus global burden estimation study. Among the laboratories that were not providing the services, two had the equipment and knowledgeable staff that could perform the analysis. Our findings confirm a huge gap in cancer diagnosis and research in Sierra Leone. Based on the demographic data, only 16% of the country's population (8.461 million (2023) live in Freetown, the capital city. This means only this fraction can access tumor markers testing services, while the others living in provinces will have to travel to access the service. The above explains the high cancer incidence and cancer mortality rates, and minimal cancer research in Sierra Leone.

Conclusion

There is a gap in cancer diagnosis and research capacity in Sierra Leone. The government, stakeholders, and researchers should commit more resources in the forms of capacity building and public-private partnerships for establishing fit-for-purpose facilities so as to prevent the alarming cases of preventable deaths due to cancer. The strategic objectives documented in the National Policy and Strategy for the Elimination of Cervical Cancer 2023-2028 should be pursued rigorously.

EACR25-2514

The Impact of Emotional Intelligence on Mental Health: A Protective Factor in Breast Cancer Survivors

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Introduction

Breast cancer (BC) is the most common neoplasm among women worldwide. Although survival rates have improved, the disease continues to negatively affect patients' mental health, with a high prevalence of depression. Emotional competencies have been identified as protective factors against affective disorders, but the specific mechanisms of their influence on the mental health of breast cancer survivors (BCS) remain unclear. This study analyzes the relationship between survival experience and depression, considering the mediating role of perceived emotional intelligence (PEI).

Material and method

A cross-sectional study was conducted with 237 women divided into two groups: 56 BCS and 181 healthy controls. The Trait Meta-Mood Scale 24 (TMMS-24) was used to assess PEI, and the Hospital Anxiety and Depression Scale (HADS) was applied to measure depression. Mediation analyses were conducted to examine the effect of PEI dimensions (emotional attention, emotional clarity, and emotional repair) on the relationship between BC and depression. Additionally, age was included as a covariate in the analyses.

Result and discussion

The results showed that survival and PEI explained 37.8% of the variance in depression, with 11.7% attributed to the direct or indirect effects of PEI dimensions. Emotional attention was positively associated with depressive symptoms, while emotional clarity and emotional repair had a protective effect. It was found that BCS exhibited lower levels of emotional clarity and higher levels of emotional repair compared to healthy controls. Additionally, reduced emotional clarity mediated the increase in depressive symptoms in BCS, while emotional repair acted as a protective factor.

Conclusion

Emotional clarity and emotional repair play a key role in regulating depressive symptoms in BCS. Interventions improving these emotional competencies may promote better psychological adjustment in this population. It is recommended to develop psycho-oncology intervention programs that strengthen emotional regulation in breast cancer survivors to reduce the risk of depression and improve their quality of life.

Radiobiology/Radiation Oncology

EACR25-0133

POSTER IN THE SPOTLIGHT

Dissecting ovarian cancer cellular responses to radioimmunotherapy with β - particle and ultra-short range conversion and Auger electron-emitting radionuclides

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Introduction

The cell surface glycoprotein L1 cell adhesion molecule (L1CAM) plays a key role in cancer stemness, promoting carcinogenesis and therapy resistance in various malignancies. Our previous study demonstrated the selectivity of anti-L1CAM radioimmunotherapy (RIT) and identified L1CAM as a potential therapeutic target in high-grade serous ovarian cancer (HGSOC) models. In targeted radionuclide therapy, terbium-161 has emerged as a promising alternative to the clinically established lutetium-177. While both radionuclides emit β - particle radiation with comparable energy, Tb-161 additionally emits short-range conversion and Auger electrons (CE/AE), which significantly enhance cytotoxicity. Despite this advantage, the radiobiological effects of CE/AE at molecular level remain poorly understood. This study provides a comparative analysis of signaling network alterations in response to ovarian cancer anti-L1CAM RIT with Tb-161 vs. Lu-177.

Material and method

The anti-L1CAM chimeric mAb chCE7 was functionalized with a DOTA bifunctional chelator, followed by radiolabeling, to produce 177Lu-DOTA-chCE7 and 161Tb-DOTA-chCE7 radioimmunoconjugates (RICs). In vitro assays confirmed the L1CAM-specificity and efficacy of the RICs. Tandem mass tagging (TMT)-based integrated phosphoproteomics and proteomics quantified 22,101 phosphopeptides and 7484 proteins, respectively. Comparative bioinformatics analyzed signaling network alterations in response to 177Lu-DOTA-chCE7 and 161Tb-DOTA-chCE7 in L1CAM-expressing ovarian cancer OVCAR8 cells.

Result and discussion

Both 177Lu-DOTA-chCE7 and 161Tb-DOTA-chCE7 treatments induced pathways involved in DNA damage response (DDR) and repair, signal transduction (including EGFR, VEGFR, and mTOR signaling), mRNA processing, cell cycle and cytoskeleton regulation, translation, apoptosis, and glycogen metabolism. Both treatments induced DNA double-strand breaks and cellular response via ATM (fold enrichment 5.3 ($P = 4.1\text{E-}06$) for 177Lu-DOTA-chCE7, and 6.2 ($P = 6.6\text{E-}08$) for 161Tb-DOTA-chCE7), and ATR pathways

(fold enrichment 3.9, ($P = 5.4\text{E-}05$) for 177Lu-DOTA-chCE7, and 5.3 ($P = 9.0\text{E-}09$) for 161Tb-DOTA-chCE7). While most alterations were common to both treatments, the phosphorylation of a few proteins associated with chromatin methylation, GTPase activation, and chromosome structure was either uniquely induced or significantly more pronounced with 161Tb-DOTA-chCE7 compared to 177Lu-DOTA-chCE7. Further bioinformatics analysis and validation studies are currently underway.

Conclusion

This study highlights signaling networks activated by Tb-161 and Lu-177, advancing the understanding of radiobiology related to β - and CE/AE emitters. In addition, these findings provide insights into potential radiation-induced molecular targets for the development of adjuvant radiosensitization strategies aimed at improving L1CAM-targeted RIT for ovarian cancer.

EACR25-0197

Experimental alpha-radioimmunotherapy for tumors that have acquired resistance to antibody therapy

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Introduction

Recent progress on radioisotope production accelerates targeted radionuclide therapy or radioimmunotherapy (RIT) for the management of cancer. A radionuclide astatine-211 emits alpha-particles in the process of its decay and has potential for cancer treatment. Uterine serous carcinoma (USC) is a rare but aggressive uterine cancer and overexpression of HER2 protein is seen in about one-third of the patients. Trastuzumab, an antibody drug reacting to HER2 protein, has been proposed as a drug against HER2-positive USC; however, the therapeutic effect is limited because of acquired resistance to trastuzumab. The aim of this study is to investigate the therapeutic potential of alpha-RIT using astatine-211-armed trastuzumab (At-211-Tras) against trastuzumab-resistant USC that metastasized into peritoneal cavity (USC-PM) in a mouse model.

Material and method

Animal experiments were carried out with permission and under the regulations of the Institutional Animal Care and Use Committee of Experimental Animals of National Institutes for Quantum Science and Technology, Japan. A mouse model of USC-PM was generated by a transplantation of luciferase genes-introduced human USC cells into peritoneal cavity of immunodeficiency mice. Tumor growth was monitored by *in vivo* chemiluminescence imaging. After generating the USC-PM mouse model and confirming tumor growth in the peritoneal cavity, antibody therapy (400 μg of trastuzumab per mouse by intraperitoneal injection, twice a week for 5 weeks) was performed. After confirming subsequent regrowth of USC cells in peritoneal cavities, a single dose of At-211-Tras (1 MBq) was administered into the peritoneal cavities. The authors declare no conflicts of interest associated with this presentation.

Result and discussion

Tumor changes were approximately 600, 500, 400, and 200 % from the initial tumor sizes at 2 weeks after treatment of PBS only, Tras, 1 MBq of At-211-HuIgG, or 1 MBq of At-211-Tras, respectively. These data suggest that a single dose of At-211-Tras suppressed the tumor growth of HER2 positive USC-PM in mice compared to other control groups. At-211-Tras showed a transient reduction of white blood cell count at 1 week after the administration, but a comparable level of white blood cell count compared to those of other groups at 4 weeks after the administration.

Conclusion

Alpha-RIT using alpha-emitting trastuzumab may be an effective treatment option for USC resistant to trastuzumab therapy.

EACR25-0206

Evaluating the role of ATM inhibitors in enhancing the efficacy of radiotherapy and their potential to improve the immunosuppressive microenvironment of glioblastoma

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Introduction

Glioblastoma (GBM) is an aggressive primary brain tumor with limited treatment options. Standard therapies include surgery, chemotherapy, immunotherapy, and radiation therapy (RT); however, GBM's infiltrative nature makes complete surgical removal nearly impossible, positioning RT as a key treatment modality. Although RT induces DNA damage, the efficiency of repair mechanisms – particularly those mediated by ataxia telangiectasia mutated (ATM) kinase – often limits its effectiveness. ATM inhibitors (ATMi) can disrupt the repair of radiation-induced DNA double-strand breaks and interfere with cell cycle progression, thereby enhancing the effects of RT and chemotherapy.

Nonetheless, the radiosensitizing effects of ATMi on GBM and their impact on the tumor immune microenvironment remain unclear. This study aims to enhance DNA damage while inhibiting repair mechanisms and to assess the combined treatment's impact on immunosuppressive cells (MDSCs, Tregs, and M2 macrophages) as well as on immune-activated cells (cytotoxic and memory T cells).

Material and method

To evaluate the therapeutic potential of ATM inhibitors (ATMi), we conducted both in vitro and in vivo experiments. In vitro, cell viability was assessed using the MTT assay to determine the impact of ATMi on RT-induced cytotoxicity, while colony formation assays

evaluated post-irradiation survival and proliferation. Flow cytometry was employed to analyze DNA damage markers (p-ATM, p-CHK2), cell cycle distribution, and immune cell populations within the tumor microenvironment. In vivo studies monitored tumor growth and immune responses following combined ATMi and RT treatment.

Result and discussion

The combination of ATMi with RT significantly impaired DNA repair and enhanced radiosensitivity. In vitro, ATMi-treated cells exhibited increased DNA damage accumulation, cell cycle arrest, and apoptosis following radiation exposure. Western blot and flow cytometry analyses confirmed a reduction in the expression of DNA repair-related proteins. In vivo, ATMi and RT co-treatment suppressed tumor growth and favorably modulated the immune microenvironment, evidenced by a decrease in immunosuppressive cells (MDSCs, Tregs, and M2 macrophages) and an increase in cytotoxic T cell activation. These results indicate that ATMi enhances RT efficacy by modulating immune responses.

Conclusion

This study demonstrates that ATMi enhances GBM radiosensitivity by disrupting DNA repair and regulating the tumor immune microenvironment. The observed reduction in immunosuppressive cells and the activation of cytotoxic T cells underscore ATMi's therapeutic potential, suggesting it as a promising radiosensitizer for future clinical application.

EACR25-0357

Personalized Tumor Models for Precision RadioTheranostics of Glioblastoma

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Introduction

Based on metabolic or targeted radiopharmaceuticals, targeted radionuclide therapy (TRT) allows for the specific irradiation of localized and disseminated disease with potentially fewer side effects than external beam radiotherapy (EBRT) (1). This may play an important role in the treatment of the most common and lethal brain tumor – glioblastoma (GBM) where despite EBRT treatment nearly 100% of patients will experience a local recurrence. The use of relevant cellular models, that can better replicate the in vivo conditions of the tumor, to investigate the biological effects of ionizing radiation is of crucial importance, namely the molecular and cellular

mechanisms involved in the radiation response in GBM. We have previously demonstrated in multicellular 3D spheroids derived from GBM cell lines that the simple theranostic radiopharmaceutical, [64Cu]CuCl₂ is able to significantly reduce spheroids' growth and viability, while also affecting cells' proliferation capacity (2).

Material and method

This work aims to provide further preclinical insights into TRT by evaluating the translational potential of [64Cu] CuCl₂ in advanced 3D culture models of GBM, in a precision therapy approach. The 3D models used were developed by the Brain Tumor Center of MDACC and are derived from patients, being classified as glioma stem-like cells (GSCs), representative of 3 of the main subtypes of GBM, according to the Cancer Genome Atlas Project: classical, proneural and mesenchymal (3). The radionuclide uptake was evaluated, as well as its cytotoxic and anti-proliferative effects on the GSCs models.

Result and discussion

Our results revealed that even though GSCs from the proneural subtype had the lowest uptake of [64Cu]CuCl₂, they also had the lowest viability and proliferative capacity after treatment, revealing a higher sensitivity of this GBM subtype to TRT. In contrast, mesenchymal GSCs exhibited the highest uptake and the most resistance to treatment.

Conclusion

Overall, this work validates the use of GBM patient-derived models, in a personalized medicine approach, benchmarking their future application for the translational evaluation and validation of new target-specific radiopharmaceuticals.

Acknowledgments: Fundação para a Ciência e Tecnologia, Portugal for the grant UID/Multi/04349/2020, PhD Fellowship 2020.07119.BD to CPinto, and for FCTAustin for grant 2022.15449.UTA to FMendes and DGrosshans. I Pouget, JP, et al., *Front Med (Lausanne)*, 2015. 2 2 Pinto CIG, et al., *EJNNMI Research*, 2024.14 3 Yuan, X, et al., *Oncogene*, 2004. 23(58)

EACR25-0369

Radiobiology of Image-Guided Radiotherapy: in vitro Impact of Computed Tomography on the Radiosensitivity of Lung Cancer

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Introduction

Lung cancer remains a leading cause of cancer-related mortality, with nearly two million cases in 2022. Approximately 76% of lung cancer patients are recommended to undergo radiotherapy (RT). The integration of image-guided RT and adaptive RT in modern treatment machines involves daily computed tomography (CT) scans before each RT session, allowing real-time adjustments to treatment plans based on changes in tumor size or patient anatomy. However, the radiobiological effect of low-dose exposure from CT followed by a high-dose RT is still not well documented.

Material and method

Two human lung cancer and one normal skin fibroblast cell lines were exposed to (a) single low doses (0.1 or 0.2 Gy) of radiation at low ([i] 50 kV, 2 mA, dose rate (DR) = 3 cGy/minute; [ii] 50 kV, 20 mA, DR = 30 cGy/minute); or [iii] high (225 kV, 13.3 mA, DR = 260 cGy/minute) energies, (b) a single high dose of 2 Gy at high energy, and (c) combinations of a single low dose followed by 2 Gy separated by time intervals ranging from 1 to 20 minutes. DNA double-strand break (DSB) repair kinetics were analyzed for each condition using immunofluorescence, focusing on key protein markers including pATM and γH2AX at pre- and post-irradiation. Cellular radiosensitivity was assessed using the clonogenic and micronuclei (MN) assays.

Result and discussion

Cells exposed to low-dose irradiation had a significant number of unrepaired DNA DSB 24 hours post-irradiation. The combination of a low dose followed by 2 Gy with a 1-minute interval resulted in significantly more γH2AX foci 24 hours post-irradiation than 2 Gy or the sum of separate doses. Similarly, the formation of MN significantly increased 24 hours post-irradiation whether at single low or high doses or combination doses. Cells treated with combination doses exhibited a decrease in the number of pATM foci 10 minutes and 1 hour after irradiation. This suggests that CT exposure can decrease the efficiency of DNA DSB repair mechanisms. These results were confirmed by the clonogenic cell survival assay. Cells exposed to a low dose followed by a high dose had a significantly lower surviving fraction than the sum of the separate single doses, suggesting that sequential irradiation has a stronger effect on survival than an additive response, with the time interval between exposures potentially influencing this response.

Conclusion

Our findings show that a low dose before a high dose can influence the DNA damage response depending on the time interval between exposures. This suggests clinical implications for adaptive RT, particularly when performed daily. This highlights the need to consider the impact of imaging exposure and time interval when planning radiotherapy.

EACR25-0402

Advanced 3D glioblastoma models for investigating radiopharmaceutical therapy

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Introduction

Glioblastoma (GBM) is the most frequent form of malignant primary brain tumor, having an extremely poor prognosis. It is characterized by an increased cell proliferation associated with erratic neovascularization, that leads to the development of hypoxic regions, known to have higher concentrations of Cu (1). Interestingly, Cu has several radioisotopes that can be incorporated into radiopharmaceuticals. Among them, ^{64}Cu has attracted interest due to its theranostic potential for PET imaging and therapy due to β^+ and β^- emissions respectively. Clinical studies with the ^{64}Cu radiopharmaceutical, $[^{64}\text{Cu}]\text{CuCl}_2$, reported its selective accumulation in GBM (2). Considering this, we aim to study the therapeutic effects of $[^{64}\text{Cu}]\text{CuCl}_2$ in GBM, also comparing them to Cu's standard therapeutic radionuclide pair, ^{67}Cu , using advanced culture models that can better replicate *in vivo* tumors. In particular, spheroids are 3D models expected to recreate crucial tumoral environmental cues and phenotypes such as the establishment of O₂, nutrients, and metabolite gradients, developing hypoxic and proliferative regions (3).

Material and method

After establishing and characterizing spheroids derived from 3 genetically and phenotypically distinct GBM cell lines (U373, U87, and T98G), we studied the uptake and distribution of ^{64}Cu within the spheroids, through autoradiography. Then we evaluated the changes in the growth, viability, production of reactive oxygen species (ROS), and the population of cancer stem-like cells (CSCs) in spheroids incubated with $[^{64}\text{Cu}]\text{CuCl}_2$. Finally, we compared the survival capacity of spheroids that were incubated with $[^{64}\text{Cu}]\text{CuCl}_2$ and $[^{67}\text{Cu}]\text{CuCl}_2$.

Result and discussion

The results revealed that ^{64}Cu is capable of penetrating spheroids from all cell lines, being able to affect spheroids' growth and viability while increasing the concentration of ROS in the most affected spheroids. Additionally, $[^{64}\text{Cu}]\text{CuCl}_2$ therapy modulated the expression of CSCs markers, particularly CD44 and CD15. Furthermore, the anti-proliferative capacity of ^{64}Cu in GBM spheroid-derived cells was relevant and comparable to the one observed in spheroids treated with ^{67}Cu .

Conclusion

Overall, our results highlighted the therapeutic potential of the simple radiopharmaceutical $[^{64}\text{Cu}]\text{CuCl}_2$, through the evaluation of its effects in advanced culture models, supporting its application as a theranostic agent for GBM.

Acknowledgments: Fundação para a Ciência e Tecnologia, Portugal for the grant UID/Multi/04349/2020 to C2TN and PhD Fellowship 2020_07119.BD to CPinto, PESSOA grant 2021_09137.CBM to FMendes and 47890XB to JPPouget, FCTAustin for the grant 2022_15449.UTA to FMendes, and EU H2020 project PRISMAP -user project 1664507870. I Cilliers, K, et al., *Anat Rec*, 2020, 303(5) 2 Panichelli, P, et al., *Cancer Biother Radiopharm*, 2016, 31(5) 3 Engrácia DM, et al., *Int J Mol Sci*, 2023; 24(15)

EACR25-0444

Ro 90-7501 Enhances The Radio-Sensitivity of Breast Cancer Cells by Impairing DNA Double-Strand Break Repair Mechanisms

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Introduction

Recent studies have shown that Ro 90-7501, a drug initially developed to inhibit amyloid β 42 fibril formation in Alzheimer's disease, can sensitize cervical cancer cells to radiation, likely by impairing DNA double-strand break (DSB) repair. This study aims to assess the radio-sensitizing potential of Ro 90-7501 in breast cancer and normal fibroblast cell lines, focusing on its impact on DNA DSB repair to optimize radiotherapy (RT) efficacy.

Material and method

Two breast cancer cell lines (MDA-MB-231, MCF7) and one normal skin fibroblast cell line (GM03652) were used in this study. Cells were treated with Ro 90-7501 (1, 3 and 10 μM), with or without 2 Gy of ionizing radiation. Cell viability, proliferation, and cytotoxicity were evaluated using MTT and Trypan Blue assays. DNA damage and repair were analyzed by quantifying γ H2AX and pATM foci at 0, 10 minutes, 1 hour, 4 hours, and 24 hours post-irradiation using immunofluorescence. Clonogenic assay was conducted to assess cell survival.

Result and discussion

Ro 90-7501 reduced viability and proliferation in breast cancer cells (p -value < 0.01) without affecting normal fibroblasts (p -value > 0.05). Following 2 Gy irradiation, Ro 90-7501 induced an increase in residual γ H2AX foci at 24 hours post-treatment (p -value < 0.001) and significantly reduced pATM foci at 10 minutes and 1 hour (p -value < 0.01) in both cancer cell lines. Clonogenic assay revealed a significant reduction in colony formation in MCF-7 cells with 3 μM and 10 μM of Ro 90-7501 (p -value < 0.01), while no effect was observed in MDA-MB-231 cells or normal fibroblasts (p -value > 0.05).

Conclusion

In conclusion, Ro 90-7501 exhibits radio-sensitizing effects on breast cancer cell lines at both the molecular and cellular levels, with minimal impact on normal cells. These findings suggest that Ro 90-7501 has potential as a

radio-sensitizer, capable of enhancing tumor response while minimizing effects on normal tissue.

EACR25-0536

Impact of Lamin Mutations on Radiation Response in Lung Cancer

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Introduction

Lamins are intermediate filament proteins that provide structural support to the nucleus by forming a meshwork of polymerized lamins. Depolymerization is induced by phosphorylation enabling cell cycle progression and cell division. Lamins also play critical roles in maintaining nuclear integrity by regulating nuclear stability.

Additionally, they have been reported to impact the DNA damage response and the organization of chromatin structure. Overall, changes in lamin expression are frequently observed, whereas lamin mutations are relatively rare in cancer. These expression changes can result in chromosomal and genomic instability, which are hallmarks of cancer. We hypothesize that the nuclear envelope network is linked to DNA damage response and cell cycle regulation upon irradiation in lung cancer cells and thus represents an important cell-autonomous mediator of radiosensitivity.

Material and method

CRISPR/Cas9-mediated approaches using site-directed gRNAs and homology-directed repair generated lamin phosphorylation mutations mimicking unfeasible or persistent phosphorylation in non-small cell lung cancer cell lines. Selection of single clones enabled further investigation of lamin function by analyzing proliferation and subcellular localization of lamins and their mutated variants using immunofluorescence. Furthermore, colony formation assays and γH2AX-stainings were performed to assess the impact of lamin mutations on nuclear stability and the DNA damage response.

Result and discussion

In total, mutations at three prominent lamin phosphorylation sites were generated and validated by sequencing. Growth differences in lamin A/C S22 mutated A549 cells indicated clonal effects independent from the mutation. Radiosensitivity of these clones was subsequently assessed by colony formation assay, which confirmed that lamin A/C S22 mutated clones exhibit increased radiosensitivity, though to varying extents. Additionally, S22D mutant cells that mimic persistent phosphorylation present altered lamin A/C localization and aberrant nuclear morphology and structure compared to the parental cells. These results confirm that phosphorylation of lamin A/C at S22 plays a crucial role for nuclear division in non-small cell lung cancer cell lines.

Conclusion

Studying the interplay between wildtype and mutant lamins and their clonal differences may provide insight into the role of the cancer-specific lamin network in the cellular damage response upon irradiation.

EACR25-0578

Radioresistance of colorectal cancer cells is driven by GPX4 and ferroptosis resistance

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Introduction

Radiotherapy is widely used for the treatment of many cancers, including rectal cancer. Despite advances in the field of radiotherapy (RT), innate and acquired radio-resistance remain the main issues leading to treatment failure and relapse. Only about 10% of rectal cancer patients fully respond to neoadjuvant treatment (RT/ chemotherapy), highlighting the need to improve treatment efficacy through novel drug combinations. Ionizing radiation (IR) delivered during treatment generates reactive oxygen species (ROS) which oxidize DNA, proteins, and lipids. Lipid peroxide accumulation at the plasma membrane triggers ferroptosis, an iron-dependent cell death. Leveraging IR-induced ferroptosis may overcome radioresistance.

Material and method

Radioresistant cells (HCT-116RR, HT-29RR) were generated through daily sublethal doses of IR (0.5 to 2Gy/day). The radioresistance of colorectal cancer cells was confirmed by cell death assay using FACS (7-AAD probe) and clonogenic assay. ROS and lipid peroxide levels were respectively measured using the CELLROX and BODIPY C11 581/591 dyes. Lipid droplets were visualized and quantified using holo-tomographic microscopy (CX-A, Nanolive). Western blot and RT-qPCR were used to confirm RNA-sequencing data performed on parental and resistant cells. CRISPR screen was performed in parental cells irradiated or not with 2Gy using a gRNA library (Addgene #191535). Sequencing of gRNA amplicons was performed by VectorBuilder.

Result and discussion

HCT116 and HT29 accumulate lipid peroxides 24 hours post-IR, a trend also observed in triple-negative breast cancer cells. HT29 cells showed less sensitivity to IR and showed lower lipid peroxide accumulation when compared to the IR-sensitive HCT116 cells. After irradiation, we observed a significant level of lipid droplets in both cell lines, which might be an adaptation response to IR. HCT116RR cells also showed resistance to GPX4 inhibitor (RSL3) when compared to parental cells. Moreover, GPX4 expression in radioresistant cells was higher. This has led us to hypothesize that a combination of RSL3 + IR before the onset of resistance to IR could improve patient response. Ongoing RNA-sequencing analysis on HCT116RR and parental cells, along with a CRISPR screen of lipid and ferroptosis-related genes in cells treated with IR will help decipher anti-ferroptotic targets driving radioresistance in colorectal cancer.

Conclusion

Taken together, our results suggest that cancer cells rely on ferroptosis resistance pathway to acquire radio-resistance. Considering this dependency of cancer cells, combining ferroptosis inducers with radiotherapy may be a promising way of improving neoadjuvant treatment efficacy for rectal cancer patients.

EACR25-0717

Tumour Volume Dynamics of Glioblastoma During Radiotherapy on 1.5T Magnetic Resonance Imaging-Linear Accelerator

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Introduction

Glioblastoma (GBM) treatment response is typically assessed using pre- and post-treatment magnetic resonance imaging (MRI), as frequent imaging can be logistically challenging and costly. MRI-guided radiation therapy (MRgRT) enables real-time high-frequency monitoring of tumour dynamics throughout treatment. Here, we quantify the daily interfraction variations in tumour volume in GBM treated on an MRI-linear accelerator (MR-linac).

Material and method

GBM patients suitable for chemoradiation using a 1.5T MR-linac were enrolled in a prospective study. T2/FLAIR hyperintensity regions were contoured at baseline and succeeding fractions. Tumour dynamics were assessed using absolute volume, percentage change in volume relative to fraction 0 (V%), and migration distance representing the linear displacement of volume relative to fraction 0. Tumour changes were compared between patients with early progression (defined as progression within 6 months post-treatment) and those without early progression, as well as between methylated and unmethylated/unknown MGMT promoter status groups.

Result and discussion

Among 26 patients, 10 received 4005 cGy/15 fractions, and 16 received 6000 cGy/30 fractions of chemo-radiation. The mean absolute volumes at Fx0, Fx1, Fx10, Fx15, Fx20, and Fx30 were 60.5 cm³, 62.1 cm³, 60.2 cm³, 57.1 cm³, 61.2 cm³, and 65.9 cm³. The corresponding mean V% at Fx1, Fx10, Fx15, Fx20, and Fx30 were 3.9%, 8.6%, 8.6%, 17.1%, and 39.2%. In early progressors, V% at these fractions were 7.8%, 22.8%, 31.4%, 57.6%, and 108.6%, while in non-early progressors, values were 3.0%, 3.1%, -1.6%, -11.0%, and -10.7%. Among methylated tumours, V% were 7.7%, 9.6%, 0.9%, -11.4%, and -7.7%, while unmethylated/unknown tumours had 1.6%, 7.9%, 13.4%, 30.1%, and 60.6%. The mean migration distances at Fx1, Fx10, Fx15, Fx20, and Fx30 were 1.0 mm, 3.3 mm, 4.9 mm, 7.1 mm, and 7.7 mm. In early progressors, migration distances were 0.9 mm, 2.7 mm, 4.4 mm, 5.0 mm, and

6.0 mm, while in non-early progressors, values were 0.9 mm, 3.5 mm, 5.1 mm, 9.5 mm, and 9.8 mm.

Conclusion

Tumour dynamics during chemoradiation may vary based on MGMT methylation status and could potentially serve as an indicator of early post-treatment progression. Early progressors showed greater volume increases, particularly after Fx10, while treatment responders had stable or decreasing volumes. Methylated tumours showed a trend toward volume stability or reduction, whereas unmethylated/unknown tumours exhibited progressive growth. Migration distance was greater in treatment responders, suggesting possible tumour deformation, while early progressors had lower migration, possibly due to continuous expansion. These findings suggest distinct tumour evolution patterns, with potential implications for treatment adaptation and response monitoring.

EACR25-0748

Activation of the E2F Pathway as a Contributor to Radioresistance in Atypical Teratoid Rhabdoid Tumor

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Introduction

Atypical teratoid rhabdoid tumor (ATRT) is a highly aggressive pediatric central nervous system (CNS) tumor with no standardized treatment and poor prognosis. The current multimodal regimen involves surgery, chemotherapy, and radiation therapy (RT). However, those approaches pose risks such as severe side effects and long-term cognitive impairments. Recently, radiation therapy has played a crucial role in the success of multimodal treatment for ATRT. However, the contentious treatment may contribute to resistance in pediatric cases. A deeper understanding of the mechanisms involved in radiation therapy could help minimize radiation doses and provide strategies to overcome resistance and recurrence in ATRT cases.

Material and method

We established a clinical protocol to generate radiation-resistant ATRT cells. Then, we examined the molecular profiles of ATRT patients and treated cells to pinpoint the specific signaling pathways impacted by radiation therapy. In-silico analysis findings were validated using cell function assays, qPCR, and western blot analysis.

Result and discussion

Our results revealed significant upregulation of HALLMARK_E2F_Targets in radiation-resistant (RES) cells, which contrasts with the findings in the single

radiation exposure. Further RNA-seq analysis of recurrent ATRT cases in our cohort who received radiation therapy also demonstrated the enrichment of E2F and cell cycle pathways, suggesting that repeated radiation cycles induce distinct gene expression profiles. Functional assays confirmed that E2F activation enhances cell proliferation and migration in RES cells. Given that E2F transcription factors regulate DNA replication and cell cycle progression, their dysregulation likely contributes to ATRT recurrence and therapy resistance.

Conclusion

Our findings highlight the critical role of E2F signaling in ATRT recurrence and radiation resistance. The repeated radiation-induced upregulation of E2F_Targets suggests that inhibiting the E2F pathway could enhance ATRT radiosensitivity, offering a potential strategy to improve ATRT treatment outcomes and survival when combined with current therapies.

EACR25-0757

Ephrin B3 as a Therapeutic Target and Radiosensitizer in Glioblastoma: New Insights

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Introduction

Glioblastoma (GB) is the most common primary brain tumour with poor overall survival despite aggressive treatment. To improve patients' response to treatment, signalling pathways, linked with therapy resistance, could be targeted. It is known that Ephrin and Eph signaling pathways are important in tumor signaling; Ephrin B3 promotes GB invasion and tumour growth, whereas EphA2 is upregulated in GB and its phosphorylation atnS897 drives progression and invasion of GB. The aim of the study was to explore the role of Ephrin B3 and EphA2 S897 signalling in GB radioresistance.

Material and method

Patient-derived GB cell lines were established from fresh tumour tissue biopsies. Clonogenic potential and cell proliferation were studied after a single dose of 10 Gy or fractionated RT with 4 x 2.5 Gy. Differences in Ephrin B3 and EphA2 S897 expression and localisation were analysed by western blot (WB) and immunofluorescence, respectively. Ephrin B3 was silenced using siRNA, following standard Lipofectamine protocol; WB analysis was done to confirm silencing. After combining siEphrinB3 with 2 Gy RT, the effect of co-therapy on clonogenic capacity of GB cells was studied.

Result and discussion

Our results showed that RT decreased clonogenic potential of GB cells in dose-dependent manner, leading to reduced number and size of colonies in RT-treated samples. Analysis of Ephrin B3 and EphA2.S897 showed that both proteins were present in GB cells prior and post RT in both RT procedures. No major difference in

expression of Ephrin B3 or EphA2.S897 was observed between irradiated and control samples in case of single dose RT, however, 2.5-fold upregulation of Ephrin B3 expression and 4-times higher levels of EphA2.S897 were detected after fractionated RT. We next silenced Ephrin B3 using siRNA. We observed that after Ephrin B3 was knocked-down, cell proliferation decreased by half, and cell morphology changed in response to treatment. Cells formed more protrusions, in which Ephrin B3 and EphA2 S897 were present in high levels. Clonogenic assay showed that plating efficiency of siEphrinB3-treated cells decreased by 40 % compared to non-target siRNA-treated cells. To reveal if Ephrin B3 silencing sensitizes GB cells to RT, we combined both treatments. After Ephrin B3 silencing in GB cells, we exposed cells to 2 Gy RT. WB analysis revealed 50 % reduction of Ephrin B3 levels after its silencing, but the effect was less prominent when combining siRNA and RT (only 20 % reduction). Clonogenic assay showed that Ephrin B3 silencing or 2 Gy RT itself reduced plating efficiency of GB cells, however, plating efficiency decreased for another 50 % when cells were exposed to the combination of siEphrinB3 and RT.

Conclusion

Our results suggest that Ephrin B3 diminishes the responsiveness of GB cells to radiation therapy, suggesting that targeting Ephrin B3 could enhance radiosensitivity in GB.

EACR25-0957

Targeting immune signaling to overcome radioresistance by increasing DNA damage in breast cancer cells

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Introduction

Around 70% of breast cancer (BC) patients receive radiotherapy (RT). However, development of resistance is a frequent cause for therapy failure. One promising strategy to overcome radiation resistance is the use of DNA damage response inhibitors to modulate the immune response and thereby increase the efficacy of immune checkpoint inhibitors in BC. Therefore, this project aims to understand how immune pathways, like cGAS/STING, can be exploited as target to overcome radioresistance by increasing DNA damage.

Material and method

Bottom-up quantitative proteomics of BRCA1-mutated MCF7 cells was performed using liquid chromatography tandem mass spectrometry. Cellular survival after irradiation (IR) and ATR inhibition (ATRi) by AZD6738 was assessed by colony formation assay. Protein expression was determined by Western Blot. PD-L1 surface expression was measured by flow cytometry. Changes in the secreted chemo- and cytokines were analyzed by real-time PCR. DNA repair capacity was analyzed by γ H2AX foci formation. cGAS positive micronuclei and IRF3 translocation were analyzed by immunofluorescence.

Result and discussion

Proteomic profiling revealed significant upregulation of interferon (IFN) alpha/gamma signaling pathway in radiosensitive compared to radioresistant BRCA1-mutated cell lines. Notably, only upon addition of ATRi, IFN signaling was upregulated in resistant cells, which was accompanied by a radiosensitizing effect. STAT1 was detected to be among the top candidates of the IFN signaling pathway, which was further confirmed by Western Blotting. STAT1 activation led to significantly increased expression of IFN-stimulated genes (IFIT1, IFIT3, OAS3 and ISG15) after IR in sensitive cell lines, whereas in resistant cells, this effect was observed only after combined IR and ATRi treatment. A similar trend was observed for several chemo- and cytokines (IFN β 1, CXCL10 and CCL5). Further, PD-L1 surface expression was upregulated after IR, however reduced after ATRi in all cell lines. Previous studies have described a CHK1-dependant PD-L1 upregulation, and our results indicate that by ATRi this might be disrupted. To identify how addition of ATRi led to an increased immune signaling in radioresistant cells, cGAS/STING activation due to increased DNA damage was investigated. γ H2AX foci revealed an increase in DNA damage in the resistant cells after combined treatment. Correspondingly, the number of cGAS-positive micronuclei and IRF3 translocation were both enhanced.

Conclusion

These results show that immune signaling was enhanced by DNA damage-induced activation of cGAS/STING signaling after combined treatment of IR and ATRi in radioresistant, BRCA1-mutated BC cells. This consequently led to a radiosensitizing effect, which may open up new avenues for the treatment of patients that developed a resistance to RT.

EACR25-1059

Evaluation of Therapeutic Efficacy of Magnolol Combined with Radiation in Non-small Cell Lung Cancer

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Introduction

Non-small cell lung cancer (NSCLC) is the leading cause of cancer-related death worldwide, and radiotherapy is a common treatment for NSCLC; however, it is often hampered by drug and radiation resistance. Magnolol is a polyphenol compound extracted from *Magnolia officinalis* bark and has multiple biological activities. Nevertheless, its specific role and mechanism in enhancing the effect of radiotherapy for NSCLC remain unclear. This study aimed to explore the synergistic effect of magnolol combined with radiotherapy in non-small cell lung cancer and its mechanism.

Material and method

CL1-5-F4 and HCC827 were treated with 15 μ M magnolol and a radiation dose of 4 Gy. Cell viability was assessed using the MTT assay, while proliferation ability was evaluated through colony formation assays. Apoptosis and DNA damage repair were analyzed using flow cytometry. Additionally, Western blotting was employed to detect the expression of apoptosis-related proteins.

Result and discussion

MTT assays demonstrated that magnolol inhibited NSCLC cell proliferation, with 15 μ M reducing cell viability. Colony formation assays revealed that the combination of magnolol and 4 Gy radiation significantly reduced colony formation compared to single treatments. Apoptosis analysis confirmed enhanced activation of caspase-3, -8, and -9, indicating the induction of both intrinsic and extrinsic apoptotic pathways. Annexin V staining showed increased early and late apoptosis in the combined treatment group. Elevated mitochondrial membrane potential loss suggested that magnolol exacerbates radiation-induced damage through mitochondrial dysfunction. Furthermore, magnolol modulated the ATM-CHK2 pathway, enhancing sensitivity to DNA damage. Western blot analysis revealed increased expression of pro-apoptotic proteins (BIM, BAK, BAX, and BID) in the combined treatment group. These findings indicate that magnolol enhances radiotherapy efficacy by promoting apoptosis and disrupting DNA repair, offering a promising strategy for NSCLC treatment.

Conclusion

Magnolol significantly enhances the sensitivity of NSCLC cells to radiotherapy. Its mechanisms likely involve the inhibition of DNA damage repair and induction of apoptosis. These results provide strong evidence supporting the potential of magnolol as a radiosensitizer in NSCLC treatment.

EACR25-1098

Upconversion Nanoparticle-Mediated Neutron Capture Therapy Lu-177 Treatment in Head and Neck Squamous Cell Carcinoma via the c-MET Signaling Pathway

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Introduction

Oral cancer has a poor prognosis due to late diagnosis

and limited treatments. Overexpressed c-Met promotes tumor progression, making it a key therapeutic target. This study developed cMApt-UCNPs, dual-functional nanoparticles for targeted theranostics. Neutron-activated UCNPs emit β radiation for therapy and γ rays for SPECT imaging while displaying upconversion fluorescence. c-Met aptamer functionalization enables selective oral cancer targeting, offering a novel precision treatment approach.

Material and method

Cell Culture FaDu and OMF cells were cultured in DMEM with 10% FBS and 1% penicillin-streptomycin at 37°C, 5% CO₂, and passaged every 2–3 days. UCNPs Synthesis and Characterization NaLuF₄:Yb,Tm@NaLuF₄ UCNPs were synthesized via high-temperature coprecipitation and characterized by XRD, TEM, UV-Vis, PL spectroscopy, FTIR, DLS, and zeta potential. DNA electrophoresis confirmed c-Met aptamer conjugation. Neutron Activation UCNPs were neutron-irradiated at THOR (10¹¹–10¹² neutrons/cm²·s, 60–120 min) to convert Lu-176 into Lu-177 for β therapy and γ SPECT imaging. Cytotoxicity and Cellular Studies Alamar Blue: Assessed UCNP cytotoxicity. Western Blot: Analyzed c-Met and apoptosis markers. Confocal Microscopy: Visualized UCNP uptake. Flow Cytometry: Measured uptake and apoptosis. Colony Formation: Evaluated long-term proliferation effects. In Vivo Imaging IVIS bioluminescence and 980 nm NIR fluorescence imaging assessed cMApt-UCNP biodistribution and theranostic potential in FaDu tumor-bearing mice.

Result and discussion

This study synthesized NaLuF₄:Yb,Tm@NaLuF₄ UCNPs for theranostics. Characterization confirmed crystallinity (XRD), morphology (TEM, 65 nm), composition (EDX), and biocompatibility (PEG, FTIR). c-Met aptamer conjugation (DNA electrophoresis) enabled tumor targeting. DLS showed sizes (210.4–241.4 nm) with stable zeta potential (-13.8 to -14.3 mV). UCNPs fluoresced at 475, 650, and 700 nm (980 nm excitation). Neutron irradiation converted Lu-176 into Lu-177, confirmed by gamma spectrum (67, 117, 206 keV), releasing β particles for therapy and γ rays for SPECT imaging. cMApt-UCNPs showed 80% biocompatibility in FaDu cells (48 hr, 1000 μ g/mL). Confocal microscopy and flow cytometry verified aptamer uptake, while Western blot showed 75% c-Met reduction (72 hr). Neutron-activated cMApt-UCNPs (1000 μ g/mL, ~100 μ Ci) reduced FaDu viability to 49%, inhibited colony formation, and induced apoptosis. IVIS imaging confirmed tumor accumulation, demonstrating strong theranostic potential.

Conclusion

This study developed cMApt-UCNPs for targeted cancer theranostics. PEG ensured biocompatibility, while c-Met aptamer enabled tumor-specific delivery. Neutron activation produced Lu-177 for therapy and imaging. In vitro and in vivo results confirmed targeting, cytotoxicity, and tumor regression, demonstrating their potential for precision cancer treatment.

EACR25-1158

Treatment of Orthotopic Human GBM Tumours in Mice Using Auger Electron-Emitting 197Hg-Gold Nanoparticles Administered by Convection-Enhanced Delivery

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Introduction

Glioblastoma multiforme (GBM) is the most common and lethal primary brain cancer. Current treatments have not improved survival, due to recurrence in almost all patients. Our group has developed a radiation nanomedicine composed of the Auger electron (AE) and γ -photon emitter, 197Hg incorporated into gold nanoparticles (AuNPs) that could be infused into GBM tumours by convection-enhanced delivery (CED) to prevent and treat recurrent disease.

Material and method

197Hg-AuNPs were produced by incorporating 197Hg into AuNPs by forming a stable mercury-gold amalgam. 197 Hg-AuNPs were modified with panitumumab to target epidermal growth factor receptors (EGFR) on GBM cells and internalize and route 197Hg-AuNPs to the cell nucleus where the AEs are most damaging to DNA. A human GBM tumor xenograft model was established by stereotaxic inoculation of U251-Luc cells into the right cerebral hemisphere in NRG mice. Two weeks later, mice were treated by stereotaxic CED of EGFR-targeted or non-targeted 197Hg-AuNPs (0.7 MBq; 7 x 10¹⁰ AuNPs; 5 μ L). SPECT/CT images were obtained up to 7 d post-infusion (p.i.) and the time integrated activity from 0 h to 7 d p.i. was determined at the intratumoural infusion site to estimate radiation absorbed doses. A dose-volume histogram (DVH) was constructed for CED of 1.0 MBq. Acute toxicity was assessed at 14 d p.i. by complete blood cell counts (CBC), blood biochemistry and monitoring body weight in mice infused with EGFR-targeted or non-targeted 197Hg-AuNPs (1.9 MBq; 9.8 x 10¹¹ AuNPs; 5 μ L). A therapy study is in progress comparing the survival of mice receiving EGFR-targeted or non-targeted 197Hg-AuNPs vs. control mice treated with non-radioactive AuNPs or normal saline.

Result and discussion

SPECT/CT images showed strong retention at the site of infusion for EGFR-targeted and non-targeted 197Hg-AuNPs, but non-targeted 197Hg-AuNPs showed greater diffusion than EGFR-targeted 197Hg-AuNPs. The DVH revealed that 60% of the tumour received >190 Gy after CED of EGFR-targeted 197Hg-AuNPs, but only 1% of the tumour received this dose for non-targeted 197Hg-AuNPs. Both EGFR-targeted and non-targeted 197Hg-AuNPs delivered >50 Gy to 95% of the tumour. Doses were highly conformal decreasing to <1 Gy at 3 mm from the infusion site, greatly minimizing irradiation of normal

brain. No significant differences in CBC and blood biochemistry or weight loss was found in treated vs. control mice indicating no general toxicity. Results of the on-going therapy study will be reported including assessment of therapeutic response by Kaplan-Meier survival and assessment of adverse effects on the brain by histopathology and T2-weighted MRI.

Conclusion

We report here for the first time that 197Hg-AuNPs were safely infused by CED into U251-Luc human GBM tumours in the brain of NRG mice and were strongly retained depositing high and conformal radiation absorbed doses in the tumour that are anticipated to be therapeutic.

EACR25-1160

Radiosensitivity and DNA Damage Response in Human Sarcoma: p53-Dependent and Independent Mechanisms Under Alpha-Particle Radiation

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Introduction

Intra-tumoral Diffusing Alpha-emitters Radiation Therapy (DaRT) harnesses high-linear energy transfer (LET) alpha particles for potent cytotoxicity and systemic antitumor immunity. Yet, the response of sarcomas, often driven by p53 mutations, remains unpredictable, marked by radiation resistance and treatment-induced adaptability. Defining how p53 status influences radiosensitivity and post-radiation plasticity is critical for optimizing DaRT's therapeutic potential. This study defines how alpha radiation differentially modulates survival, proliferation, and adaptability across p53-variant sarcoma models, revealing key mechanistic drivers of resistance and potential therapeutic vulnerabilities.

Material and method

Five sarcoma cell lines – U2OS (wild-type p53), SAOS2 (p53-null), RD18 (mutated p53, rhabdomyosarcoma), A673 (mutated p53, Ewing sarcoma), and SK-LMS (mutated p53, leiomyosarcoma) – were irradiated with alpha particles from a sealed Americium-241 (Am-241) source (0–4.2 Gy). Colony formation assays determined survival fractions (SF) and D_0 values, Incucyte live-cell imaging tracked proliferation, γ -H2AX immuno-fluorescence staining assessed double-strand break (DSB) repair kinetics, and wound healing assays evaluated

Result and discussion

Radiosensitivity followed a p53-dependent hierarchy, with the wild-type p53 model demonstrating higher sensitivity, while p53-deficient sarcomas exhibited progressive resistance, requiring greater exposure for comparable cytotoxicity. Notably, A673 and SK-LMS

required nearly three times the radiation dose for similar survival reduction, reinforcing p53 dysfunction as a key driver of radioresistance. Proliferation assays showed dose-dependent suppression, yet p53-deficient models like RD-18 maintained growth at intermediate doses, suggesting stress-induced survival tied to p53 dysfunction. DSB repair dynamics varied significantly, revealing distinct genomic stress responses across sarcoma subtypes. Additionally, low-dose irradiation (0.35–0.7 Gy) enhanced motility in A673 and RD18, suggesting radiation-induced cytoskeletal remodeling, while SAOS2 exhibited a paradoxical response, motility inhibition at 1.4 Gy but recovery at 4.2 Gy, indicating nonlinear stress adaptation.

Conclusion

These findings highlight the paradox of alpha radiation in p53-deficient sarcomas, inducing lethal DNA damage while simultaneously driving adaptive survival responses. The implications for DaRT therapy extend beyond direct tumor eradication, highlighting the need to consider radiation-induced motility and DNA repair capacity when optimizing treatment strategies. Future studies will explore p53 restoration as a potential strategy to enhance radiosensitivity and limit radiation-driven adaptability in resistant sarcoma subtypes.

EACR25-1233

Measurement of radiosensitivity in cervical cancer patients, optimisation of the RILA method

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Introduction

Radiotherapy causes severe side effects in 5–10% of patients. Radiation-Induced Lymphocyte Apoptosis (RILA) appears to be a promising clinical method for detecting radiosensitivity. Threshold values can vary by patient group, that's why our goal is to establish the method's threshold, improve workflow efficiency, and clinically validate it in cervical cancer patients. We investigated the impact of different incubation times on RILA values. We also tested the necessity of the CO₂ addition during the culturing step. We are also testing the elimination of the pre-irradiation time to reduce the total test duration from 4 days to 3 days, making the method more practical for clinical use. Furthermore, we examined the possibility of replacing irradiation with

bleomycin to eliminate the need of an irradiator, which is typically the linear accelerator used to treat the patients.

Material and method

We collect blood from patients before radiotherapy and at 3 months, 1 year, and 2 years after treatment completion. At these time points, we assess side effects using EMBRACE II, EORTC-QoL-C30, and EORTC-Cx24 questionnaires. We quantify gastrointestinal side effects with citrulline measurements. We mix the blood with RPMI (20% FBS) and irradiate it with 8 Gy, 100 MU/minute, 6MV radiation. Two days later, we label lymphocytes with CD8-FITC and CD4-eFluor506 antibodies. After erythrolysis, we treat the samples with propidium iodide and RNase, then analyze them using flow cytometry.

Result and discussion

We measured radiation-induced apoptosis levels (8 Gy) in CD8+ and CD4+ lymphocytes in samples from 147 patients scheduled for definitive radiochemotherapy. Our current threshold values for CD8+ and CD4+ lymphocytes are 5.78% and 3.86%, respectively. The pre-irradiation time, post-irradiation time, and increase in FACS lag affect the RILA values. However, this can be resolved if the samples are always irradiated and the processing is initiated at the same time. In the case of CD8+ lymphocytes we demonstrated that the use of CO₂ during culturing can be omitted. We are currently testing the removal of the pre-irradiation time, reducing the entire test from 4 days to 3 days. Our initial results show similar outcomes as historical controls (7.4% CD8+ RILA), but validation against side effects is still needed. We examined the replacement of radiation using bleomycin. The addition of 70 and 80 µg/ml bleomycin to the samples for 5 hours, 24 hours before flow cytometry measurements, provided values most similar to irradiation.

Conclusion

We have successfully established the threshold for the RILA method in cervical cancer patients at our center, but validation against side effects is still required. Further development of the method is ongoing to improve workflow efficiency and facilitate its introduction into clinical practice.

EACR25-1271

Unsupervised Clustering of Histopathological Images: A Promising Tool for AI-based Profiling of Head and Neck Cancer Xenograft Models

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Introduction

Locally advanced head and neck squamous cell carcinomas (HNSCC) are commonly treated with radio-chemotherapy, but improving treatment efficacy and patient quality of life remains a challenge. Reliable preclinical models are essential for testing new therapeutic strategies, biomarkers and understanding radiation response. In this work, we utilized histopathological imaging and unsupervised learning techniques to cluster histopathological slides of 10 HNSCC xenograft models, and assessed how the results correlate with radiosensitivity data from previous in-house experiments.

Material and method

In prior experiments, athymic nude mice were xenotransplanted with ten HNSCC tumor models of varying radiosensitivity. These models, ordered from radiosensitive to radioresistant based on tumor control dose 50% (TCD50) values obtained from fractionated radiotherapy, include: XF354, UT-SCC45, SAT, UT-SCC14, UT-SCC8, UT-SCC15, CAL-33, FaDu, SAS and UT-SCC5. A total of 71 hematoxylin and eosin (H&E) whole slide images (WSI) were obtained from excised untreated control tumors (4-10 slides per model). For each WSI, we considered the entire tissue area, from a resolution of 0.44 µm x 0.44 µm, to extract tiles of size 224x224. A publicly available deep-learning model pretrained on large histopathological datasets (UNI) was used to extract 1024 deep features from each tile. The features were standardized using Z-score normalization.

Dimensionality reduction was then performed using principal component analysis (PCA). The reduced features were subsequently clustered using Gaussian Mixture Models (GMM) into five clusters, a number determined empirically. We calculated the percentage of each cluster within individual slides to compare the clustering distribution across the tumor models, and assessed the correlation with the TCD50 values to examine how they align with radiosensitivity.

Result and discussion

Clustering results showed a relatively good visual separation of the clusters in the 2-dimensional space (PCA), in addition to similar cluster distribution within the slides of each tumor model. Visual inspection of the tiles revealed that one cluster likely represents necrotic and stromal areas, while another corresponds to keratin presence in the tissue. The remaining clusters represent tumor regions, though no definitive distinctions were made. Notably, the median percentage of the keratin cluster in each model showed a negative correlation with the TCD50 values, with a Spearman coefficient of -0.75 (p-value = 0.01).

Conclusion

Unsupervised clustering shows promise for HNSCC xenograft models profiling, offering a potential complement to the TCD50 information. Future work will investigate feature interpretability, and undergo further validation and clinical relevance assessment.

EACR25-1300

Combining Hypoxia and Stemness Biomarkers with Hematoxylin and Eosin Stained Images improves Radiosensitivity Classification in Head and Neck Cancer Preclinical Models

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Introduction

Predictive biomarkers have a significant role in developing personalized treatment in head and neck squamous cell carcinomas (HNSCC) patients. Despite recent advances, there is still a crucial need for reliable biomarkers for radiosensitivity. Therefore, we investigated combining hypoxia and stem cell markers with predictive scores derived from a deep learning (DL) model based on hematoxylin and eosin (H&E) stained images for classifying the radiosensitivity of HNSCC xenograft models.

Material and method

In prior in-house experiments, athymic nude mice were xenotransplanted with 10 HNSCC tumor models of varying radiosensitivity. These models can be classified into radiosensitive: XF354, UT-SCC45, SAT, UT-SCC14, UT-SCC8 and radioresistant UT-SCC15, CAL33, FaDu, SAS, UT-SCC5, according to the tumor control dose 50% (TCD50) values obtained from fractionated radiotherapy. In parallel to radiotherapy, slices of untreated control tumors were stained with hematoxylin and eosin (H&E), hypoxia markers (Pimonidazole, CAIX and CD31) and stem cell markers (CD44, CD98 and CD31). H&E and immunofluorescence (IF) images were obtained for a total of 63 mice (4 to 8 per model). IF images were analyzed using QuPath software to derive marker scores, defined as the percentage of positive cells for each marker and for each sample. The percentages of double positive cells for CAIX-Pimonidazole and CD44-CD98 were considered as additional scores. For modeling, we considered a binary classification approach. A DL model based on an open source feature extractor (CtransPath) and attention-based multiple instance learning was trained on H&E slides. A logistic regression with l2 regularization was trained using the marker scores. We combined both models (A) using the DL model output as a feature in logistic regression, and (B) averaging the probabilities from both methods. 5 repetitions of 5-fold validation were performed, where in each iteration a different combination of one radioresistant and one radiosensitive xenograft model was held out.

Result and discussion

Across 5 repetitions, the DL model achieved averages of 0.80 and 0.82, while the logistic regression achieved 0.77 and 0.80, for accuracy and F1-score, respectively.

Combining the two models led to an improved performance, with an average accuracy and F1-score of 0.82 and 0.85 in (A), and 0.84 and 0.87 in (B). Pearson correlation was calculated between the deep learning scores derived from the validation sets and the marker scores. The strongest correlation was observed for the CD44-CD98 score with an average coefficient of -0.50 (p-value < 0.05 across 5 repetitions).

Conclusion

Integrating biomarkers related to hypoxia and stemness with H&E-trained models improves radiosensitivity prediction in HNSCC xenograft models, suggesting a potential path towards more personalized treatment for patients.

EACR25-1384**Novel biomarkers for Radiotherapy response in Small Cell Lung Cancer**

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Introduction

Thoracic radiotherapy (TRT) has been widely used in the treatment of extensive-stage small cell lung cancer (ES-SCLC). However, phase III trials evaluating chemotherapy (CT) + anti-PD-L1 in the first-line setting excluded this treatment approach. More recently, the use of TRT as a means of boosting the anti-tumour immune response through the activation of innate immune DNA/RNA sensors has been reported. However, what role TRT in combination with CT plays in switching SCLC to immune-responsive type remains unclear. Here, we characterised multiple omics data in SCLC cell lines and peripheral immune cells from SCLC patients undergoing TRT treatment with respect to rare germline variants and the transcriptional profile following treatment with RT alone or in combination with CT.

Material and method

We enrolled patients with a diagnosis of SCLC receiving one of the following treatments: chemotherapy (cisplatin) and/or TRT. Within 2 h of blood draw, PBMCs were isolated by using gradient centrifugation. NGS analysis, alignment and filtering analysis were performed. We then performed an analysis of germline variants which led to loss of function (LoF). We performed GSEA on RNAseq data obtained from n=6 SCLC cell lines before and after treatment with CT (cisplatin 0.5 uM) and/or RT (4 Gy) for 72h and from PBMCs of SCLC patients before and after receiving TRT treatment. Innate immune biomarkers expression in SCLC cells was explored by immunofluorescence, flow cytometry and western blot to validate transcriptomic data.

Result and discussion

GSEA revealed that RT-treated SCLC cells were enriched for DNA Damage Repair (DDR) gene sets (BRCA1, BRCA2, ATM) and Notch gene sets in the RT-treated SCLC cells compared to CT. EMT genes were significantly downregulated in the RT SCLC cells compared to CT. The combination of RT+CT resulted in enrichment of cell adhesion molecules (CAMS) and upregulation of type I Interferon (IFN) gene sets compared to CT alone in SCLC cells, suggesting a concomitant reduction of EMT features and increase of immune-responsive phenotype. Furthermore, CT+RT combination treatment affected SCLC transcriptomic profile by decreasing EMT, glycolysis, oxidative phosphorylation, stemness and lung cancer poor survival gene sets compared to CT alone. The NE marker ASCL1 was also significantly downregulated in SCLC cells after RT+CT treatment. In parallel, in vitro analysis of innate immune markers in PBMCs from SCLC patients undergoing TRT showed an activation of STING preferentially in post-RT setting.

Conclusion

Taken together, these data suggest a potential pro-immune role for TRT in chemotherapy-treated ES-SCLC patients.

EACR25-1594**Investigating the Synergistic Effects of HDAC and ABC Efflux Transporter Inhibitors in Radiation-Resistant Triple Negative Breast Cancer**

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Introduction

Triple Negative Breast Cancer (TNBC), an aggressive subtype of breast cancer, accounts for approximately 15% of cases. Radiation resistance remains a major therapeutic challenge, necessitating a deeper understanding of its underlying mechanism. The present study investigates the synergistic therapeutic potential of a FDA approved histone deacetylase inhibitor (HDACi), Romidepsin, an epigenetic modulator, in combination with the ABC drug efflux inhibitor, Verapamil, in radioresistant TNBC cells, aiming to elucidate their mechanism of action.

Material and method

Radiation resistant TNBC cells (RR) were generated by exposing MDA-MB-468 cells to a clinical fractionated radiation dose of 40 Gray. These cells were characterized using colony-forming, migration scratch assay and FACS analysis. Cell viability assay was performed to generate dose response matrices and synergy score plot for RR cells in combination with romidepsin and verapamil. qRT-PCR and immunoblotting assays confirms the mechanistic insights into the radiation resistance and mode of drug action were analysed through apoptotic assays, FACS based assays.

Result and discussion

RR cells exhibited increased radiation resistance, proliferation, and migration potential. Among the tested HDACis, (Romidepsin, SAHA, Belinostat), Romidepsin displayed a promising cytotoxic effect but was less effective in RR cells as compared to the parent radiation-sensitive (RS) cells. Gene expression analysis demonstrated upregulation of Endothelial to Mesenchymal Transition (EndMT) markers (N-Cad, ZEB1, TWIST, FIBRONECTIN), Cancer Stemness regulatory markers (OCT4, SOX2, NANOG) and ABC efflux transporters (ABCC1, ABCC2, ABCG2) in the RR cells. Hoechst efflux assays confirmed increased transporter activity in TNBC-RR cells. Romidepsin treatment alone did not significantly alter H3K9ac/K27ac acetylation levels in RR cells. However, Inhibition of efflux transporters by verapamil lowered romidepsin efflux, restored H3K9/K27 acetylation, and resensitized RR cells to Romidepsin. The combination of Romidepsin and Verapamil inhibited cell viability of TNBC-RR in a dose dependent synergistic manner. Additionally, an increase in the sub-G1 population upon combinatorial treatment

indicated activation of cell death pathways in TNBC-RR cells. Ongoing *in vivo* studies aim to validate these findings in mouse models of radiation-resistant TNBC.

Conclusion

The synergistic effect of Romidepsin and Verapamil shows promising cytotoxic response in radiation-resistant TNBC cells, highlighting the potential of ABC transporter efflux inhibitors to overcome HDACi resistance. This study suggests a promising therapeutic strategy for improving treatment outcomes in radiation-resistant TNBC.

EACR25-1683

Enhancing cancer radiotherapy efficacy using NanOx, a novel oxygenating nanoemulsion that reverses tumour hypoxia

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Introduction

Radiotherapy is used to treat over 50% of cancer patients and is used in combination with surgery, chemotherapy, and immunotherapy, for cancers of the breast, lung, oesophagus, and rectum [1-3]. Ionising radiation exerts its anti-cancer effect through both direct DNA damage and indirectly through the production of reactive oxygen species via water radiolysis. This DNA damage is made permanent in the presence of molecular oxygen; however, it is reversible under hypoxia [4]. Therefore, hypoxia confers significant radiotherapy resistance and given that it is a common feature of most solid tumours it offers a unique tumour vulnerability to exploit to improve radiotherapy efficacy. To address this, we have developed a biocompatible, oxygenating perfluorocarbon nanoemulsion with imaging capacity (NanOx) that has potential to increase the radiosensitivity of hypoxic oesophageal adenocarcinoma (OAC) cells with acquired radioresistance.

Material and method

NanOx biocompatibility was assessed *in vitro* using 2D and 3D HepG2 cells, and *in vivo* using zebrafish embryos. NanOx oxygen delivery kinetics were assessed using the Seahorse bioanalyzer and via HIF-1α expression by Western immunoblot. Radiosensitisation efficacy studies were conducted using an isogenic model of acquired radioresistance in OAC and included colony forming assays, DNA damage and repair via 53BP1 foci immunofluorescence, cell cycle kinetics, and apoptosis.

Result and discussion

NanOx was biocompatible across *in vitro* and *in vivo* model systems, and significantly increased supernatant oxygen levels and significantly reduced HIF-1α

expression with 30 min treatment. NanOx, in combination with radiotherapy, significantly reduced the surviving fraction of hypoxic OAC cells with acquired radioresistance with a sensitiser enhancement ratio of 2.22. Combining NanOx with radiotherapy did not alter the level of DNA damage but significantly impaired the repair of this DNA damage, evident through increased 53BP1 foci persistence. NanOx combined with radiotherapy significantly increased the number of radioresistant OAC cells in G2/M and the number of apoptotic cells, in comparison with either treatment alone.

Conclusion

Hypoxia is a common feature of solid malignancies and significantly impairs treatment efficacy, particularly radiotherapy. We have invented a novel oxygenating perfluorocarbon nanoemulsion with imaging potential which can significantly improve response to radiotherapy in hypoxic OAC cells with acquired radioresistance.

EACR25-1929

Hypoxic tumor cells acquire a cellular quiescence phenotype that protects them against radiation-induced cell death

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Introduction

Previously, with the use of a lineage-tracing hypoxia reporter system we showed that pre-treatment hypoxic cells enrich in the regrowing spheroids and xenograft tumors following irradiation (IR). Here, we aim to understand how hypoxic tumor cells evade radiation-induced cell death.

Material and method

The lung AdenoCa H1299, head and neck SCC FaDu and the murine GBM LA6 cell lines originally carrying the hypoxia lineage tracing reporter (UnaG) (1), were transduced with the Fluorescent Ubiquitination-base cell cycle reporters (Fucci) to visualize the different cell cycle phases. Colony forming assays were performed in normoxic, chronic hypoxic or reoxygenated prior to IR conditions. To estimate the DNA repair capacity of the cells at different conditions, immunofluorescent staining for DNA damage response (DDR) proteins was performed and subsequently, the 53BP1 protein was endogenously tagged with a Halo tag to visualize the radiation-induced DNA double-strand breaks (DSBs) by means of live cell imaging.

Result and discussion

Tumor cells cultured in 1% O₂ atmosphere are progressively arrested in the G1/G0 phase of the cell cycle and display higher radioresistance compared to normoxic cells. Interestingly, hypoxic cells that were re-oxygenated prior to IR (3h) remain radioresistant despite the fact that induction of DNA damage in terms of nuclear foci is not different. Sorting FUCCI cells based on the cell cycle profile revealed that cells in the G1-arrested state are more radioresistant compared to S and G2 phase cells upon re-oxygenation, suggesting that the hypoxia-induced quiescence is a protective mechanism. In live cell imaging, irradiated re-oxygenated cells displayed slower progression to the cell cycle, less catastrophic events and less chromosomal miss-aggregations during mitosis compared to normoxic cells. To link clearance of DNA damage with cell cycle progression and the retention of cellular fitness in a single cell level we tagged endogenously 53BP1 and we are currently performing live-cell imaging. In 3D spheroids, hypoxic tumor cells are in a quiescence state and upon irradiation exhibit a survival advantage and enrich in regrowing spheroids. Irradiated hypoxic cells remain in a non-proliferative state for prolonged period, before they acquire a proliferative profile and contribute to spheroid regrowth. Importantly, splitting the total radiation dose and re-irradiate at the regrowing phase of the sphere led to higher spheroid control probability by irradiation, suggesting that re-irradiation at the time that hypoxic cells exit their quiescent state might be an appealing novel therapeutic strategy.

Conclusion

Our data suggests that hypoxic tumor cells acquire a quiescent state and this is linked with higher capacity to maintain cellular fitness upon radiation-induced damage even upon re-oxygenation.

EACR25-2136

Exploring Synergistic Effects of Ionizing Radiation and Asparaginase to Treat Solid Tumors

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Introduction

Combination therapies targeting cancer metabolic vulnerabilities and enhancing radiosensitivity offer promising strategies to improve treatment efficacy. L-asparaginase (L-ASNase) is a clinically established chemotherapy agent used to treat acute lymphoblastic leukemia (ALL): the treatment strategy is to exploit asparagine auxotrophy of cancer cells, by depriving them of asparagine (Asn) and glutamine (Gln). However, its possible application in solid tumors remains under-explored. This study investigates the combined effects of L-asparaginase II (EcAII) and ionizing radiation (IR) across selected solid tumor *in vitro* models, focusing on quantitative synergy assessments, building on preliminary findings demonstrating EcAII's efficacy in reducing proliferation in adenocarcinomas and triple-negative breast cancer (TBNC) cell lines.

Material and method

In vitro models included adenocarcinoma (A549 lung, 786-O renal, Caco-2 colorectal) and TNBC (BT549) cell lines, which were treated with EcAII (0.05–3 U/ml) and/or X-rays (1–5 Gy). Synergy was quantified using two complementary approaches: short-term proliferation (72-h growth assays) and long-term survival (colony formation assays). Dose-response curves for monotherapies (EcAII or X-rays only) were generated investigating clinically relevant doses centered on IC₅₀ values for each agent; data were fitted via Hill's equation. Synergy was assessed using the Bliss Independence model, generating Combination Indexes (CI, where CI < 1 indicates synergy) across effect levels. Secondary analyses included cell cycle profiling and mTOR pathway modulation.

Result and discussion

Bliss analysis revealed context-dependent interactions. Caco2 colorectal cells exhibited significant synergy (CI < 0.68) at low EcAII concentrations (0.05–0.1 U/mL) and moderate IR doses (3–5 Gy). In contrast, A549 and 786-O models showed additive effects (CI ≈ 1). TNBC models displayed cytostatic responses to EcAII monotherapy, characterized by G1-S arrest and mTOR pathway suppression, though the combination with IR yielded variable effects. These findings underscore the interest of tumor-specific context in designing combination regimens. While the Caco2 synergy highlights therapeutic potential of metabolic targeting in colorectal adenocarcinoma, variability in other models emphasizes the need for precision-based approaches.

Conclusion

This study provides a quantitative framework for evaluating the possible synergy for combined EcAII-IR treatments, identifying colorectal cancer as a promising candidate for metabolic radiosensitization. Limitations include *in vitro* model constraints and CI threshold interpretation; ongoing work employs 3D/organoid models to refine the predictive framework. These insights advance quantitative synergy evaluation in combined chemo-radiotherapy, offering strategies for optimizing combination therapies in solid tumors.

EACR25-2154

Assessement of the combination of iron oxide nanoparticles and erastin to radiosensitize head and neck cancer cells

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Introduction

Head and neck cancers (HNC) rank as the seventh most common malignancy worldwide, contributing

significantly to cancer-related mortality. In 2022, more than 940.000 cases of HNC were reported and more than 480.000 deaths were recorded. In locally advanced stages (III and non-metastatic IV), radiochemotherapy is the standard treatment, administered either alone or as an adjuvant therapy following surgery. Despite advances in radiotherapy, HNC remain challenging due to the complexity of the anatomical region. In this context, iron oxide nanoparticles (IONPs) have been explored as potential radiosensitizers for HNC treatment, aiming to enhance radiotherapy efficacy while minimizing damage to surrounding healthy tissues. These nanoparticles are hypothesized to amplify cancer cell radiosensitivity by increasing reactive oxygen species (ROS) production and decreasing antioxidant defences. Furthermore, erasin, a known ferroptosis inducer, has been investigated in combination with IONPs to enhance the antitumor effects through oxidative stress generation.

Material and method

In this study, IONPs coated with 3-(triethoxysilyl)propyl succinic anhydride (TEPSA) were used. Experiments using radiotherapy were performed using a 6 MeV beam. Clonogenic assays were conducted to assess the impact of IONPs on cell survival following exposure to various doses of radiation (2–8 Gy) and pre-treatment or not with nanoparticles. ROS generation, glutathione depletion, and thioredoxin reductase (TrxR) enzyme activity were measured to evaluate oxidative stress dynamics.

Additionally, the effects of erasin were also evaluated alone and in combination with IONPs regarding cell viability, ROS production, and ferroptosis induction.

Result and discussion

Results indicate that IONPs alone do not significantly reduce cell viability or enhance ROS production, though they are efficiently internalized across all tested HNC cell lines. The radiosensitizing effects of IONPs appear to be variable depending on the cell line and experimental conditions, and their overall impact remains insufficient. Interestingly, the combination of IONPs with erasin – at doses that are non-toxic when used separately – induces a marked decrease in cell viability and a concomitant reduction in ROS levels, suggesting a potential ferroptotic mechanism.

Conclusion

These findings highlight the promising role of IONPs and erasin in HNC treatment. Further investigations integrating radiotherapy are necessary to confirm their radiosensitizing potential and to elucidate the underlying molecular pathways, particularly ferroptosis induction.

EACR25-2166

The alpha-emitter radium-223 modulates migration, invasion and angiogenesis in metastatic prostate cancer preclinical models

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Introduction

Radium-223 dichloride ($^{223}\text{RaCl}_2$) is an alpha-emitting radiopharmaceutical approved for the treatment of metastatic castration-resistant prostate cancer (mCRPC) with bone metastases. While its primary therapeutic effect is through targeted bone deposition and induction of DNA damage in tumor cells, emerging evidence suggests potential effects beyond the bone micro-environment, including modulation of tumor cell migration, invasion, and angiogenesis. However, these effects remain poorly understood. This study aimed to investigate the effects of $^{223}\text{RaCl}_2$ on cancer cell

migration, invasion, and angiogenesis using 2D and 3D *in vitro* models.

Material and method

To investigate the impact of $^{223}\text{RaCl}_2$ on tumor progression *in vitro*, we used PC3 prostate cancer cells as 2D monolayers and 3D spheroids, plus mouse aortic rings for angiogenesis studies. Cell models were exposed to $^{223}\text{RaCl}_2$ at doses of 0.88–110.66 mGy (24 h irradiation) or 0.3–3.1 mGy (10-minute irradiation). Migration was assessed in monolayers (scratch assay) and spheroids (in extracellular matrix assay), while invasion was evaluated in spheroids (after 5 and 7 days). Angiogenesis was measured using aortic ring assay. Gene expression of migration and invasion regulators was analyzed in spheroid mRNA (after 8 d).

Result and discussion

Our long-term results indicated that $^{223}\text{RaCl}_2$ significantly reduced PC3 cell migration and invasion in a dose-dependent manner, both in monolayer and spheroid models. The migration area, compared to control (5 days post-irradiation), was significantly reduced in scratch assay as well as the migration distance in spheroid migration assay when exposed to 55.33 and 110.66 mGy. PC3 spheroid invasion capacity (maximum invasion distance and sprout density) was significantly reduced when evaluated in the same endpoint after exposure to equal doses of $^{223}\text{RaCl}_2$. Short-term exposure to $^{223}\text{RaCl}_2$ significantly impaired angiogenesis by significantly reduced sprout distance (48 h post-irradiation) and mean migration distance (5 d post-irradiation). A significant downregulation of migration-related genes, especially integrin- β 1 were determined after exposure to 0.88, 55.33, and 110.66 mGy.

Conclusion

These findings showed that $^{223}\text{RaCl}_2$ suppressed metastatic potential by inhibiting cellular migration, invasion, and angiogenesis in prostate cancer models. Even short-term exposure reduced sprouting and vascular network formation, highlighting its broader impact on the tumor microenvironment and potential role beyond targeting bone metastases.

Funding: FCT support CIBB - Strategic Projects UIDB/04539/2020 (doi.org/10.54499/UIDB/04539/2020); UIDP/04539/2020 (doi.org/10.54499/UIDP/04539/2020); Associated Laboratory funding LA/P/0058/2020 (doi.org/10.54499/LA/P/0058/2020).

EACR25-2337

Targeting Protein Disulfide Isomerases for Radiation Sensitization in Prostate Cancer

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Introduction

Prostate cancer frequently managed with radiotherapy faces challenges from radioresistance, leading to recurrence and poor prognosis. Mechanisms contributing to radiation resistance include antiapoptotic pathways, enhanced DNA repair, cancer stem cell maintenance, hypoxia, and ROS detoxification. Antioxidants have been implicated in radioresistance with varying degrees of emphasis. Protein disulfide isomerases (PDI) are chaperone proteins regulating cell viability and oxidative

stress by modulating cellular redox balance. Increased PDI levels occur in multiple cancers, including prostate cancer. This study investigates PDI inhibitors as radiation sensitizers in prostate cancer models with different radiation sensitivity profiles.

Material and method

We evaluated pan-PDI inhibitors (PACMA31, 35G8, E64FC26) alone and combined with ionizing radiation (2 Gy) in four prostate cancer cell lines (IGR-CaP1, 22rv1, PC3, and DU145) representing varied radiation sensitivity. Cell viability was assessed using WST-1 assays, and proliferation was monitored by real-time IncuCyte imaging. Sequence-dependent effects were examined by administering PDI inhibitors either before or after radiation treatment.

Result and discussion

PACMA31 demonstrated potent anti-proliferative activity across all prostate cancer cell lines with nanomolar EC₅₀ values (10–250 nM). Efficacy and optimal timing were cell-line dependent. In radioresistant cell lines (PC3 and DU145), PACMA31 before radiation showed enhanced growth inhibition compared to post-radiation administration. In radiosensitive 22rv1 cells, PACMA31 demonstrated synergy when given after radiation, reducing proliferation to near-complete inhibition. 35G8 showed particular efficacy in radio-sensitive cells. For IGR-CaP1 cells, PACMA31 showed significant efficacy both before and after radiation, with stronger inhibition in post-radiation protocols. Importantly, PDI inhibitors remained effective against chemoresistant prostate cancer cells, suggesting potential applications in both chemotherapy-naïve and chemoresistant disease settings.

Conclusion

PDI inhibitors, particularly PACMA31, can effectively enhance radiation response in prostate cancer cells, with optimal timing dependent on intrinsic radioresistance profiles. Radioresistant models benefit from PDI inhibition before radiation, while radiosensitive models respond better when PACMA31 follows radiation. Results also suggest PDI may influence cells' ability to resist oxidative stress through interaction with glutathione systems, thioredoxin, and antioxidant enzymes, thus constituting an interesting Achilles heel to target radioresistant cells. Tailored personalized administration sequence of PDI inhibition and radiation therapy based on tumor radioresistance characteristics could significantly improve treatment outcomes in prostate cancer.

EACR25-2419

111In-Radioconjugates Carrying DNA Intercalators and PSMA Inhibitors for Improved Auger Electron Therapy of Prostate Cancer

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Introduction

Prostate specific membrane antigen (PSMA) has emerged as one of the most promising molecular targets for prostate cancer (PCa), which motivated the study of PSMA inhibitors radiolabeled with different imaging and/or therapeutic radionuclides in PCa theranostics. In particular, Auger electron (AE) emitters, such as ^{111}In , are attractive tools in targeted radionuclide therapy to selectively irradiate tumour cells while sparing healthy tissues [1]. However, short range AEs must be emitted close to radiosensitive organelles to obtain efficient therapeutic effect [2]. Considering that nuclear DNA is the canonical target for biological damage induced by AEs, we have synthesized and proceeded with the pre-clinical evaluation of dual-targeted ^{111}In -complexes carrying a PSMA inhibitor and an acridine orange (AO) DNA intercalator. The designed dual-target radio-complexes were expected to undergo a selective uptake by PSMA(+) PCa cells with potential accumulation of the radionuclide in the nucleus (Figure 1).

Material and method

DOTA-based chelators bearing a PSMA inhibitor with the Glu-urea-Lys binding motif and AO groups were synthesized and labeled with ^{111}In . The preclinical evaluation of the resulting radioconjugates included cellular uptake and internalization, PSMA-blocking assays and nuclear uptake in cell lines expressing different levels of PSMA, as well as the evaluation of radiobiological effects (e.g., clonogenic survival, cell viability and g-H2AX assays) in the same cell lines. It also comprised the evaluation of biodistribution and pharmacokinetics of ^{111}In -labeled conjugates in healthy female mice. The study was done in comparison with single-targeted congeners carrying uniquely the PSMA inhibitor or AO group.

Result and discussion

The ^{111}In -complexes were obtained with high radiochemical yield, purity and in vitro stability. The single- and dual-targeted radiocomplexes carrying the PSMA inhibitor displayed high and PSMA-specific cell uptake and internalization in the PSMA(+) PC3 PIP cells while presenting a negligible uptake and internalization in the PSMA(-) PC3 flu cells. The radiobiological studies indicated that the dual-targeted radiocomplexes compromise cell viability and survival in a dose-dependent manner, being more efficient to induce DNA double-strand break formation than the single-targeted congeners. The animal studies demonstrated a high in vivo stability and favourable pharmacokinetic profile for dual-targeted complexes.

Conclusion

The dual-targeted ^{111}In -complexes exhibited high and specific internalization in PSMA(+) PCa cells and extensive radiotoxicity in the same cell lines revealing their potential for Auger therapy of PCa cancer.

EACR25-2426

Normal Human Epithelial-Fibroblast 3D Spheroid Cultures for Modeling Radiation-induced Lung Injury

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Introduction

Lungs are among the most sensitive organs to ionizing radiation, and this high intrinsic sensitivity is dose-limiting for thoracic radiotherapy (RT). RT-induced lung injury (RILI) includes acute inflammation and repair, which can develop into chronic inflammation and fibrosis. The classically used mouse model of RT-induced pneumopathy –like other animal models- only inadequately reflect clinically relevant dose sensitivities and characteristics of human RILI. We investigated direct spheroidal co-cultures of epithelial cells and fibroblasts, which can be considered as an *in vivo*-approximated culture method, and analyzed the cell-type specific RT response in combination with potential radioprotective strategies.

Material and method

Spheroidal co-cultures of human epithelial cells and HS5 fibroblasts were optimized concerning cell numbers and culture conditions. The RT response was further investigated (growth/retardation and cell fate e.g., viability, cell cycle, cell death and senescence) with or without co-applied modifier treatment (Src inhibitor Dasatinib; antidiabetic drug metformin).

Result and discussion

After irradiation, the growth of spheroids decreases while the cells remain viable. In these cultures, a rather low induction of apoptosis of 10% can be observed post RT, but an induction of senescence (to approx. 35%) can be observed, which is equally distributed between epithelial and fibroblastic cells. Purely epithelial spheroids show neither a reduction in growth after RT nor an induction of apoptosis and senescence, which on the one hand reflects an inherently resistant character and on the other hand shows that co-cultured fibroblasts influence the cell fate of the epithelial cells. A co-applied metformin treatment seems to lower the growth in general with no further decrease post RT, while no effects on the cell-type specific apoptosis and senescence level could be observed. A co-applied Dasatinib treatment lowers the spheroid growth significantly under non-irradiated conditions with no (further) impact post RT. growth in general but especially in combination with RT, while no effects on the cell-type specific apoptosis and senescence level could be observed. The degree of RT-induced senescence was not affected but apoptosis induction was limited in fibroblasts following Dasatinib co-treatment while increased in respective HBEC cells.

Conclusion

Using normal bronchial epithelial cells together with fibroblasts resulted in complex lung structures consisting of different epithelial and mesenchymal lung cell types. Differential cell fates post RT revealed the fibroblasts being more sensitive and fostering senescence of co-cultured epithelial cells. Currently we are validating respective findings using different epithelial-fibroblast pairs. Supported by grants of BMBF (16LW0293) and by the DFG RTG2762.

EACR25-2473**Exploiting replication stress as a novel therapeutic target in CIC-rearranged sarcomas**

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Introduction

CIC rearranged sarcomas are a relatively recently recognized, aggressive round cell sarcoma that are distinct from Ewing Sarcoma. While there is no consensus standard of care approach, the treatment for newly diagnosed CIC rearranged sarcomas usually consists of multimodal therapy including neoadjuvant and/or adjuvant polychemotherapy, in combination with surgery and often radiotherapy. Nevertheless, the prognosis remains very poor with a high rate of relapse/progression. ATR (Ataxia Telangiectasia Mutated and Rad-3 Related protein kinase) is activated by single-stranded DNA such as can be produced during replication stress, double-strand break (DSB) resection, or DNA cross-links. Cerelastib (AZD6738) is an ATP competitive, commercially available ATR inhibitor (ATRi) that blocks phosphorylation of CHK1 proteins disrupting DNA replication, inducing DNA damage, and preventing DNA repair, finally leading to cell death. We hypothesized that CIC rearranged sarcomas with high levels of replication stress may therefore be sensitive to ATR inhibition and would be radiosensitized by ATRi.

Material and method

A comparative transcriptional analysis was performed between cell lines (RSP7-C1, TE-441.T and IB120) harbouring an oncogenic CIC::DUX4 fusion and their counterpart with siRNA based CIC::DUX4 knock down. Differential genes expression and enrichment analysis were performed. Each line was treated, with increasing concentration of ATRi (AZD 6738 : 10 nM-5 µM) and/or RT (2 Gy-4 Gy). Tumoroids were then tested with increasing concentration of AZD 6738 (100 nM-2 µM) and/or radiation therapy (2 Gy-4 Gy).

Result and discussion

Enrichment analysis showed that terms involved in replication and cell cycle were related to CIC::DUX4 fusion. All cell lines demonstrate sensitivity to AZD6738 with IC50 of 490 nM (IC95 : 410-590 nM), 510 nM (IC95 : 480-540 nM) and 980 nM (IC95 : 820-1200 nM) for RSP7-C1, TE-441.T and IB120 respectively. Radiosensitization with AZD6738 was consistent between all cell lines and non cytotoxic concentration of AZD6738 showed a significant radiosensitization effect.

Conclusion

CIC::DUX4 oncogenic fusion induce transcription of genes involved in replication stress. To the best of our knowledge, this is the first study showing a cytotoxic effect of ATR inhibition in CIC::DUX4 sarcoma in vitro, particularly in three tumoroid models. Moreover, non cytotoxic concentration of ATRi showed a significant radiosensitization effect in both monolayer and tumoroid models. These data supports the importance of targeting replication and DNA damage pathways in CIC rearranged sarcomas. Further investigation is required to

determine the potential mechanisms of differential sensitivity, and determine the efficacy in vivo.

EACR25-2516**Response of non-small lung cancer cells to irradiation with helium ions**

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Introduction

Radiotherapy is an important treatment option for oncologic patients. Compared to conventionally used photon irradiation, hadron beam radiotherapy (hadron-therapy) has emerged as a more promising treatment approach due to its improved precision and better sparing of the surrounding tumor tissue, also causing fewer side effects. Radiotherapy with protons or carbon ions, already used in clinical settings, is proven to maximize the dose delivered to the tumor within the Bragg peak region. Helium ion beams are considered another promising alternative for radiation therapy, especially for the treatment of deep-seated and pediatric tumors, due to their favorable physical and biological properties over protons and carbon ions. This study aimed to examine the effects of helium ion radiation on radioresistant lung cancer cells, as well as to compare the efficiency of helium ion with the previously obtained proton irradiation.

Material and method

Human non-small lung cancer cell line HTB177 was irradiated with 62 MeV/u helium ions, with the irradiation position in the middle of the Spread-Out Bragg Peak. Clonogenic cell survival analysis was performed to detect changes in cellular radiosensitivity concerning different radiation doses applied. The dynamics of DNA double-strand break (DSB) repair was estimated by γ-H2AX foci analysis. The irradiation-induced changes in the cell cycle by helium ion beams were analyzed as well.

Result and discussion

Radiobiological data showed that HTB177 cells are more radiosensitive to helium ion irradiation compared to the corresponding data obtained following proton irradiation. The analysis of γ-H2AX foci showed that helium ions induced a comparable number of DSB as proton irradiation, which does not entirely correlate with the cell survival data. These results could be explained by complex DNA damages that may arise following helium ion irradiation, which reduce the cellular clonogenic potential. According to the obtained profile of cell cycle distribution, an increase in the subG1 cell cycle phase was more prominent for cells irradiated with helium ions compared to protons. These results point to stronger lethal effects of helium ions than protons, which is in agreement with the analysis of cell survival data. To further elucidate the cellular response to helium irradiation, an in-depth analysis of the complexity of the DNA lesions and DNA damage response will be performed.

Conclusion

Due to the growing interest in finding novel and more effective approaches in hadrontherapy, the study outlined here will enable prospective in vitro studies to be carried out on radioresistant cancer cells, such as pancreatic cancer cells, using helium and lithium-ion beams of different energies.

EACR25-2519

Therapeutic antitumor potential of brachytherapy combined with TLR7 agonist imiquimod

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Introduction

Brachytherapy (BR, contact radiotherapy) is one of the radiation methods of treating patients with cancer. It destroys cancer cells and also affects the tumor microenvironment (tumor blood vessels and immune cells). A dose of 10Gy acts as an "in situ" vaccination leading to the development of a robust antitumor immune response. Late effect after radiation is characterized by arise of numerous areas of underoxygenation (hypoxia) which weakens the anticancer effect of RT. Imiquimod (IMQ), the toll-like receptor 7 (TLR7) agonist, acts as an immunostimulant and vascular normalizing agent. In this study, we evaluated the effect of IMQ on the long-term antitumor response of BR in a mouse model of melanoma. The combination of IMQ with BR should elicit synergistic effect in cancer treatment.

Material and method

Mice with well-developed B16-F10 melanoma tumors were treated with IMQ and 5 days later with BR. IMQ was administered subcutaneously at a vascular normalized dose of 50 µg. BR was used at dose of 10Gy. The dose was planned using CT scans on the dedicated commercial platform. Irradiation was performed in the shielded therapeutic room with a high-dose rate after-loader equipped with an iridium-192 radioactive source (Microselectron, Nucletron). The dose per fraction was planned to be specified 2–3 mm from the applicator surface. The time of fraction delivery was adjusted depending on the source activity (3–10 Ci).

Result and discussion

Therapeutic anticancer effect of IMQ and BR combination was studied. We observed 70% tumor growth inhibition following monotherapy with the use of IMQ or 10Gy dose radiotherapy at 22th day of therapy. Combined therapy (IMQ with BR) inhibited tumor growth the most effectively compared to the other groups (tumor growth inhibition was 90% to control group and 70% to monotherapies). In a long-term observation we noticed tumor progression after IMQ treatment and large necrosis areas in tumors treated with BR. In mice treated

with combination of IMQ+BR we observed prolonged tumor growth inhibition. We also checked the therapy's impact on the hematological system parameters. Combined therapy decreased the number of lymphocytes with increase of granulocytes and eosinophils share in blood of treated mice. The proportion of eosinophils in peripheral blood was 10-times higher after combined therapy, compared to the other groups.

Conclusion

The combination of the vasculature normalizing dose of IMQ with BR elicit synergistic antitumor effect in melanoma treatment. Eosinophils mediated anticancer immunity after combined therapy may be essential for the therapeutic antitumor effect. Our data indicate that it is reasonable to use a drug that will prevent the changes occurring in the tumor microenvironment in combination with radiotherapy.

This work was financed by the National Science Centre (Poland), the project no. UMO-2018/31/D/NZ5/01754.

EACR25-2544

Revolutionizing Pancreatic Cancer Treatment: The Game-Changing Duo of Radiotherapy and PARP Inhibitors

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Introduction

Pancreatic cancer (PanC) is one of the most lethal types of cancer worldwide, with a 5-year survival rate of approximately 5%, requiring novel and more effective treatment approaches. Poly(ADP-ribose) Polymerase inhibitors (PARPi) can prevent cancer cells from repairing their damaged DNA. Therefore, the combination of PARPi with external beam radiation therapy can trigger an accumulation of DNA damage that surpasses the repair mechanisms of cancer cells, enhancing treatment effectiveness. This study aimed to investigate the combination of external beam radiation

therapy with PARPi to improve PanC treatment outcomes.

Material and method

Olaparib (OLA), a PARPi, was combined at a fixed non-cytotoxic concentration (1 μ M) with 6 MV X-rays in the MIA PaCa-2, PANC-1, and Hs 766T pancreatic cancer cell lines. The clonogenic assay was used to evaluate cell survival and calculate survival parameters, such as dose to achieve 10% or 50% of the survival fraction (D10 or D50), as well as the survival fraction at 2Gy (SF2) and the mean inactivation dose (MID). The radiosensitization effect of OLA was quantified by the sensitization enhancement ratio at D10 (SERD10), D50 (SERD50), SF2 (SERSF2), and MID (SERMID). Genotoxicity was also evaluated 72 hours after treatment using the Cytokinesis-Block Micronucleus assay. Additionally, the presence of DNA double-strand breaks (DSBs) was evaluated using the gamma-H2AX foci assay at 2, 4, 8, 12, and 24 hours after treatment.

Result and discussion

OLA combined with X-rays showed a synergistic effect in all cell lines, with all the survival parameters evaluated being lower in the OLA and X-rays combination compared to X-rays alone. Overall, OLA demonstrated a radiosensitizing effect in the three pancreatic cancer cell lines, with SER values ranging from 1.2 to 1.4. Notably, this radiosensitizing effect was observed regardless of the tumor origin or cell line radioresistance. Furthermore, an increase in the percentage of binucleated cells with micronuclei was observed with the combined treatment, indicating that it induced more DNA damage than the isolated treatments. Preliminary results also showed a slight increase in the number of gamma-H2AX foci per nucleus in the combined treatment, 24 hours post-treatment in the PANC-1 cell line, suggesting an increase in DSBs.

Conclusion

This study demonstrates the potential of combining OLA with X-rays as a future therapeutic approach for PanC, representing an alternative to the therapeutic options currently used in clinical practice. The induction of DNA damage by X-rays with the consequent inhibition of their repair by OLA may help explain the synergistic and radiosensitizing effects observed.

Funding: CIBB strategic projects 10.54499/UIDB/04539/2020 and 10.54499/UIDP/04539/2020, and Associated Laboratory funding 10.54499/LA/P/0058/2020 from FCT. Scholarship grant from FCT and European Social Funding 2021.05543.BD.

Signaling Pathways

EACR25-0078

New insights into the effects of coffee extracts on colon cancer and healthy human cells

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Introduction

The scientific community is concerned with the quality and safety of the raw materials and the content of bioactive substances beneficial to health. Coffee contains more than a thousand chemical compounds, some of them have bioactive potential, such as polyphenols known for antioxidant and anti-inflammatory effects. The fatty acid composition of coffees Burgundy processed by fermentation was previously described. The main risk factor for colorectal cancer has always been diet. The focus of our research was monitoring the effect of different methodological approaches of roasted coffee and green coffee on colon cancer tumor cells and compared to fibroblasts in vitro. We focused on monitoring the anti-proliferative effect of the extracts and profiling individual signaling pathways due to the influence of the extracts used.

Material and method

Cell cultures were used for Label-Free Protein Quantification analysis consisting of BJ-5ta healthy human cells and HCT116 colon cancer cells. The effect of coffee extract from naturally roasted coffee, anaerobically roasted coffee, naturally unroasted coffee, and anaerobically unroasted coffee was investigated. Determination of the proteins was characterized by UHPLC/MS analysis and then searched against the Uniprot database, followed by PCA and heatmap analysis and Gene Set Enrichment Analysis. We examined proliferation capacity, response to therapy and signaling pathway.

Result and discussion

In total, 1371 proteins were identified and quantified in BJ-5ta cells and 1528 in HCT116 cells. The differentially expressed genes (DEGs) obtained among the control (C), coffee natural roasted (CNR), coffee anaerobic roasted (CAR), coffee natural unroasted (CNU), and coffee anaerobic unroasted (CAU) showed that 989 proteins in BJ-5ta cells and 51 in HCT116 cells showed significant changes. Fibroblast cells exhibited significant differences between the CNR group and groups (C, CAR, and CAU). The significant was an decrease in the proliferation capacity. qRT-PCR analysis revealed the changes in the expression of genes associated with the inhibition of proliferation.

Conclusion

This study provides a comprehensive overview of the effect of coffee extracts after different preparations (roasted and unroasted) on tumor tissue and the effect on healthy cells. The key pathway enrichment of the gene sets across the C, CNR, CAR, CNU, and CAU groups in BJ-5ta and HCT116 cell cultures was examined using GSEA in the KEGG database. Our study demonstrates that the most significant pathways across all groups were the Pentose phosphate pathway and the Ribosome pathway. The Gap junction pathway was found to be downregulated in BJ-5ta cells.

Acknowledgments: This work was supported by the Scientific Grant Agency of the Ministry of Education of the Slovak Republic by grant No. VEGA/0500/23, VEGA 1/0038/25 and Slovak Grant Agency KEGA 024UPJŠ-4/2024.

EACR25-0097

SoxC transcription factors are oncoembryonic regulators of colon development and cancer

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Introduction

Colorectal cancer is a leading cause of death worldwide and thus a major unmet medical need. The standard of care upon diagnosis consists of surgical removal and chemotherapy. Existing targeted therapies are suitable only for a small subset of patients with specific molecular features. Therefore, the identification of new genes and proteins involved in tumor progression as potential targets is necessary to improve therapeutic outcome. Recently, the reactivation of embryonic programs in tumors has emerged as a mechanism used by cancer cells to acquire malignant features. Understanding this reactivation at the molecular level may allow to identify new targets to attack cancer-specific features, avoiding toxicity to healthy cells.

Material and method

Colorectal tumors, healthy adult colon and developing colon of the mouse were compared at the transcriptional level, to identify “oncoembryonic” programs active during development and in cancer but not in homeostasis. Using a combination of scRNAseq, ATACseq and ChIPseq, the genetic networks and hierarchies inside these oncoembryonic programs were defined. The results were then validated in colon development using knock-out mouse models and in colorectal cancer using a colonoscopy-based injection of genetically modified and control cancer organoids. The clinical relevance of the results was assessed by analyzing human tumor data and comparing the expression of oncoembryonic genes and survival rates.

Result and discussion

We identified the family of SoxC transcription factors as critical regulators within these oncoembryonic programs. Indeed, developing colons depleted of SoxC displayed an impaired phenotype. The genetic network regulated by SoxC during colon development included genes such as Mdk, Tead2, and Klf4. In the murine colorectal cancer model, depletion of SoxC hindered tumor growth and affected similar downstream target genes as during development. The expression of SoxC transcription factors and their downstream target genes also correlated with poor survival in human colorectal cancer patients, underscoring the therapeutic potential of targeting SoxC-

regulated pathways in colorectal cancer treatment.

Conclusion

SoxC transcription factors emerged as key regulators of oncoembryonic programs in colon development and colorectal cancer. Targeting SoxC-regulated pathways in colorectal cancer may therefore be an innovative approach to hit cancer cells without harming healthy adult cells. More broadly, systematically comparing development and cancer thus emerged as a powerful framework to identify new molecular vulnerabilities in cancer cells with a strong translational potential.

EACR25-0135

Structural characterization of ligand binding to stabilin-2, the Hyaluronic Acid Receptor for Endocytosis (Stab2/HARE)

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Introduction

Studies of the past decades have demonstrated that endothelium lining the blood vessels in the liver, spleen, pancreas, and other organs and tissues plays an important scavenging role and acts as a gatekeeper of immunity. The function of one of the scavenger receptors, stabilin-2 (Stab2) or Hyaluronic Acid Receptor for Endocytosis (HARE), seems to be especially important in these processes, as well as in other physiological and pathologic states, including cancer. Stab2 is expressed by sinusoidal endothelium and other types of cells, including macrophages, acting as a scavenger receptor of more than 10 ligands. In terms of its importance in cancer, blocking Stab2 function effectively opposed cancer metastasis in mice. The decreased level of metastasis of melanoma cancer cells in the Stab2 knock-out mice was demonstrated, accompanied by a dramatic increase in the circulating HA level. A similar effect was observed after the administration of monoclonal antibodies, which suggests that blocking Stab2 function provides an interesting strategy to prevent metastasis. Moreover, the homotypic interaction of Stab2 plays a critical role in lymph node metastasis of tongue cancer, and the lack of expression of Stab2 in endothelial cells in peritumorous tissues was correlated with increased patient survival. Apart from HA, Stab2 is also a receptor for Advanced Glycation End-products (AGEs). AGEs constitute a non-homogenous, chemically diverse group of compounds formed either exogenously or endogenously on the course of various pathways in the human body. They gained the scientific community's interest due to the increasing evidence of their involvement in many pathophysiological processes and diseases, such as cancer, diabetes, aging, cardiovascular and neuro-degenerative diseases. The negative impact of the accumulation of excessive AGE levels is connected with inflammation and oxidative stress.

Material and method

Stab2 fragments were expressed and purified in bacterial or baculovirus system. The structure of the recombinant Fas1 Stab2 domain was solved by X-ray crystallography. Molecular modelling (homology modelling, docking) was used for ligand-receptor interaction characterization.

Result and discussion

I hereby present the high-resolution (1.48 Å) crystal structure of the seventh FAS1 domain of Stab2, which provided the first and only experimental information about the three-dimensional fold of a fragment of this receptor. Molecular models of putative HA and AGEs binding sites at Stab2 are also proposed.

Conclusion

The deciphering of the structural aspects of Stab2-ligand (HA, AGEs) interactions could facilitate the understanding of the signalling pathways triggered by this receptor and potentially serve as a novel protein target in anticancer strategy.

EACR25-0339

Receptor-like Tyrosine Kinase as a Promising Therapeutic Target in Pancreatic Cancer: Counteracting Wnt/β-catenin and Sonic Hedgehog/GLI1 Signaling

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Introduction:

Pancreatic ductal adenocarcinoma (PDAC) is a highly aggressive malignancy with an exceptionally poor prognosis, as reflected in a 5-year survival rate of less than 8%. The complex molecular landscape underlying PDAC aggressiveness, which is characterized by genetic alterations, hyperactivation of receptor tyrosine kinases (RTKs), and dysregulated embryonic developmental signaling, poses a significant challenge to single-targeted therapies. Targeting key regulators of tumor signaling networks is essential for effective PDAC therapy.

Material and method

Cell viability was assessed using the MTT assay. A phospho-receptor tyrosine kinase (p-RTK) array assay was conducted to profile basal RTK activity and evaluate drug effects in pancreatic cancer cells. Co-immunoprecipitation assays were performed to examine the interaction between receptor-like tyrosine kinase (RYK) and EGFR. Small interfering RNA (siRNA) transfection was utilized to achieve RYK knockdown. The therapeutic efficacy of the treatment was evaluated in a pancreatic cancer xenograft mouse model.

Result and discussion

In the PANC-1 PDAC cell line, p-RTK array revealed that phosphorylated EGFR exhibited the highest basal activity, followed by IGF-1R and RYK. Co-immunoprecipitation assays demonstrated that RYK was cross-phosphorylated by EGFR. RYK knockdown led to increased phosphorylation of glycogen synthase kinase-3 beta (GSK3β) at serine 9 (S9), an inhibitory modification, without altering phosphorylated β-catenin levels. In addition, RYK silencing promoted nuclear translocation of GLI1, a key effector of the Sonic Hedgehog (Shh) signaling. Targeting the RYK-GSK3β axis with a 2,4-dimethylpyridin-3-ol analog of the multi-RTK inhibitor sunitinib induced cancer cell-selective cytotoxicity and exhibited potent antitumor efficacy in PDAC xenograft mouse model. Notably, unlike other multi-RTK inhibitors

such as sunitinib and gefitinib, especially the latter of which inhibited both EGFR and RYK, the sunitinib analog only suppressed Sonic Hedgehog (Shh)/GLI1 signaling. Moreover, this modulation reprogrammed macrophage polarization toward M1 phenotype, thereby enhancing cancer cell death in the tumor microenvironment.

Conclusion

Our study identifies RYK as a novel EGFR cross-phosphorylation target and functions as an upstream regulator of GSK3β. The RYK-GSK3β axis plays a critical role in counterbalancing both canonical Wnt signaling and RTK-mediated non-canonical Wnt/β-catenin signaling, as well as canonical and KRAS-driven non-canonical GLI1 activity. Pharmacological inhibition of the RYK-GSK3β using a 2,4-dimethylpyridin-3-ol analog of sunitinib effectively suppressed tumor growth, reprogrammed macrophage phenotypes, and inhibited metastasis, underscoring its potential as a promising therapeutic strategy against PDAC.

EACR25-0370

Elucidating receptor-mediated modulation of cannabidiol activity in prostate cancer

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Introduction

The five-year survival rate for metastatic prostate cancer (PCa) is only 30%. While androgen deprivation therapy, the standard of care treatment, initially proves effective, inevitable development of castration resistance renders the disease incurable. Therefore, novel therapeutic approaches are urgently needed to improve treatment options & outcomes for aggressive PCa. Cannabinoids have shown promising anticancer properties & potential to enhance efficacy of existing therapies. Cannabidiol (CBD), a major non-psychotropic cannabinoid, reduces PCa cell viability & alters expression of key cell cycle proteins. However, the mechanism by which CBD enters PCa cells remains unclear. This study aims to identify receptors involved in modulating CBD activity in PCa cells.

Material and method

mRNA expression of cannabinoid receptor 1 (CNR1), CNR2, transient receptor potential vanilloid 1 ion channel (TRPV1), TRPV2, G protein-coupled receptor 55 (GPR55), GPR18, GPR19, TRP ankyrin 1 (TRPA1), TRP melastatin 8 (TRPM8), peroxisome proliferator-activated receptor gamma (PPARG), & fatty acid amide

hydrolase (FAAH) in 7 prostate cell lines (6 cancerous; DU145, PC-3, LNCaP, 22Rv-1, C4, LNCaP Abl, and 1 non-cancerous; PWR1E) was established via RT-qPCR. DU145 cells were seeded in a 6-well plate & transfected with 10 nM non-targeting pool (control) or PPARG siRNA for 24hr. After 24hr, cells were trypsinised & re-seeded in a 96-well plate. Cells were treated with the vehicle (DMSO) or CBD IC₅₀ value (11.07 μM). After 72hr treatment, cell viability was measured using the MTT assay.

Result and discussion

CNR1, CNR2, GPR55, & GPR18 were not expressed in any cell lines. TRPV1 & TRPV2 were expressed across all cell lines at low-to-medium levels ($5 < \Delta Ct < 20$). TRPM8 was expressed exclusively in androgen receptor (AR)-positive PCa cell lines (LNCaP, 22Rv-1, C4, & LNCaP Abl) at low levels ($10 < \Delta Ct < 20$). TRPA1 was solely expressed in the non-cancerous cell line ($\Delta Ct = 12.22$). GPR19 & FAAH were expressed to varying extents across all cell lines. Finally, PPARG was widely expressed at medium-to-high levels ($4 < \Delta Ct < 10$), with AR-negative cell lines (DU145 & PC-3) having the highest expression ($\Delta Ct = 7.61$ & 4.54 respectively). siRNA-knockdown of PPARG had no effect on the efficacy of 72hr CBD treatment on DU145 cells.

Conclusion

Having established gene expression levels of 2 known & 5 novel cannabinoid receptors in PCa cells, the receptors will be systematically knocked down & treated with CBD to assess the role of receptors in the mechanism of action of CBD. Results indicate that PPARG is not involved in CBD's mechanism of action in DU145 cells. Receptors may be knocked down in pairs &/or groups depending on results of individual knockdown experiments. These findings provide a foundation for further investigation into receptors mediating CBD's effect on PCa cells, which may ultimately inform the development of targeted therapeutic strategies for advanced prostate cancer.

EACR25-0404

Role of intracrine VEGFA-VEGFR1 signaling in BRAF mutated melanoma

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Introduction:

Despite recent advances in prevention and treatment, melanoma remains an aggressive and deadly malignancy. Moreover, therapy-driven resistance is still a major obstacle in the clinical management of the metastatic disease. These findings highlight the need to further elucidate mechanisms underlying melanoma growth and progression. VEGF/VEGF receptors axis represents an attractive target for combinatorial cancer therapy. It has been demonstrated that its role is not limited to angiogenesis and immuno-suppression, but VEGFA mediated signalling may also occur in tumour cells. However, how VEGF receptors contribute to tumorigenesis and tumor progression remains largely unclear. Here we

investigated the biological role of VEGF receptor 1 (VEGFR1) in melanoma cells.

Material and method

VEGFR1 loss-of-functions strategy was based on gene silencing (shRNA) in BRAF-mutated melanoma cell lines D4M, YUMM 3.3 and A2058. Cell proliferation was assessed by CellTiter Glo assay. Individual cell migration was investigated using time-lapse video microscopy. Tumor spheroids were produced according to the hanging drop methods. In situ proximity ligation assay (PLA) was executed with Duolink® Detection Reagents kit (Merck). VEGFR1 localization was assessed by Subcellular protein fractionation kit (Thermo).

Result and discussion

We observed that VEGFR1 was highly expressed in different melanoma cell lines and its silencing reduced in vitro growth and motility in 2D culture, independently of exogenous VEGFA stimulation. We demonstrated that VEGFR1 was needed for fast and directional cancer cell migration, through actin re-organization. In addition, we observed that VEGFR1 silencing altered melanoma invasive activity in 3D spheroids, decreasing cell protrusions into the extracellular matrix. Furthermore, PLA confirmed the intracellular VEGFA-VEGFR1 association and cell fractionation showed a nuclear localization of VEGFR1. These results suggested that VEGFR1 may be involved in gene expression regulation. Then, we performed RNA-seq that validated the role of VEGFR1 in the control of cell proliferation and cell migration. Moreover, RNA-seq revealed also the involvement of VEGFR1 in the amino acids metabolism. In particular, VEGFR1 silencing impaired the expression of genes involved in serine and proline metabolism (PYCR1, ALDH18A1, PHGDH, PSPH). Interestingly, both the pathways are mainly regulated by ATF4 (activating transcriptional factor 4) that we found expressed in close proximity to VEGFR1, also in the nucleus.

Conclusion

Collectively, these findings suggest that VEGFR1 is involved in growth, spreading and metabolism of tumor cells. Since melanoma cells were never treated with recombinant exogenous VEGFA, we suggest that VEGFR1 is activated by VEGFA in an autocrine fashion. Furthermore, the nuclear expression of VEGFR1 and its colocalization with ATF4 indicates a possible role of VEGFR1 as a coactivator of transcriptional program.

EACR25-0405

Self-antigen presentation drives T-cell leukemia development in a TCR transgenic mouse model

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Introduction

T cell acute lymphoblastic leukemia (T-ALL) is a malignant disorder characterized by development arrest of immature T cells, followed by their rapid proliferation and dissemination. The T cell receptor (TCR), plays an essential role in T cell development, since its interaction with MHC-bound self-antigens defines T cell fate during

positive and negative selection in the thymus. Since many T-ALL cases exhibit TCR on their leukemic cells, we aimed to understand the TCR role in leukemia development, and the impact of leukemic cell antigenic stimulation.

Material and method

A cohort of Rag2^{-/-} mice expressing transgenic TCR OT-I, which is responsive to the SIINFEKL ovalbumin peptide (OVA), were generated and followed for 12–14 months. Leukemic cells underwent immunophenotypic analysis by flow cytometry and, to confirm their malignant nature, OT-I leukemic cells were injected into Rag2^{-/-} recipient mice. Both leukemic and non-leukemic OT-I T cells were stimulated *in vitro* with OVA to activate the TCR pathway. Activation of the TCR pathway was also achieved through PMA/Ionomycin and anti-CD3 monoclonal antibody. Activation status was assessed by induction of T cell activation marker CD69 by flow cytometry.

Result and discussion

Around 60% of TCR OT-I mice developed a T-ALL-like disease, characterized mainly by thymic lymphoma, increase of white cells in the blood and dissemination to lymphoid and non-lymphoid organs. This malignant transformation occurred through basal TCR signaling, since the ablation of this interaction by β2-microglobulin gene knockout impaired disease development. Malignant cells were found to express CD90, CD4, CD8, CD24, CD5 and TCRβ, confirming the development of a T cell leukemia. When injected in recipient mice, these cells led to a rapid development of fatal leukemia. Surprisingly, when treated with OVA, most OT-I leukemic cells did not upregulate CD69 and those that did had low levels compared to healthy OT-I lymphocytes. The responsiveness patterns were similar when the TCR pathway was activated by anti-CD3 or PMA/Ionomycin.

Conclusion

These findings show that transgenic TCR OT-I leads to T cell leukemia through basal TCR signaling by presentation of self-peptides to the receptor. However, upon malignant transformation, the TCR is less responsive to antigenic stimulation. Further research is needed to understand why leukemic cells do not upregulated CD69 when OT-I TCR is stimulated.

EACR25-0443

Novel interactions of Mixed-Lineage Kinase 4: identifying key binding partners in breast cancer

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Introduction

Mixed-Lineage Kinase 4 (MLK4) is frequently over-expressed in various cancer types and linked to poor prognosis. MLK4 can enhance migration, invasiveness, DNA damage response, and chemoresistance in triple-negative breast cancer (TNBC). Despite growing evidence implicating MLK4 kinase in tumorigenesis, the

underlying molecular mechanisms of MLK4 signaling in cancer progression remain largely unexplored, emphasizing the necessity for further investigation. Hence, this study aims to investigate the MLK4 interactome to uncover its molecular mechanisms driving cancer advancement and aid therapy development.

Material and method

To achieve this goal, we employ coimmunoprecipitation (co-IP) coupled with mass spectrometry to investigate the MLK4 interactome in TNBC cells – HCC1806. Then, the candidates are validated by co-IP followed by immunoblotting, immunofluorescent and fractionation experiments. An *in vitro* kinase assay using inactive GST-MLK4β kinase domain is applied to determine whether any candidates are MLK4 direct substrates. Moreover, phenotypic assays such as colony formation, migration and comet assay are conducted to elucidate the functional implications of these interactions in cancer cells.

Result and discussion

The 54 new interactors of MLK4 were identified. They were involved in cell cycle, PI3K/Akt and MAPK signaling. These hits were mostly present in cytosol, nucleus or plasma membrane in cellular component terms. Afterward, 14-3-3 – the anchored protein, GNL3 – a nucleolar protein, MLK1 – another MLKs member, RAN – a RAS-related nuclear protein and MYBBP1A - a transcriptional regulator, were validated as potential MLK4 binding partners. MYBBP1A is chosen for further validation because it modulates p65 (NF-κB) activity, which is regulated by MLK4 in DNA damage and chemoresistance in TNBC cells (Marusiak 2019, Mehlich 2021). The interaction of MLK4 and MYBBP1A localizes in the nuclei, which was confirmed by co-staining and fractionation experiments. Interestingly, silencing of MLK4 decreased the binding of MYBBP1A and p65, suggesting that MLK4 may act as a molecular anchor, bridging this interaction. Notably, this interaction is disrupted under doxorubicin treatment, indicating a dynamic regulatory response to chemotherapy.

Conclusion

The interaction of MLK4-MYBBP1A was selected for deeper validation to understand the molecular mechanisms of MLK4 signaling promoting cancer progression. We confirmed the interaction between MLK4 and MYBBP1A in the nuclei under basal conditions. We also observed that MLK4 enhanced the interaction of MYBBP1A and p65. Moreover, this interaction may be interrupted upon therapeutic treatment. Further study will investigate the functional roles of this network in DNA damage response through p65 signaling and the novel nuclear function of MLK4 in regulating cancer cell behaviors.

EACR25-0621

H19 Non-Coding RNA expression in Bladder Cancer Tissue as a marker of progression and therapy resistance

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Introduction

Bladder cancer (BC) is one of the most common urological malignancies, with high recurrence and progression rates. Long non-coding RNAs (lncRNAs) have emerged as key regulators of cancer development, with H19 being one of the most studied lncRNAs in various cancers, including BC. H19 is implicated in tumor proliferation, invasion, and drug resistance. This study aims to evaluate the expression levels of H19 in bladder cancer tissues using the real-time PCR (qPCR) method and assess its potential role as a biomarker for BC.

Material and method

Bladder cancer tissue samples and adjacent normal tissues were collected from patients undergoing surgical resection. Total RNA was extracted, and cDNA synthesis was performed. The expression of H19 was quantified using real-time PCR with specific primers and normalized to housekeeping genes. Statistical analyses were conducted to determine the correlation between H19 expression and clinicopathological characteristics.

Result and discussion

H19 was significantly upregulated in bladder cancer tissues compared to adjacent normal tissues. H19 expression correlated with higher tumor grade, advanced stage, and progression ($p = 0.0117$), but not with the increased risk of recurrence ($p = 0.05$). It was confirmed that survival predictions are significantly different in the groups of patients with high-grade and low-grade cancers ($p = 0.02101$). The real-time PCR method provided highly sensitive and reproducible quantification of H19 expression levels.

Conclusion

In summary, lncRNA H19 could be a critical factor in bladder cancer progression and therapy resistance. Understanding its mechanisms offers avenues for developing targeted therapies to improve treatment efficacy. Real-time PCR is a reliable method for detecting H19 expression, highlighting its potential application in BC diagnosis and prognosis. Further studies are needed to explore the functional role of H19 and its therapeutic implications in bladder cancer.

EACR25-0665

APRIL-induced feedback loop regulating BCMA expression in Multiple Myeloma

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Introduction

The B-cell maturation antigen (BCMA; TNFRSF17), primarily expressed on both healthy and malignant plasma cells, has become a prominent target for immune cell therapies in B-cell malignancies and diseases such as multiple myeloma (MM) and certain autoimmune diseases. These therapies include chimeric antigen receptor (CAR) T cells and bispecific T cell engagers (BiTEs) targeting BCMA. MM growth and survival are mediated by APRIL (A proliferation-inducing ligand) binding to BCMA, triggering NF-κB signaling. Here, we investigate a potential feedback mechanism in MM that enhances BCMA expression via APRIL-induced NF-κB activation.

Material and method

To investigate the functional relationship between NF-κB activity and BCMA expression, NF-κB activation and suppression were modulated using either cytokines and APRIL or small-molecule inhibitors and doxy-inducible shRNA knockdown (KD). The analysis was performed at both the mRNA and protein levels using quantitative PCR (qPCR) and immunoblotting, respectively. To visualize APRIL-mediated NF-κB signaling, we examined nuclear translocation through cellular fractionation followed by immunoblotting and complemented this with immunofluorescence (IF) analysis. Genomic analysis was performed to identify potential NF-κB binding motifs in regions regulating TNFRSF17 transcription. Chromatin immunoprecipitation (ChIP) in MM cells in response to APRIL stimulation was used to investigate binding of NF-κB to the selected regions of BCMA. NF-κB binding to targeted genomic regions was quantified by q-PCR.

Result and discussion

We observed that enhanced NF-κB activity was associated with increased BCMA mRNA and protein levels, while suppression of NF-κB resulted in a corresponding decrease in MM cells. Notably, APRIL-induced activation of NF-κB and its subsequent nuclear translocation led to significant upregulation of BCMA protein levels, an effect abolished by either pharmacological inhibition of NF-κB or tet-on shRNA-mediated KD of RELA (NF-κB, p65). ChIP experiments revealed that NF-κB regulates TNFRSF17 transcription, a process enhanced upon APRIL stimulation.

Conclusion

Here, we identify a previously unexplored positive feedback loop in which BCMA promotes its own expression through APRIL-induced NF-κB activation. These findings suggest that this specific feedback mechanism and its interplay with the bone marrow niche may contribute to MM progression and survival.

EACR25-0694

Decipher the Roles of IL-6 Signaling in CTPS Filament-mediated Cancer Progression

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Introduction

CTP synthase (CTPS) filament forms in cancer cells, which reprograms nucleotide metabolism for stress adaptation under glutamine (Gln) deprivation. While research on the physiological effects of CTPS filament is developing, only a few regulators of CTPS filament formation have been identified. Here, we propose that IL-6, a extracellular protein overproduced by HEp-2 cancer cell under Gln-free (Gln(-)) and serum-free (S(-)) conditions, served as an external signal to enhance the CTPS filament formation. IL-6 is a pro-tumor cytokine that remodels the activities and differentiation of immune cells, but few studies discuss its influence on the protein compartmentalization.

Material and method

We performed a screen by comparing the extracellular proteins secreted by the cells cultured in the Gln(-)S(-) conditions with those in the Gln(+)S(+) conditions, and identified IL-6 as a top elevated protein. We used IL-6 antibodies to block effects of IL-6 signaling in the HEp-2 and HCT116 cancer cells and examined their CTPS filament formation. We also added exogenous recombinant human IL-6 into the conditional medium to enhance IL-6 signals. Small molecule inhibitors targeting IL-6 downstream pathways were used to determine the critical signals regulating CTPS filament formation.

Result and discussion

We identified a role of IL-6 in enhancing, but not inducing, CTPS filament formation in HEp-2 and HCT116 under Gln(-)S(-), and it is mediated by IL-6 downstream signaling pathway. Our data indicate that IL-6 is a critical factor to regulate CTPS filament formation, therefore we are currently dissecting its molecular mechanism by investigating the connection between CTPS filament-associated proteins and ERK downstream signatures.

Conclusion

Here we demonstrate that the IL-6-downstream signaling axis regulates CTPS filament formation, which may facilitate the adaptation of cancer cells in nutritional stress. The value of therapeutic strategy by hindering CTPS filament formation is brought out with its universally-conserved feature in different types of cancer cell. We will further decipher the detailed molecular mechanism and to provide a widely-effective anti-cancer therapeutic strategy combining CTPS filament formation and IL-6 signaling.

EACR25-0838

SRF fusions act as hyperactivated transcription factors in pediatric tumors

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Introduction

Soft tissue tumors expressing muscle markers, including perivascular tumors, myofibroma, and rhabdomyosarcoma, have been associated with chromosomal translocations leading to SRF fusion genes. The Serum Response Factor (SRF) is a transcription factor that regulates muscle development via TCF or MRTF cofactors. We characterized four SRF fusion genes: SRF::RELA, SRF::FOXO1, SRF::ICA1L, and SRF::

PDGFRB, to assess their molecular mechanisms and oncogenic potential.

Material and method

To investigate the properties of SRF fusion proteins, we transfected their constructs into mammalian cells and analyzed their subcellular localization using fluorescence microscopy. Their transcriptional activity was evaluated through luciferase reporter assays, while co-immunoprecipitation assays assessed dimerization. To study their oncogenic potential, we performed cell proliferation assays and tested their sensitivity to imatinib. Muscle differentiation was studied by fluorescence microscopy, examining the expression of differentiation markers. Transcriptomic analyses were conducted to compare gene expression profiles between cell lines and tumor samples carrying SRF fusions. Additionally, BioID experiments were performed to identify protein interaction partners of the fusion proteins.

Result and discussion

All SRF fusion proteins localized to the nucleus and exhibited constitutive transcriptional activity, independent of MRTF or TCF cofactors, unlike wild-type SRF. This required the SRF MADS box for DNA binding and the partner transactivation domain. Interestingly, we identified a cryptic transactivation domain in the cytosolic protein ICA1L. In contrast, SRF::PDGFRB functioned differently, relying on its tyrosine kinase domain for activity. It transformed Ba/F3 cells, demonstrating oncogenic potential, and was inhibited by imatinib, suggesting therapeutic relevance. Most SRF fusions increased cell proliferation and altered muscle differentiation genes, except SRF::PDGFRB, which induced STAT1 signaling and an interferon-like response. We observed a correlation in gene expression between cell line and tumors bearing the same SRF fusion.

Conclusion

Most SRF fusions act as hyperactivated transcription factors, driving oncogenesis in myoid soft tissue tumors by deregulating muscle differentiation and increasing cell proliferation. The MADS box is crucial for dimerization and transcriptional activity. SRF::PDGFRB, acting as a tyrosine kinase-driven oncogene, is sensitive to imatinib. Gene expression similarities between cell models and tumors highlight their biological relevance.

EACR25-0963

The Serine/Threonine phosphatase POPX2 and its effects on cell mechano-sensing

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Introduction

POPX2 is a serine/threonine phosphatase from the PP2C family. It has been reported that POPX2 levels positively correlate to the invasiveness and motility of cancer cells. POPX2 dephosphorylates and downregulates p21-activated kinase (PAK) activity. Through POPX2's interaction with the PAK-interacting guanine nucleotide exchange factor (PIX), it is thought to participate in the maintenance of focal adhesions (FAs) and actin stress fibers (SFs). FAs are formed from the recruitment of FA

proteins (FAPs) to the cell surface membrane upon the interaction of integrin receptors with the extracellular matrix (ECM). Actin SFs which are linked to the FAPs allow cells to exert pulling forces on the ECM. The physical characteristics of the ECM are then fed back to the cells through the FAs in a process known as mechano-sensing. In this study, we investigated the effects of POPX2 on mechano-sensing.

Material and method

Control (ctrl-3T3) and POPX2-overexpressing NIH3T3 (X2-3T3) mouse embryonic fibroblasts were seeded onto fibronectin-coated polyacrylamide gels of 4 different rigidities: 6kPa, 14kPa, 31kPa, and 61kPa. Changes in cell morphology, FA, and SF formation were observed using immunofluorescence microscopy. Traction force microscopy was performed to measure changes in cell-generated traction stress. Cell lysates were prepared for Western blot analysis to identify changes in pathways implicated in mechano-sensing.

Result and discussion

Our results show that X2-3T3 cells display higher cell spreading area, actin intensity, FA area, and FA number compared to ctrl-3T3 cells across all substrate rigidities. We also confirmed that cells plated on stiffer ECM are more well spread and contain more FAs. X2-3T3 cells were found to generate greater traction stress compared to ctrl-3T3 cells across a range of substrate rigidities. Our data suggest that POPX2 could facilitate rigidity sensing. We are now in the process of exploring different mechanosensors which may be regulated by POPX2 signalling.

Conclusion

In this study, we found that NIH3T3 cells expressing high levels of POPX2 exhibit a more well spread morphology, more robust actin SFs and contain more FAs. Our observations also suggest that POPX2 could promote rigidity sensing.

EACR25-0964

IL-6/STAT3 Regulates Solid Stress Enhanced Migration and Invasion in Breast Cancer Cells

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Introduction

While tumour cells are known to respond to biochemical signals, the influence of physical and mechanical cues is increasingly more recognised. Solid stress compression, a prevalent biomechanical force in the tumour micro-environment that accumulates alongside tumour growth, has been associated with enhanced invasive and metastatic phenotypes in various cancer cells. When a tumour mass rapidly expands within the body, it pushes on surrounding tissues, which in turn exerts an equal and opposite force on the tumour. This phenomenon is known as solid stress compression. In this study, we investigated the effects of solid stress compression on breast cancer cells.

Material and method

We applied solid stress compression on MBA-MD-231 breast cancer cells using a 2D in-vitro compression setup.

To mimic the varying solid stress pressures experienced by cells at the periphery of the tumour mass, three incremental solid stress compressions were applied: 386.5 Pascal (Pa), 773.0 Pa (the estimated breast tumour microenvironment pressure), and 1546.0 Pa. The cells were plated on transwell insert membrane of 0.4 μm pore size. The compression weights are cushioned with 2% agarose disks of ~3mm thickness to ensure even pressure on the cell monolayers. After 16 hours of compression, the cells were harvested for further experiments. Cell lysates were prepared for western analysis and RNA was extracted for RNA-sequencing. Migration assays were also conducted to monitor the effects of compression on cell migration.

Result and discussion

Our findings revealed that MDA-MB-231 cells under incremental solid stress compression exhibited enhanced migration and followed a biphasic trend. Migratory and invasive capacities were elevated at 386.5 Pa, peaked at 773.0 Pa, and reduced but remained elevated at 1546.0 Pa as compared to uncompressed control cells. Global transcriptome analysis found upregulation of many genes, including Interleukin-6 (IL-6), a vital cytokine in breast cancer progression and metastasis. Western blot analysis demonstrated that solid stress upregulated protein levels of IL-6 and SNAI1, as well as IL-6 secretion. STAT3, the main transducer of IL-6 signalling, also showed elevated phosphorylation status with increasing solid stress. Our data suggest solid stress compression leads to the activation of the IL-6/IL-6 receptor/STAT3 signalling pathway.

Conclusion

This study, for the first time, links solid stress compression to the upregulation of IL-6. In the context of breast cancer, solid stress appears to elicit a more aggressive phenotype through the upregulation of genes such as IL-6 and SNAI1. Solid stress compression also leads to increased secretion of IL-6, which might act in autocrine and paracrine manners to activate IL-6 signalling and upregulate downstream gene targets such as SNAI1.

EACR25-0973

Characterizing the Transition from Well-Differentiated to Anaplastic Thyroid Cancer Using Digital Spatial Profiling

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Introduction

Anaplastic thyroid cancer (ATC) is a rare, aggressive form of thyroid cancer with a mortality rate exceeding 80%. Unlike differentiated thyroid cancers (DTC), ATC are refractory to standard therapies. Research suggests that ATC typically develops from pre-existing DTC, accumulating molecular alterations and becoming undifferentiated and aggressive. The mechanisms driving this transition are poorly understood, and further investigation is crucial for developing targeted therapies and personalized prognostic tools. Recent studies, including some of our own, have identified potential drivers of ATC development, but a comprehensive understanding of their interconnected roles remains elusive.

Material and method

We leveraged the GeoMx™ Digital Spatial Profiler, enabling us to precisely separate and analyze the ATC and DTC components within the same biphasic samples with high spatial resolution. Using PanCK and TTF1 as distinguishing markers, we identified 203 regions of interest (ROIs) across seven samples and sequenced the transcriptome of each ROI using the Whole Transcriptome Atlas panel. We performed RNA-seq on a larger, independent cohort of FFPE samples from pure DTC and pure ATC to validate these findings.

Differential analyses were performed to identify relevant altered pathways while, to investigate the TFs driving the transcriptional program, we used different motif search algorithms. The biological role of these putative drivers will be validated using knockdown (KD) techniques to mimic the observed alterations. Specifically, TFs upregulated or associated with ATC will be silenced in ATC cell lines to assess whether KD can mitigate aggressiveness. Conversely, TFs upregulated or associated with DTC will be silenced in DTC cells to determine if KD can induce dedifferentiation and increase aggressiveness.

Result and discussion

Differential analysis comparing DTC and ATC ROIs identified 2193 differentially expressed genes (DEGs) in ATC, with 1339 UP-regulated and 854 DOWN-regulated. Gene Ontology analysis revealed five key pathway clusters altered in the transition, providing insight into the underlying biological processes: EMT, cell cycle, thyroid hormone-related processes, immune system, and metabolism. We also focused on the TFs driving the transcriptional program underlying this transition. We considered both TFs present in the DEGs list and TFs upstream of these DEGs, identified through a bioinformatics approach. Following manual and literature-based curation of these two lists, we generated a shortlist of 20 candidate TFs, equally divided between TFs associated with UP-DEGs and those associated with DOWN-DEGs.

Conclusion

This study will provide a detailed characterization of the DTC-ATC transition by identifying all relevant interconnected pathways and the TFs most likely to play a pivotal role in the transition, thus also revealing potential therapeutic targets for restraining ATC aggressiveness.

EACR25-1023

Histone deacetylase inhibitors (HDACi) downregulate HER2 protein expression in gastric cancer, leading to pronounced HDACi sensitivity in tumor cells with high basal HER2 expression

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Introduction

Gastric carcinoma represents a major cause of cancer-associated deaths. Cytoreductive chemotherapeutic drugs and targeted therapeutics established for this entity usually show only rather unsatisfactory effects and the use of immunotherapeutics is only promising in a small proportion of patients. In gastric carcinoma with HER2 overexpression (about 10–20% of cases), the use of HER2 inhibiting therapeutics has shown some effectiveness, although resistance almost always occurs during the course of the disease. The present study aimed at investigating the effect of histone deacetylase inhibitors (HDACi) on the expression and signal transduction of HER2 in gastric carcinoma and elucidating differences in susceptibility to HDACi in gastric carcinomas with high vs. low HER2 expression.

Material and method

Basal or HDACi-altered HER2 expression was investigated in a panel of human gastric carcinoma cells and in tissue slice cultures of cell line- and patient-derived xenografts. The phosphorylation of AKT and the expression of p21 were recorded as downstream signaling pathways. The antitumor effect of HDACi was delineated by formazan-based WST assays, colony forming assays, cell cycle analyses and annexin V/PI staining.

Result and discussion

Treatment with HDACi led to a significant reduction in HER2 protein levels in the investigated cells. In line with this reduced HER2 protein expression, a reduction in AKT phosphorylation and p21 induction was observed as well. Interestingly, the proliferation/cytotoxicity assays showed that cell lines with a high basal HER2 expression (before treatment with HDACi) were significantly more sensitive to HDACi than cell lines with a low basal HER2 level.

Conclusion

The HER2 expression level of gastric cancer cells represents a factor affecting the susceptibility against HDACi. Of note, previous clinical trials (which were unsuccessful) on the efficacy of HDACi in gastric cancer patients did not include patients with high HER2 expression in the tumor tissue; this strategy may have excluded those patients who could in particular benefit from HDACi due to their additional HER2 inhibitory action.

EACR25-1066

Uncovering the paraspeckle-dependent and independent role of NONO in transcriptional regulation in Multiple

Myeloma

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Introduction

Multiple myeloma (MM) is a malignant disease of bone marrow plasma cells (PCs) with variable outcomes. Despite advances in treatment, MM remains incurable. LncRNA NEAT1, a scaffold for the paraspeckle (PS) organelle, plays a critical role in DNA repair and cell survival in MM. NONO, a protein involved in NEAT1 stability and PS, is upregulated in MM and correlates with poor overall survival and progression free survival. Besides its role in PSs, NONO may have independent functions in MM cells. We plan to investigate NONO's role in modulating the transcriptome of MM PCs through NGS and assess its contribution within and independently of PSs.

Material and method

RNA was extracted from NONO-KD, NEAT1-KD, and scramble AMO1 and LP1 human MM cell lines (HMCLs). RNA-seq libraries were prepared using Illumina's Stranded TotalRNA PrepLigation protocol. Sequencing was done on an Illumina Novaseq. CoMMpass data were retrieved from the Interim Analysis 15a.

Result and discussion

RNA-seq data from NONO-KD or NEAT1-KD AMO1 and LP1 HMCLs were compared to identify overlapping pathways. Significant downregulation was observed in gene sets associated with chromatin modifications, WNT/β-catenin, and NOTCH signalling in NEAT1 and NONO silenced cells. RNA-seq data from AMO1 HMCL over-expressing NEAT1 and PSs confirmed a significant and positive modulation of the same pathways. Further validation was obtained by stratifying samples from the CoMMpass dataset based on NONO expression levels, comparing the expression profiles between the two extreme quartiles, and conducting GSEA on the list of differentially expressed coding genes. Since NONO is essential for protecting NEAT1 from degradation, its silencing results in a marked downregulation of NEAT1 expression levels, thereby impacting the transcriptome of NONO-silenced cells in a NEAT1-dependent manner, making it impossible to distinguish NONO's effects independent of PSs. However, the analysis of data from the extreme quartile of NONO in CoMMpass dataset enabled the identification of NONO specific pathways not shared with NEAT1-KD HMCLs, which may suggest pathways that NONO regulates independently of PS in MM cells. This analysis revealed NONO's involvement in RNA splicing or maturation, cellular RNA trafficking to the cytoplasm, as well as its role in mitochondrial biogenesis and cell-matrix adhesion.

Conclusion

NONO plays complex transcriptional roles in MM, influencing pathways critical for disease progression. The in-silico validation further supports the clinical relevance of these findings, highlighting the value of the HMCLs model in advancing our understanding of MM.

EACR25-1199

Protective Role of Thymosin Beta 4 Against Sarcopenia via Regulation of PI3K/Akt/mTOR and NLRP3 Signaling Pathways in C2C12 Myotubes

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Introduction

Sarcopenia, characterized by progressive skeletal muscle loss and functional decline, is a major health concern associated with aging and chronic diseases such as diabetes. Oxidative stress and glucocorticoid exposure are known contributors to muscle atrophy. Thymosin beta 4 (Tβ4), a multifunctional peptide with anti-inflammatory and cytoprotective properties, has shown potential as a therapeutic agent; however, its role in sarcopenia remains unclear. This study aimed to evaluate the protective effects of Tβ4 against D-galactose- and dexamethasone-induced muscle atrophy in C2C12 myotubes and to elucidate the underlying molecular mechanisms.

Material and method

C2C12 myoblasts were differentiated into myotubes over six days and treated with Tβ4 (0.1, 0.4, and 1.6 μg/mL) for 24 hours. Sarcopenia-like conditions were induced using D-galactose (50 mM) and dexamethasone (100 μM) for an additional 24 hours. Gene and protein expressions were analyzed via RT-PCR and Western blotting to assess myogenic factors (MyoD, Myogenin, Myo1D), atrophy-related genes (MuRF1, Atrogin-1), autophagy markers (p62, LC3A/B, Atg3), and components of the PI3K/Akt/mTOR pathway. The involvement of the NLRP3 inflammasome (NLRP3, IL-1β, IL-18, TNF-α), apoptotic regulators (Caspase-1, Bax, Bcl-2), and IGF-1 was also examined.

Result and discussion

Tβ4 treatment significantly alleviated muscle atrophy in C2C12 myotubes. It restored the expression of MyoD and Myogenin while reducing MuRF1 and Atrogin-1 levels. Tβ4 modulated autophagy by decreasing p62 accumulation and LC3A/B ratios and suppressed NLRP3 inflammasome activation, as evidenced by decreased levels of NLRP3, IL-1β, and IL-18. Additionally, Tβ4 reduced pro-apoptotic markers Bax and Caspase-1 and increased anti-apoptotic Bcl-2 expression. IGF-1 levels were also enhanced, suggesting improved anabolic signaling and muscle regeneration. These results indicate that Tβ4 protects muscle cells from atrophy through coordinated regulation of protein degradation, inflammation, and apoptosis.

Conclusion

In conclusion, Tβ4 demonstrates protective effects against sarcopenia-like stress in C2C12 myotubes by modulating key signaling pathways, including PI3K/

Akt/mTOR, autophagy, and the NLRP3 inflammasome. These findings support further *in vivo* studies to explore Tβ4's therapeutic potential in diabetic sarcopenia and other muscle-wasting conditions, highlighting its promise as a novel intervention for age-related muscle degeneration.

EACR25-1266

A novel regulator of Hedgehog signaling and stemness in prostate cancer

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Introduction

The disruption of tissue organization is a major contributing factor of the malignant transformation of glandular epithelium, including prostate glands. Physiologically relevant models of tissue organization, specifically three-dimensional organotypic cultures, have led to the discovery of molecular mechanisms underlying malignant progression. Through our screening, we identified ACTL6A as a potential oncoprotein in prostate cancer (PCa). To further investigate its role, we employed patient-derived organoid (PDO) models and PCa cell lines to evaluate its expression and functional significance.

Material and method

This study uses MicroWestern Array analysis to screen for ACTL6A as a potential oncoprotein of PCa. IB and co-IP analysis alone with Quantitative reserve transcription PCR (qRT-PCR) analysis were used to evaluate ACTL6A expression and its association with GLI-1 in PDO models of normal and malignant prostate epithelium, as well as in PCa cell line. Bioinformatics analysis was conducted to interrogate public datasets for the expression of ACTL6A in benign and malignant prostate tissues. Orthotopic mouse model of PCa was also used to assess the expression of ACTL6A in tumor tissues.

Result and discussion

The protein abundance level of ACTL6A is upregulated in disorganized prostate epithelial organoids and PCa cells compared to its expression in normal prostate cells and organoids. Echoing this finding, the expression of ACTL6A is upregulated in human PCa compared to normal prostate and is correlated with poor clinical prognosis. At the functional level, small hairpin RNA (shRNA)-mediated knockdown of the expression of ACTL6A in PCa cells attenuated stemness and tumorigenesis *in vitro* and *in vivo* in an orthotopic mouse model of PCa progression. To gain mechanistic insights into how ACTL6A regulates PCa oncogenesis, we screened several development-associated signaling pathways for their reporter activities in ACTL6A-deficient PCa cells and thereby uncovered the Hedgehog (Hh) pathway whose activity was affected by the loss of ACTL6A expression. Consistently, ACTL6A deficiency led to the reduced expressions of Hh-pathway target genes in PCa cells. We identified the marked reduction in the protein

abundance level of GLI1, the key transcriptional factor in Hh-pathway, in ACTL6A-deficient PCa cells, implicating its potential role as a downstream mediator of ACTL6A-regulated Hh-pathway activity. We continue exploring the in-depth molecular and biochemical mechanisms behind the effect of ACTL6A on GLI1 expression.

Conclusion

In conclusion, our study illuminated a novel oncogenic mechanism mediated by the ACTL6A-GLI1-Hh signaling axis that contributes to PCa progression, which may have diagnostic and therapeutic implications.

EACR25-1276

Elevated temperatures induce PDCD1 expression via HSF1 activation

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Introduction

Elevated body temperatures (regardless of the cause) lead to the activation of HSF1 (heat shock factor 1), which is a major mediator of transcriptional responses to proteotoxic stress (including heat stress), frequently overexpressed in cancer. PDCD1 (Programmed cell death protein 1) is a cell surface receptor that inhibits the excessive response of antigen-activated T cells to prevent autoimmune tissue damage. Thus, the PDCD1 has been named an “immune checkpoint”, referring to its role as a gatekeeper of immune responses. In chronic infections or cancer, lasting antigen exposure leads to permanent PDCD1 expression that can limit immune-mediated clearance of pathogens or degenerated cells.

Consequently, blocking PDCD1 can enhance T cell function, which is the basis of cancer immune checkpoint therapy.

Material and method

The effect of proteotoxic stress (heat shock, bortezomib) on PDCD1 expression was studied in various human cell lines, peripheral blood mononuclear cells (PBMC), and mouse tissues by RT-qPCR, western blot, or flow cytometry. HSF1 binding to chromatin was studied by chromatin immunoprecipitation. Functional HSF1 knockout was obtained using the CRISPR/Cas9 editing system.

Result and discussion

PDCD1 expression can increase as early as a few hours after temperature elevation. We observed this at the mRNA and protein levels in human leukemic and lymphoblastoid cell lines (such as Jurkat, THP1, HL-60, and GM07062). Transcriptional upregulation of the PDCD1 gene was associated with the binding of heat shock factor 1, HSF1, to the promoter. In contrast, HSF1 knockout in HL-60 cells resulted in the inhibition of PDCD1 activation. Although PDCD1 transcript levels increased in the thymus and spleen of heat-shocked mice,

PDCD1 mRNA did not increase in human PBMC and NK-92 (natural killers cell line). Nevertheless, we observed a heat shock-dependent increase in glycosylated (and therefore active) PDCD1 protein levels also in these cells. This is associated with PDCD1 exposure on the cell membrane and may lead to the loss of cytotoxic properties of NK-92 cells. We propose different mechanisms of heat-induced up-regulation of PDCD1 in cancer and normal cells: at the transcriptional and post-transcriptional levels.

Conclusion

Our observation suggests that the immune response could be attenuated in various physiological conditions accompanied by increased temperatures (infection, heat stroke, etc.). For this reason, fever (as well as pharmacological fever reduction) can have unexpected consequences depending on the disease state. This observation may have clinical implications, and therefore, further research is warranted to understand the importance of fever and PDCD1 in various disease states, as well as their interaction with treatment.

EACR25-1289

Generation of an In Vitro Model for the Study of the Aryl Hydrocarbon Receptor Pathway in Adrenocortical Carcinoma Cell

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Introduction

The Aryl Hydrocarbon Receptor (AhR) is a sensor of environmental stimuli and a mediator of their harmful effects on health. It recognizes specific sequences known as Xenobiotic Responsive Elements (XRE), through which it controls a wide set of genes, such as the CYP450 cytochrome family. Despite its role in xenobiotics metabolism, AhR has many pathophysiological functions, including a role in cancer, where it exerts pro-oncogenic or onco-suppressive properties depending on the tumor type. Adrenocortical carcinoma (ACC) is a rare and aggressive tumor, for which mitotane, a derivative of DDT pesticide, is the only approved drug. Of note, compounds similar to these insecticides can modulate AhR. Furthermore, a connection with mitotane-based therapy is highlighted in our recent work, where we observed that mitotane activates both the AhR molecular pathway and the endoplasmic reticulum stress via ATF4. Interestingly, ATF4 pathway activation and related cellular stress are listed as one of the AhR-mediated tumor suppressor mechanisms. Given this evidence and the lack of pharmacological alternatives to mitotane, we generated and validated an in vitro model to elucidate the involvement of AhR pathway in ACC and its possible use as future therapeutic target.

Material and method

To generate our in vitro tool, all components of the AhR-mediated transcription modulation were integrated with

lentiviral transduction into the H295R cell line, the cell model of choice for ACC. We obtained stable H295R clones expressing Firefly-luciferase under the control of xRE (5x 5'-TTGCGTGASAA-3'), with different basal luciferase expression levels. To validate the model, cell clones were treated with some AhR modulators, and the receptor activity was evaluated with luciferase assay and, at transcriptional level, with qPCR experiments.

Result and discussion

To validate our in vitro model, we correlated the experimental luciferase results with the expression of CYP1A1, a gene commonly used as an indicator of AhR activation. Our results showed the congruence between CYP1A1 expression and reporter gene activation, with a fold change $>\pm 1$. Our model can respond to AhR activation/inhibition mediated by modulators of this receptor. We measured an increase in luciferase production compatible with the activation of AhR after treatment with agonists and a decrease with antagonists. Of note, the different behavior of some compounds compared to literature could suggest a tissue or cell type dependent effect, underlining the complexity of this pathway.

Conclusion

Our cell model serves as a tool to explore AhR role in ACC, independently and in conjunction with other molecular pathways. Our system enables a rapid observation of changes induced by various pathophysiological or pharmacological stimuli, facilitating the identification of novel molecular pathways for diagnostic or therapeutic applications.

EACR25-1393

FOSL1 controls chromosomal instability in non-small cell lung cancer (NSCLC) via the β -catenin/TCF4 complex

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Introduction

Chromosomal instability (CIN) is a hallmark of cancer, characterized by a high rate of chromosomal alterations associated with tumor heterogeneity and cancer progression. In non-small cell lung cancer (NSCLC), CIN is associated with poor prognosis and immune evasion. Transcription factors play a crucial role in regulating genomic stability by modulating key pathways involved in cell division. However, the underlying mechanisms of CIN induction in NSCLC cells remain unclear, and tools for CIN detection are missing.

Material and method

To investigate the role of individual transcriptional regulators, inhibition of AP1 constituents (FOSL1, JUN, JUND) and WNT signaling (β -catenin and TCF4) in A549 cells was followed by next-generation sequencing (NGS). The perturbation of AP1 activity and the β -catenin/TCF4 complex was achieved using gene-specific siRNAs or inhibitors (SR11302 and iCRT3, respectively). Quantitative PCR (qPCR), Western blotting, and

chromatin immunoprecipitation were utilized to confirm the transcriptional regulation of genes. A CIN gene signature served as an indicator for the presence of CIN. TCGA expression data from NSCLC patients was analyzed, and immunohistochemical staining of NSCLC tissue microarrays was performed for FOSL1 and pH2AX.

Result and discussion

FOSL1 and the β -catenin/TCF4 complex regulate gene expression relevant to mitosis and associated with CIN. In contrast, JUN and JUND do not yield similar results. Notably, FOSL1 significantly induces β -catenin expression and interacts with the β -catenin/TCF4 complex, suggesting that FOSL1 may affect β -catenin/TCF4 complex activity in a bimodal manner. In NSCLC tissues, a significant correlation between nuclear FOSL1 and the CIN marker pH2AX was observed, suggesting a relationship between FOSL1 activity and CIN in lung cancer development. Lastly, NGS reanalysis identified long non-coding RNAs (lncRNAs) regulated by FOSL1 and TCF4, which may be biomarkers for CIN activity in NSCLC. The association between a lncRNA signature and the expression of CIN-associated genes can be validated by data from NSCLC patients.

Conclusion

This study suggests an essential role of the FOSL1/ β -catenin/TCF4 axis in regulating CIN in NSCLC. Moreover, we identified a FOSL1/ β -catenin/TCF4-driven lncRNA signature as a potential biomarker for CIN. Thus, monitoring this lncRNA signature may offer new diagnostic tools for NSCLC patients. Future studies will investigate whether lncRNA signatures can be detected in the serum of NSCLC patients with FOSL1/ β -catenin/TCF4 activation and CIN.

EACR25-1568

Decoding EGFR-Driven Transcriptional Networks in NSCLC

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Introduction

The Epidermal Growth Factor Receptor (EGFR) signalling pathway activates multiple downstream sub-pathways which are frequently mutated in non-small cell lung cancer (NSCLC). While these cytoplasmic sub-pathways have been extensively characterized, our understanding of how each node along a given sub-pathway regulates transcription factors (TFs) and their target genes remains incomplete. Previous research has identified a limited subset of TFs (such as AP1, ELK1, and STAT3) downstream of EGFR, however activity-based evidence is lacking, with most conclusions being drawn from abundance-based assays. Moreover, the relative impact of how multiple nodes influence TF activity is largely unknown.

Material and method

We have developed and validated a multiplexed reporter system which simultaneously measures 100 TF activities in a single experiment. Each reporter consists of a TF-specific response element driving expression of a barcoded mRNA, optimized through testing 36,000

different designs across multiple cell types and conditions. We will apply this system to NSCLC cell lines with diverse genetic backgrounds (including EGFR and KRAS constitutively activate mutants). We will systematically perturb nodes within a given sub-pathway using clinically relevant inhibitors, to identify the differential TF activity downstream of the EGFR signalling pathway.

Result and discussion

Initial data reveal a distinct and overlapping sets of TFs controlled by each node within a single sub-pathway. For example, by treating EGFR constitutively active cells with an EGFR inhibitor (Osimertinib) or MEK inhibitor (Trametinib) revealed unique TF activity profiles, despite the often-linear representation. These observations are consistent across KRAS driven cells, despite the driver mutation differences.

Conclusion

This comprehensive mapping of EGFR-driven transcriptional networks will enhance our understanding of resistance mechanisms, identify novel drug targets beyond kinase inhibitors, and may inform rational drug repurposing strategies. Future applications include exploring tissue-specific variations in EGFR-TF networks across cancer types and crosstalk between receptors.

EACR25-1651

Wound Healing Analogy in Medulloblastoma Response to Gap Junction Inhibition and Cytotoxic Therapy

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Introduction

Intercellular communication is a significant factor in chemotherapy resistance in malignant brain tumors. Connexin-43 (Cx43)-based gap junctions (GJs) facilitate this communication by allowing intercellular ion transfer and small molecule exchange. The impact of GJs in medulloblastoma as the most common malignant pediatric brain tumor is unknown. In the present study, we analyzed the potential of GJs as a putative novel therapeutic target in medulloblastoma. Here, we utilized meclofenamate (MFA), an FDA-approved drug with gap junction-inhibitory properties, which had never been used in medulloblastoma research before.

Material and method

Immunofluorescence and Western blot analysis for Cx43 were used to evaluate intercellular GJ expression in human group 3 and 4 medulloblastoma cell lines. A CRISPR/Cas9 gene knockdown of Cx43 was used to explore cellular effects related to the inhibition of GJs. Realtime-imaging fluorescence-guided measurements of GJ-mediated cell-to-cell cytoplasm transfer was

performed for the CRISPR/Cas9 Cx43 knockdown model and MFA treatment. We used RNA-sequencing to study downstream signalling cascades in response to GJ inhibition. DNA-fragmentation served as readout for cell death and was assessed by flow cytometric analysis of propidium iodide-stained nuclei.

Result and discussion

All medulloblastoma group 3 and 4 cell lines showed an expression of intercellular Cx43-based GJs. We observed a significant reduction of intercellular cytoplasm transfer via GJs in both the CRISPR/Cas9 Cx43 knockdown model and in MFA-treated medulloblastoma cells. MFA-mediated inhibition of GJs profoundly rendered medulloblastoma cells susceptible for lomustine-mediated cell death. Cytotoxic effects were similar to those observed in CRISPR/Cas9 Cx43 cells. In RNA sequencing, we observed not only an upregulation of programmed cell death pathways but also a significant upregulation of wound healing pathways when MFA was combined with lomustine treatment.

Conclusion

This study suggests that inhibiting intercellular connectivity makes medulloblastoma cells more susceptible to lomustine-induced cytotoxic effects. This disruption of tumor networks can be compared to the initial phase of wound healing, where the cellular network is broken apart. Understanding these processes provides insight into therapeutic strategies that target both the destruction and regrowth phases of tumor cell networks. Consequently, gap junction-targeted approaches might offer a novel treatment strategy for this malignant pediatric brain tumor.

EACR25-1659

The Alcatraz-Strategy: a roadmap to break the connectivity barrier in malignant brain tumours

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Introduction

Several discoveries both on the morphological and functional cellular level have fundamentally changed our understanding of glioblastoma biology over the last years. Most notably, tumor microtubes (TMs) as ultralong membrane protrusions enable glioblastoma cells to assemble to a syncytial microinvasive communicating network. TM-supported neurogliomatous synapses (NGS) have been shown to integrate glioblastoma cells into neural circuits which foster malignant

growth and cellular invasiveness. The authors speculate to what extent these novel insights into the malignant network might impact future standard of care and attempt to conceive a multimodal treatment regime based on current translational efforts and ongoing clinical trials.

Material and method

A therapeutic outlook was conceptualized with regard to the following basic principals deduced from above-mentioned discoveries: 1) Morphological isolation on the single cell level via inhibition of TM-based spatial network architectures. 2) Functional isolation on the single cell level via inhibition of TM-mediated intercellular cytosolic exchange and inhibition of NGS activation. 3) Greatest possible removal of the micro-invasive tumor cell front far beyond MRI-detectable abnormalities.

Result and discussion

Three multicenter clinical trials in the light of above-mentioned principles are being designed, are recruiting or are at the stage of initiation: The MeCmeth/NOA-24 phase I/II trial evaluates meclofenamate (MFA) as a potential TM-targeted drug. MFA has recently been shown to exert a morphological and functional breakdown within TM-based glioblastoma networks by an inhibition of TM outgrowth and TM-mediated intercellular cytosolic traffic. The PERSURGE trial will analyze the effects of perampanel as a NGS-inhibitory drug regarding functional connectivity and radiological tumor growth kinetics. The ATLAS/NOA-29 trial is designed as the first prospective trial to evaluate supramarginal resection for a potential survival benefit compared to a gross-total resection regime in temporal glioblastoma.

Conclusion

Reminiscent of the Alcatraz Federal Penitentiary, where inmates had to face 1) a spatial isolation in single cells, 2) a functional isolation by the ban of interpersonal communication and 3) a safety-margin to the mainland by the sea as an insuperable barrier, the authors propose the Alcatraz-Strategy as a multimodal therapeutic approach – aimed at fighting glioblastoma’s long-distance connectivity and microinvasive capacity.

EACR25-1660

Hypericum alpestre phytochemicals and L-NAME downregulate PI3K/AKT/mTOR signaling and activate apoptosis in A549 lung adenocarcinoma cells

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Introduction

Lung adenocarcinoma remains one of the most aggressive cancer types, often exhibiting resistance to conventional therapies. The PI3K/AKT pathway is crucial in promoting tumor survival, angiogenesis, metastasis, and inflammation. Natural compounds with inhibitory effects on this pathway may provide new

therapeutic opportunities. *Hypericum alpestre* (HA), a medicinal plant rich in bioactive polyphenols, has demonstrated anticancer potential. When combined with L-NAME, a nitric oxide synthase (NOS) inhibitor, its efficacy may be further enhanced. This study aimed to investigate the effects of HA extract, phytochemicals and L-NAME (combined and separately) on PI3K/AKT/mTOR signaling and associated inflammatory and angiogenic mediators in A549 lung adenocarcinoma cells and in breast cancer rat models.

Material and method

The cytotoxic effects of HA and L-NAME were assessed via MTT assay. ELISA, ICC/IF and Western blot analyses were conducted to evaluate the expression levels of key signaling molecules, including PI3K, AKT, mTOR, TNF α , VEGF α , COX-2, and MMP-2. Apoptosis induction was confirmed through Caspase-3 activity assays and Hoechst 33258 nuclear staining.

Result and discussion

HA extract and its combination with L-NAME reduced the mTOR, total and phosphorylated forms of PI3K and AKT, reducing TNF α and VEGF α levels, thereby limiting inflammation and angiogenesis. The combination of HA with L-NAME resulted in a more pronounced suppression of COX-2 and MMP-2, which are key mediators of tumor progression and metastasis. Furthermore, Caspase-3 activation and apoptotic cell death were significantly enhanced, suggesting that HA and L-NAME exert their effects through both signaling inhibition and apoptosis induction. Notably, this combination therapy exhibited superior efficacy compared to 5-Fluorouracil and Dactolisib (PI3K/AKT inhibitor). To further clarify the potential anti-metastatic effect of the proposed therapeutic model, *in vivo* studies were conducted in rats with 7,12-dimethylbenzanthracene (DMBA)-induced breast cancer, focusing on the VEGF α /MMP-2 axis in lung tissue. Consistent with findings in A549 cells, the results demonstrated inhibition of VEGF α /Akt/MMP-2 signaling in the lung tissue of rats with breast cancer. Additionally, histological analysis revealed the cessation of lung cancer-associated changes, further supporting the anti-metastatic and anti-angiogenic efficacy of the HA and L-NAME combination therapy.

Conclusion

These findings suggest that the strong effects of HA extract and L-NAME on the PI3K/AKT/mTOR signaling pathway and downstream targets hold promise for developing novel more effective therapeutic strategies against cancer.

EACR25-1666

Glucocorticoid signaling regulates luminal A and triple negative subtypes of breast cancer differently on the epithelial-mesenchymal transition pathway

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Introduction

Glucocorticoid (GC) plays an important role in human pathophysiology as an anti-inflammatory and ubiquitous stress-activated steroid hormone. Clinically, it is commonly used as a co-treatment for cancer therapies with high efficacy in inducing apoptosis of leukemia and lymphoma. However, in breast cancer treatments, GC can sometimes produce controversial effects that induce drug resistance to chemotherapy and even promote breast cancer recurrence. To understand underlying mechanisms, we focused on investigating the specificity of GC signaling in different subtypes of breast cancer cells in this study.

Material and method

Triple negative breast cancer (TNBC) cell line MDA-MB-231 and lumina A subtype breast cancer cell line MCF-7 were used in this study. Dexamethasone (Dex, a synthetic GC) was used for treatments with optimized conditions. Quantitative Western blotting was carried out to analyze protein expression, and breast cancer specific qRT-PCR array (RT² Profiler™ PCR Array, Qiagen) was used to quantify the expression of target genes. CRISPR technique was utilized to down-regulate gene expression, and the real-time label-free technique (ECIS, Applied Biophysics) was employed to measure cell migration.

Result and discussion

When MDA-MB-231 and MCF-7 cells were treated by Dex, in addition to inducing apoptosis slightly, GC regulated cell migration more sensitively in MDA-MB-231 cell than in MCF7 cell. Gene expression profiling by qRT-PCR array revealed that the GC/GC receptor (GR) signaling regulated gene expression differently between these two breast cancer cells; MDA-MB-231 cell showed more genes up- or down-regulated than MCF7 cell, among which Snai2/Slug (a transcriptional factor involved in EMT) was identified to be highly activated in both cell lines. Western blotting analysis confirmed that MDA-MB-231 cells had higher levels of Snai2 than MCF-7 cells upon Dex treatment. GR response elements (GREs) were also identified in the promoter region of Snai2 bioinformatically, thus confirming Snai2 as a new target gene of GR in breast cancer. When Snai2 was knocked out by CRISPR, it was found that the migration rates were decreased in both cell lines, indicating Snai2 as a mediator to regulate breast cancer cell migration in response to GC/GR signaling.

Conclusion

The findings from this study shed new light on the differential use of GC in breast cancer therapies depending on different subtypes of breast cancer, wherein TNBC should be precautionary.

EACR25-1685

The extracellular matrix produced by VEGFR2R1032Q altered melanoma cells modifies adhesive and migratory characteristics of endothelial cells

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Introduction

The VEGF/VEGFR2 axis is frequently altered in cancer where it regulates cell metabolism, proliferation, motility, and stemness. Recent pan-cancer mutational analysis identified the alteration VEGFR2R1032Q as the most frequent point mutation in VEGFR2. Although this mutation reduces the kinase activity of VEGFR2, its expression in melanoma cell lines (SK-MEL-31) promotes tumor growth and vascularization. Preliminary functional data and transcriptomic analysis suggested an alteration in extracellular matrix (ECM) organization. In cancer, continuous ECM remodelling affects tumor cell-ECM and cell-cell interactions. Altered ECM may also influence tumor vasculature by modulating endothelial cells (ECs)-ECM interactions.

Material and method

To dissect the effects of tumor-derived ECM, an in vitro decellularization protocol was established. Decellularized matrices (d-ECM) were characterized by mass spectrometry and used for functional assays. In particular ECs seeded onto VEGFR2WT SK-MEL-31 and VEGFR2R1032Q -SK-MEL-31-(d-ECM) were assessed in static culture conditions and in response to unidirectional laminar flow (6 dyne/cm² for 24 h) by high resolution microscopy. Also FLIM/FRET biosensors were used for the characterization of mechano-transduction signaling.

Result and discussion

Mass spectrometry analysis revealed significant alterations in VEGFR2R1032Q -SK-MEL-31-d-ECM, which exhibited higher haptotactic activity on ECs. ECs plated on VEGFR2R1032Q-derived matrix displayed impaired spreading, increased number and dimension of focal adhesions (FAs) compared to EC plated onto VEGFR2WT-SK-MEL-31-dECM. Also, FLIM/FRET-based FAT-FAK sensor highlighted a reduced FAK activity in FAs in ECs plated onto VEGFR2R1032Q -SK-MEL-31-derived matrix. According to this, adhesion on VEGFR2R1032Q -SK-MEL-31-dECM increased intracellular traction forces in FAs as demonstrated by VinTS -based mechano-sensor and reduced the amount of primary cilia, a structural mechano-sensor. These tensile and adhesive features were coupled with an increased expression of genes involved in the endothelial to mesenchymal transition. Under laminar flow conditions, ECs seeded on VEGFR2WT SK-MEL-31-derived matrix elongated and polarized in response to shear stress. In contrast, ECs on VEGFR2R1032Q-derived ECM exhibited a delayed polarization and a reduced sensitivity to shear stress, as confirmed by cytoskeletal organization analyses. Time-lapse imaging over 24h further validated these findings under flow conditions and highlighted a reduction in the fluidity of cell monolayer.

Conclusion

These findings suggest that differences in tumoral ECM composition and organization impact EC behavior, mechano-transduction, and shear stress responses. Understanding these mechanisms could unveil novel therapeutic targets providing advancements in cancer treatment.

EACR25-1763

Downregulation of Frizzled-5 in Gastric Cancer: Tumor Suppressor Potential and the Role of Fibroblast-Derived WNT5A

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Introduction

The Wingless (Wnt) signaling pathway is involved in gastric carcinogenesis originating from gastric cancer stem cells. Frizzled-5 (FZD5), the Wnt-receptor mediating non-canonical WNT5A activity, seems to be involved in this process and the WNT5A-FZD5 signaling axis is crucial in gastric inflammation and regeneration. Studies suggest that increased FZD5 expression indicates tumor-suppressing effects, while targeted deletion inhibits stem cell activity in alveolar epithelial cells. Within the tumor microenvironment (TME) of gastric cancer both cancer-associated fibroblasts (CAFs) and tumor-associated macrophages (TAMs) are known as key pro-tumor mediators and discussed as WNT5A source. This study aims to identify FZD5- and WNT5A-expressing cells in the stomach and their role in human gastric carcinogenesis.

Material and method

Tissue samples of 50 gastric cancer patients were analyzed. Immunohistochemistry and RT-qPCR were used to assess the expression of FZD5, ATP4A for parietal cells, and PGA5 for chief cells. To investigate the role of WNT5A expressing cells in gastric cancer, we isolated fibroblasts from both healthy gastric tissue and gastric tumor tissue obtained from surgical resections. M2 macrophages, polarized from THP-1 cells using IL-4, were co-cultured with the isolated fibroblasts. WNT5A expression levels were assessed by RT-qPCR.

Additionally, the levels of WNT5A in the co-culture supernatant were measured by ELISA (Enzyme-Linked Immunosorbent Assay).

Result and discussion

We show that FZD5 is the highest expressed receptor in human gastric tissue in healthy mucosa as well as in tumor tissue. FZD5 is expressed by ATP4A-positive parietal cells but not by chief cells (PGA5) in the normal gastric epithelium. In gastric cancer tissue, FZD5 expression is significantly reduced and the expression of ATP4A and PGA5 is lost. Further investigation is necessary to clarify the molecular mechanisms underlying the selective loss of parietal cells while preserving FZD5-positive cell populations in the tumor.

Investigating this pathway, we were able to identify human fibroblasts isolated from fresh mucosa and tumor tissue as a source of WNT5A and confirmed by RT-qPCR that WNT5A is one of the most relevant ligands in

human gastric tissue. Additionally, an *in vivo* gastric cancer model using *Helicobacter*-infected Wnt5a knock-out mice revealed progressive loss of parietal cells and chief cells, as well as a reduced expression of Fzd5 with persisting infection.

Conclusion

These findings suggest that a loss of FZD5-positive cells is one of the drivers in gastric carcinogenesis, mediated by WNT5A. We conduct further experiments, including scRNA sequencing and more comprehensive *in vivo* studies, to gain deeper insights into the development and progression of gastric cancer and the role of the WNT5A-FZD5 signaling cascade in this process. Modulating this pathway could potentially serve as a treatment option in gastric cancer therapy.

EACR25-1775

The Impact of IK Deficiency on Cancer-Related Signaling Pathways: Insights from Zebrafish and Cellular Models

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Introduction

IK is a splicing factor that facilitates spliceosome activation and plays a crucial role in the assembly and catalytic activation of the spliceosome during pre-mRNA splicing. Loss of IK in cells is known to halt cell division, leading to apoptosis in cancer cells, and causes genetic instability by impairing the splicing of DNA damage repair genes. Consequently, IK deficiency is likely to influence cancer development; however, the specific genes and mechanisms involved remain largely unknown. This study aims to explain the impact of IK deficiency on cancer-related gene expression and the underlying mechanisms by analyzing transcriptome data from IK-deficient zebrafish embryos.

Material and method

To investigate the molecular consequences of IK deficiency, we performed real-time PCR (RT-PCR) to analyze the mRNA expression of genes associated with GTPase activity, EGFR signaling, and GPCR activity. mRNA sequencing was conducted on RNA extracted from wild-type and IK-deficient zebrafish embryos. Additionally, Western blot analysis was carried out to assess protein expression levels relevant to the correlation between IK deficiency and cancer. To further elucidate the role of IK in cancer pathways, alternative splicing mechanism differences across cancer types were compared using human cancer cell lines.

Result and discussion

mRNA sequencing of IK-deficient zebrafish embryos revealed altered regulatory mechanisms of transcriptome related to GTPase activity, EGFR signaling, and GPCR activity, suggesting a role for IK in cancer-related pathways. Changes in alternative splicing mechanisms involved in cell proliferation, survival, and apoptosis indicate that IK deficiency may contribute to the regulatory mechanism of multiple steps in cancer initiation and progression. Western blot analysis confirmed protein-level changes consistent with

alternative splicing variation, supporting IK's influence on transcriptional and post-transcriptional regulation. *In vivo* experiments using zebrafish embryos further demonstrated disruptions in key signaling pathways, reinforcing the link between IK deficiency and cancer progression. These findings suggest that IK plays a critical role in tumorigenesis by affecting transcriptional regulatory mechanisms driving cancer growth.

Conclusion

This research demonstrates that IK plays an essential role in the regulation of alternative splicing through cancer-related signaling pathways, influencing cell proliferation, survival, and apoptosis. Further studies are needed to validate candidate genes and investigate the potential of IK as a therapeutic target in cancer progression.

EACR25-1825

The Androgen Receptor-Serum Response Factor Network As a Pharmacological Target in Castration Resistant Prostate Cancer

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Introduction

Prostate cancer (PCa) is one of the most common cancers in men worldwide. Current treatments focus on targeting the Androgen Receptor (AR) with androgen deprivation therapies (ADT) such as enzalutamide. Although initially effective, ADT's create a selection pressure resulting in treatment resistance in a form of advanced PCa called castration resistant prostate cancer (CRPC). This emphasises the need for alternative therapeutic approaches that will bypass this resistant mechanism, continue to disrupt AR signalling without targeting AR itself. One such method includes targeting co-regulators in the AR signalling network such as the Serum Response Factor (SRF), which has been identified as being crucial to PCa progression. Previous co-immunoprecipitation studies combined with mass spectrometry have identified common interactors between AR and SRF intracellular network, including HSP70, HSP90 and members of the PI3K/Akt pathway. Our research focuses on targeting the co-regulators within the AR-SRF network as a pharmacological approach for patients with CRPC.

Material and method

We investigated targeting the AR-SRF interactome in four PCa cell lines: LNCaP (ADT-sensitive), LNCaP Abl and C4 (ADT-resistant), 22Rv1 (ADT-resistant and ARv7 positive) and in a non-cancerous prostate cell line (PWR1E). Small molecule inhibitors include SRF inhibitors (CCG1423, Lestaurtinib), AR/ARv7 inhibitors (Enzalutamide, EPI7170) and common co-factor inhibitors (VER-15508, JG-98, Ganetespib, Ipatasertib, Alpelisib). MTT assays and IncuCyte proliferation analysis were performed for single and combination treatments, with CompuSyn software analyzing combination profiles for antagonism, additivity, or

synergy. Western blotting assessed phosphoproteomic changes after treatment.

Result and discussion

We have shown that inhibiting AR, SRF and common co-factors, singly and in combination, decreases cell viability and proliferation in the PCa cell line panel. Furthermore, we show that the following combinations are synergistic, even at IC₁₀ concentrations: Lestaurtinib+Ipatasertib, CCG1423+EPI7170, EPI7170+Ipatasertib and EPI7170+Lestaurtinib. Lestaurtinib and Ipatasertib were shown to alter the global phosphorylation landscape in 22Rv1. To better understand the molecular mechanisms behind the synergy, proteomic and phosphoproteomic analysis are currently ongoing.

Conclusion

To conclude, our results indicate that targeting the AR-SRF intracellular network holds promise as a treatment option for patients with CRPC. Combination treatments were shown to bypass resistance mechanisms in our CRPC cell line models and are synergistic at lower concentrations. Furthermore, by studying the proteomic and phosphor-proteomic landscape before and after treatments, we can further understand disease and resistance mechanisms in PCa as well as find potential novel targets which can be used for the disease.

EACR25-1864

SP1 Downregulates ADAMTS-8 mRNA Expression in Colorectal Cancer Model

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Introduction

A Disintegrin and Metalloproteinase with Thrombospondin Motifs (ADAMTS) proteases play a crucial role in forming and remodeling the extracellular matrix (ECM). The ADAMTS family, consisting of 19 members, is involved in key biological processes such as angiogenesis, migration, proliferation, and cancer progression. ADAMTS-8, an anti-angiogenic member of this family, is frequently downregulated in various cancers and is a potential tumor suppressor in colorectal cancer (CRC). Specificity Protein 1 (SP1), a zinc finger transcription factor that binds to GC-rich sequences, is highly expressed in human CRC tissues. This suggests that SP1-regulated genes may contribute to CRC progression and metastasis. This study aims to investigate the transcriptional regulation of ADAMTS-8 by SP1 in CRC.

Material and method

The promoter region of the ADAMTS-8 gene was analyzed using the PROMO TF Search program. Bioinformatic analysis identified multiple SP1 binding motifs within the ADAMTS-8 promoter, which were mapped and visualized. To evaluate the effect of SP1 on the transcriptional regulation of ADAMTS-8, SP1 was overexpressed in SW480 cells. The impact of SP1 on ADAMTS-8 mRNA expression was analyzed using a qRT-PCR-based strategy.

Result and discussion

Multiple SP1 binding sites were identified within the ADAMTS-8 promoter. Ectopic expression of SP1 decreased ADAMTS-8 mRNA expression in SW480 cells.

Conclusion

These findings indicated that SP1 negatively regulates ADAMTS-8 expression in the SW480 CRC model. Further research is required to elucidate the precise mechanisms underlying this regulatory interaction and the potential contribution of SP1-mediated ADAMTS-8 downregulation to CRC progression.

EACR25-2097

11q Loss Meets 17q Gain: Molecular Insights into Neuroblastoma Treatment Resistance

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Introduction

Neuroblastoma (NB) is the most common solid tumor in early childhood, responsible for around 15% of cancer deaths in this age group. NB tumors are highly heterogeneous, and segmental chromosomal alterations are related to the more aggressive cases. MYCN amplification is the most significant alteration in 40% of high-risk tumors. In addition, 35–40% of primary tumors have an 11q deletion, which is strongly related to refractory recurrences. This study aims to identify copy number alterations (CNAs) in NB tumor samples to identify alterations associated with tumor resistance and recurrence.

Material and method

A retrospective study used data and tumor samples from pediatric patients diagnosed with NB and treated at the Pequeno Príncipe Pediatric Hospital (HPP), Curitiba, Brazil, between 2004 and 2014 [1]. DNA was isolated from FFPE samples, and CNA analysis was performed using an oligonucleotide a-CGH platform (SurePrint G3 Human CGH Microarray 8x60K; Agilent Technologies Inc.). CNA was defined as Agilent Cytogenomics guidelines. The number of calls and affected cytobands were obtained from the generated aberration interval base reports (Agilent Cytogenomics v. 5.0). The list of the genes from the affected cytobands were used to create pre- and post-CT protein-protein integration (PPI) networks for each patient using the STRINGdb R package.

Result and discussion

Out of 76 patients initially included in the study, three cases had paired pre- and post-CT FFPE samples and presented 11q deletion. Systems biology analysis revealed that the PPI networks in the post-CT samples showed fewer connections than the pre-CT networks. Five genes in the post-CT networks were common in all patient samples, which may be related to treatment resistance: BIRC5, BRCA1, PRKCA, SUMO2, and GPS1, all located in the 17q region. Gains in 17q are

common on NB tumors and are associated with poor disease prognosis. Also, The PPI analysis showed that BIRC5 acts as a Bottleneck and Switch, suggesting it may be a central gene for different molecular pathways such as DNA damage repair, apoptosis, and chromatin remodeling.

Conclusion

This study reinforces the importance of BIRC5 as a key gene in cancer progression and highlights its impact on the most resistant tumors. This work opens the perspective of the validation of BIRC5 as a biomarker in high-risk neuroblastoma.

[1] This study was approved by the institution's ethics committee (approval number 33.573.221).

EACR25-2181

Tissue factor augments TGF β signaling in cancer cells by modulating receptors dynamics and plasma membrane tension

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Introduction

Tissue Factor (TF) is a protein mediator of platelet-cancer cross talk: it is indeed the key activator of blood coagulation, while it is highly expressed on human aggressive malignancies. Tumor growth factor beta (TGF β) is the most abundant biomolecule secreted by platelets and the most potent promoter of the epithelial to mesenchymal transition (EMT). Distribution analysis of TF on human lung cancer biopsies reveals that it is predominantly expressed on the epithelial edge of aggressive tumors, indicating that TF expression correlates with cell invasion and migration. Based on these initial observations, we sought to determine the role played by TF in the intracellular TGF β signaling and cell phenotype.

Material and method

CRISPR/Cas9-mediated TF knocked out in human non-small cell lung carcinoma PC9 cells was performed and EMT indicators such as cell shape and size were evaluated by confocal microscopy and protein analysis. To investigate TGF β signaling, Smad protein phosphorylation was evaluated by luciferase reporter system and TGF β phosphoarray was performed. TGF β receptor 1 and 2 expression and function were analyzed by flow cytometry and Proximity Ligation Assay (PLA). Spectrophotometry analysis followed by in silico simulation, were employed to gain insight into the interactome of TF. Finally, Flipper-TR associated with confocal live cell imaging was employed to study how TF modulates membrane tension.

Result and discussion

Depletion of TF resulted in acquired epithelial features such as smaller size, round shape, reduced Smad2/3 phosphorylation and augmented E-Cadherin expression. The ability of TF-depleted cells to produce and activate TGF β is unaltered. Although TGF β receptors 1,2 and 3 transcripts remain unchanged, our data show that TF

supports their surface expression and activity. Moreover, PLA assays show reduced TGF β receptors tetrameric structure formation. The spectrometry analysis unveils that TF interacts with Ezrin and in silico studies suggest that the intracellular TF domain stabilizes Ezrin dimer and inhibits its phosphorylation. Finally, we observed that TF Knocked out cells display a reduced plasma membrane tension, which might explain the affected TGF β receptors dynamics.

Conclusion

Taken together, these data suggest that TF enhances TGF β signaling by augmenting the availability and dynamics of TGF β receptors. Ezrin, the master regulator of plasma membrane architecture and tension, is one of the candidate to orchestrate such an underlying mechanism. The role of TF in the modulation on plasma membrane tension and TGF β signaling transduction, is a novel concept and more studies are needed to investigate its function in receptors dynamics. Moreover, like TGF β receptors, the scenario that TF/Ezrin interaction might control the expression and dynamics of multiple cancer-related surface proteins is under investigation and may highlight novel mechanisms for drug resistance.

EACR25-2184

The effects of 17 β -estradiol on the expression and functionality of large-conductance voltage- and Ca $^{2+}$ -activated potassium channels in human glioblastoma cells

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Introduction

Gliomas are a type of brain tumor that is more common in men than in women suggesting that sex hormone levels, including estrogens, are important for glioma-gensis. However, the mechanism of estrogen action on the metabolism and pathogenesis of gliomas is not yet fully understood. In our work, we focused on the effects of 17 β -estradiol (E2) on the expression and functioning of large-conductance voltage- and Ca $^{2+}$ -activated potassium channels (gBK) present in human U87-MG cell line. gBK channels can contribute to cell growth and extensive migration in glioblastoma. Moreover, these channels are overexpressed in malignant gliomas in comparison to nonmalignant cortical tissues, and their expression level correlates positively with the malignancy grade of the tumor. Thus, changing gBK expression levels or altering their activity should significantly affect cancer cell behavior.

Material and method

U-87 MG cells were treated with various concentrations (from ng/ml to μ g/ml) of E2 for 24 or 48h. Then we determined cell viability using the CCK-8 assay and evaluated the expression levels of α and β (1-4) subunits of gBK channels using the RT-qPCR technique. The promoter regions of the genes under study were analyzed for E2 response elements using the online program AliBaba2.1. We examined the gBK channels

functionality based on the single-channel patch-clamp recordings obtained at two E2 concentrations (i.e., a physiological-like 0.0018 µg/ml and pharmaceutical 1,8 µg/ml) and the appropriate controls. Membrane potentials were fixed at -50 mV, -25 mV, +25 mV, +50 mV, +75 mV.

Result and discussion

The results showed that the expression of both β3 and β4 subunits of gBK channels depends on the E2 concentration, with the most significant increase observed for the β3 stimulated by physiological-like concentration of E2. The observed changes suggest that at least some of the estrogen response elements present in genes encoding gBK subunits are functional. E2-induced changes in the expression of gBK subunits can result in differences in their protein levels, which can affect the gBK channel functionality and can shape their functional hormone-dependent hallmarks in glioblastoma. The patch-clamp results indicated the decrease of the open-state probability (pop) of gBK channel (inhibition) as a consequence of E2 administration. The observed decrease of pop depended on E2 concentration and applied voltage, with the well-pronounced effects at membrane depolarization (e.g. at 50 mV, control: pop = 0.45 ± 0.06, E2physiol.: pop = 0.11 ± 0.03 and E2pharm.: pop = 0.14 ± 0.03). E2 stimulation resulted in a decrease of U87-MG viability.

Conclusion

E2 is an effective functional inhibitor of gBK channel activity and a potent expression regulator of its subunits in glioblastoma U-87 MG cells. Moreover, E2 affects the viability of these cells. This study reveals the novel aspects of glioblastoma biology which enlighten the role of K⁺ channels modulation by E2.

EACR25-2185

The effects of progesterone on the expression and functionality of large-conductance voltage- and Ca²⁺-activated potassium channels in human glioblastoma cells

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Introduction

Progesterone (P) is a steroid hormone involved in the regulation of many cellular processes. It can act via the classical and non-classical pathways by binding to the progesterone receptor. In glial cells, progesterone affects proliferation, migration, and the cell cycle. Progesterone levels are also associated with cancer development, but the exact mechanism of action is not yet understood. In our study, we examined the effect of P levels on the expression and functioning of large-conductance voltage- and Ca²⁺-activated potassium channels (gBK) in the human U87-MG cells, and its effects on cell viability. The gBK can regulate cell growth and extensive migration in glioblastoma. Therefore, changing gBK

expression levels or altering their activity should significantly influence cancer cell biology.

Material and method

We treated U87 cells with various concentrations (from ng/ml to µg/ml) of progesterone for 24 or 48h. We then determined cell viability using the CCK-8 assay and determined the expression levels of α and β subunits of gBK channels using the RT-qPCR technique. The promoter regions of the genes under study were analyzed for progesterone response elements using the online program AliBaba2.1. For functional gBK channel analysis, the single-channel patch-clamp recordings were obtained at two P concentrations (i.e., 0.025 µg/ml and 25 µg/ml) at membrane potentials ranging from -50 mV to +75 mV with 25 mV step.

Result and discussion

The expression of the gBK channel subunits depends on the P concentration, with the highest effects observed for β3 and β4 subunits. The changes in relative expression levels of regulatory subunits in E2-stimulated cells can result in differences in their protein levels, which can affect the gBK channel functionality (activation, inactivation, Ca²⁺-sensitivity), and consequently shape their functional hormone-dependent hallmarks in glioblastoma. The patch-clamp results indicated the decrease of the open-state probability (pop) of the gBK channel (inhibition) as a consequence of the P administration. The pop decrease depended on P concentration and applied voltage, with the well-pronounced effects at membrane depolarization (e.g. at 50 mV, control: pop = 0.45 ± 0.06, P0.025 µg/ml: pop = 0.07 ± 0.03 and P25 µg/ml: pop = 0.08 ± 0.03). P stimulation resulted in a decrease of U87-MG viability, with a nonlinear dose-dependent relation (it ranged from 0.65 ± 0.01 to 0.91 ± 0.03 for the examined P concentrations).

Conclusion

Progesterone is an effective functional inhibitor of gBK channel activity and a potent expression regulator of its subunits in glioblastoma U-87 MG cells. It also changes the viability of these cells. This study reveals novel aspects of glioblastoma biology which enlighten the role of K⁺ channels modulation by sex-hormones.

EACR25-2200

Unveiling the Molecular Mechanisms of KCa3.1 in Pancreatic Cancer Development

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Introduction

Ion channels mediating ion flux across biological membranes are gaining attention as potential oncology targets, as their aberrant expression and function contribute to multiple cancer hallmarks. Ion channels play crucial roles in cancer progression, not only by mediating ion flux but also by modulating intracellular signalling pathways. The intermediate conductance Ca²⁺-activated potassium channel (KCa3.1) is highly expressed in various cancers, including pancreatic ductal adenocarcinoma (PDAC) where its dysregulation

correlates with poor prognosis underscoring its significance in pancreatic malignancy. However, the molecular mechanisms linking KCa3.1 to pancreatic cancer remain incompletely understood. To investigate its role, we characterized a PDAC cell line lacking KCa3.1 and applied TurboID-based proximity labeling to map its interactome, identifying key interactors and pathways associated with KCa3.1 channel activity.

Material and method

To demonstrate the involvement of KCa3.1 in tumorigenesis through its participation in signaling complexes, we first investigated its interactome using TurboID proximity labeling in KPC intact cells. To further establish the relevance of KCa3.1 in tumorigenesis, we generated a CRISPR/Cas9 knock-out (KO) KPC cell line and characterized it both *in vitro* and *in vivo*.

Result and discussion

TurboID analysis revealed that the KCa3.1 channel interacts with several cancer-related signal transduction pathways including those associated with integrins and WNT/β-catenin signaling. We next validated these functional interactions with some of the identified partners, taking advantage of KCa3.1 KO pancreatic cancer cells, which exhibited remodeled gene expression, reduced proliferation *in vitro*, and formed significantly smaller tumors *in vivo*.

Conclusion

The analysis of the interactome of KCa3.1 using TurboID proximity labeling has, for the first time, allowed us to identify novel putative interacting proteins of the channel in the context of pancreatic cancer. These interactions may explain the channel's ability to modulate specific signaling pathways involved in cancer-promoting mechanisms. In conclusion, our findings provide new insights into the molecular landscape of KCa3.1 in pancreatic cancer and highlight its potential as a therapeutic target.

EACR25-2232

Regulation of MBD4 expression and cell-cycle mediated Interactions with the DNA Mismatch Repair (MMR) pathway

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Introduction

Uveal melanoma (UM) is an ocular neoplasm that predominately affects those of European origin. Loss of MBD4 is implicated in UM. Germline mutations in MBD4 also predispose to cancer syndromes such as Lynch Syndrome, which commonly results from germline mutations in DNA mismatch repair (MMR) genes. MBD4 and TDG encode base excision repair (BER) glycosylases responsible for the repair of G:T mismatches caused by spontaneous deamination of 5-methylcytosine. MBD4 deficiency has been associated with an increase in tumor mutational burden. Here, we aim to understand MBD4 regulation and characterize physical interactions throughout the cell cycle.

Material and method

To evaluate potential regulatory sensors affecting MBD4 expression, the ratio between mature and nascent MBD4 RNA was determined using quantitative PCR (qPCR) and RNA-seq data in isogenic HAP1 cell lines wild-type or knockout for MBD4. Protein expression of BER glycosylases (MBD4 and TDG) and MMR components (MLH1, MSH2, MSH6 and PMS2) was evaluated by western blotting in RPE1 or HAP1 cell lines. Cells in different phases of the cell cycle were sorted based on fluorescent cell cycle indicators (FUCCI) or by DNA content. We aim to establish the interaction between BER and MMR proteins during the G1 and S phases of the cell cycle by co-immunoprecipitation, using epitope-tagged MBD4 as bait.

Result and discussion

Through qPCR and RNA-seq analysis on HAP1 cell lines, MBD4 loss-of-function mutations showed minimal effect on MBD4 transcription. This either indicates a weak effect of nonsense-mediated decay or transcriptional upregulation. The proportion of immature relative to mature MBD4 transcripts was higher in MBD4-mutated cell lines. However, our work found no evidence to support increased expression of unspliced transcripts as the cause. Evaluation of protein levels in the context of the cell cycle revealed that MBD4 protein levels were not cycle-regulated, although we observed strong reduction of TDG levels in S-phase cells. Current literature suggests that MLH1 and PMS2 physically interact with MBD4, suggesting a role of non-canonical MMR in 5mC deamination repair. Here, we will aim to clarify if this interaction is cell cycle-regulated.

Conclusion

Our work demonstrates some key features of MBD4 gene and protein regulation that have not been previously characterized. In MBD4 deficient cell lines, we demonstrated that increased expression of MBD4 is not the result of increased expression of immature MBD4 transcripts. Furthermore, MBD4 protein level is not cell cycle-regulated. Our ongoing work seeks to build on this knowledge to clarify the interplay between BER and MMR pathways.

EACR25-2315

SUPT5H influences Cell Identity in Pancreatic Ductal Adenocarcinoma

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Introduction

Approximately 85% of pancreatic ductal adenocarcinoma (PDAC) patients are diagnosed at a locally advanced stage, eventually developing chemotherapy resistance during the course of treatment in most cases. The intrinsic ability of PDAC to develop therapy resistance is likely attributable to the tremendous phenotypic plasticity of PDAC tumor cells, in combination with the transition of the classical to the basal PDAC subtype, where the classical subtype exhibits better response to standard chemotherapy treatment compared to the basal subtype.

Since the subtypes exhibit only minor differences at the genomic level, their distinctions appear to be primarily driven by epigenetic factors, such as transcriptional regulation and chromatin modulation. Therefore, understanding the epigenetic mechanisms that govern the molecular identity of PDAC subtypes is crucial for identifying novel therapeutic targets, potentially enhancing treatment outcomes. In PDAC, cell identity is governed by gene expression, where RNA Polymerase II (Pol II) regulation and enhancer-promoter interactions shape subtype-specific transcription in a context-dependent manner. The release of promoter-proximal Pol II pausing, an essential checkpoint in gene expression, plays a crucial role in regulating classical subtype-specific genes. Within this transcriptional regulatory network, SUPT5H, a key subunit of the DRB Sensitivity-Inducing Factor (DSIF) complex, is a central player in controlling Pol II pausing. In this study, we investigate if SUPT5H governs PDAC subtype identity and therapeutic responsiveness by modulating promoter-proximal Pol II pause release and transcriptional elongation.

Material and method

We employed siRNA-mediated knockdown of SUPT5H followed by mRNA sequencing and Chromatin Immunoprecipitation sequencing (ChIP-seq) for SUPT5H, Pol II, GATA6, p63 and relevant chromatin marks.

Result and discussion

Results of this study indicate that siRNA-mediated depletion of SUPT5H in basal-like PDAC specifically results in downregulation of basal-specific genes, including KRT5, FAT2, MIR205HG and TP63. ChIP-seq analysis after SUPT5H depletion revealed a global reduction in Pol II occupancy across downregulated genes, while at unregulated and upregulated genes, Pol II levels decreased at the transcription start site but accumulated in the 5' downstream region. Further ChIP-seq analysis of subtype-specific active distal regions could unravel SUPT5H's potential role in subtype-specific enhancer regulation, in turn influencing subtype-specific gene expression in PDAC potentially.

Conclusion

The results of this study suggest that SUPT5H depletion alters Pol II distribution, potentially leading to transcriptional dysregulation in a gene-specific manner.

EACR25-2379

Differential gene expression in immune and metabolic pathways in right and left normal colonic mucosa from Lynch syndrome carriers

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Introduction

Lynch Syndrome (LS) is the most common inherited cancer syndrome, characterized by development of mainly right-sided colorectal cancers (CRC), among other types of tumors. It is caused by germline pathogenic variants in mismatch repair (MMR) genes. In tumors and preneoplastic lesions, loss of MMR system associates with high neoantigen burden that is recognized by the immune system. A distinctive immune microenvironment has been recently described in normal colonic mucosa from healthy LS carriers. The aim of this study is to provide novel insights on the early molecular events in normal colonic mucosa that underlie LS colon cancer predisposition.

Material and method

39 LS carriers (13 survivors – with previous CRC or advanced adenomas – and 26 previvors – without previous CRC or advanced adenoma) and 10 healthy non-LS individuals were included. Fresh-frozen biopsies of normal appearing mucosa were obtained from left and right colon during routine surveillance colonoscopies. RNA was extracted and mRNA stranded libraries were sequenced on a NovaSeq 6000 (Illumina) in paired-end mode, with a read length of 2x151 bp. Differential gene expression, gene set enrichment and cell type deconvolution analyses were assessed according to the anatomical localization of the analyzed sample and the germline mutated MMR gene.

Result and discussion

Normal colonic mucosa from LS individuals showed a distinct transcriptomic profile compared to non-LS individuals, with 2774 differentially expressed genes (DEG) (p adjusted value (p_{adj}) < 0.05), of which 24 with a $|FC| > 2$. The most enriched biological processes in LS samples were related to metabolism, cellular respiration and T cell activation. Sharp differences in gene expression were observed between right and left colon mucosa samples in both control (1593 DEG, $p_{adj} < 0.05$) and LS (7053 DEG, $p_{adj} < 0.05$) groups, mainly related to B cell activation and antimicrobial response in colon. The most enriched biological processes in the right colon from LS compared to controls were related to metabolism, cellular respiration and T cell activation whereas in the left colon additional pathways were observed such as DNA replication and proliferation. Specific immune cell types (CD4, CD8, Treg and dendritic cells) were particularly abundant in the right colon in LS previvors. Distinct transcriptomic profiles according to the MMR germline mutated gene were also identified.

Conclusion

Differentially expressed genes and pathways have been identified in both right and left normal colonic mucosa from LS carriers. Enhanced immunosurveillance is enriched in the right colon of LS previvors, which may play a role in elimination of (pre)malignant cells. Our findings suggest a novel role of the MMR system in metabolic processes.

EACR25-2432**Coordinated Changes in Midkine Expression and Its Receptor Network in Gliomas***R. Zagożdżon¹, K. Zieliński¹, A. Góźdz², P. Szpak³,**I. Kalaszczyńska³, M. Lachota⁴**¹Medical University of Warsaw, Laboratory of Cellular and Genetic Therapies, Warsaw, Poland**²Medical University of Warsaw, Department of Histology and Embryology, Warsaw, Poland**³Medical University of Warsaw, Department of Histology and Embryology, Warsaw, Poland**⁴Children's Memorial Health Institute, Department of Ophthalmology, Warsaw, Poland***Introduction**

The diverse functions of midkine (MDK) are harnessed in cancer to support tumor development and invasion. However, despite previous reports of MDK presence in gliomas, its specific roles and the significance of each of its receptors remains inadequately understood.

Material and method

To investigate the role of MDK, we integrated four bulk RNA-Seq datasets to create a comprehensive glioma dataset comprising over a thousand adult gliomas. Next, we collected fresh human glioblastoma tumor samples and established glioblastoma stem cell cultures to examine MDK expression and secretion patterns as well as surface expression of MDK receptors. Furthermore, we correlated MDK serum levels with its mRNA expression in primary tumors. Finally, we conducted an in-depth cell-cell communication analysis using single-cell RNA-Seq data from 44 tissue fragments of different grade glioma tumors.

Result and discussion

We identified divergent MDK expression patterns in IDH1wt and IDH1mut gliomas. MDK was associated with negative prognostic value which was more pronounced in IDH1wt gliomas compared to IDH1mut tumors. Integrated bulk, single-cell RNA-seq and proteomic data indicate that malignant glioma cells are the primary source of MDK in the glioma micro-environment with MDK exerting multidirectional protumorigenic effects. While the mechanisms responsible for high MDK expression in gliomas remain unknown, cell-cell communication analysis and flow cytometry profiling of MDK receptors has allowed for improved understanding of the role of the MDK's complex receptor network.

Conclusion

Collectively, these findings provide novel insights into the role of MDK and its receptor network in glioma, allowing for informed targeting of MDK and its signaling in MDK-high subset of gliomas.

EACR25-2551**Novel insights into CDH1 non-coding regulatory elements: effects on E-Cadherin expression, metastasis, and patient outcomes***R. Barbosa-Matos¹, C. São José¹, S. Lobo¹, M. Ferreira¹, N. Martins¹, I. Gullo¹, J. Senz², J. Bessa³, S. Mundlos⁴,**C. Oliveira⁵**¹i3S, Expression Regulation in Cancer, Porto, Portugal**²University of British Columbia, Department of Pathology and Laboratory Medicine, Vancouver, Canada**³i3S, Vertebrate Development and Regeneration, Porto, Portugal**⁴Max Planck Institute for Molecular Genetics, RG Development & Disease, Berlin, Germany**⁵i3S, Porto, Portugal***Introduction**

Regulation of CDH1 expression relies on regulatory elements (RE) located between the 5'-UTR and intron 2. However, the effects of disrupting these RE in cancer cells, and their link to patients' outcomes remain poorly defined. Here, we explore the consequences of single and combined disruptions of non-coding RE at the CDH1 locus, assessing both local and global gene expression changes.

Material and method

METABRIC tumor samples (n=2085) were classified based on intron 2 deletions or amplifications, examining clinical-pathological features and disease-specific survival. The regulatory potential of coding exon 2, REa, and REb was assessed by luciferase assays, zebrafish enhancer detection, and lacZ reporter assays in mice. Their functional impact was evaluated by CRISPR/Cas9 perturbation, RNA-seq, ATAC-seq, 4C-seq with a viewpoint at the CDH1 promoter, as well as lung colonization assays via mice tail vein injections. Gene ontology analysis from differentially expressed genes (DEG) was integrated with ATAC-seq to identify key genes and relevant pathways. KMPlotter was used as an independent cohort to explore candidate genes expression in metastasis-free survival.

Result and discussion

Breast cancer patients with homozygous deletions spanning CDH1 intron 2 RE showed better outcomes and lower tumor grades compared to other tumors, including those with intron 2 amplification. Intronic REa, REb and Exon2 showed regulatory properties. Combined disruption of REa and REb led to CDH1 loss of function, producing the strongest impact on transcriptome and global chromatin accessibility profiles, but cells were not able to colonize the lung. Cells with REb disruption alone displayed half CDH1 expression, and less ability to colonize the lung than Exon2 deletion or wild-type cells, highlighting an hypomorphic nature. Cells with combined disruption of REa and REb revealed DEG impairing extravasation, supporting their lack of lung colonization. Reduced expression of two candidate genes associated with extravasation and colonization correlated with better patient's outcomes in an independent cohort.

Conclusion

Impairment of intron 2 RE induces global changes in 3D-chromatin architecture, accessibility and expression profiles that prevent cell extravasation, hampering lung colonization. This mechanism supports the favorable outcomes of breast cancer patients with homozygous CDH1 RE deletions, despite E-cadherin loss.

EACR25-2602

Receptor tyrosine kinases as suitable molecular targets in the precision treatment of giant cell tumor of bone

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Introduction

Giant cell tumor of bone (GCTB) is a benign but locally aggressive osteolytic tumor that typically originates in the epimetaphyseal region of long bones. It is characterized by a high recurrence rate and contains multinucleated osteoclast-like giant cells and neoplastic mononuclear stromal cells. To minimize the risk of recurrence, various adjunctive therapies have been employed, including cryosurgery, phenol, bone cement, and argon beam therapy. Systemic treatments such as bisphosphonates, interferon alpha, and denosumab are also used. Denosumab, a human monoclonal antibody, inhibits the activity of osteoclast-like giant cells, but it does not affect the neoplastic mononuclear stromal cells. Therefore, there is a need for new therapies targeting the neoplastic stromal cells to reduce recurrence and improve patient outcomes.

Material and method

A phospho-protein array was used to detect phosphorylation of 49 RTKs in tumor tissues obtained from diagnostic biopsies or post-treatment surgery.

Subsequently, protein expression was verified on tumor tissue samples and derived stromal cell lines by western blotting. Based on those results, we have identified druggable molecular targets involved in RTK signaling. Selected FDA-approved low-molecular weight inhibitors were used to test their in vitro effects on cell viability and cell cycle.

Result and discussion

After denosumab treatment, phosphorylation of specific RTKs, including EGFR, PDGFR α , PDGFR β , and IGF-IR, was detected in tumor tissues. A similar profile was observed in tumor-derived cell lines. MTT assay and live cell imaging demonstrated a significant dose-dependent effect on cell viability in vitro after the use of specific RTK inhibitors (e.g., sunitinib, axitinib, crizotinib, ceritinib, and trametinib). In addition, flow-cytometric analysis demonstrated induction of cell death as indicated by an increase in the sub-G1 peak. Among the tested inhibitors, trametinib, a specific MEK1/2 kinase inhibitor, showed the best effect in all stromal cell lines used.

Conclusion

These findings suggest that RTK signaling plays a crucial role in promoting stromal cell proliferation within GCTB tumors. Targeted therapy with inhibitors of specific

RTKs or downstream signaling proteins could serve as a valuable addition to current GCTB treatment strategies.

This study was supported by project no. NU22-10-00054 from the Ministry of Healthcare of the Czech Republic and by the project National Institute for Cancer Research (Programme EXCELES, ID Project No. LX22NPO5102)—Funded by the European Union—Next Generation EU.

Translational Research

EACR25-0015

Superclustering-induced activation of death receptors using nanopatterned TRAIL-decorated DNA origami nanostructures

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Introduction

Clustering of type-II tumour necrosis factor receptors (TNFRs) is required to induce intracellular signaling. Current methods for receptor clustering lack precise control over ligand valency and spatial organization, potentially limiting optimal TNFR activation, biological insight, and therapeutic efficacy. DNA nanostructures provide nanometer-precise control over molecular arrangement, allowing control of both ligand spacing and valency.

Material and method

Here, we produce a DNA nanostructure decorated with controlled numbers of engineered single-chain TNF-related apoptosis-inducing ligand (sc-TRAIL) trimers. These trimers cluster death receptor 5 (DR5), enabling investigation of the geometric parameters influencing apoptotic pathway activation.

Result and discussion

We show that cell killing is affected by both valency and separation of sc-TRAIL trimers, which can be utilized to induce cell killing in human primary pancreatic and colorectal cancer organoids.

Conclusion

Together, our data shows that precise control of receptor clustering through spacing and valency enhances our understanding of receptor activation mechanisms and informs the development of more effective cancer therapies.

EACR25-0041

The role of Phytocannabinoids in Triple Negative Breast Cancer

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Introduction

Breast cancer is the most frequently diagnosed cancer in women worldwide. Triple negative tumors (TNBC) are deficient for well-defined molecular targets making chemotherapy, which is non-specific and cytotoxic, the most common treatment option. Patients with TNBC tend to develop metastasis and recurrence after treatment as well as lower survival compared to patients with other subtypes of breast cancer. Hence, there is a need for innovative therapeutic interventions to help women with breast tumors. Cannabinoids are products of Cannabis sativa. They were first introduced as palliative medicinal products, aiding in reducing emesis resulting from chemotherapy for cancer patients. Cannabinoids possess anti-tumoral activity in breast cancer cell lines. In this study, to elucidate the role of CBD and CBDA (Cannabis oils extracted by cannabis inflorescence) in tumor growth progression in TNBC, we performed in vitro studies on MDA-MB231 cells, and in vivo studies on heterotopic mice of TNBC.

Material and method

In vitro assays were performed on triple-negative MDA-MB-231 cells treated with CBD and CBDA, alone and in combination. The effects of CBD and CBDA on viability were determined by wound healing and MTT assays, cell migration was assessed by transwell migration and in vitro apoptosis by flow cytometry. Xenograft mouse model of TNBC was generated by a subcutaneously injection of MBA.MB231 cells into the right-side flank area of BALB/c mice (n total = 24 mice). After the randomization mice were divided into 3 groups according to the different types of treatment: 1) Normal Saline (vehicle); 2) CBD and 3) CBDA injected peri-tumorally every day for 3 weeks. The animals were sacrificed 2 weeks later. Half of the tumor tissue was formalin-fixed and paraffin-embedded for immunohistochemistry for CD31, for immunofluorescence localization of Ki67 protein, and routine H&E staining. Western blotting analysis was performed according to standard protocols on protein extracted from breast tumor tissues to detect the expression of proteins P53 and Bcl2.

Result and discussion

We demonstrated that both CBD and CBDA, can inhibit cell proliferation of MDA MB 231 cells by enhancing the apoptosis. In vivo studies performed on xenograft mouse model of TNBC, revealed that tumors of mice treated with CBD and CBDA are smaller than those observed in the controls. CBD Modifies the Expression of Tumor

Development Markers Ki67, Bcl2 and P53. Our results suggest that CBD and CBDA, can be viewed as promising agents for inhibiting TNBC progression, which has scarce therapeutic options and is featured by inauspicious prognosis and low survival rates.

Conclusion

Our results suggest that phytocannabinoids, CBD, and CBDA, can be viewed as promising agents for inhibiting TNBC progression.

EACR25-0074

Natural stilbenes in prostate cancer targeted interception and therapy: pre-clinical studies

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Introduction

Nutritional chemoprevention and interception are a particularly promising approach for prostate cancer due to its slow progression and predominance in elderly men. Plant-derived stilbenoids are a class of natural polyphenols with multiple positive pharmacological properties including antioxidant, anti-inflammatory, and pro-apoptotic effects. Metastasis-associated protein 1 (MTA1), a master transcriptional co-regulator and chromatin modifier, is strongly associated with prostate cancer progression and metastasis. The importance of MTA1-targeted interventions to block prostate cancer progression will be discussed.

Material and method

We established multiple MTA1 loss-of-function prostate cancer cell lines for mechanistic studies and developed various preclinical models of prostate cancer, including xenografts and unique transgenic mouse models reflecting different stages of prostate cancer to test the anti-inflammatory and anticancer effects of resveratrol, pterostilbene, and Gnetin C by different routes of administration. Immunohistochemistry, real-time PCR, western blot analysis, colony formation, wound healing, ELISA, to mention some, were used for evaluating MTA1 signaling (PTEN, p53, pAkt/Akt, mTOR, Cyclin D1, Notch2, Ets2, E-cadherin, IL-1 β) targeting results.

Result and discussion

Our in-dept in vitro and in vivo experiments demonstrated remarkable interceptive/therapeutic potential for stilbenes, specifically for the most potent dimer-resveratrol Gnetin C, in constraining MTA1 signaling and blocking progression of prostate cancer in preclinical models. Stilbene-supplemented diets diminished the progression of prostate cancer in transgenic mice by reducing inflammation, cell proliferation, angiogenesis and inducing apoptosis. Combination of natural compounds with clinically approved drugs showed promising results in combating castrate-resistant prostate cancer. Challenges in combinatorial approaches using natural compounds with approved drugs will be discussed.

Conclusion

Due to known reasonable safety, natural stilbenes may become the most promising MTA1-targeted strategy for prostate cancer chemoprevention, interception, and therapy. Well-controlled clinical trials are needed to

confirm novel nutritional intervention for untreated patients under active surveillance and novel combined therapy for more advanced disease.

EACR25-0108

Stress-Reducing Intervention in Patients With Colorectal and Breast Cancer

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Introduction

The prognostic relevance of heart-rate variability (HRV) among cancer patients has stimulated scientific exploration into its therapeutic utility. This prospective interventional phase III randomised clinical trial with open-label approach aims to assess the therapeutic efficacy of the HRV biofeedback intervention (HRV BI) in patients with solid tumors as a supplementary therapeutic approach. The trial aims to evaluate the impact of HRV BI on various clinical and psychosocial parameters compared to standard of care (SOC) alone.

Material and method

Trial design Participants in the intervention group will receive the SOC treatment along with HRV BI, involving four sessions led by a trainer. They will learn about the prognostic significance of the vagal nerve in cancer, as well as techniques for reducing distress and pain through deep paced breathing with HRV monitoring. Subsequently, they will perform daily HRV BI sessions for three months with online monitoring and support. The participants in the non-interventional group will receive the SOC alone. Cohort A- patients with pathologically confirmed breast cancer diagnosis planned for neo-adjuvant chemotherapy +/- targeted therapy +/- immunotherapy approach defined by standard of care in current European Society for Medical Oncology (ESMO) guideline. Cohort B- includes patients with pathologically confirmed colon cancer diagnosis planned for adjuvant chemotherapy +/- targeted therapy +/- immunotherapy defined by standard of care in current ESMO guideline. The study's primary endpoints include the effect of HRV biofeedback on inflammation (IL-6, Tumor necrosis factor α , IL-10 and Tumour Infiltrating Lymphocytes), QoL (measured by EORTCQLQ-C30), executive functions (measured by Stroop task test), effect on stress relief (measured by salivary cortisol slopes), sleep quality (measured by actigraphy and self-report data), HRV (indexed by the root mean square of successive differences between heartbeats – RMSSD) and working memory (measured by digit span test score). The study's secondary endpoints include evaluation of the Relapse-free Survival (RFS), Overall survival (OS), Serious Adverse Events (SAE) and the rates of

Pathologic Complete Response (pCR) and Residual Cancer Burden (RCB) in the cohort A.

Result and discussion

Clinical trial identification NCT06281145

This study was financially supported by VEGA 1/0090/22 and APVV-22-0231 grants.

EACR25-0165

CT Scan Identified Sarcopenia and MST and AND/ASPEN Malnutrition Guidelines in Gastrointestinal Cancer Patients

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Introduction

Sarcopenia, a debilitating muscle-loss disorder, worsens quality of life and raises morbidity and mortality, particularly in gastrointestinal (GI) cancer patients. Although up to 89% of cancer patients have sarcopenia, no clear consensus exists on risk screening, including the use of malnutrition tools. While Computed Tomography (CT) is considered the gold standard for diagnosing sarcopenia and is routinely performed in GI cancer care, there is currently limited utilization of CT scans for sarcopenia, clinically. This study used routine CT scans to assess how well the Malnutrition Screening Tool (MST) and the Academy of Nutrition and Dietetics and American Society for Parenteral and Enteral Nutrition (AND/ASPEN) malnutrition guidelines detect sarcopenia in a GI cancer population.

Material and method

In this retrospective chart review, CT scans were used to diagnose sarcopenia utilizing ImageJ software. Demographic, MST scores, and malnutrition data were collected from the subjects' medical record at their initial oncology visit. GI cancers evaluated included colon, rectal, small bowel, gastric, esophageal, and pancreatic. Fisher's exact tests, independent t-tests, receiver operating characteristic curves, and logistic regression were used to examine the relationship between the two tools and sarcopenic and non-sarcopenic groups.

Result and discussion

At baseline, 50 participants were included in the study. The population was 56% (n = 28) male, 92% (n = 46) white, and the median age of the group was 65.5 (IQR = 16.0) years old. Of GI cancers, 48% (n = 24) had colon or rectal cancers, with pancreatic cancer being the next most prevalent (28%, n = 14). The median body mass index (BMI) of the group was 26.9 kg/m² (IQR = 6.8). 50% (n = 25) of participants were identified as sarcopenic. Baseline demographics were not statistically different between groups. The odds of being diagnosed with sarcopenia increased by 153% for each one unit increase in the MST (OR 1.53, 95% CI: 1.00, 2.33; p-value = 0.050). There was no statistically significant relationship when comparing malnutrition status using the AND/ASPEN guidelines with CT diagnosed sarcopenia.

Conclusion

CT scans identified sarcopenia in half of the population, offering additional clinical utility of routinely accessible

scans. There may be a relationship between MST and sarcopenia, showing potential as a clinical prediction tool of sarcopenia risk. Given the small sample size, further larger powered studies are needed to confirm the relationships between more commonly used nutrition assessment tools and their predictive value in identifying sarcopenia.

EACR25-0342

Treatment response and survival of patients with early-onset compared to older-onset colorectal cancer in the Netherlands

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Introduction

The incidence of patients with early-onset colorectal cancer (eoCRC) (<50 years) is rising. The aim of this study is to investigate response to current treatment and survival in patients with eoCRC compared to older-onset CRC.

Material and method

Clinicopathological data, including treatment response and survival of patients diagnosed between 2016 and 2022 were requested from the Netherlands Cancer Registry. EoCRC patients were compared to patients 50-70 and ≥70 years of age. Additionally, eoCRC patients were matched with patients 50-70 and ≥70 years based on tumor stage, RAS/BRAF mutations, tumor sidedness and therapy. To compare groups, two sided X2 and Fisher's exact tests were used. Overall survival (OS) was analyzed with Kaplan Meier and median survival times were compared with the log-rank test (R software). P-values < 0.05 were considered significant.

Result and discussion

Between 2016 and 2022, 81,769 patients were diagnosed with CRC, including 3,948, 34,918, and 42,930 patients <50, 50-70 and ≥70 year, respectively. Patients <50 and ≥70 years had worse OS compared to patients aged 50-70 years ($p < 0.001$; HR, 1.14 (95% CI, 1.07-1.21); HR, 2.06 (95% CI, 2.06-2.11)) with a 5-year OS of 69%, and 54%, compared to 73%, respectively. EoCRC patients with high risk stage II CRC (T4N0) have a similar OS compared to patients 50-70 years, but better than patients ≥70 years ($p = 0.02$; HR, 2.19 (95% CI, 1.15-4.16); $p = 0.03$; HR, 1.46 (95% CI, 1.04-2.05), respectively). EoCRC patients with high-risk stage III (T4 and/or N2), have worse OS compared to patients 50-70 years ($p = 0.03$; HR, 0.81 (95% CI, 0.67-0.98)), while OS in low-risk stage III (T1-3/N1) was similar. Patients ≥70 years have worse OS compared to patients 50-70 years in both high- and low risk stage III CRC ($p < 0.001$; HR, 1.41 (95% CI, 1.28-1.55), $p < 0.001$; HR, 1.80 (95% CI, 1.58-2.06), respectively). EoCRC patients with pMMR stage IV disease at diagnosis have better OS, while patients ≥70 years of age have worse OS compared to patients 50-70 years ($p < 0.001$; HR, 0.56 (95% CI, 0.79-0.93), $p < 0.001$; HR, 1.55 (95% CI, 1.47-1.64), respectively). EoCRC patients more often received triple therapy

(fluoropyrimidine + oxaliplatin + irinotecan +/- bevacizumab) in first line compared to older patients, (20%, 10% and 3% for patients <50, 50-70 and ≥70 years, respectively). Patients ≥70 years more often received fluoropyrimidine monotherapy +/- bevacizumab (8%, 14% and 40%, respectively). After matching no significant difference in OS was observed between eoCRC patients and patients 50-70 years. OS of patients ≥70 years remained worse.

Conclusion

OS of eoCRC patients with high-risk stage II and low risk stage III was similar to patients 50-70 years, but OS was worse in high-risk stage III eoCRC. EoCRC patients with pMMR stage IV CRC have better OS but also receive more intense systemic therapy. OS difference between eoCRC and patients 50-70 years disappeared after matching. Survival of patients ≥70 years remained worse.

EACR25-0416

SWOT Analysis for Maintaining the Sustainability of Newly Established Rectal Cancer Biobank at the Institute for Oncology and Radiology of Serbia

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Introduction

Rectal cancer biobank (RCB) is the first human-sample, research-based repository in Serbia established by following highly controlled internationally approved biobanking criteria at the Institute for Oncology and Radiology of Serbia (IORS). The World Health Organization reported 42,000 new cancer cases in Serbia in 2022, which provides a rationale for further expansion of the IORS biobank with collections of other tumor types and locations besides rectal cancer. To implement best practices for effective strategic planning and management, we performed a SWOT analysis to address possible challenges in the process of IORS biobank development.

Material and method

A comprehensive evaluation of internal and external determinants that may impact the future growth and performance of the IORS biobank has been performed.

Result and discussion

Accessibility of patients with different tumor types, good logistic management between clinicians and biobank staff and a well-integrated bioethical framework at IORS,

allow extensive collecting strategy. Those advantages together with biobank team members who hold advanced knowledge and skills for various methodologies in processing of biospecimens and data annotation are major strengths that biobank growth can rely on. On the other hand, weaknesses include currently limited storage and working space that could not be easily improved considering project-based funding and a lack of legal clauses for implementation of a self-sustainability plan, considering the four dimensions of sustainability: operational, financial, social and environmental. Being the first official cancer biobank at the national level and also tightly collaborating with several international scientific groups and international biobanks, make opportunities for IORS biobank to take a significant place in contributing to cancer research in the region and beyond. However, the major threats to IORS biobank prosperity are lack of knowledge about biobanking from layman population, researchers and clinicians, deficiencies of national legislative regulations regarding the development of non-transplant tissue biobanks, national accreditation path, and the obstacles to enroll additional administrative and laboratory personnel without IORS executive department consent.

Conclusion

The results of the conducted SWOT analysis serve as a valuable foundation for development planning, assisted us to establish priorities, and challenge risky assumptions that could lead to misconceptions and failure of IORS biobank objectives. Complementary tools related to business planning and data management planning will be crucial to achieving excellence in national biobanking.

[1] The RCB was established within STEPUPIORS Horizon Europe project No 101079217 according to guidelines recommended by international expert institutions (ISBER, BBMRI, ERIC).

EACR25-0423

Cardiac Remodelling and Incident Cardiovascular Risk in Haematological Cancer Survivors from the UK Biobank

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Introduction

Haematological cancer survivors are at increased risk of cardiovascular disease (CVD) due to cancer-related biological processes and cardiotoxic cancer therapies. Cardiovascular magnetic resonance (CMR) provides detailed organ-level information about cardiovascular health. However, population-level studies examining long-term cardiovascular health in this population are limited. This study integrates clinical and CMR data from the UK Biobank to define the excess risk of specific CVDs and cardiovascular remodelling patterns in survivors of haematological cancer.

Material and method

Participants with a record of haematological malignancy prior to baseline recruitment were identified using linked

hospitalisation and cancer registry data. Each cancer-exposed participant was propensity score matched to two non-cancer controls on an extensive range of socio-demographic, lifestyle, baseline morbidity, and clinical biomarker variables. Missing covariate data were imputed using predictive mean matching. Cox regression was used to calculate hazard ratios for the following CVD outcomes: chronic ischaemic heart disease, non-ischaemic cardiomyopathy, heart failure, myocardial infarction, atrial fibrillation, pericardial disease, and venous thromboembolism. Incident outcomes were prospectively ascertained from linked hospital and death registry records over a median of 13.6 years. In participants with CMR data available, linear regression was used to examine the association of cancer exposure with CMR-derived metrics of cardiovascular structure and function, and myocardial tissue character. These included ventricular and atrial volumes and function, arterial stiffness strain, and myocardial T1.

Result and discussion

A total of 2,166 haematological cancer survivors [median age 61, (IQR: 53–65) years; 56% women] and 4,331 controls were included in the analysis. Cancer survivors demonstrated a significantly increased incident risk for all selected CVDs, with the highest risks observed in relation to pericardial disease [HR = 6.38, 95% CI: 3.18–12.83]. Participants with haematological cancer had significantly large left ventricular volumes with poorer function (lower ejection fraction, worse global longitudinal strain), and pronounced myocardial fibrosis (higher T1).

Conclusion

Haematological cancer survivors have a significantly elevated risk of a range of incident CVDs. CMR analysis reveals adverse cardiac remodelling, comprising larger and poorer functioning left ventricle with greater myocardial fibrosis in haematological cancer survivors, compared to matched controls. These findings highlight the excess long-term cardiovascular risk of this cohort and the potential role of imaging biomarkers in risk stratification and understanding underlying mechanisms.

GA is supported by the Wellcome Trust (218584/Z/19/Z). JC, CM-G, and DG-C are supported by Barts Charity (G-002389, G-002777).

EACR25-0479

Genomic Profiling of Colloid Carcinoma of the Pancreas

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Introduction

Colloid carcinoma (CC) of the pancreas is a rare and distinct subtype of pancreatic cancer (PDAC), representing only 1–3% of all pancreatic tumors. Unlike the more common PDAC, CC is distinguished by the abundant extracellular mucin produced by tumor cells. CC often arises from intraductal papillary mucinous neoplasms (IPMNs), particularly of the intestinal subtype. While CC shares similarities with other pancreatic tumors in terms of symptoms, diagnosis, and treatment, it has a significantly better prognosis than PDAC. At the molecular level, CCs are associated with frequent

mutations in GNAS, followed by mutations in KRAS and TP53, with MSI cases typically being KRAS wild-type. However, most molecular studies focus primarily on PDAC, with relatively few cases of CC. The reasons behind its improved prognosis remain unclear, highlighting the need for further research to uncover potential therapeutic targets.

Material and method

We used DNA-targeted sequencing (174 genes) and immunohistochemistry (IHC) analyses to profile this cohort.

Result and discussion

This study analyzed 46 patients, including 27 with IPMN-associated CC (1 with intraductal oncocytic papillary neoplasm, IOPN; 26 with IPMN) and 19 with CC alone. Histological grading was performed based on immunophenotypic classification to characterize these tumors. The IHC analyses confirmed that IPMNs are mostly high-grade intestinal lesions, while the IOPN exhibited only high-grade dysplasia. Among the invasive tumors, 31 out of 46 (67%) were classified as mucinous and low-grade tumors, whereas 15 out of 46 (33%) displayed an infiltrative glandular component and high-grade tumors. DNA sequencing revealed that the most common genetic variations affected KRAS (48%), GNAS (46%), TP53 (35%), ATM (22%), RNF43 (17%), CDKN2A/SMAD4 (15%). Interestingly, 4 out of 9 ATM mutations were germline, as confirmed by Sanger sequencing. In addition to these germline mutations, one patient had a CHEK2 mutation, and another carried two different germline mutations affecting NF1 and ATM. Two patients of 46 showed microsatellite instability (MSI) with high tumor mutational burden (TMB). Lastly, it was found patients harboring amplification in CCND1 (1/46), CCND2 (4/46), CCND3 (1/46), CCNE1 (1/46), and IDH2 (1/46), which may be sensitive to targeted therapies (CDK4/6 inhibitors, CDK2 inhibitor or IDH2 Inhibitors). Overall, this study emphasizes the value of genomic profiling to identify patients with 1) potentially druggable mutations, gene amplifications or MSI; and 2) germline mutations sensitive to platinum-based therapies and/or PARP inhibitors.

Conclusion

To date, this study includes the largest cohort of patients with CC. This may help shed light on the factors contributing to the improved outcomes of CC compared to PDAC.

EACR25-0500

Breast cancer immunotherapy using magnetised oncolytic virus

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Introduction

Oncolytic viruses (OV) are encouraging new immunotherapies for cancer. OV, replicate in cancer cells inducing immunogenic cell death (ICD) and activating antitumour immunity. To date, clinical use has focused on intratumoural delivery due to concerns over inadequate tumour targeting following systemic administration. We hypothesize that magnetising OV

and magnetic guidance strategies will improve systemic delivery by protecting the viruses from inactivating immune mechanisms and non-specific adsorption.

Material and method

To investigate this, we magnetised oncolytic viruses and delivered these to tumours *in vitro* and *in vivo* using human and bacterial cell delivery systems.

Characterisation of the physical, chemical and oncolytic potential of our magnetised viruses (MAG-OV) was performed in combination with magnetic guidance strategies in preclinical mouse models of mammary cancer.

Result and discussion

Stable MAG-OV complexes of ~90nm diameter successfully infected human and murine breast cancer cells in a dose-dependant manner, and induced tumour oncolysis. Following MAG-OV infection, a significant increase in viral replication (ICP0, gB, ICP8), ICD (HMGB1, CALR, ATP) and apoptotic (CASP 3, CASP8, FASL) signals were detected. Intravenous delivery of MAG-OV resulted in reduced tumour burden in the presence of magnetic guidance and an increase in tumour-infiltrating T-cells, NK cells and neutrophils. Furthermore, MAG-OV complexes were protective in the presence of neutralising viral antibodies.

Conclusion

This study indicates that magnetising oncolytic viruses results in viral protection from neutralising antibodies and in combination with magnetic guidance reduces tumour burden and induces antitumour immunity.

EACR25-0524

In vitro strategy to discover therapeutic targets in PDAC

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Introduction

Pancreatic ductal adenocarcinoma (PDAC) is a leading cause of cancer death worldwide with increasing incidence over the years. Due to late onset of symptoms in early stages, early metastasis, limited therapeutic options and high rate of therapy resistance, overall prognosis remains dramatically poor (10% 5-year survival rate). One of the primary reasons for the low success rate is the complex tumor microenvironment of PDAC, characterized by a highly fibrotic and dense stroma surrounding the tumor cells which not only supports tumor progression but also increases resistance to treatment. Thus, developing innovative ex vivo culture modalities that accurately reflect the complexity and heterogeneity of PDAC represents a significant challenge for the precise evaluation of potential therapies.

Material and method

Our experimental approach lies on the set-up and characterization of various models derived from patient samples including tumoral biopsies or resections from

primary tumors or liver metastases, blood and ascites. Comprehensive characterization of patient avatars involves pharmacotyping with standard-of-care (SoC) and advanced multi-omics investigations, such as single-cell transcriptomics, proteomic, and genomics. In recent years, culture of patient-derived explants have emerged as a promising approach for preclinical studies. This model maintains the full tumor microenvironment with its intact architecture. Taking advantage of this highly relevant model, we investigate drug response in tissue slices along with paired control samples (basal state) through histological analyses to evaluate quality and tumoral content. Samples of interest are then evaluated through multiplex and/or multi-omics investigations in the context of associated clinical data. Matched blood samples will allow us to investigate circulating mediators as potential biomarkers of treatment response.

Result and discussion

We have completed the set up of our slicing method and culture conditions. We are currently in the process of sample collection and selection for the different readouts. These investigations are expected to significantly impact our understanding of PDAC complexity and response to treatment

Conclusion

This research project represents a crucial step towards enhancing our understanding of PDAC heterogeneity and refining experimental models for translational studies. The ultimate objective is to employ these avatars as an asset for bench-to-bedside translation for 1) identifying SoC vulnerabilities to uncover new therapeutic targets 2) guiding the selection of optimal therapeutic strategies and 3) identifying or positioning Servier's assets in this indication based on a more nuanced comprehension of the disease. This approach will also facilitate the identification of biomarkers and monitoring of therapeutic responses, further contributing to personalized medicine in pancreatic cancer care.

EACR25-0526

Protein Tyrosine Phosphatase Receptor type Psi (PTPRU) in clinical breast cancer

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Introduction

Receptor-type Protein tyrosine phosphatase Receptor Type U (PTPRU) is a member of the protein tyrosine phosphatase (PTP) family, a family known to have roles in regulating cell growth, cell adhesion and differentiation. PTPRU has been shown to have a possible impact on focal adhesion complex and may interact with the cell adhesion regulator CTNNB1 (b-catenin). It has been shown to influence the development of bone and central nerve system (CNS). However, little is known for the role of PTPRU in cancer and particularly in clinical

cancers. The present study investigated the expression pattern of PTPRU and its clinical significance in clinical breast cancer.

Material and method

Expression levels of PTPRU in a cohort breast cancer tissues including normal and tumour tissues were tested by way of transcript analysis. Patient's clinical, pathological, hormone receptor status, molecular subtypes and clinical outcomes (with a 10 year followup) were analysed against PTPRU expression.

Result and discussion

Breast cancer tissues had a marginally increased expression of the PTPRU transcript ($p = 0.081$) compared with normal mammary tissues. There was a marked reduction of PTPRU transcripts in breast tumours which developed local recurrence ($p = 0.022$, compared with those who remained disease free). ER positive (ER+) tumours had a significantly higher PTPRU than ER negative (ER-) tumours ($p = 0.0049$). PGR negative tumours also had raised levels compared with PGR negative tumours ($p = 0.0106$). Patients who had high levels of PTPRU had a significantly longer disease-free survival (DFS) than those with low levels ($p = 0.046$) and also have a longer overall survival (OS) although this was yet to be statistically significant ($p = 0.056$). The connection between PTPRU and DFS was more prominent in Her3 positive tumours ($p = 0.039$). In the Her2(+)ER(-) molecular subtype, high levels of PTPRU were associated with both longer DFS and OS. Interestingly, PTPRU does not show a correlation with CTNNB1 (a-catenin) in cancer tissues ($r = 0.111$, $p = 0.291$), but significantly correlated with E-cadherin ($r = 0.289$, $p = 0.005$) and a-catenin ($r = 0.208$, $p = 0.046$).

Conclusion

The expression of Protein tyrosine phosphatase receptor type U (PTPRU) has a significant correlation with favourable clinical outcome of patients with breast cancer, a relationship with a strong connection with the ER status of cancer cells. PTPRU thus has a hallmark of being a tumour suppressor in breast cancer and warrant further investigation for its prognostic values in breast cancer.

EACR25-0565

Harnessing Patient-Derived Organoids to Personalise Chemotherapy for Advanced Gastric Cancer

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Introduction

Gastric cancer (GC) is a challenging disease with a 5-year survival rate of <6% for advanced disease with current, standard care treatment. Patient-derived organoids (PDOs) are an emerging technology that has shown promise in guiding personalised treatment for cancer patients. A limited number of studies have evaluated PDO for GC, and existing studies test only a very few chemotherapeutic options with low culture success rates. We thus aimed to culture GC PDOs and perform drug testing using standard care and alternative chemotherapeutics to evaluate the efficacy of PDOs in treatment prediction and alternate chemotherapy choices.

Material and method

Tumour tissue samples were obtained from locally or systemically advanced GC patients who underwent tumour resection, and these were cultured as PDOs and expanded. In vitro, drug testing was performed using standard care chemotherapeutics FLOT (Fluorouracil, leucovorin, oxaliplatin, docetaxel), FOLFIRI (Fluorouracil, leucovorin, irinotecan), FOLFOX (fluorouracil, leucovorin, oxaliplatin) and alternate treatments. Cell viability was determined by whole-well ATP quantification using CellTiter-Glo 2.0 and normalized to vehicle-only controls. PDO response to FLOT was correlated with the patient responses using CT/PET imaging.

Result and discussion

GC patients were recruited, and PDOs with varying morphologies and growth characteristics were established. PDOs were treated with three combination chemotherapy regimens with an average treatment duration of five weeks from sample processing. Varying half maximal inhibitory concentrations (IC₅₀) with FLOT were observed, and the viability of PDOs from responders was lower compared to non-responders to FLOT in the clinic.

Conclusion

To date, the establishment of GC PDOs has shown a slightly higher culture success rate at our site than published previously. The heterogeneity of GC has been apparent through varied GC PDO morphologies, growth characteristics, and IC₅₀ drug viability values. PDOs have successfully recapitulated clinical responses, indicating their potential as a reliable model for guiding precision medicine within a clinically relevant timeframe.

EACR25-0585

Targeting PI3K-Akt Signalling Pathway in the Search for Novel Therapeutic Strategies for Treatment Resistant High-Grade Serous Ovarian Cancer

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Introduction

Ovarian cancer is the most lethal gynecological cancer, and high-grade serous ovarian cancer (HGSOC) is the most aggressive and common form of it. HGSOC is associated with high disease heterogeneity and distinct enriched pathways that mediate progression and resistance towards the current standard-of-care. The standard treatment is primary debulking surgery combined with platinum and taxane-based chemotherapy to which 80% of patients gain resistance towards within a few years. After this there is no additional serious treatment options, giving an urgent need for developing new treatment strategies. Since PI3K-Akt pathway is one of the main survival pathways in chemo resistant HGSOC, research on its inhibition could assist development of novel therapies for chemo resistant HGSOC.

Material and method

Our recent collaborative work is a tumor evolution study based on whole-genome and RNA sequencing data of longitudinal, prospective and multiregional collected tumor samples. This study suggests targets for drug intervention for the relapsed cases, such as inhibition of PI3K (Lahtinen et al. *Cancer Cell* 2023). In this study we have chosen to use a cohort of 2 control patient derived tumor organoids (PDTOS) with baseline PI3K expression and 4 case PDTOS with amplified PI3K expression. We have chosen PDTOS as our model system to gain insight closely resembling the reality of the patient tumor. We evaluated the response to PI3K inhibition through cell viability, proliferation and phosphorylation of Akt1 under a 3D setup with ImageXpress Confocal HT.ai. Image-based quantification was done with MetaXpress analysis software.

Result and discussion

PDTOS that resist chemotherapy become dependent on specific proliferative pathways that are drivers for relapses. We have found a PI3K inhibitor, alpelisib, to successfully kill 50% of the cells in the PDOs from the case group, while having only minor effect on the control group. Quantification of phosphorylation of Akt1 after treatment with alpelisib shows a significant decrease of Akt1 phosphorylation at each of its distinct phosphorylation sites T308 and S473 in the case group. For the control group there is only a significant decrease of phosphorylation of S473, but not for T308. This difference in phosphorylation might be an indicator of how the alpelisib specifically targets the PI3K-amplification in the case group of PDTOS. Future experiments will focus on exploring the synergistic effects of combining alpelisib with other targeted therapies with the focus on exploring the phosphorylation status of Akt1.

Conclusion

The PI3K-Akt pathway pose a great potential target for future treatments, but the need for highly effective treatments killing preferably all tumor cells is crucial when working with a heterogeneous and fast relapsing cancer such as HGSC. Our ultimate goal is to translate

these findings into clinical applications, providing new therapeutic options for patients with refractory cancers.

EACR25-0644

POSTER IN THE SPOTLIGHT

Ovarian cancer Zebrafish Avatars for personalized multiple drug testing

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Introduction

Ovarian Cancer is the 1st cause of death amongst gynecological cancers. Treatments are chosen with no evidence of the tumor's response to therapy, exposing patients to unnecessary toxicities. Until now, there is no test to assess the best option for each patient. To solve this unmet need we developed the Ovarian cancer zebrafish Avatar-test (zAvatars).

Material and method

Cells were isolated from ovarian cancer patients, after obtaining their informed consent. zAvatars from 27 patients were generated by injecting cells in the perivitelline space of 2 days post fertilization zebrafish embryos. zAvatars were challenged with the same therapy as their donor patient as well as second line therapeutic options, to evaluate drug sensitivity, in a non-interventional retrospective clinical study.

Result and discussion

Our data shows that, patients that were sensitive to treatment, their matching zAvatars were also sensitive; whereas patients that were resistant their zAvatars also showed resistance to treatment. By plotting the results in a confusion matrix we obtained a positive predictive value of 88% and a negative predictive value of 91%, which corresponds to an overall correlation of 89%. Moreover, through Kaplan-Meier survival curves we demonstrate that patients with a sensitive zAvatar-test have significantly longer PFS and OS ($p<0.0001$) than patients with a resistant zAvatar test. Analysis of the metastatic capacity showed that tumor cells from patients with accumulation of malignant ascitic or pleural effusion fluid, had a higher metastatic potential, regardless of isolating cells from the peritoneal wall or the fluid. These results are in line with the literature that considers that patients with fluids have poorer prognosis despite having the same follow up and treatment as patients without fluid accumulation.

Conclusion

With this clinical study we were able to demonstrate that zAvatars predict the patient response to therapy and thus can be used as a screening method to guide patient therapy. As a next step, we are performing a Multicentric Randomized Clinical Trial (EU CT 2023-509598-22), to demonstrate the clinical benefit of using the zAvatar test

as a screening tool to guide patient therapy and thus improve patient survival and wellbeing.

This research received support from Champalimaud Foundation and FCT (FCT-PTDC/MEC-ONC/31627/2017).

EACR25-0654

Malignant Ascites Derived Organoids from Ovarian Cancer Patients for Understanding Platinum Resistance and Approaching Targeted Therapy

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Introduction

Ovarian cancer (OC) is a leading gynaecological cause of death, with high-grade serous ovarian carcinoma (HGSOC) as the most common and aggressive subtype, often diagnosed at an advanced stage. Despite optimal surgery and initial response to platinum-based chemotherapy, most patients develop resistant disease, leading to recurrence with peritoneal carcinomatosis and malignant ascites. These ascites contain metastasis-initiating cell aggregates that drive dissemination and therapy resistance. In order to overcome the limited number of clinically-relevant HGSOC cancer models, we are developing 3D in vitro models of organoids from malignant ascites for the study of intrinsic and acquired therapy resistance mechanisms.

Material and method

Malignant ascites from advanced HGSOC patients is collected during surgery to generate patient derived organoids (PDOs). The protocol consists in separating the cells, followed by erythrocytes depletion and cells seeding as 3D in drops embedded in basement membrane extract (BME) with an optimized PDO culture media. Successfully generated organoids are serially passaged and are established after four passages. PDOs are characterized by immunohistochemistry (IHC) and immunofluorescence (IF) using E-Cadherin, EpCAM, Vimentin, F-actin, Ki67 and PAX8 for understanding the 3D complexity and origin. High throughput drug screenings through specialized machinery of Multidrop Dispenser and D300 Digital Dispenser are performed to assess platinum resistance, generating robust data that is correlated with patients' clinical information.

Result and discussion

We successfully generated PDO models from 9 ascites samples, with a 50% of generation success rate. PDO generation from malignant ascites is more challenging than from tumour tissue due to the low presence of epithelial tumour cells (~1-2%) and the predominance of immune cells together with other cell types. IF of established organoids confirmed the epithelial origin and the 3D structure composed of different cell types of our models, while IHC showed strong pathological and molecular correlation with patient tumours. Three PDO models were tested for Carboplatin, Olaparib, and Niraparib response and were able to successfully mimic the platinum-treatment response.

Conclusion

In this study, we demonstrated that PDOs derived from malignant ascites of HGSOC patients can recapitulate the histologic and molecular features of the patients' tumours, and can predict treatment response. These viable and relevant preclinical models are valuable tools to deep on the understanding of therapy resistance and to advance in precision medicine approaches for resistant HGSOC patients.

EACR25-0688

BQ323636.1 Overexpression Drives mTOR Signaling and Lipid Metabolism via ACSL4 in ER-positive Breast Cancer

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Introduction

BQ323636.1 (BQ), a splice variant of NCOR2, its overexpression in estrogen receptor (ER)-positive breast cancer shown to be predictive of disease recurrence and/or metastases. Previous studies revealed that BQ overexpression enhances polyunsaturated fatty acid (PUFA) levels and represses oxidative stress via NRF2 modulation. As mTOR signaling is critical in tumorigenesis, we investigated how BQ sustains energy production and mTOR activation through lipid metabolism reprogramming.

Material and method

RNA-sequencing assessed BQ overexpression effects. Targeted metabolomics quantified fatty acids, while Western blot and qPCR analyzed protein and gene expression. Lipid, acetyl-CoA, and ATP levels were measured alongside oxygen consumption rates. ACSL4 was inhibited using siRNA, rosiglitazone, and PRGL493. Tumor growth was evaluated in nude mice.

Result and discussion

ACSL4 emerged as a central regulator of BQ-mediated lipid metabolism reprogramming. BQ overexpression increased PUFA levels, confirmed by metabolomics, while ACSL4 knockdown reduced PUFA, acetyl-CoA, and ATP in BQ-overexpressing cells. PUFA susceptibility to peroxidation was repressed by NRF2-induced antioxidant gene expression, lowering ROS and lipid peroxidation. NRF2 knockdown triggered ferroptosis – a lipid peroxide- and iron-dependent cell death – in BQ-overexpressing cells, which ACSL4 inhibition prevented. BQ enhanced mitochondrial oxygen consumption, elevating ATP and GTP via the TCA cycle,

activating mTOR through Rheb. ACSL4 suppression abolished these effects. In vivo, ACSL4 inhibition reduced tumor growth in mice, validating its role.

Conclusion

BQ exploits ACSL4 to elevate PUFA levels, fueling acetyl-CoA and GTP synthesis via the TCA cycle for mTOR activation. NRF2 prevents ferroptosis by limiting lipid peroxidation. Targeting ACSL4 emerges as a promising therapeutic strategy to avert disease progression in BQ overexpressing ER-positive breast cancer.

EACR25-0778

Trametinib for NRAS-mutated tumors: Results from the Drug Rediscovery Protocol (DRUP)

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Introduction

NRAS mutations occur in about 3.1% of all patients (pts) with cancer. To date, no FDA or EMA-approved targeted therapies exist for the treatment of NRAS-mutated (NRASmut) cancer. Trametinib, currently registered for BRAF V600-mutated melanoma and NSCLC in combination with dabrafenib, blocks the RAF/RAS pathway through inhibition of MEK1/2. Therefore, it is hypothesized that this small molecule inhibitor could elicit clinical benefit (CB) in pts with NRASmut tumors. Here, we present the efficacy and safety of trametinib in NRASmut tumors.

Material and method

Pts were treated as part of Drug Rediscovery Protocol (DRUP, NCT02925234), a single-arm, nonrandomized clinical trial treating pts with cancer with anti-cancer therapy based on their tumor molecular profile, outside of their registered indication. Eligible adult pts with progressive advanced or metastatic treatment-refractory tumors harboring a pathogenic, activating NRAS mutation received trametinib 2 mg QD in 28-day cycles until disease progression or unmanageable toxicity. Primary endpoints included CB, defined as confirmed objective response (OR) (partial or complete response

(PR; CR)) or stable disease (SD) ≥ 16 weeks, and safety. Pts were enrolled using a Simon-like two-stage design.

Result and discussion

Twenty-four pts were evaluable for response, two of whom were still on treatment at time of analysis. Nine different tumor types were treated, of which 13/24 were NSCLC (54.1%). The majority of pts (19/24; 79.2%) harbored a p.Q61 NRASmut, the remaining (5/24; 20.8%) a p.G12/G13 NRASmut. With 1 PR (4.2%) and 7 SD at ≥ 16 weeks (29.2%), the resulting CB-rate was 33.3% (95% CI: 15.6–55.3%), and the OR-rate was 4.2% (95% CI: 0.1–21.1%). The median progression-free survival and overall survival amounted to 3.7 months (95% CI: 3.3–5.3 months) and 9 months (95% CI: 5.6–13.5 months), respectively. The pt who achieved PR had an NRAS p.Q61R mutated NSCLC with concomitant BRAF (p.N581S) and TP53 (p.R110C) mutations. No statistically significant survival difference was observed between NSCLC-pts and other tumor types, nor between NSCLC pts harboring a p.Q61 or a p.G12/G13 NRASmut. Notably, 8/24 pts (33.3%) had unconfirmed SD (7/24; 29.2%) or unconfirmed PR (1/24; 4.2%) at the 8-week interim evaluation, that they were unable to sustain at the 16-week primary endpoint assessment. In 10/24 (42%) pts, 15 treatment-related adverse events (trAE) grade ≥ 3 were observed, mainly related to skin (5/15 trAE; 33.3%) and left ventricular function (3/15 trAE; 20%). Together, the trAE grade ≥ 3 led to treatment discontinuation in 3 pts (12.5%) after 27, 97 and 101 days of treatment.

Conclusion

Trametinib showed CB in a subset of NRASmut pts, reaching the primary study endpoint. However, the observed effect was moderate given rapid progression and a short progression-free survival. The high percentage of unconfirmed responses could imply a fast resistance mechanism following treatment with trametinib.

EACR25-0798

Investigating the Therapeutic and Prognostic Utility of Long Non-Coding RNA Targets and their Association with Tumour Microenvironment in KRAS-Mutant NSCLC

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Introduction

Lung cancer is the leading cause of cancer incidence and mortality. 85% of cases are classed as non-small cell lung cancer (NSCLC). Of these, 40% bear an oncogenic KRAS mutation (KRASmt) which is associated with

poor prognosis. Some KRAS-targeting therapies are approved but exhibit isoform specificity and resistance. Further, immunotherapy efficacy is limited due to immune contexture heterogeneity between KRAS isoforms. Novel strategies to target other key oncological drivers in KRASmt tumours must be explored. Long non-coding RNAs regulate gene expression at epigenetic, transcriptional, and translational levels, are implicated in several tumourigenic pathways and associated with distinct immune phenotypes. Despite this, >98% remain uncharacterised. UCD researchers have identified 80 candidate lnc-RNA targets for KRASmt NSCLC. This project will examine the clinical expression and immune contexture associated with a subset of these candidates.

Material and method

11 lncRNA candidates were selected for further analysis based on predicted toxicity and efficacy. Antisense oligonucleotides (ASOs) were designed to knockdown (KD) target lncRNA expression, as well as ASOs targeting MALAT1, a lncRNA routinely implicated in NSCLC, and non-targeting ASOs. Per candidate, 5 ASOs were transfected into A549 cells and KD efficacy assessed via qRT-PCR. The anti-proliferative effect of the two most efficient ASOs was analysed in a live cell imaging system. In order to investigate target expression and prognostic utility in archival formalin-fixed paraffin-embedded (FFPE) patient tissue, the BaseScope in situ hybridisation (ISH) protocol was optimised for each target and applied to clinical cohorts. To investigate the immune microenvironment associated with the targets in KRASmt tumours, a multiplex immunofluorescence (mIF)-based macrophage panel, designed by UCD researchers is being adapted to lung cancer tissue. Following this, a co-detection technique combining mIF and ISH will be implemented.

Result and discussion

Of the 11 candidate lncRNAs brought forward, 7 displayed anti-proliferative effects in live-cell analysis. BaseScope ISH protocols have been optimised for both MALAT1 and one of the 7 candidates, GCAWKR in FFPE tissue. These protocols are now being applied to a wide cohort of patient tissue. Regarding immune contexture analysis, preliminary optimisation has been completed in lung tissue.

Conclusion

Several lncRNAs have demonstrated potential therapeutic effect on a cell line level. However, critical to their success as therapeutic targets, and potential prognostic utility, is their expression profile in patients and the correlation to patient outcome. Further, by establishing the relationship between target expression and the immune contexture we can better predict patient response to current and future immune targeting strategies, and offer novel strategies in cases where immunotherapy targets are absent.

EACR25-0890

Canine Melanoma as a Translational Model for Novel Immuno-Nanotherapy and Focal Adhesion Kinase-targeted Strategies in Human Oncology

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Introduction

Melanoma in dogs serves as a well-recognized model for human melanoma due to shared biological and molecular features. Canine melanoma (CM) exhibits aggressive biological behavior and affected dogs have a post-surgical survival time of less than one year. Given the conserved molecular pathways between canine and human melanoma, insights gained from this study may support the development of novel nanoparticle-based immunotherapies and focal adhesion kinase inhibitors (FAKi) for human melanoma patients. On one hand, nanoparticles demonstrate great potential as delivery systems for cancer vaccines, enhancing the co-delivery of tumor antigens and toll-like receptor (TLR) agonists to dendritic cells (DC), resulting in more effective immune system activation. On the other hand, FAK is a protein critical for several cellular processes, including cell survival, invasion, and migration. FAK is associated with advanced stages of cancer development, the presence of metastatic cells, and their increased mobility. Currently, there are no studies in veterinary research that investigated FAK expression in CM, neither the benefits of co-delivery of CM antigens and TLR using a nanoplatform or of FAKi for these patients.

Material and method

This project aims to test the potential of a canine glycoprotein 100 mannosylated-nanoparticle (Gp100-NP) and FAKi using *in vitro* and *ex vivo* models of CM. The polymeric Gp100-NP was formulated using the double emulsion technique. Its physicochemical characteristics were described, and *ex vivo* assays with canine erythrocytes and bone marrow-derived DC evaluated its biocompatibility. FAK expression in CM was assessed using molecular and fluorescent methods. Gp100-NP uptake by primary canine DC was evaluated through flow cytometry and confocal microscopy. The impact of three different FAKi on CM was assessed through cell metabolic activity and migration assays using the TLM-1 cell line.

Result and discussion

Gp100-NP exhibited the targeted characteristics necessary to promote DC uptake ($-5 < \text{Zeta Potential} < +5$; Polydispersity index < 0.2 ; and Size $< 200 \text{ nm}$). Moreover, canine DC successfully and selectively internalized the Gp100-NP. It was found that FAK expression is higher in CM compared to healthy tissue. Mirroring findings in human melanoma, it reinforces FAK's role as a potential therapeutic target. The tested FAKi were able to reduce TLM-1 cell function and viability and appeared to delay cell migration capacity. The observed impact of FAKi on cell viability and migration suggests a conserved mechanism that may be exploited in future translational studies.

Conclusion

Further studies involving canine DC and primary T-cell cultures, alongside co-cultures with CM spheroids, will contribute to examining the benefits of this dual approach for treatment, enhancing its relevance and facilitating comparisons with human cases in support of a One Health strategy for broader benefit.

EACR25-0916

Detection of low frequency variants from low input FFPE samples with comprehensive tumor/normal exome sequencing

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Introduction

Molecular profiling of solid tumors through comprehensive tumor/normal exome sequencing represents a new paradigm in oncology research. As sequencing capabilities expand and costs decline, Whole Exome Sequencing enables comprehensive screening of tumor samples to identify novel biomarkers and actionable drug targets. Formalin-fixed, Paraffin-embedded (FFPE) tissues are used for retrospective and prospective studies in oncology labs. However, the fixation and processing of the tissues can damage and/or modify DNA making tumor genetic analysis challenging, especially for detecting low abundant somatic mutations.

Material and method

Illumina FFPE DNA Prep with Exome 2.5 Enrichment was tested on FFPE DNA. Libraries were prepared from 40 ng input from 12 tumor–normal pairs using this kit and the Twist Bioscience for Illumina Exome 2.5 Panel. Sequencing was performed on the NovaSeqTM 6000 System using the S1 flow cell.

Result and discussion

Libraries from High-mid quality tumor and matched normal DNA achieved $>100\text{M}$ and $\sim 28\text{M}$ single-end reads respectively. Tumor libraries from mid- to high-quality FFPE samples showed mean target coverage depth above $130\times$ and $\geq 90\%$ targets with $\geq 50\times$ coverage. All libraries achieved $>95\%$ aligned reads and above 70% read enrichment. SNVs and Indels at $\sim 5\%$ VAF, and low abundance CNV were detected at $\geq 90\%$ sensitivity. The performance of the kit detecting key oncology Biomarkers; TMB, MSI and HRD was also assessed.

Conclusion

Illumina FFPE DNA prep with Exome 2.5 enrichment is a novel enrichment library preparation method that uses Unique Molecular Identifiers (UMI) for error correction and demonstrates exceptional performance. For Research Use Only. Not for use in diagnostic procedures.

EACR25-0933

Evaluation of the Therapeutic Efficacy and Immunomodulatory Effects of Iron Oxide-Conjugated Photosensitizer Nanoparticles in Glioblastoma

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Introduction

Glioblastoma (GBM) is an aggressive CNS tumor with a median survival of less than 15 months. A major challenge in nanoparticle (NP)-based GBM therapy is limited drug delivery due to the blood-brain barrier (BBB) and the immunosuppressive tumor micro-environment. To address this, we developed a novel sugar (CG) coated and photosensitizer methylene blue (MB) conjugated iron oxide (IONP) based NP, IONP@CG@MB. We investigated whether IONP@CG@MB, in combination with laser exposure, can suppress GBM progression by (i) inducing ferroptosis and apoptosis through reactive oxygen species (ROS) accumulation (ii) remodeling the immunosuppressive tumor micro-environment and (iii) enhancing nanoparticle delivery to GBM cells, ultimately leading to tumor suppression.

Material and method

IONP@CG@MB nanoparticles were synthesized via a hydrothermal reaction by loading iron oxide (Fe_3O_4 , IONP) with ortho-nitrophenyl- β -galactoside (CG) and subsequently functionalizing the surface with photosensitizer MB to generate photodynamic therapy (PDT). In vitro, GBM cell proliferation and apoptosis were assessed using cell viability assays, flow cytometry, and immunofluorescence staining, while ferroptosis was confirmed via Western blotting. A PBMC/U87-MG co-culture system was utilized to evaluate T-cell activation through flow cytometry. Statistical significance was determined using Student's t-test and ANOVA ($p < 0.05$).

Result and discussion

IONP@CG@MB combined with PDT significantly reduced GBM cell viability, and Ki67 staining indicated an attenuation in cell proliferation ability. Flow cytometry and immunofluorescence analyses confirmed an increase in both intrinsic and extrinsic apoptosis, along with elevated ROS accumulation. Western blotting further revealed the downregulation of GPX4 and Nrf2, consistent with ferroptosis induction. In a PBMC/U87-MG co-culture model, treatment with IONP@CG@MB enhanced CD3+ T-cell proliferation and activated cytotoxic T lymphocytes (CD8+Granzyme B+), indicating a pronounced immunostimulatory effect.

Conclusion

This study demonstrates that IONP@CG@MB, in combination with PDT, effectively suppresses GBM progression by inducing ferroptosis and apoptosis, enhancing ROS production, and remodeling the immunosuppressive tumor microenvironment. The treatment directly eliminates tumor cells and stimulates anti-tumor immunity, offering a promising strategy for GBM therapy. Future research will optimize nanoparticle design, elucidate the interplay between ferroptosis and

immune activation, and evaluate clinical translation potential.

EACR25-0968

M2 macrophages promote survival and proliferation of patient-derived gastric cancer organoids in a tumor biology-dependent manner

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Introduction

Gastric cancer (GC) is one of the leading causes of cancer-related mortality worldwide, with high incidence rates and poor prognosis due to its molecular heterogeneity and therapeutic resistance. In this regard, the use of functional models such as patient-derived tumor organoids can shed light on and allow these issues to be addressed on a patient-specific basis. In addition, tumor microenvironment plays a critical role in GC progression by modulating the immune response and facilitating tumor evasion. In particular, tumor-associated macrophages can adopt pro-inflammatory (M1) or anti-inflammatory (M2) phenotypes, with the latter promoting tumor aggressiveness.

Material and method

A prospective biobank of gastric tumor organoids (GTOs) derived from advanced GC patients was generated according to an in-house protocol from January 2019. The human monocytic THP1 cell line was used and differentiated into macrophages, which were subsequently polarized into M1 macrophages using IFN γ + LPS, and M2 macrophages using IL4. Macrophage polarization was assessed measuring M1 (TNF; CXCL10) and M2 (CD206; MS4A4A) markers by real-time quantitative PCR (RT-qPCR). The conditioned media from M1 (M1CM) and M2 (M2CM) macrophages were used to culture the organoids. To assess the effect of each conditioned medium on organoid viability and proliferation, flow cytometry apoptosis/necrosis and cell cycle assays were performed, respectively. M2 macrophages were repolarized into M1 phenotype by treatment with IFN γ and LPS.

Result and discussion

A biobank of more than 40 GTO lines has been generated covering all gastric cancer subtypes. For this study, 7 GTOs were used: 3 intestinal, 2 diffuse, 1 mixed and 1 undifferentiated, according to Lauren's classification;

with 2 of them being HER2-positive and 2 others presenting microsatellite instability. THP1 cells were differentiated into macrophages and properly polarized into M1 and M2 macrophages. A differential effect of M1CM and M2CM on GTOs viability and proliferation were observed. Concerning GTOs viability, M1CM promoted organoid cell death, significantly increasing the number of cells undergoing apoptosis and necrosis. With regard to proliferation, distinct effects were observed. While most GTOs exhibited an increase in the number of cells in the G2+S cell cycle phases in M2CM compared to M1CM, others experienced no difference, suggesting that the influence of M2 macrophages on tumor cell proliferation depends on tumor-specific biology. Treatment of M2 macrophages with IFN γ and LPS repolarized them toward M1 phenotype. Conditioned medium from these repolarized macrophages reversed the observed effects of M2CM on GTOs.

Conclusion

M2 macrophages promote survival and proliferation in most GTOs but showing an interesting heterogeneous effect possibly due to tumor-specific biology. Therapeutic strategies focused on repolarizing M2 macrophages into an M1 phenotype may improve GC management.

EACR25-0976

Single-cell spatial atlas of high-grade serous ovarian cancer unveils MHC class II as a key driver of spatial tumor ecosystems and clinical outcomes

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Introduction

The tumor microenvironment (TME) is a complex network of interactions between malignant and host cells, yet its orchestration in advanced high-grade serous ovarian carcinoma (HGSC) remains poorly understood.

Material and method

To investigate the relationship between the TME ecosystem topography, clinical prognosis, and molecular profiles in HGSC, we applied a multi-omics analysis combined with high-plex imaging at subcellular resolution across 280 advanced HGSC samples. By integrating DNA methylation, gene copy number, and mutational profiling, we established clinico-molecular profiles underpinning critical dynamics within the tumor ecosystem. We then used our image analysis pipelines to resolve the spatial TME at the single-cell level, identifying orchestrated multicellular domains that influence patient outcomes. To integrate single-cell spatial, molecular, and survival data, we developed CEFIIRA (Cell Feature Importance Identification by Random Forest), a custom machine learning tool that uncovers survival-associated features. We further exploited advanced single-cell and spatial transcriptomic

data and immunopeptidomic analyses to uncover the mechanisms underpinning immune activation.

Result and discussion

We present a comprehensive single-cell spatial atlas of metastatic HGSCs, integrating high-dimensional imaging, genomics, and transcriptomics. Utilizing 929 single-cell maps, we identified distinct spatial domains characterized by phenotypically heterogeneous cellular compositions. We demonstrated that immune cell co-infiltration at the tumor-stroma interface significantly influences clinical outcomes. Through spatial profiling of approximately 15.1 million cells across two independent cohorts, we observed that MHCII+ cancer cells within the immunogenic tumor and tumor-stroma interface (TSI) neighborhoods generate immune hotspots. These hotspots showed evidence of coordinated immune activity, particularly with T cells and antigen-presenting myeloid cells, emphasizing the pivotal role of cancer cell-intrinsic MHC class II (MHCII) expression at the TSI in driving immune landscapes. Our findings highlight that MHCII-positive cancer cells contribute to the formation of immune hotspots marked by increased activation of immune cells, impacting prognosis independently of clinicomolecular profiles. Validation with external datasets confirmed that MHCII-expressing cancer cells associate with immune infiltration and shape spatial tumor-immune interactions, further supporting the significance of MHCII in structuring the spatial TME and influencing patient outcomes.

Conclusion

Our comprehensive multi-omics atlas decodes the interplay between tumor and immune cells, shedding light on mechanisms of immune evasion thus paving the way for targeted interventions in HGSC.

EACR25-1014

Exosomes from urine: A multi-purpose system for urine liquid biopsy

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Introduction

Liquid biopsy is a method of collecting blood or other body fluids for the analysis of molecular alterations, tumor cells, and metabolites. Recently, urine liquid biopsy has gained in importance because of its less invasive sampling. Urine samples, in comparison to blood, allow more real-time monitoring, especially for cancer patients. Nevertheless, urine can rapidly hydrolyzed in a short time, which can result in the production of a large amount of ammonia and degradation of urine components requiring fast processing or stabilization.

Material and method

Urines were collected from 5 healthy volunteers. Each participant provided 10 mL aliquots of urine, that were stabilized in PAXgene Urine Liquid Biopsy Tubes at

room temperature or maintained non-stabilized at 4°C up to preliminary centrifugation procedures. Exosomes were isolated from stabilized and non-stabilized urines by ultracentrifugation. Biophysical characterization by AFM and DLS, protein analysis by Western-Blot, and RNA isolation were carried out in exosomes from stabilized and non-stabilized urines at the time of collection and after 7 days of storage.

Result and discussion

Protein detection showed a higher amount of proteins at the time of collection in non-stabilized samples in comparison to stabilized ones, but after 7 days, the profile reversed which indicates a degradation of proteins in unstabilized samples. AFM analysis confirmed the presence of exosomes both in stabilized and non-stabilized samples at t=0 and after 7 days. After 7 days an increase in aggregates (particles larger than 200 nm in diameter) was observed both in stabilized and non-stabilized urine. In stabilized samples, a higher number of particles/ vesicles than in the non-stabilized samples was detected as a possible sign of decay in the latter ones.

Conclusion

PAXgene Urine Liquid Biopsy Tubes showed to be a multi-purpose collection device for urine liquid biopsy allowing the sampling of urine at room temperature for exosome isolation even after 7 days of storage at room temperature.

EACR25-1055

Chemotherapy during pregnancy – a prospective study of short- and long-term cardiac effects

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Introduction

Pregnancy-associated cancer poses a unique clinical challenge, requiring a delicate balance between risks and benefits to ensure the safety of both the mother and the fetus. The most commonly used chemotherapy classes during pregnancy are Anthracyclines, predominantly Doxorubicin (DXR), being the primary treatment used during the second and third trimester. However, clinical evidence regarding the short and late term effects of in utero exposure to chemotherapy is limited. The underlying mechanism of DXR-induced cardiotoxicity had been widely studied in cancer patients, there is paucity of data regarding cardiac effects of in utero exposure to chemotherapy. We had formerly demonstrated that DXR induces placental vascular toxicity. In the current study we aimed to characterize the short and long-term cardiac effects of in-utero exposure to chemotherapy.

Material and method

Pregnant ICR mice were injected with DXR (10 mg/kg) or saline on day E14.5 of pregnancy, which is equivalent to the second trimester in human. The in utero exposed mice were followed up from birth until full puberty at three time-points: day 0 (birth), 10 weeks and 6 months. Cardiac performance was evaluated at 10 weeks and 6 months mice via Transthoracic Echocardiography followed by euthanasia and cardiac tissue harvesting at all three-time points. Conventional echocardiographic measurements were obtained, as well as fractional

shortening (FS) calculation and 2D speckle tracking echocardiography. Tissue fibrosis and cardiac hypertrophy were evaluated by histological biomarkers by immunohistochemistry (IHC); Masson's trichrome (TRI) and wheat germ agglutinin (WGA) staining were utilized to evaluate cardiac collagen percentage and calculated cell area, respectively. Ethical approval of animal experimentation was received from the ethical committee of the Rapaport Faculty of Medicine, Thechnion.

Result and discussion

Echocardiogram imaging revealed at 10-week-old and 6-month-old mice reduced FS and thinning of the left ventricular (LV) wall compared with controls. 10-week-old male mice showed decreased ejection fraction (EF) and global longitudinal strain (GLS), accompanied by enhanced fibrotic markers reflected by TRI in IHC section. 10-week-old DXR exposed females displayed LV hypertrophy as evidenced by increased cell area calculated by WGA staining. At 6 months, both male and females presented lengthening of the left ventricular internal dimension (LVID), indicating LV dilation.

Conclusion

In utero exposure to DXR may result in a latent cardiac effect, manifested by reduced cardiac contractility and hypertrophy, in a sex-specific manner, as studied in a pre-clinical model. Future studies should focus on late-term surveillance in in utero exposed children to reveal the potential cardiac outcomes.

EACR25-1087

Incomplete ovarian function suppression (OFS) in premenopausal patients with oestrogen receptor positive breast cancer receiving GnRH agonists/antagonists whilst being treated with CDK4/6 inhibitors

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Introduction

Guidelines suggest ovarian function suppression (OFS) can be used in combination with endocrine therapies in the treatment of premenopausal ER+ breast cancer. OFS can be achieved through the use of GnRH agonists/ antagonists (GnRHa). Estradiol (E2) measurements are frequently used to define incomplete OFS, however there

is a lack of consensus regarding appropriate cut-off levels or assays. Incomplete OFS has important potential implications on the efficacy of treatment in the premenopausal setting. We are therefore conducting a systematic review to assess the risk of incomplete OFS in premenopausal women with ER+ breast cancer receiving OFS. A case report [1] was identified which described a possible interaction between abemaciclib, a CDK4/6 inhibitor, and a chemiluminescent microparticle immunoassay, which may have resulted in falsely elevated E2 readings. We present analysis of rates of incomplete OFS, as well as assay details, of a subset of studies identified within our systematic review where patients received GnRHa and CDK4/6 inhibitors.

Material and method

The full systematic review protocol has been registered with PROSPERO [2]. A search of MEDLINE and EMBASE was carried out to identify studies which included premenopausal women with ER+ breast cancer receiving treatment with GnRHa. The outcome of interest was measurement of incomplete OFS based on any definition. Titles and abstracts were screened by two authors, with no restriction on study type. Full text screening was completed by two of the authors, any disagreements were discussed with a third author.

Result and discussion

We have identified 125 papers in total for inclusion. Of these, several trials have been identified which included patients receiving both GnRHa and CDK4/6 inhibitors. These include the PENELOPE-B trial, the MONALEESA-7 trial and the PALOMA-3 trial. We will present analysis of rates of OFS, definitions of OFS, and E2 measurement techniques in the subset of included papers including patients treated with both GnRHa and CDK4/6 inhibitors.

Conclusion:

At least a subset of patients receiving GnRHa have incomplete OFS. There has been at least one documented case in the literature of elevated E2 levels which may have been due to an interaction between the assay and concurrent abemaciclib treatment. We summarise the available literature identified in the process of a systematic review in order to further understand current assays in use and rates of incomplete OFS in patients receiving CDK4/6 inhibitors and GnRHa.

[1] Kessler A J, Patel R, Gallagher E J, Shao T & Fasano J (2023) Discrepancies in estradiol levels in a premenopausal woman receiving abemaciclib despite ovarian function suppression and bilateral salpingo-oophorectomy. *Current Problems in Cancer: Case Reports* 9, doi: doi.org/10.1016/j.cpcr.2023.100224

[2] and is available at: www.crd.york.ac.uk/PROSPERO/view/CRD42023395920

EACR25-1101

BR55: Clinical Development of the First Ultrasound Molecular Imaging Agent for angiogenesis

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Introduction

Microbubbles (MB) have revolutionized ultrasound imaging by enhancing contrast for better visualization of

blood flow and tissues. They enable real-time imaging, are safe, cost-effective, making them an invaluable tool in modern medical imaging. MB can be engineered to target specific tissues expressing molecular markers, enhancing the ability to diagnose and monitor diseases at a molecular level, an approach known as molecular imaging. Bracco has been actively involved in this field, with its advanced UltraSound Molecular Imaging agent BR55, a propirety molecular imaging contrast agent of angiogenesis targeting the Vascular Endothelial Growth Factor Receptor 2 (VEGFR2). This lecture will summarize BR55's key achievements, updates on the Phase 2b clinical trial completed in 2024, and outline the next steps towards potential commercialization.

Material and method

The affinity of the VEGFR2 binding peptide was evaluated on immobilized recombinant human and mouse receptors, while the binding efficiency of BR55 was tested on cells expressing VEGFR2 and in vivo tumor models. In vivo imaging performance of BR55 was compared to non targeted clinically approved ultrasound contrast agent. In animals receiving anti-angiogenic treatment, the tumor response was monitored using BR55. Finally, Bracco sponsored the clinical evaluation of the molecular imaging performance of BR55 in ovarian, breast, prostate and thyroid cancer.

Result and discussion

BR55 is a targeted lipid-shelled microbubble specifically developed for molecular imaging of angiogenesis thanks to the use of a high affinity targeting peptide. The binding specificity was demonstrated on recombinant proteins as well as on human and mouse endothelial cells. BR55 demonstrated potential in detecting various cancers in animal models by highlighting areas of angiogenic vessels expressing VEGFR2. Additionally, preclinical models indicated that BR55 could provide early indications of tumor response to antiangiogenic therapies. The clinical exploratory phases across different disease conditions, demonstrated BR55 safety and ability to localize malignant lesions in prostate, breast, and ovarian cancers. Recently, BR55 completed a Phase 2b clinical trial, involving five different diseases (breast, ovarian, and thyroid cancers, rheumatoid arthritis, and Crohn's disease). This trial further validated its efficacy and safety in a larger patient cohort ($n = 250$). The study showed an excellent concordance between BR55 enhancement in ultrasound molecular imaging and VEGFR2 expression in immunohistochemistry.

Conclusion

BR55's ability to provide detailed molecular imaging supports the move towards personalized medicine, where treatments can be tailored based on patient's tumor specific characteristics. The results from the clinical trial are expected to pave the way for potential clinical approval and ultimately commercialization.

EACR25-1130

PET imaging of PD1-positive MOLT-4T-cell lymphoblastic leukemia tumours in NRG mice using 89Zr-labeled pembrolizumab

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Introduction

Pembrolizumab binds to programmed cell-death protein-1 (PD1) on T-cells and inhibits its interaction with PDL-1 on tumour cells which enables a cytotoxic T-cell response. Pembrolizumab labeled with zirconium-89 (89Zr) could be used for PET imaging of pembrolizumab delivery to tumours which may help predict tumour response. The aim of this study was to evaluate and image the PD1-mediated delivery of 89Zr-labeled pembrolizumab to a human T-cell lymphoblastic leukemia tumour in NRG mice.

Material and method

Pembrolizumab was conjugated to deferoxamine (DFO) to complex 89Zr. PD1-positive MOLT-4 tumours were established s.c. in NRG mice. B-cell deficiency in NRG mice results in the mice having very low levels of IgG which leads to high spleen uptake of i.v. injected radioimmunoconjugates. The spleen uptake may be blocked by injecting an excess of non-specific IgG prior to the radioimmunoconjugate. Additionally, PD1 specific tumour uptake can be assessed by injecting an excess of unlabeled pembrolizumab to block PD1 on tumour cells. NRG mice with MOLT-4 tumours were injected i.v. with 2.0–2.5 MBq of 89Zr-DFO-pembrolizumab (20 µg) with or without a 40x excess of IgG to block spleen uptake or excess pembrolizumab to block PD1. PET imaging was performed 72 hours post-injection and the tumour, blood and normal tissues were collected and the percent injected dose per gram (%ID/g) was calculated.

Result and discussion

Pembrolizumab was conjugated to 5.4 ± 0.8 DFO and labeled with 89Zr to a specific activity of 0.15 MBq/ug. The labeling efficiency was $96.2 \pm 0.2\%$. Spleen uptake was very high in mice that did not receive excess IgG (50.3 ± 13.3 %ID/g). Spleen uptake decreased with the administration of excess IgG and excess pembrolizumab (4.0 ± 0.1 %ID/g; $p = 0.0008$ and 3.7 ± 1.2 %ID/g; $p = 0.0008$, respectively). Tumour uptake was modest without excess IgG (6.0 ± 1.0 %ID/g). However, tumour uptake significantly increased to 26.0 ± 4.4 %ID/g ($p = 0.0002$) when spleen uptake was blocked by IgG. This high tumour uptake significantly decreased with the administration of excess pembrolizumab (3.0 ± 1.0 %ID/g; $p < 0.0001$) demonstrating PD1-specific binding of 89Zr-DFO-pembrolizumab to MOLT-4 cells in vivo. PET images corroborated the biodistribution data as there was high spleen uptake that was greatly reduced by the excess IgG and excess pembrolizumab. Additionally, tumours were well-visualized by PET in mice that received excess IgG and the intensity of tumour uptake on the images decreased with the administration of excess pembrolizumab.

Conclusion

PD1-positive MOLT-4 T-cell lymphoblastic leukemia tumours were imaged by PET in NRG mice using 89Zr-labeled pembrolizumab. Achieving high tumour uptake required blocking of spleen sequestration by administering excess non-specific IgG while PD1-

specific uptake was demonstrated by administering excess pembrolizumab. These results are promising for PET imaging of T-cell malignancies using 89Zr-DFO-pembrolizumab.

EACR25-1133

Melting the Iceberg: Nanomedicines Overcoming Stromal Barriers and Recruiting Effector T Cells in Pancreatic Cancer

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Introduction

While immune checkpoint inhibitors (ICIs) have revolutionized cancer treatment, pancreatic cancer (PDAC) remains one of the most ICI-resistant tumors [1]. Its unique dense stroma and highly immunosuppressive tumor microenvironment (TME) limit immune cell infiltration and therapeutic efficacy. Here, we demonstrate how nanotechnology-based platforms overcome these barriers to drive an effective anti-tumor response against PDAC. We developed a crosslinked nanoconjugate carrying a focal adhesion kinase inhibitor (FAKi) to remodel PDAC stroma and a nanovaccine to recruit effective immune cells into the TME.

Material and method

We engineered a star-shaped, crosslinked polyglutamate FAKi (St-FAKi) conjugate to enhance FAKi solubility and promote higher accumulation in the TME. Using mannose-grafted PLA/PLGA polymers, we prepared a dendritic cell-targeted nanovaccine (NV) co-entrapping PDAC-associated antigens and immune regulators. NV physicochemical properties, including size, charge, and antigen loading, were extensively characterized. The anti-tumor effect of the nanomedicines was evaluated in vivo using the PDAC KPC-bearing mouse model. Antigen-specific responses and TME immune profiling were thoroughly analyzed.

Result and discussion

Stable and safe nanotechnology-based platforms were achieved. The combination of St-FAKi and nanovaccine led to significant tumor growth suppression, marked by the recruitment of effective CD8+ T cells and CD103+ DC, and modulation of the cancer-associated fibroblast phenotype. In the PDAC in vivo model, our nanomedicines achieved 100% survival at 80 days post-inoculation.

Conclusion

By unlocking the dense, immunosuppressive TME, these nanotechnology-based platforms significantly enhanced anti-tumor immune responses, resulting in tumor regression and improved survival rates in PDAC.

EACR25-1147**Tiny Warriors Against Tumors: A Nanovaccine Approach for Melanoma Brain Metastases**

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Introduction

Despite advancements with immune checkpoint inhibitors for metastatic melanoma, their benefits are limited to a small percentage of patients, with many experiencing immune-mediated side effects or disease relapse. These challenges stem from tumor-driven immunosuppression and poor T cell infiltration. To overcome these barriers, we developed a precision nanovaccine (NV) targeting dendritic cells (DC). This strategy aims to enhance melanoma-immune cell interactions and broaden the range of targeted immune cells in a site-specific manner, offering a novel approach to improve outcomes.

Material and method

We synthesized and characterized poly(lactic acid) and poly(lactic-co-glycol) (PLA/PLGA)-based NV using mannose-grafted polymers to deliver combinations of melanoma neoantigen and immunoregulators. NV physicochemical properties were fully characterized, including size, surface charge and morphology. The amount of melanoma antigens entrapped within NV was determined by HPLC. Immature DC were used to evaluate the impact of NV on cell viability. NV ability to target and trigger the activation of DC, was assessed in the lymph nodes of immunized mice. The NV anti-tumor effect was evaluated *in vivo* in two primary melanoma-bearing mouse models, and in a melanoma brain metastasis (MBM) mouse model, which included the immune profiling within tumor site by flow cytometry, before and after treatment.

Result and discussion

Polymeric NV were shown to have spherical shape with an average diameter of 180 nm, narrow polydispersity index, near-neutral surface charge, and high loadings of the immune regulators. Both subcutaneous and intranasal immunization induced the activation and maturation of DC within draining lymph nodes and triggered the systemic activation of neoantigen-specific cytotoxic T cells. Treatment with the combination of NV with PD-L1 modulators *in vivo* led to increased tumor inhibition in primary melanoma-bearing mice, with minimal systemic toxicity. Combination of the NV with the anti-PD-L1 antibody was the most effective, with maximal tumor growth inhibition, translated into high infiltration of cytotoxic CD8+ T cells into tumor microenvironment (TME) and reduced expression of immunosuppressor cells. At the metastatic disease, intranasal immunization of the NV combined with anti-PD-L1 led to the prevention of melanoma brain metastases. The

combination strategy led to 100% surviving at 52 days followed intracranial tumor inoculation, and recapitulation into a T-cell inflamed brain TME.

Conclusion

Altogether, the synergy between the nanovaccine and the anti-PD-L1 antibody provides essential insights to devise alternative combinations regimens to improve the efficacy of immune checkpoint inhibitors in metastatic melanoma, thus opening a new line for polymeric nanovaccines as potential tools to advance clinical response to advanced melanoma.

EACR25-1180**Beating Breast Cancer Brain Metastases: Leverage Nano-Immunotherapy to Re-educate Host Immunity**

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Introduction

Breast cancer remains the most common cancer worldwide and is the second leading cause of brain metastases after lung cancer. Breast cancer brain metastases (BrM) can emerge up to a decade after the successful treatment of the primary tumor, representing a significant clinical challenge. In addition, BrM patients face poor prognoses due to the difficulty of crossing the blood-brain barrier with current systemic therapies [1]. Cancer vaccines aim to re-educate the patient's immune system by stimulating T-cell responses to eliminate tumor cells while generating long-term immune memory to prevent metastasis and disease progression [2]. However, most cancer vaccines have not demonstrated clinical benefits as monotherapy for patients with advanced-stage cancers since their anti-tumor efficacy is blocked by compensatory immune escape mechanisms within the tumor microenvironment (TME). Although immune checkpoint blockades are approved for triple-negative breast cancer and other solid tumors, brain malignancies frequently exhibit resistance to anti-PD-1/L1 therapy, making disease management difficult [3]. Here, we hypothesized that the activation of host immunity using a dendritic cell-targeted nanovaccine, while modulating the focal adhesion kinase (FAK) function together with immune checkpoint blockade, can contribute to re-shape the "cold" suppressive BrM-immune-stroma network to a "hot" BrM-permissive landscape, allowing the extensive infiltration of effector T cells to reactivate the anti-tumor immunity.

Material and method

The synergistic immunotherapeutic potential of our nanovaccine, isolated and in combination with the FAK inhibitor (FAKI) and the immune checkpoint inhibitor αPD-L1 monoclonal antibody, was assessed in both primary and BrM immunocompetent EO771 mouse models.

Result and discussion

Mannose nanovaccine combined with FAKi strongly restricted EO771 primary tumor growth, and synergized with PD-L1 blockade, leading to long-term survival when compared with nanovaccine alone. This data was further validated in BrM immunocompetent-bearing mice.

Conclusion

This innovative approach discloses the synergy among the targeted cancer nanovaccine and immune modulatory and checkpoint therapies within the cold and immune-suppressive TME, which overall outcome may constitute a promising nano-immunotherapy for BrM patients.

- References: [1] Thulin A, et al. *Breast*. 2020;50:113-24. doi: 10.1016/j.breast.2020.02.007.
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- Acknowledgements: The project that produced these results received funding from the "la Caixa" Foundation under the grant's agreements LCF/PR/HR22/52420016 and LCF/HR24/52440018, in addition to funds from FCT-MCTES (UIDB/04138/2020, UIDP/04138/2020, PTDC/BTM-SAL/4350/2021).

EACR25-1187

Results of a Neuroscience Education Program in Adults with Cancer Pain: A Controlled Clinical Trial

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Introduction

Pain is an unpleasant sensory and emotional experience that may or may not be associated with tissue damage. It is the second most frequent symptom during cancer treatment due to toxicity, surgery, radiotherapy, and other factors. Given its multidimensional nature, pain involves cognitive mechanisms that require additional intervention strategies. Advances in pain neuroscience have led to a better understanding of this phenomenon, contributing to improved management of various painful conditions. The objective of this study was to determine the effectiveness of a neuroscience education program on pain compared to conventional management in individuals with cancer pain.

Material and method

An experimental study was conducted through a randomized controlled clinical trial with parallel groups and evaluator blinding, following CONSORT guidelines and registered in ClinicalTrials.gov [1]. An educational protocol based on the principles of pain neuroscience was developed, consisting of nine sessions covering topics such as the pain system, the alarm system, pain

modulators, fatigue, anxiety and stress, current treatment models, as well as goal setting and achievements (Ordoñez-Mora et al., 2024). Patients in both the control and intervention groups were evaluated at 10 weeks (Ordoñez-Mora et al., 2023).

Result and discussion

A total of 66 patients with cancer pain were included, with breast cancer being the most prevalent type. Regarding cancer staging, 63% of patients were in stage III, 31.8% in stage IV, and 4.5% in stage V. Pain intensity was assessed using the Visual Analogue Scale (VAS). In the intervention group, the mean pain score at T1 (Time 1) 5.41 (± 2.13), which decreased to 4.0 (± 2.06) at T2 (Time 2), representing a reduction of 1.40 points in pain intensity ($p = 0.00$), a statistically significant and clinically relevant result. In the control group, T1 was 5.47 (± 2.30) and T2 a result of 5.39 (± 2.15), resulting in a difference of 0.078 without statistical significance ($p = 0.79$).

Conclusion:

This study demonstrated that an intervention based on pain neuroscience education leads to significant reductions in pain intensity, with a decrease of one point on the VAS, a change considered clinically relevant.

[1] Identifier: NCT05581784

EACR25-1190

PET-based and glycolytic biomarkers reveal 18F-FDG uptake/heterogeneity phenotypes and predict metabolically active disease in aggressive B-cell lymphomas

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Introduction

Radiomic features derived from 18F-fluorodeoxyglucose positron emission tomography (18F-FDG PET) non-invasively quantify 18F-FDG uptake and intratumoral heterogeneity in intact tumor regions. Despite evidence supporting the prognostic relevance of radiomic features, their biological underpinnings and potential to identify poor responders remain underexplored in aggressive B-cell lymphomas (ABCL). This study investigates associations between baseline radiomic features and glycolysis-related proteins, and their role in predicting metabolically active disease (MAD) on end-of-treatment (EoT) 18F-FDG PET.

Material and method

The immunohistochemical expression of glucose transporters (GLUTs) 1, 3, and 4, hexokinase 2 (HK2), lactate dehydrogenase A (LDHA), carbonic anhydrase 9 (CA9), and cluster of differentiation 147 (CD147) was assessed in 90 ABCL samples, including tumor tissue

and benign cells in the tumor microenvironment (BCTME). Sixteen textural and five semantic radiomic features were extracted from baseline 18F-FDG PET. Patients with Deauville scores 1–3 on EoT 18F-FDG PET were classified as having complete metabolic response, while scores 4–5 indicated metabolically active disease (MAD). Associations between radiomic features and glycolysis-related proteins were evaluated, while similarity network fusion integrated semantic and textural features to reveal distinct uptake and heterogeneity phenotypes. Key predictors of MAD were identified through univariate logistic regression, followed by relevance-based redundancy filtering and random forest (RF) feature importance ranking. Machine learning models, including RF and support vector machine (SVM), were trained with the top-ranked predictors, using 10-fold cross-validation.

Result and discussion

GLUT3 and LDHA expression in BCTME was associated with a low-image-heterogeneity pattern. GLUT3 was associated with increased angular second moment and decreased entropy values, while LDHA was predominantly expressed in instances with low sum variance and sum average values. A higher proportion of CA9-positive and CA9/CD147-positive BCTME was observed in cases with low metabolic tumor volume (MTV) and high image heterogeneity (low-uptake/high-heterogeneity phenotype). The top-ranked predictors of MAD included the textural feature inverse difference moment (IDM), MTV, staging, and HK2 expression in both tumor and BCTME. The SVM model combining these five features to predict MAD achieved an AUROC of 0.850 (95% confidence interval: 0.811–0.889) in cross-validation folds.

Conclusion

GLUT3, LDHA, CA9, and CD147 expression is associated with semantic and textural features of baseline 18F-FDG PET. Moreover, HK2 and IDM act as potential molecular and imaging biomarkers for MAD prediction in ABCL.

EACR25-1192

Engineered v5 PE6A-VLPs Enable In Vivo PCSK9 Gene Editing to Suppress Hepatocellular Carcinoma Progression and Modulate Cholesterol Metabolism

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Introduction:

The sixth-generation prime editor A (PE6A) is a precise and compact genome-editing tool capable of introducing targeted mutations, deletions, or insertions in living cells. Proprotein convertase subtilisin/kexin type 9 (PCSK9), a critical regulator of low-density lipoprotein receptor (LDLR) degradation and serum cholesterol levels, is also associated with hepatocellular carcinoma (HCC) progression. While targeting PCSK9 to manage hyperlipidemia is currently effective, a long-term gene editing therapy that offers patients the possibility to discontinue lifelong medication is still lacking. The naturally

occurring PCSK9 Q152H mutation, which reduces PCSK9 secretion and lowers serum cholesterol, presents therapeutic potential in modulating HCC growth that remains unexplored.

Material and method

We engineered PE6A for packaging into virus-like particles (VLPs) to achieve in vivo delivery. The expression levels of PCSK9 in tumor and adjacent tissues of HCC patients were analyzed by immunoblotting, qPCR, and immunohistochemistry in our clinical cohort. In vitro, PCSK9 knockout, overexpression, and mutation cell lines were constructed to explore the mechanism of PCSK9's effect on HCC cell growth. In vivo efficacy was assessed in mouse models via tail vein injection, evaluating serum cholesterol reduction.

Result and discussion

Technologically, we integrated the sixth-generation prime editing system with the fifth-generation VLP packaging system, showcasing improved production yield and robust editing efficiency in human and mouse cell lines. From a clinical perspective, elevated PCSK9 expression in HCC tissues was found to be associated with poorer overall patient survival. In vitro experiments revealed that PCSK9 knockout or Q152H mutagenesis led to increased LDLR protein levels and inhibited HCC cell proliferation. Moreover, in vivo studies demonstrated that PE6A-VLP administration resulted in decreased mouse serum PCSK9 levels and LDL-cholesterol.

Conclusion

PE6A-VLPs enable efficient PCSK9 editing in vivo, simultaneously lowering LDL-cholesterol. These findings position PCSK9 as a potential dual therapeutic target for metabolic and oncologic interventions.

EACR25-1207

Establishment and Characterization of a Diabetic Sarcopenia Model in Aged Mice: Insights into Metabolic and Muscular Dysfunction

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Introduction

Diabetic sarcopenia, a multifactorial condition characterized by concurrent muscle atrophy and metabolic dysregulation, is increasingly prevalent in the aging population. However, appropriate animal models to study the interaction between aging, diabetes, and sarcopenia remain limited. This study aimed to develop and validate a diabetic sarcopenia model in aged mice to assess the combined effects of glucocorticoid-induced muscle atrophy and diet-induced diabetes on muscle function, metabolism, and organ pathology.

Material and method

C57BL/6 mice aged over 12 months and young controls (6 weeks old) were allocated into eight experimental groups, including control, diabetes, sarcopenia, and diabetic sarcopenia models. Diabetes was induced via a high-fat diet (60% kcal from fat) for eight weeks, while sarcopenia was triggered through dexamethasone administration. Body weight, food intake, fasting blood glucose levels, and oral glucose tolerance were

monitored. Muscle strength was evaluated by grip strength and limb hang tests. Following euthanasia, organ weights were recorded, and muscle tissues were collected for gene expression analysis of muscle atrophy-related markers via RT-PCR and Western blot.

Result and discussion

In the aged diabetic sarcopenia (ODS) group, body weight gain was limited compared to the diabetes-only group but higher than the sarcopenia-only group, indicating metabolic disruption alongside muscle loss. Grip strength and limb endurance were significantly reduced in sarcopenia and diabetic sarcopenia groups, with the ODS group showing the most severe decline, suggesting compounded impairment in muscle function due to aging, diabetes, and dexamethasone-induced stress. Muscle loss rate analysis revealed the highest reduction in the ODS group, while organ index analysis indicated increased liver and fat mass in diabetic conditions, highlighting systemic metabolic alterations. Blood glucose analysis confirmed exacerbated hyperglycemia and impaired glucose tolerance in diabetic sarcopenia models, supporting the hypothesis that sarcopenia aggravates metabolic dysfunction. These findings demonstrate that the combination of aging, diabetes, and sarcopenia accelerates muscle atrophy and metabolic deterioration, providing a robust platform for therapeutic evaluation.

Conclusion

We successfully established a diabetic sarcopenia model in aged mice that mimics the complex interplay between muscle degeneration and metabolic abnormalities. This model offers a valuable tool for investigating pathophysiological mechanisms and evaluating potential therapeutic strategies targeting muscle preservation and metabolic regulation in aging populations with diabetes.

EACR25-1223

Engineering M13 Bacteriophages for Targeted Sonodynamic Therapy of Cancer

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Introduction

Sonodynamic therapy (SDT) is an emerging non-invasive approach for cancer treatment based on molecules, known as sensitizers, that once activated by ultrasound generates reactive oxygen species (ROS), leading to tumor ablation. However, low accumulation of sensitizer in tumoral mass have significantly reduce SDT therapeutic potential. This study explores the potential use of M13 bacteriophages as targeted nanovectors for selective sonodynamic eradication of cancer cells.

Material and method

M13 phages were genetically engineered to display the 7D12 nanobody, which specifically binds the Epidermal Growth Factor Receptor (EGFR), frequently over-expressed in various cancers. The modified phage,

M13D12, was further functionalized with fluorophores, enabling selective recognition of EGFR-overexpressing A431 cells while minimizing interactions with EGFR-negative controls. To enhance its therapeutic potential, M13D12 was chemically conjugated with Rose Bengal (RB), a well-characterized sonosensitizer, via the pVIII major coat proteins. The resulting nanovector, M13D12-RB, was designed to deliver hundreds of RB molecules per phage particle. Upon ultrasound activation, RB generates reactive oxygen species (ROS), leading to oxidative stress and subsequent cancer cell death. The therapeutic efficacy of M13D12-RB was evaluated in both 2D and 3D tumor models, focusing on cell viability and tumor spheroid integrity.

Result and discussion

M13D12-RB demonstrated strong specificity for EGFR-overexpressing cancer cells, leading to selective accumulation of RB within tumor-like structures. Upon ultrasound activation, significant sonodynamic cytotoxicity was observed, resulting in extensive cell death in 2D cultures and substantial disruption of tumor spheroids in 3D models. Notably, ex vivo imaging revealed deep tumor penetration and complete spheroid disaggregation following treatment. These findings highlight the advantages of M13 phage-based nanovectors, including their ability to efficiently target tumor cells and deliver a high payload of therapeutic agents. The combination of targeted phage display and ultrasound-triggered ROS generation provides a highly selective and minimally invasive approach, potentially overcoming limitations of conventional sonodynamic therapy.

Conclusion

The engineered M13D12-RB nanovector represents a promising platform for targeted sonodynamic therapy. Its high specificity, deep tumor penetration, and effective ultrasound-activated cytotoxicity suggest a novel and versatile strategy for treating hard-to-reach malignancies, including glioblastoma. Additionally, M13 phages are cost-effective and highly modular, allowing for easy functionalization with different targeting ligands or therapeutic agents. These advantages position phage-based nanovectors as a scalable and adaptable approach for precision oncology applications.

EACR25-1259

The Impact of Nanoparticle Size on the Enhanced Permeability-Retention Effect in Breast Cancer

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Introduction

Breast cancer remains the most commonly diagnosed malignancy and a major cause of cancer-related deaths among women. Nanoparticles (NPs) offer promising strategies for improving cancer diagnostics and enabling tumour-targeted drug delivery, often relying on the enhanced permeability and retention (EPR) effect.

However, the full potential of EPR and the optimal NP size for efficient tumour accumulation remains uncertain due to rapid opsonization in plasma, which alters NP size and surface properties. To address this challenge, we developed non-opsonizing brush polymers as NP surrogates and systematically investigated how NP size influences pharmacokinetics, biodistribution, and tumour accumulation in a triple-negative breast cancer (TNBC) mouse model.

Material and method

We synthesized heavily PEGylated, fluorescently labelled polymers with three distinct hydrodynamic diameters within the 15–60 nm range. A TNBC model was established by inoculating female Balb/c mice with 100,000 4T1 cells in the fourth mammary fat pad. All procedures were approved by the Latvian Animal Protection Ethical Committee of the Food and Veterinary Service (Riga, Latvia). Once palpable tumours formed, mice were intraperitoneally injected with equivalent doses of different-sized NPs ($n = 6$ per group) for assessment of pharmacokinetics (PK) and biodistribution (BD). Blood samples were collected at multiple time points over a 14-day period. After two weeks mice were euthanized and tissues (heart, kidney, liver, spleen, lung, tumour, and brain) were harvested and homogenized. Fluorometric analysis was performed to quantify NP concentrations in blood and tissues.

Result and discussion

The plasma clearance half-lives of NPs in tumour-bearing mice were indistinguishable from those in healthy controls, with the smallest NP exhibiting the longest circulation time (clearance $t_{1/2} = 3.0$ days). The tumour was the primary site of NP accumulation across all tested materials. Tumour-to-liver selectivity showed an inverse relationship with NP size, with the smallest NP achieving the highest tumour/liver concentration ratio of 4:1. Notably, tumour size did not significantly influence NP accumulation. Interestingly, NP concentrations in the spleens of tumour-bearing mice were significantly lower (2- to 4-fold) compared to healthy controls. Biodistribution to other organs remained largely unaffected by tumour presence.

Conclusion

The non-opsonizing nature of the investigated polymers facilitated prolonged circulation times, enabling an effective assessment of the EPR effect as a function of NP size. Tumour tissue consistently served as the primary site of NP accumulation, independent of tumour size, and did not significantly alter plasma clearance rates. These findings highlight the potential of non-opsonizing materials as valuable tools for studying tumour permeability and optimizing nanomedicine design for targeted chemotherapeutic delivery.

EACR25-1305

Positioning of MS-AP-030 as a clinical candidate in an adjuvant setting to delay metastasis in colorectal cancer

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Introduction

Given that 90% of cancer-related deaths worldwide are due to metastasis and that current anti-cancer drugs do not target metastasis, we were driven to explore the underlying biology of this process. The failure of clinical candidates aimed at metastasis in clinical trials has raised valid concerns regarding the effectiveness of target-based therapies and our understanding of metastasis biology. To address this issue, we took a different approach. Our research focuses on a phenotypic and translational strategy, backed by prospective clinical samples, retrospective clinical trials and animal models. We identified critical steps in metastasis and selected a few potential targets. Using AI-based *in silico* modelling, we identified several FDA-approved drugs for non-oncology indications that bind to at least two of our identified targets. One such drug, MS-AP-030, approved for chronic hyperlipidaemia, was shown to delay metastasis onset.

Material and method

We utilized our proprietary AI-ML platform, METSCAN®, to identify critical targets involved in metastasis. Next, we used AI-based protein-small molecule binding platforms to identify FDA-approved drugs, commonly applied for non-oncology purposes, that bind to at least two of these identified targets. These selected drugs were evaluated using our METAssay® platform to assess their anti-metastatic properties in colorectal cancer cell lines and primary colorectal cancer patient samples. Finally, the efficacy of MS-AP-030 was tested in animal models using the METVivo® platform.

Result and discussion

In vitro studies were performed at non-cytotoxic concentrations. MS-AP-030, the most effective anti-metastatic compound, promoted epithelial-to-mesenchymal transition (EMT) and invasion – key steps in metastasis. Additionally, we observed increased reactive oxygen species (ROS) levels and elevated HIF-1 α expression. Despite these effects, MS-AP-030 inhibited extravasation and blocked mesenchymal-to-epithelial transition and colony formation. In the Drosophila model, the compound significantly reduced metastasis with some impact on the primary tumour volume. Similar results were observed in the mice xenograft model, where MS-AP-030 treatment led to a notable reduction in lung and liver lesions.

Conclusion

Our findings suggest that MS-AP-030 promotes a mesenchymal phenotype to such an extent that the cells lose the ability to revert to an epithelial phenotype, ultimately preventing secondary site growth. We propose repositioning MS-AP-030 as a clinical candidate to delay metastasis in an adjuvant setting.

EACR25-1456

Whole exome sequencing of non-small cell lung cancers in a cohort of patients enriched for genetic tumor risk

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Introduction

The influence of inherited genetic risk on non-small cell lung cancer (NSCLC) and its impact on somatic mutation profiles remains unclear. As part of the National Network Genomic Medicine Lung Cancer, the Task Force 5 (TF5) "Genetic Tumor Risk" evaluates the molecular landscape of NSCLC in Germany to improve molecular diagnostics and personalized treatment strategies. With a cohort enriched for patients with multiple tumor diseases (45%), family history of cancer (71%) and 28% never smokers, our goals include investigating germline mutations, rare somatic oncogenic drivers and complex molecular alterations that are not yet well characterized in NSCLC, where the predominance of smoking-related risk often masks hereditary factors. Therefore, TF5 integrates whole genome sequencing (WGS) of blood-derived DNA and whole exome sequencing (WES) of formalin-fixed paraffin-embedded (FFPE) tumor samples, a promising yet technically challenging avenue for refining molecular profiling in clinical oncology.

Material and method

A new workflow was developed, integrating Sarek v3.2.2 from nf-core with TheSarekSnake, a containerized pipeline. It integrates multiple small variant callers with an ensemble approach for variant detection in samples with a tumor cell content (TCC) as low as 20%. An adaptive filtering strategy enhances sensitivity while prioritizing calls in known lung cancer hotspots. The analysis focused on 966 cancer-associated genes. In the exome, tumor mutational burden (TMB) and microsatellite instability (MSI) were assessed in samples with at least 40% TCC. Components evaluating somatic loss of heterozygosity, copy number variations, and mutational signatures are being developed for further therapeutic stratification.

Result and discussion

To date, 330 patients have been enrolled in TF5, of which 91 have already been analyzed for somatic alterations and had sufficient coverage. The most frequently mutated genes are TP53 (48.4%), EGFR (25.3%), CSMD3 (24.2%), KRAS (19.8%), LRP1B (18.7%), FAM135B (15.4%), and SPTA1 (14.3%). A total of 75 samples could be assessed for complex genomic alterations; 26.6% were TMB-H (≥ 10 mutations/Mb) and all but one of those patients had smoking history. Among the TMB-H, two had high MSI scores; one was confirmed as mismatch repair deficient by immunohistochemistry.

Conclusion

This ongoing prospective study provides insights into the molecular landscape of selected patients with NSCLC and suspected genetic tumor risk using a comprehensive sequencing approach of tumor and normal tissue. Thus far, our findings support the feasibility of using blood WGS as a reference for FFPE tumor WES in somatic variant detection in a clinical context, although tailored

filtering is needed to accommodate coverage differences while preserving sensitivity for actionable mutations. Our preliminary analysis suggests comparable mutation frequencies between our germline risk-enriched cohort and general NSCLCs.

EACR25-1501

Challenges in the Collection of Biological Samples from the Operating Room - The Champalimaud Foundation Biobank Experience

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Introduction

Biobanks in the health sector are fundamental to the advancement of biomedical research. However, the effectiveness of a Biobank depends on the quality, quantity and accessibility of these samples, as well as compliance with ethical and legal requirements, such as informed consent. The Champalimaud Clinical Centre (CCC) is an oncological Institution with associated research laboratories; thus, the Champalimaud Foundation Biobank (CFB) is a key infrastructure, mainly focused on cancer. The aim of this study is to perform a retrospective analysis of the logistic challenges encountered when collecting biological material from the operating room (OR) and elucidate actions to increase samples' accrual which could be effectively implemented.

Material and method

The CFB generates a list of "possible collections (PC)" every week after receiving the OR schedule, which are used for internal organizational purposes. For this study, all PC lists were reviewed as well as the procurement forms from 2023 and compared the number of samples collected (tissue and blood) with the PC. Then all the causes for not collecting were annotated.

Result and discussion

We have identified a total of 517 surgeries of interest, mostly in the digestive unit, for 2023, of which 82 were canceled for various reasons, resulting in 435 surgeries of interest performed. A total of 613 samples were collected: 217 tissue samples and 396 blood samples; thus, collection rates resulted in 49,89% and 91,03%, respectively. The most frequent reason for the lack of tissue collection was that the resected specimens were placed in formalin in the OR 90,09% (200/222), followed by 6,31% (14/222) due to the small tumor size, 2,25% (5/222) due to modification of surgery approach, 0,90% (2/222) due to the absence of macroscopic tumor and 0,45% due to the lack of identifiable consent at the moment of surgery. This analysis revealed practices in the OR that could be easily avoided by having a closer communication with the Biobank, such as adding formaldehyde for sample fixation and not sending fresh specimens for biobanking. The inability to identify the informed consent at the moment of surgery, although minimal in our case, prevents the ethical use of the samples and could be guaranteed with a better control of

the consenting workflow. Collection was not possible due to external Biobank factors in almost 10% of the cases.

Conclusion:

This analysis reinforces the need for improvement in collection practices, most of which are feasible with better organization.

EACR25-1527

Deciphering intestinal zonation and its role in tumour initiation, evolution and progression in mouse models of colorectal cancer

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Introduction

The spatial regulation of Wnt signalling and stem cell dynamics across intestinal regions remains incompletely understood, in particular the transition away from normal tissue homeostasis during tumour development. Previous studies, primarily using single markers to define overall pathway activation, have suggested that Wnt activity and canonical stemness are elevated in the small intestine (SI) compared to the colon.

Material and method

In this current study, we integrate bulk and single-cell RNA-seq, with spatial transcriptomics to assess phenotypic changes along the full intestine during normal-precancer-cancer transitional stages. To investigate how genetic alterations shape intestinal biology and tumour evolution, we analysed a novel mouse tissue cohort spanning normal, pre-cancer, and cancer states across 24 genetically engineered mouse models (GEMMs).

Result and discussion

The transcriptomics analysis of normal intestine indicated that, Wnt activity is lower in the SI compared to the colon. Furthermore, while bulk RNA-seq indicates higher Wnt activity in the left-sided colon, epithelial-enriched spatial transcriptomics reveals a more uniform distribution across colonic regions, resolving inconsistencies caused by stromal contamination in bulk analyses. Phenotypic interrogation of the tumour transcriptomic data reveals that the primary driver of biological variation is tumour location (SI vs. colon) rather than genotype, with further class discovery identifying three biologically distinct tumour subtypes within each location. By comparing the phenotypic signalling landscapes of these tumour subtypes to their corresponding normal intestinal regions, we can segregate tumours and genotypes into those that either enhance their native microenvironment or override it entirely, leading to divergent molecular profiles. To maximize impact, we developed a Shiny-based data portal under FAIR principles, bridging mouse models with human colorectal cancer to enhance translational research and precision oncology.

Conclusion

By integrating multi-omics data, we propose an updated model of Wnt signaling and stemness zonation, capturing longitudinal (SI to colon) gradients. Our analysis of

normal and precancerous stages suggests that tumours from SI and colon develop through distinct regulatory mechanisms. By tracking tumour evolution from normal and precancerous states, we uncover key regulatory pathways missed in endpoint analyses, offering insights for targeted therapies.

EACR25-1704

Improving intraoperative tumor margin assessment in lung cancer surgeries using the MaSpec Pen technology

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Introduction

Curative-intent oncological lung surgery aims to enhance patient outcomes by achieving histologically negative resection margins. In clinical practice, tumor margins are determined by a histopathological assessment using frozen sections. However, in lung cancer this approach is challenging due to the use of surgical staples for tissue resection, prohibiting sectioning of the stapled margin. MSPen technology is a promising alternative for surgical margin evaluation due to its ability to characterize rich molecular profiles from tissue rapidly and non-destructively. Here, we describe the use of MSPen for intraoperative analysis of the stapled margin of resected lung cancer tissues to enhance the accuracy of margin evaluation during surgery.

Material and method

The MSPen employs a droplet of water to directly analyze a tissue site upon contact. Here, we used a handheld MSPen to analyze freshly resected human wedges and lobes. Multiple MSPen analyses, above and below the stapled margin, were performed. Mass spectrometry analyses were performed in negative ion mode using a QExactive Exploris Orbitrap mass spectrometer at mass-to-charge ratio (m/z) range of 100-1000. Sampled areas were marked, sectioned, and histologically stained, followed by pathological evaluation. Mass spectra were subjected to statistical classification using the least absolute shrinkage and selector operator (LASSO).

Result and discussion

Mass spectra obtained from lung tissue analyses were rich in metabolite and lipid species, particularly in the parenchymal aspect. For example, analysis taken from a tissue region below the stapled margin, close to the tumor, showed high relative abundance of lipids in the m/z 600-1000 range. In the stapled line, a putative negative margin, lipids such as phosphatidylglycerol (PG) (34:1) and PG (36:2) were seen at high relative abundance. Analysis of the tumor tissue showed high relative abundance of (PE) (38:4) and phosphati-dylinositol (PI) (38:4). However, analysis along the stapled margin on the visceral pleura had lower relative abundances of lipids commonly detected between m/z 600-1000. In the m/z 100-400 region, metabolites

including glutamine, glutamic acid, and fatty acids (FA) such as FA (18:1) and FA (18:0) were also detected. A predictive model of the MSPen data was generated using LASSO and trained on datasets acquired from fresh frozen lung specimens. Results showed agreement with histopathology, with a prediction sensitivity of 91%, specificity of 95%, and overall accuracy of 93%. We are currently evaluating classification performance on MSPen data from lung surgeries.

Conclusion

Our results demonstrate the feasibility of the MSPen for rapid and accurate intraoperative assessment of lung cancer margin, highlighting its potential in guiding surgical decision making.

EACR25-1765

Equitable access to Oncology clinical trials in Europe

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Introduction

Less than 8% of adult patients affected by cancer globally participate in oncology clinical trials, and up to 15–25% in hospitals conducting the trials. Participation in clinical trials improves patients' outcome, healthcare organizations in general, country readiness for clinical trials, therapeutic innovations and economy. Inequalities in access to trials stem from patient identification, screening, and participation procedures. Moreover, 30% of the European population live close to borders that persists to be a major barrier for the access to clinical trials.

Material and method

We propose a vision where existing European consortia create a favorable environment to improve patients' access to clinical trials, supported by optimised organisations and technologies that respects individuals.

Result and discussion

We will present how the use of various data types can enable concrete advances in AI in the health sector, with various impact on patients, specifically:

- CGI-clinics, Trial Match 2, Klineo and DigitalECMT that focus on language and molecular models. They are deploying a suite of AI-powered devices in the hospitals and online to inform patients and caregivers about relevant clinical trials, and bring patients to trials.
- Spiderweb, PCM4EU and PRIME-ROSE that open and expand academic or industry promoted personalized

medicine clinical trials in up to 25 countries in Europe, to bring trials to patients.

We will present the AI and medical devices developed in these projects. We will discuss how it can be organised as a suite of solutions to improve molecular testing coverage in Europe, data interpretation and communication between Hospitals, and ultimately patients' inclusion in clinical trials.

Conclusion

These projects promote AI and medical devices to improve the information, access and experience of patients in personalized oncology clinical trials in Europe. They have the potential to increase country readiness to Oncology clinical trial and therapeutic innovations and can benefit from a community awareness.

EACR25-1770

Enhancing Anti-Tumor Activity in Pancreatic Cancer by Targeting PI3Kδ and Autotaxin

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Introduction

Pancreatic ductal adenocarcinoma (PDAC) is a highly aggressive malignancy characterized by a dense desmoplastic stroma and an immunosuppressive tumor microenvironment (TME), both of which limit therapeutic efficacy. Immune evasion and fibrosis within the TME create substantial barriers to effective treatment. Key immunosuppressive cell populations, such as regulatory T cells and Arginase-1 (Arg1)-expressing M2 macrophages, suppress anti-tumor immunity primarily by inhibiting cytotoxic T cell activity. A critical enzyme regulating the immunosuppressive TME is phosphatidylinositol 3-kinase δ (PI3Kδ), which enhances regulatory T cell activation and migration while driving cytotoxic T cell exhaustion. Additionally, lysophosphatidic acid (LPA) signaling, driven by the enzyme autotaxin (ATX), impairs CD8+ T cell activation and proliferation. Elevated ATX levels in PDAC patients not only reinforce immune suppression but also promote fibroblast and immune cell migration, further exacerbating tumor fibrosis.

Material and method

Here, we used two clinically relevant ATX and PI3Kδ inhibitors to simultaneously enhance anti-tumor immunity and remodel the tumor microenvironment (TME). To investigate the role of PI3Kδ and ATX in patient prognosis, we first analyzed human PDAC tissue microarrays. Additionally, we utilized a genetically defined KrasG12D-driven pancreatic cancer mouse

model (KPC mouse model) and an orthotopic mouse model, followed by histological examination and immunohistochemistry stainings of dissected tissues.

Result and discussion

The combination of ATX/PI3K δ treatment improved immune response, diminished fibrosis, and increased activated cytotoxic T cells, ultimately leading to decreased tumor progression and prolonged survival in PDAC. Moreover, combination of ATX/PI3K δ inhibitors with chemo- and immunotherapy further extended survival.

Conclusion

Our findings highlight the potential of this multitargeted approach to enhance anti-tumor immunity and improve therapeutic outcomes.

EACR25-1786

Loss of the tumor suppressor NUMB drives aggressive bladder cancer via hyperactivation of the RHOA-ROCK-YAP pathway: implications for personalized prognosis and targeted therapy

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Introduction

Advances in the personalized management of bladder cancer (BCa) have been hampered by the lack of predictive biomarkers and targeted therapies. This study highlights a previously uncharacterized role for the loss of the tumor suppressor NUMB as a hallmark of biologically and clinically aggressive BCa disease.

Material and method

The relevance of NUMB expression variations to naturally occurring BCa was investigated in longitudinal retrospective patient cohorts of non-muscle invasive BCa (NMIBC) and muscle-invasive BCa (MIBC). In a mouse transgenic NUMB knock-out (KO) model, we explored the contribution of NUMB loss, both as a primary factor and in cooperation with carcinogenic insults, to the neoplastic transformation of the normal urothelium. Comparative transcriptomic and functional analyses in NUMB-proficient versus NUMB-deficient models, including the use of primary mouse and established human BCa cell lines in *in vitro* 3D-Matrigel organoid and *in vivo* xenograft studies, were used to reveal the molecular mechanisms underlying NUMB loss-driven bladder tumorigenesis and identify actionable vulnerabilities to reverse the aggressive phenotypes of NUMB-deficient BCa cells.

Result and discussion

Our clinical studies show that low NUMB expression in primary tumors correlates with increased risk of muscle invasion progression in NMIBC patients and poor survival in MIBC patients. In the NUMB-KO model, NUMB loss induces spontaneous malignant transformation of the normal urothelium, accelerating tumor onset and progression in response to carcinogenic insults. Mechanistically, NUMB loss disrupts the Hippo pathway, activating the YAP oncogene via a RHOA/ROCK-dependent axis, triggering an EMT program and aggressive proliferative and invasive phenotypes. Pharmacological and genetic inhibition of the RHOA/ROCK/YAP-TEAD axis selectively blocks proliferation, invasion, and tumorigenic potential of NUMB-deficient BCa cells, positioning the RHOA/ROCK/YAP axis as a promising vulnerable target for therapeutic intervention in highly aggressive NUMB-deficient human BCa. We also identify a 27-gene NUMB-less signature, characteristic of the NUMB-defective condition, which independently predicts risk of NMIBC-to-MIBC progression and associates with improved BCG (Bacillus Calmette–Guérin) treatment response in a cohort of 535 NMIBC patients enrolled in the UROMOL trial. These findings establish NUMB loss as a prognostic biomarker of aggressive BCa and a predictor of response to targeted RHOA/ROCK/YAP-TEAD therapy.

Conclusion

By identifying NUMB as a tumor suppressor in BCa and exploring the underlying biology of NUMB-deficient BCa aggressiveness, our findings pave the way to the development of new prognostic tools and targeted treatments, including the repositioning of already clinically available anti-ROCK/YAP drugs, thus advancing precision oncology for high-risk NMIBC and MIBC patients.

EACR25-1927

Enhanced Spatial Proteomics: High-Precision Analysis of the Tumor Microenvironment

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Introduction

Spatial biology is essential for understanding cancer progression and treatment by transforming complex cellular interactions into actionable spatial signatures. Protein biomarkers are key targets for spatial analysis, yet achieving high-plex spatial proteomics has been challenging. To address this, we developed VistaPlex™ Panels using EpicIF™ technology, offering a streamlined and scalable solution for spatial proteomic analysis. EpicIF™ technology enables efficient signal removal and iterative multiplexing without compromising sample integrity.

Material and method

VistaPlex Panels are used for multiplex immuno-fluorescence (mIF) analyses to precisely characterize the tumor immune microenvironment (TiME). These modular panels target key biomarkers for immune and tumor cell segmentation, tissue structures, and functional

markers. The panels are validated for sensitivity, specificity, and reproducibility using the CellScape™ Precise Spatial Proteomics platform across various carcinoma tissues. The workflow includes the EpicIF™ technology allowing rapid and complete photobleaching of fluorophores, ensuring high-fidelity signal removal between staining cycles.

Result and discussion

The VistaPlex™ panels enabled robust segmentation of immune, tumor, and stromal cell populations, supporting comprehensive spatial analysis of the TiME. We identified distinct spatial neighborhoods with immune infiltrates, including B-cells and T-cells, showing heterogeneous expression of developmental and activation markers. The assays mapped cellular interactions within the TiME, revealing immune infiltration patterns and tumor-immune dynamics. EpicIF™ technology ensured consistent signal removal, allowing iterative multiplexing without fluorescence carryover, thereby improving assay reliability and reproducibility.

Conclusion

By integrating VistaPlex™ Assays with advanced spatial proteomics and leveraging EpicIF™ technology for optimized signal removal, we provide a scalable solution for high-plex tissue analysis. This approach streamlines spatial biology workflows, enabling researchers to generate detailed spatial insights with minimal technical barriers. These assays set a new standard for tumor microenvironment characterization, accelerating discoveries in oncology and immunotherapy research.

EACR25-1934

Innovative Multiomic Spatial Biology Assay for Comprehensive Immune Profiling of the Tumor Microenvironment

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Introduction

Spatial biology has significantly advanced our understanding of cancer biology, highlighting the extensive transcriptomic and proteomic heterogeneity within the tumor-immune microenvironment (TiME). Traditionally, most analytical and diagnostic methods provide only a single type of biological readout per tissue section, making the generation of true multi-omic data challenging. To overcome these assay-based limitations and streamline the integration of multiplex experiments, we developed Enhanced Photobleaching in Cyclic Immunofluorescence (EpicIF™) technology. EpicIF™ technology allows for the quick, gentle, and complete removal of common photostable fluorophores *in situ*, enabling the rapid development and customization of novel spatial omics assays on the CellScape™ spatial biology platform. This technology facilitates the integration of multi-omic analyte detection within the same tissue sections. We successfully combined multiplex immunofluorescence (mIF) with the detection of RNA targets via HCR™ Gold RNA-FISH and the

detection of protein-protein interactions via *in situ* proximity ligation assay (isPLA), creating a tri-omic same-section experiment.

Material and method

This workflow centers around the new signal removal strategy, EpicIF™ technology, which effectively and rapidly removes fluorophores while leaving tissue and probe chemistries unaffected. Using this workflow on the CellScape™ platform, we iteratively performed spatial proteomic, transcriptomic, and interactomic assays on the same tissue sections. Formalin-fixed, paraffin-embedded tissue sections were subjected to standard histological processing and incubated with primary antibodies against PD1 and PD-L1. These were then treated with oligo-nucleotide-modified secondary probes designed for an *in-situ* Proximity Ligation Assay (isPLA) to detect PD1 and PD-L1 interactions. Next, RNA targets were detected using 12 HCR™ HiFi Probes, visualized by adding target-specific fluorescently labeled HCR™ Gold Amplifiers (RNA-FISH). Finally, spatial proteomic labeling was conducted with VistaPlex™ multiplex immunofluorescence (mIF) panels targeting over 30 biomarkers.

Result and discussion

The multi-omic assay was developed and tested on breast cancer samples of various subtypes. High-resolution imaging with the CellScape platform allowed for precise detection and quantification of isPLA, RNA-FISH, and mIF signals on the same slide. The combined assay revealed differential biomarker expression and interaction profiles associated with immune-regulatory processes in the tumor samples, showcasing remarkable inter- and intra-tumoral heterogeneity.

Conclusion

This spatial multi-omics approach provides a comprehensive insight into the complex interplay of immune and non-immune cell populations in the TiME.

EACR25-1966

Artificial intelligence predicts prognostic features of patient survival related to microvascular invasion and molecular classes of hepatocellular carcinoma

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Introduction

Hepatocellular carcinoma (HCC) is the most common form of liver cancer, with a 5-year survival rate of 30%. While surgical resection is an option for 20–25% of patients with early-stage HCC, ~50% experience recurrence within 3 years. This study aims to use artificial intelligence (AI)-driven tools to predict microvascular invasion (mVI) and HCC molecular subclasses from digitalized histology slides.

Material and method

A transformer-based deep-learning (DL) model was developed using digitized H&E slides from 431 surgically resected HCC patients (in-house training cohort). Here, 5-fold cross-validation was applied, and the model was then deployed on two test sets: TCGA-LIHC (TCGA) ($n = 363$) and advanced-stage HCC (aHCC) ($n = 64$). Cell-type fractions were estimated from bulk RNAseq data using BayesPrism algorithm.

Result and discussion

The pathological evaluation of mVI in the in-house training cohort was an independent predictor of overall survival (OS) ($p < 0.05$) in both uni- and multivariable analyses vs. other prognostic factors. For mVI prediction, the DL model achieved a median area under the curve (AUC) of 0.70 ± 0.08 in internal cross-validation and exhibited acceptable generalizability to the TCGA (AUC = 0.62 ± 0.07). Additionally, TCGA patients with predicted mVI had significantly worse median OS (4.9 vs. 7.6 years for mVI absence; $p = 0.003$). Moreover, HCCs with predicted mVI exhibited higher levels of proliferative hepatocyte-tumour cells and pro-tumorigenic immune cells, including SPP1+ macrophages ($p < 0.001$). These results were paralleled in HCCs with pathologically detected mVI, aligning with the known aggressive phenotype and immunosuppressive features of tumours with mVI. Furthermore, a separate DL model trained to predict molecular subclasses of HCC successfully distinguished poor-prognostic proliferative S1/S2 subclasses, from good-prognostic non-proliferative S3 subclass. Internal cross-validation achieved AUCs of 0.75–0.79 for S1, S2 and S3, with performance replicated in two test sets: AUCs of 0.72–0.80 in TCGA and 0.76–0.81 in aHCC. Notably, predicted S3 was linked to better survival in both TCGA (5-year OS: 52% vs. 44% in S1/S2; $p = 0.02$) and aHCC (3-year OS: 89% vs. 42% in S1/S2; $p = 0.02$). Predicted S3 HCCs displayed significantly higher levels of non-proliferative hepatocyte-tumour cells ($p < 0.01$), but less immune cells, including immunosuppressive TREM2+ macrophages and regulatory T cells (Tregs) ($p < 0.001$). Consistently, these results were mirrored in molecular subclasses of transcriptomically profiled HCCs.

Conclusion

Our AI models demonstrated capability to distinguish patient outcome-related biological features such as mVI and molecular profiles in HCC from a single H&E slide. These models have the potential of providing valuable prognostic insights to stratify patients with favourable or poor tumour biology and pave the way for AI-driven precision oncology in HCC.

EACR25-1973

Patient-derived HER2-positive gastric cancer organoids display differential response to targeted therapies correlating with patients' outcomes and serving as models to unravel treatment resistance mechanisms

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Introduction

HER2-positive (HER2+) gastric cancer is an aggressive form of gastric cancer (GC) characterized by an amplification of the ERBB2 gene and is found in approximately 10–20% of cases. Its identification is critical because it allows the use of targeted therapies that have improved patient survival. However, intratumoral heterogeneity and treatment resistance remain major challenges. Therefore, it is essential to develop tumor models that reflect the diversity and behavior of tumors in patients. In this context, patient-derived gastric tumor organoids (GTOs) represent an innovative preclinical model that allows the study of tumor biology and novel therapeutic strategies.

Material and method

A biobank of GTOs was established following an in-house laboratory protocol, including two HER2+ organoids (GTO20 and GTO50). To assess whether the GTOs maintained the primary tumor characteristics, an evaluation of HER2 expression was performed by immunohistochemistry (IHC) and copy number variation (CNV) analyses. Drug sensitivity assays to targeted therapy agents were performed through cell viability experiments and compared with patients' clinicopathological data to assess potential correlations in treatment response. To focus on the anti-HER2 therapy sensitivity, mass spectrometry-based proteomics and differential expression analysis was performed between resistant and sensible models.

Result and discussion

ERBB2 amplification was confirmed in both organoid models by CNV analysis. IHC further revealed heterogeneous HER2 overexpression in the organoid culture,

successfully reproducing the intratumoral heterogeneity observed in patient tissue. Drug sensitivity assays showed increased sensitivity to HER2-targeted therapies, trastuzumab and lapatinib, on HER2+ organoids compared to other organoids, but a striking differential response between them, with GTO20 being more resistant than GTO50. Indeed, GTO20 response was consistent with the patient's clinical history, as it was derived from a relapse after the patient became resistant to trastuzumab. Unfortunately, in the case of GTO50 no correlation could be established since the patient passed away before starting trastuzumab treatment. Exploring deeper into the possible molecular mechanisms underlying anti-HER2 drugs response in our models, quantitative proteomic profiling identified 272 differentially expressed proteins between GTO20 and GTO50. A network analysis grouped these proteins into 18 functional clusters, revealing enrichment in several biological processes, including protein folding, signal transduction, and DNA repair, which may play a role in the response to anti-HER2 therapies.

Conclusion

Patient-derived HER2+ GTOs could represent valuable preclinical models, as they accurately recapitulate the pathological characteristics of patients and allow functional studies on therapy resistance.

EACR25-1997

Enhanced photobleaching for fast and highly multiplexed immunofluorescence staining with broad reagent and assay compatibility

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Introduction

The demand for high-plex biomarker detection *in situ* has grown significantly in recent years, driving the development of various cyclic immunofluorescence staining techniques to expand biomarker plexity. These methods rely on the removal of fluorescence signals after each detection cycle, typically through chemical stripping of antibodies, photobleaching of fluorophores, or the use of barcoded antibodies. However, each approach presents unique limitations that pose challenges for the development, validation, and customization of multiplex immunofluorescence assays. To address these issues, we developed "Enhanced Photobleaching In Cyclic Immunofluorescence (EpicIF™ technology), an improved signal removal method that enables rapid, gentle, and complete bleaching of common photostable fluorophores *in situ*.

Material and method

In vitro and *in situ* experiments first assessed the effectiveness and specificity of the EpicIF solution. On formalin-fixed paraffin-embedded (FFPE) slides we performed multiplexed immunofluorescence staining using the CellScape™ platform. We then studied EpicIF solution on signal removal efficacy, as well as its effect on sample integrity and target stability by comparing signal intensity and signal-to-noise ratios between non-

bleached control slides and slides treated with EpicIF method.

Result and discussion

We introduce EpicIF™ technology to enhance fluorophore signal elimination by more than 30-fold compared to conventional photobleaching. This method effectively decolorizes a broad range of commercially available photostable dyes (e.g., AlexaFluor™, Atto-Tec™) both *in vitro* and *in situ*, overcoming their resistance to signal removal in iterative multiplexed staining applications. Additionally, we demonstrate its application in iterative multiplexed detection of protein targets using fluorescently labeled antibodies. Our results confirm that this approach preserves sample integrity and tissue antigenicity, even after multiple rounds of signal removal.

Conclusion

EpicIF™ technology enables rapid and virtually unlimited multiplexing in iterative immunofluorescence staining. By effectively eliminating signals from photostable fluorophores, this method expands the range of commercially available fluorescently labeled antibodies suitable for highly multiplexed *in situ* applications. Importantly, its ability to reliably remove fluorescence signals, regardless of the biomarker assay used, opens the door for a wide range of multiomic multiplexing approaches beyond immunofluorescence.

EACR25-2002

Preclinical combination of selenium nanoparticles and a nanovaccine demonstrates immunotherapeutic properties against luminal B breast cancer

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Introduction

Selenium (Se) is an element crucial for human health, with anticancer properties. Although bare SeNPs are unstable in aqueous solutions and prone to aggregation, stabilized SeNPs have been demonstrated to induce apoptosis in cancer cells and to have immunostimulatory properties. Therefore, the aim of this work was to access the main immune cell populations responsible for SeNPs immunotherapy against luminal B breast cancer.

Material and method

SeNPs production and stability: SeNPs were produced using sodium selenite, ascorbic acid as reducing agent and bovine serum albumin (BSA) as stabilizing agent. The stability of BSA-SeNPs was performed in human plasma, cell medium, pH 7.4 and 5.5. Therapeutic Intervention Study Design of the Combination Treatment of BSA1-SeNPs and the KRASwt Nanovaccine: An E0771 luminal B breast cancer orthotopic model was established to evaluate the antitumor efficacy of the combinational treatments of the KRASwt nanovaccine

and the BSA-SeNPs. Once the average volume of tumors reached \approx 50–100 mm³, mice were randomly divided into a control group and 4 treatment groups ($n = 6$ animals per group). KRASwt nanovaccine were subcutaneously administered to mice via injection proximal to both left and right sides inguinal lymph, on days 7 and 14 following tumor inoculation. BSA1-SeNPs were both intratumorally (i.t.) or i.v. administered at 1.25 mg kg⁻¹ every 2 days. Tumors and spleens were collected from mice ($n = 6$ animals per group) after euthanasia and homogenized in a single-cell suspension in cold sterile PBS. Cells were seeded in 96-well plates, washed with PBS, and incubated with Ghost Dye Red 780. Afterwards, cells were stained with extracellular and intracellular fluorochrome-labeled anti-mouse antibodies.

Result and discussion

SeNP were spherical, smaller than 50 nm, and with a narrow size distribution and stable in medium, plasma, and at physiological pH, maintaining their size for a prolonged period. Moreover, the combination of BSA-SeNPs with a KRAS-loaded nanovaccine resulted in a strong tumor growth reduction. Indeed, the synergistic effect of KRASwt nanovaccine combined with SeNPs was confirmed by the tumor growth inhibition of 62.2%, compared to 34% and 16.6% for SeNPs (i.t.) and KRASwt nanovaccine, respectively. This synergistic anticancer effect of the combined treatment significantly increased the tumor infiltration of both B, NK, and CD8+ T effector cells. Furthermore, the tumor infiltration of Tregs and PD1-expressing T cells were decreased for the combined treatment with the SeNPs and the nanovaccine.

Conclusion

Stable SeNPs at physiological pH and plasma were produced. Also, SeNPs presented anticancer properties in EO771-bearing mice, presenting synergy with a KRASwt nanovaccine. Therefore, this study offers valuable insights for the development of innovative combinatorial approaches using SeNPs to improve the outcomes of cancer immunotherapy.

EACR25-2012

Hyaluronic Acid-Coated Nanoparticles for Targeted Delivery in Colorectal Cancer: Unlocking the Potential of CD44-Dependent Therapy

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Introduction

The development of targeted cancer therapies is crucial to improve treatment outcomes and minimize side effects. CD44, a transmembrane glycoprotein, is overexpressed in many cancers, including colorectal cancer (CRC), where it plays an important role in tumor progression and metastasis. Nanoparticles (NPs) coated with hyaluronic acid (HA) have shown promise as vehicles for selectively targeting CD44-expressing cells and offer a potential approach for cancer therapy.

Material and method

We investigated the behaviour of HA-coated NPs (HA-NPs) in a translational model using peripheral blood mononuclear cells (PBMCs) from healthy donors and CRC patients. The safety and internalization of the HA-NPs were assessed by flow cytometry. The expression of CD44 and the CD44v6 variant, a specific isoform involved in cancer cell migration and invasion, was evaluated in colon biopsies and on PBMCs from CRC patients by immunofluorescence staining. CD44 was activated by pro-inflammatory stimulation and its effects on HA-NPs uptake were investigated. The study on human biopsies was approved by the clinical research ethics committee of the University of Naples Federico II and A.O.R.N. Antonio Cardarelli. All patients enrolled in this study provided written informed consent.

Result and discussion

HA-NPs did not affect the viability of PBMCs from both healthy volunteers and CRC patients, indicating a favourable safety profile. Furthermore, we observed that PBMCs from CRC patients had a significantly higher uptake of HA-NPs compared to those from healthy volunteers. More specifically, 28.81% of PBMCs from CRC patients internalized the NPs, whereas only 7.32% of PBMCs from healthy donors did. To investigate whether the tumor microenvironment (TME) could enhance NP uptake in PBMCs, we stimulated healthy PBMCs with phorbol myristate acetate (10 ng/mL) plus ionomycin (1 μ g/mL) to simulate the TME. Stimulated PBMCs showed a significant increase in HA-NPs uptake, which was correlated with increased expression of CD44 and CD44v6. These results suggest that the TME promotes the expression of CD44 and CD44v6. Indeed, we also found that CD44 and CD44v6 expression was higher in PBMCs from CRC patients, and this expression correlated with their levels in CRC biopsies from the same patients.

Conclusion

In conclusion, our results indicate that HA-NPs have a safety profile in PBMCs from healthy volunteers and CRC patients. In particular, PBMCs from CRC patients showed a significantly higher uptake of HA-NPs compared to those from healthy donors. Moreover, HA-NPs internalization was enhanced in healthy PBMCs when they were stimulated to replicate the TME. These results support the idea that the TME plays a crucial role in modulating HA-NPs uptake and could be exploited for more effective therapeutic strategies.

EACR25-2016

Building precision oncology via real-world data standardization, privacy preserving infrastructures, and collaboration among IT and clinical experts: TheDIGICORE network experience

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Introduction

Precision oncology necessitates personalized treatments maximizing efficacy while minimizing toxicity. Real-

World Data (RWD) presents a powerful, yet underutilized, resource to achieve this, offering insights complementary to clinical trials and facilitating cross-national comparisons of treatment effectiveness and practice variations. However, significant obstacles persist: data sparsity, unstructured formats, and limited digitization; complex and internationally diverse data privacy regulations hindering collaboration; and heterogeneous IT infrastructure and expertise across institutions.

Material and method

The DIGICORE (Digital Institute for Cancer Outcome Research) consortium – a network of European hospitals and companies – directly addresses these challenges. Its initiatives, among the DIGIONE research plan, include:

- (1) the definition of a set of minimum data elements related to electronic health record systems required to setup RWD studies (MEDOC)
- (2) a standardized survey assessing the digitalization of these key cancer-related data elements and their OMOP-standard adherence;
- (3) AI-based tools such as natural language processing to support data digitization;
- (4) a federated learning infrastructure, leveraging the Dutch Personal Health Train initiative, for privacy-preserving clinical research;
- (5) the development of standards for comprehensive cancer center infrastructure,
- (6) the establishment of disease-focused working groups to foster collaboration, and
- (7) dedicated training programs (IDEAL4OMOP) for clinicians to enhance their IT knowledge.

Result and discussion

To date, three projects have been completed, with additional 10 projects underway. The completed projects are:

Pan-cancer Study: This research, which involves 124,682 patients across Belgium, Germany, Italy, Norway, the UK, and the Netherlands, investigated the impact of the COVID-19 lockdowns on the number of new cancer diagnoses and estimates 12-month survival rates.

Lung Cancer Study: Conducted in Germany, Italy, Norway, the UK, and the Netherlands, this study included 1,294 patients. It aims to explore survival rates in patients with metastatic non-small cell lung cancer based on the location of metastases, treatment patterns by therapy line, and to benchmark care quality according to ESMO guidelines.

Breast Cancer Study: This study, involving 5,000 to 10,000 patients across Belgium, Italy, and the UK, focuses on HR+/HER2– metastatic breast cancer (mBC). It seeks to describe the demographic, clinical, molecular, and next-generation sequencing (NGS) characteristics of patients in two cohorts: those with de novo mBC and those with recurrence after locoregional disease.

Additionally, it compares HER2-low and HER2-zero cases within these cohorts.

Conclusion

DIGICORE's multi-faceted approach promises to unlock the transformative potential of RWD in advancing personalized cancer care

EACR25-2048

3D printing in microfluidic device prototyping for cancer research

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Introduction

Three-dimensional printing has enabled the fabrication of master molds for casting PDMS-based microfluidic devices used in in vitro cancer microenvironment simulation. This study presents a novel PDMS microfluidic platform, fabricated via a high-resolution 3D printed mold, designed to replicate complex cancer microenvironments using various human cancer cell lines.

Material and method

The master mold was manufactured using a Anycubic Mono 5s resin printer, achieving a printing resolution of approximately 10 µm in the z-axis. Numerous resins were tested during the development phase, with the best results obtained using TR250LV, biomedical resin, and 8k resin; among these, TR250LV proved to be the cost- and time-efficient option. The 3D printed mold features 15 chambers specifically designed to accommodate human cancer cells and includes all peripheral components such as tube adapters, degassers, and other essential features. The printing parameters provided by the resin producer for a 50 µm resolution were adjusted by reducing them by 40% and extending the resin flow time beneath the build platform, thereby achieving the desired 10 µm resolution. PDMS was subsequently cast onto the mold, cured, and peeled off to form the final microfluidic device.

Result and discussion

The PDMS microfluidic device derived from the 3D printed mold demonstrated excellent replication of the mold's intricate features, achieving channel resolutions approaching 10 µm. The combination of high-resolution 3D printed molds with PDMS casting offers a cost-effective and reproducible method for fabricating microfluidic devices that accurately mimic the cancer microenvironment.

Conclusion

This study successfully demonstrates the use of a commercially available 3D printer, the Anycubic Mono 5s, to fabricate a high-resolution master mold for PDMS-based microfluidic devices. The integration of 15 chambers and detailed peripheral components provides a robust platform for in vitro cancer microenvironment simulation.

Funding: "Innovative methods of biomedical data analysis and classification – the future of diagnostics" as part of the project "Supporting students in enhancing their competencies and skills", European Funds for Social Development Program 2021-2027, W131 - DB, AS. The "Innovative system for testing chemical compounds

(especially drugs) on human and animal cells based on microfluidics" project is carried out within the Proof of Concept (PoC FENG) programme of the Foundation for Polish Science co-financed by the European Union under the European Funds for Smart Economy 2021-2027 (FENG) – SS, MP.

EACR25-2094

Characterisation of the Colon Adenocarcinoma microenvironment with a focus on Natural Killer Cells

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Introduction

Colorectal Cancer (CRC) is the 3rd most common cancer worldwide. In patients with advanced CRC, standard treatment strategies have shown low effectiveness; thus, novel therapeutic approaches are needed for such patients to improve outcomes. Recently, Natural Killer (NK) cells have appeared as an alternative to T-cells as adoptive cell therapy due to their lesser secondary effects, strong cytotoxicity, and tumour microenvironment (TME) regulatory capacity. The main aim of this study is to evaluate the immune infiltrates in CRC tissue specimens, with a focus on NK cells, to better understand the role of these immune cells in tumour control.

Material and method

41 patients (18 females, 23 males) with mean diagnosis' age of 69.5 years (37-89 years) underwent surgery for CRC. Tumour location corresponded to cecum (6), ascending colon (14), hepatic flexure (5), transverse colon (1), descending colon (1) and recto-sigmoid transition (12). Formalin-Fixed Paraffin-Embedded specimens were analysed using multispectral microscopy to assess NK cells, their subtypes and functionality, using NCAM1 /CD56 (NK cells), CD16 (cytotoxic NK cells), NKG2D (activated NK cells), CD3 (T cells), and Pan-Cytokeratin (tumour cells) as biomarkers. For each specimen, 10 regions of interest were acquired using a multispectral camera. The images were generated with Nuance software and analysed using Fiji/ImageJ. Three tumour compartments (intraepithelial, intratumoral and periphery) were examined and correlated with clinicopathological patient data. 34 patients are alive without disease, 2 alive with disease, 3 dead of disease, 1 lost to follow-up and 1 dead of other causes after a mean follow-up of 72.1 months (13.4-123.5 months).

Result and discussion

Intraepithelial and periphery compartments displayed significantly less NK cells across all NK cells' subtypes than intratumoral regions. However, greater variation in NK cells number between patients was observed in the intratumoral area. As expected, most of the NK cells identified in the three compartments of the TME corresponded to regulatory NK cells. A significant correlation was found between the number of periphery NK cells ($p = 0.0456$) and periphery regulatory NK cells ($p = 0.0085$) with pathological stage, decreasing NK cells' numbers in higher stages. Notably, low numbers of intraepithelial activated NK cells were statistically associated with shorter survival ($p = 0.0491$). Although

non-significant ($p = 0.10$), low numbers of periphery NK cells correlated with poorer outcomes.

Conclusion

Results obtained in this pilot analysis revealed that a decrease in NK cell infiltration in CRC TME is significantly associated to disease progression and overall survival, highlighting the fact that analysis of NK cells may be an important prognosis biomarker in CRC. Furthermore, increasing the numbers of NK cells in advanced CRC could be an interesting tactic to control tumour progression and improve patient outcomes.

EACR25-2104

Tissue-Engineered Human 3D Tumor Models for Predictive Preclinical Anti-Cancer Therapy Assessment

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Introduction

Preclinical dropout rates in oncology remain alarmingly high (95%), largely due to the limitations of standard in-vitro systems, which fail to account for spatial orientation and essential stromal components. Chimeric animal models, while commonly used, often yield inaccurate results in humans, highlighting the need for better in vitro methods that replicate tumors and their micro-environment. To address this, we developed a biological xenogeneic matrix from decellularized porcine jejunal segments. This matrix has been successfully applied to various cancer types, including lung, colorectal, breast and pancreatic cancer. However, its biological nature presents challenges for platform standardization. Therefore, the development of novel scaffold materials is crucial to enable both efficient scalability and the preservation of the model's predictive capabilities.

Material and method

A conventional electrospinning process was modified, resulting in a highly porous scaffold. Since this approach allows for cell migration, matrix remodeling, and extracellular matrix synthesis, the scaffold provides an ideal platform for the generation of soft tissue equivalents. Within this study we compared the capacity of the novel material platform to generate tumor models to the previous established biological matrix. Through this comparative analysis, we aimed to assess its suitability for replicating key tumor characteristics and its potential for further applications in oncology research.

Result and discussion

With the successful generation of melanoma models on the novel scaffold material, we took an important step towards improving preclinical efficacy assessment of anti-melanoma therapies. These models, spanning different tumor stages and complexities, closely resemble physiological conditions and the *in vivo* situation with melanoma forming naturally at the basal layer of the stratified epidermis. Additionally, the 3D model captures

key aspects of the respective microenvironment, in which the melanomagenesis takes place. Building on this foundation, this approach was successfully extended to other tumor types, including breast, colon and pancreatic cancer. Overall, niche-specific responses and a stronger correlation to clinical outcomes were observed compared to traditional 2D and animal models.

Conclusion

The innovative scaffold serves as an industry-compatible platform for the generation of advanced in vitro tumor models, offering a reliable alternative to biological matrices. This diverse range of tumor models aligns with the 3R principle – Replace, Reduce and Refine – by minimizing animal testing, reducing experimental models, and enhancing preclinical study relevance. By meeting key preclinical testing requirements, this platform holds great potential for improving drug development and personalized medicine approaches in oncology research.

EACR25-2131

Nerve growth factor inducible (VGF) is a secreted mediator for metastatic breast cancer tropism to the brain

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Introduction

Brain metastases are one of the most serious clinical problems in breast cancer (BC) progression, associated with lower survival rates and a lack of effective therapies. Significant efforts have been made to identify players involved in this process and to understand how cancer cells directly modulate the brain PMN. However, it remains an unexplored and very challenging area of research. Therefore, in this work, we aimed to decipher the soluble factors secreted by BC cells responsible for the remodeling of the brain pre-metastatic niche (PMN), and how these modifications can impact the brain metastatic capacity of cancer cells.

Material and method

As models, brain organotropic BC cells (without or with HER2 overexpression), derived from the parental 231, were used. To control for brain metastatic specificity, the results were always compared with data obtained with 231.Lung and 231.Bone organotropic BC cells. Organotrophic BC cells were maintained in collagen-embedded 3D cultures and their secretome was collected for proteomic analysis. In parallel, the secretomes were used in the modulation of different components of the brain pre-metastatic niche, in vitro and in vivo, through the measurement of the blood-brain barrier (BBB) integrity and microglia activation, using complementary methods such as functional assays, western blot and immunofluorescence. VGF nerve growth factor inducible mRNA and protein expression was analyzed in the TCGA

database and in a series of primary tumors and metastases from patients with BC.

Result and discussion

We found that BC cells with specific tropism to the brain caused significant blood-brain barrier (BBB) disruption, as well as microglial activation, in both in vitro and in vivo models. Further, we searched for a brain-organo-tropic metastatic signature, as a promising source for the discovery of new biomarkers involved in brain metastatic progression. Of relevance, we identified VGF (nerve growth factor inducible) as a key mediator in this process, also impacting the BBB and microglial functions both in vitro and in vivo. In a series of human breast tumors, VGF was found to be expressed in both cancer cells and the adjacent stroma. Importantly, VGF-positive tumors showed a significantly worse prognosis and were associated with HER2 (human epidermal growth factor receptor 2) overexpression and triple-negative molecular signatures. Further clinical validation in primary tumors from metastatic BC cases showed a significant association between VGF and the brain metastatic location, clearly and significantly impacting on the prognosis of BC patients with brain metastasis.

Conclusion

Our study reveals a unique secretome signature for BC with a tropism for the brain, highlighting VGF as a crucial mediator in this process. Furthermore, its specific impact as a poor prognostic predictor for BC patients with brain metastasis opens new avenues to target VGF to control the progression of brain metastatic disease.

EACR25-2156

Organotypic Slice Cultures as a Feasible Predictive Model for Carboplatin Response in High-Grade Serous Ovarian Cancer (TOSCA Study)

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Introduction

Resistance to platinum-based therapies in High-Grade Serous Ovarian Cancer (HGSOC) is observed in 20–40% of patients and no predictive biomarkers exist. This represents a critical unmet need. To address this, we developed an ex vivo 3D organotypic slices culture

(OSC) model that preserves tumor architecture, aiming to provide a fast-track predictive tool.

Material and method

Surgical specimens were collected during primary debulking or explorative laparoscopic surgery from patients with advanced HGSOC treated at Fondazione Policlinico Gemelli IRCCS. Samples were embedded in 4% agar and sectioned into 350 µm-thick slices using a tissue chopper. Slices were cultured in an air-liquid interface for up to six days and treated with Carboplatin (Cp) at 100µM, which was the identified IC50 dose-response assay. The treatment was administered either as a single dose or a double dose (T0: day of surgery, T1: 72 hours post-surgery). Cell viability was assessed at the end of treatment using the CellTiter-Glo 3D assay. For histopathological analysis, samples were formalin-fixed and paraffin-embedded (FFPE), then stained with Hematoxylin and Eosin. Key parameters – including sclerosis, necrosis, fibrosis, tumor cell percentages, and immune composition – were evaluated. Patients' clinical outcomes were monitored to validate the model's predictive accuracy.

Result and discussion

OSCs were successfully generated from five patients. Cytotoxicity assays revealed that three patients exhibited high sensitivity to Cp, with cell viability dropping below 20% after the double dose. One patient showed an intermediate response, with viability decreasing from 62% after a single dose to 34% after the double dose. Notably, one patient displayed resistance to Cp, as cell viability unexpectedly increased from 44% with a single dose to 90% with the double dose – consistent with the patient's clinical outcome. Histopathological analysis aligned with these cytotoxic responses. In samples with reduced cell viability, FFPE-embedded organotypic slices showed decreased tumor cellularity and increased necrosis. Conversely, the resistant sample, which retained higher cell viability, exhibited preserved tumor cellularity without significant histopathological changes. Follow-up data from two patients – one sensitive and one resistant – further supported these findings, reinforcing the correlation between histopathological changes, cell viability, and response to Cp.

Conclusion

Organotypic slice cultures represent a promising ex vivo model for HGSOC, with potential for improving drug screening. Our findings highlight the model's ability to capture the heterogeneity of Cp response. However, validation in a larger patient cohort is needed. If confirmed, this model could be integrated into clinical trials to assess its effectiveness in guiding therapy selection.

EACR25-2178

Advanced Biomonitoring System for Colorectal Cancer Survivors: Enhancing Early Detection and Continuous Monitoring of Anastomotic Dehiscence

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Introduction

Colorectal cancer is the 3rd most incident and the 2nd deadly cancer worldwide, with surgery being the only potentially curative treatment. Within patients submitted to colorectal cancer surgery, anastomotic dehiscence (AD) is a significant cause of postoperative morbidity and mortality, after gastrointestinal tract reconstruction. Around 24% of patients undergoing distal rectal surgery are affected, with a mortality rate of approximately 7%. The need for intensive care and revision surgeries to treat complications arising from AD significantly prolongs hospitalization time and increases overall treatment costs. Early detection of AD is critical given the need for diagnosis in order to initiate timely treatments minimizing complications and associated costs. Therefore, we propose the development of a new implantable electronic system, not only for early identification of AD, but also for monitoring the anastomosis over time. This innovative biomonitoring system consists of an advanced adhesive in which sensors made of conductive metal alloys with highly elastic properties will be integrated. This study aimed to evaluate the in vitro cytotoxicity of several sensors with different components, as well as the biocompatibility in vivo of the most promising ones.

Material and method

According to ISO-10993, the sensors' cytotoxicity was evaluated in the VERO cell line using two methodologies, the direct contact and the extraction methodologies. The MTT and the SRB assays were used to evaluate the metabolic activity and protein content 24h after the cell's treatment. Furthermore, the two selected most promising sensors were subcutaneously implanted in 8–12 weeks old male Wistar rats, and the short-term local toxicity was evaluated 2 weeks after implantation [1].

Result and discussion

Our sensors composed of different metal alloys showed no considerable cytotoxicity in vitro in the two tested

methodologies. The two most promising sensors tested in vivo demonstrated a safe profile with minimal local toxicity observed.

Conclusion

These results support the suitability of the sensors for use in the proposed implantable biomonitoring system, demonstrating their potential. Nonetheless, further studies, including genotoxicity assessments and in vivo long-term toxicity evaluations, are necessary to fully validate their application. This innovative approach offers significant potential for enhancing early detection and continuous monitoring of AD in colorectal cancer survivors, paving the way for improved patient outcomes and quality of life, as well as reduced healthcare costs.

Funding: CIBB strategic projects 10.54499/UIDB/04539/2020 and 10.54499/UIDP/04539/2020, and Associated Laboratory funding 10.54499/LA/P/0058/2020 from FCT. Scholarship grant from FCT and European Social Funding 2021.05543.BD.

[1] Animal studies were approved by institutional and national authorities and were conducted according to the ISO 10993-2.

EACR25-2271

Intraductal Carcinoma of the Prostate- A Precursor Lesion?

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Introduction

Intraductal Carcinoma (IDC) of the prostate is in an in-situ lesion that has been well associated with worse prognoses. However, the pathogenesis of IDC is yet to be determined. The conventional retrograde invasion theory proposes that the malignant glands invade back into preexisting glands, forming the in-situ lesions.

Alternatively, we suggest that IDC is a precursor lesion that further invades the stroma. Characterising these transition areas between in situ lesions and invasive prostate cancer (PCa) glands could help confirm which hypothesis is more likely.

Material and method

75 samples from 46 PCa patients were selected from the IDC database at the Erasmus MC with the approval of the Erasmus MC Ethical Committee. The scoring of the samples was conducted using the H&E slides of the samples. Immunohistochemistry staining for basal cells (34BE12) was performed on these samples. Additionally, six tissue punches of regions containing transitions from Prostatic Intraepithelial Neoplasia (PIN), Atypical Intraductal Proliferation (AIP) or IDC to invasive cancer were fluorescently stained for Keratin 5 and Keratin 8/18 and then cleared. The z-stack was then imaged using an upright Leica SP5 confocal microscope.

Result and discussion

PIN occurred adjacent to IDC in 88.9% of the cases. Additionally, the basal cell stainings indicated that the invasive-like glands lying next to the in-situ lesions were also surrounded by basal cells, similar to in-situ lesions. These glands would have typically been classified as invasive, but due to the presence of basal cells, which are absent around invasive glands, we refer to them as invasive-like glands. Using 3D imaging, we captured spatial continuity between these in-situ lesions and invasive-like glands. Basal cells were also observed to be

scattered around these invasive-like glands and progressively disappeared around farther invasive glands. Based on these results, we propose a new precursor lesion model. According to the ‘Repetitive Invasion, Precursor Progression’ (RIPP) model, PIN is the primary precursor lesion. Upon accumulating enough genomic aberrations, PIN possesses the ability to repetitively invade into the stroma to establish invasive-like glands that eventually lose basal cells upon further proliferation and invasion. Simultaneously, PIN can accumulate more genomic alterations and form IDC structures within pre-existing glands. AIP is a transient stage between PIN and IDC. The newly formed in situ lesions can invade the stroma and form invasive PCa.

Conclusion

This study characterises the transition areas between in situ lesions and invasive PCa. We identified spatial continuity between in-situ lesions and invasive-like glands that eventually transitioned to invasive glands. We present the RIPP model based on these 2D and 3D imaging revelations.

EACR25-2352

Cancer cell therapy using gold-doped carbon dots for biorthogonal catalysis

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Introduction

Aiming to reduce off-target toxicity, recent advances in oncological pharmacology have focused on smart nanocarrier systems that enable controlled drug release at tumor sites, reducing. Carbon dots (CDs) have gained increasing prominence due to their excellent fluorescence properties, biocompatibility, and high cellular internalization. In parallel, biorthogonal catalysis has emerged as a cutting-edge strategy for targeted activation of pro-drugs directly in the tumor microenvironment, enhancing therapeutic precision while minimizing systemic toxicity.

Material and method

Gold-doped CDs (Au@CDs) were synthesized by a microwave-assisted method at 180 °C for 5 minutes. The synthesized CDs were purified and characterized using UV-Vis and fluorescence spectroscopy, FTIR, TEM, AFM, and XPS. The catalytic activity of Au@CDs was evaluated by activating the non-fluorescent pro-dye, pro-Rhodamine 110 (RH 110), using sodium ascorbate (SA) as a reducing agent. Cell viability was assessed on A375 melanoma cells using MTT assays, and confocal microscopy studies were conducted to visualize cellular uptake and provided insights into the intracellular localization of Au@CDs after 24-hour incubation.

Result and discussion

In the catalytic activation of pro-RH 110, fluorescence emission was detected immediately upon adding Au@CDs to the pro-dye solution, pointing to promising biorthogonal catalytic activity. Preliminary in vitro studies revealed that Au@CDs exhibited minimal toxicity to cells at concentrations up to 100 µg/mL. However, upon adding pro-RH 110 and SA, a significant reduction in cell viability occurred. This result can be attributed to the generation of reactive oxygen species (ROS) induced by SA, amplified by the gold nanoclusters present in Au@CDs. Due to high cell death, fluorescence conversion of pro-RH 110 could not be verified. Cells treated with Au@CDs exhibited strong intracellular fluorescence, especially in the nuclear region, suggesting effective cellular uptake and nuclear affinity.

Conclusion

This study explores Au@CDs in biorthogonal catalysis, specifically for activating pro-RH 110, a non-fluorescent derivative of RH 110. Preliminary results showed the catalytic potential of Au@CDs. Future research should optimize SA concentration and alternative synthesis methods for pro-RH 110 without reducing agents. Upon successful activation, subsequent steps will investigate pro-drug activation within cells.

FCT supports CIBB (10.54499/UIDB/04539/2020; 10.54499/UIDP/04539/2020; 10.54499/LA/P/0058/2020); CQC (10.54499/UIDB/00313/2020; 10.54499/UIDP/00313/2020; TEMA (10.54499/UIDB/00481/2020), UIDP/00481/2020 (10.54499/UIDP/00481/2020); Projects CarboNCT (10.54499/2022.03596.PTDC) and Chem4LungCare (10.54499/PTDC/QUI-QOR/0103/2021); PhD Scholarship from FCT and European Social Fund to CA-F (10.54499/2022.12228.BD).

EACR25-2367

A controllable and integrated pump system for high-precision microfluidics used for drug testing and cell cultures

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Introduction

Drug testing and cell culturing require precise environmental control, often dependent on specialized microfluidic equipment. A critical component of these systems is a high-precision pump regulating fluid substance movement. However, research is frequently hindered by outdated and unsupported software, limiting the usability of existing equipment. To address this, we

developed custom software compatible with modern systems while expanding functionality.

Material and method

We focused on two key devices: the New Era Pump System Model NE-1000 and the CorSolutions ECO nano Flow Meter. Both connect to a control computer via an RS-232 to USB adapter, enabling bidirectional data exchange. The control software, written in Python using the pyserial package, imports configuration parameters from a TOML file for easy customization. It features a graphical user interface (GUI) for accessibility and a text-only mode for automation. Commands can be sent interactively or as predefined sequences executing research programs with multiple phases. The system operates in a closed-loop configuration, dynamically adjusting flow rates based on real-time feedback. The GUI displays live flow rate data, active program phases, last executed commands, and system logs for streamlined supervision.

Result and discussion

The developed software restores the full functionality of the outdated equipment, integrating it seamlessly into research workflows. The intuitive GUI reduces user errors and enhances real-time decision-making. The software improves workflow efficiency and system scalability by consolidating multiple devices under a single lightweight program. Its modular architecture allows future expansions and easy adaptation to new research requirements.

Conclusion

Funding: "Innovative methods of biomedical data analysis and classification – the future of diagnostics" as part of the project "Supporting students in enhancing their competencies and skills", European Funds for Social Development Program 2021–2027, W131 - MO, AS. The "Innovative system for testing chemical compounds (especially drugs) on human and animal cells based on microfluidics" project is carried out within the Proof of Concept (PoC FENG) programme of the Foundation for Polish Science co-financed by the European Union under the European Funds for Smart Economy 2021–2027 (FENG) – SS.

EACR25-2395

A Multiomic and Highly Multiplex Method of Imaging Metabolites, Targeted Proteins and Targeted Transcripts on the Same Lung Cancer Tissue Microarray Using Novel Mass-Tagged Affinity Probes

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Introduction

Lung cancer is the second most common cancer and the leading cause of cancer-related deaths worldwide. Most lung cancer (80–85%) is non-small cell (NSCLC), comprising mostly adenocarcinoma and squamous cell carcinoma, while roughly 10–15% of lung cancer is small cell lung cancer (SCLC). Accurate subtype identification, for example, is critical for determining treatment.

However, conventional analyses such as immunohistochemistry (IHC) are limited to small numbers and classes of biomarkers imaged, thereby providing a narrow view of the tissue biology. More broadly, understanding the full breadth of tumor biology requires a spatial multiomic approach which can image all biomolecular classes, and

on the same sample, for a truly ‘holistic’ view of tumor microenvironments and tumor heterogeneity. Towards this end, mass spectrometry imaging (MSI) is uniquely capable of detecting small molecules in tissues including metabolites (e.g., lipids), glycans and even xenobiotics such as drugs/drug metabolites. However, macro-molecular MSI of proteins and mRNA presents several challenges. These include the need for *in situ* proteolytic digestion which generates highly complex biomolecular mixtures imaged at each “pixel” in the tissue. This results in signal suppression effects, often restricting detection to only the most abundant targets. While cross-platform approaches such as combining MSI with multiplexed immunofluorescence or spatial transcriptomics partially address this problem, image co-registration is difficult, and these approaches can require serial tissue sections comprising different cell populations.

Material and method

We report a unique, fully multiomic and highly multiplexed MSI approach on the same tissue section that encompasses label-free untargeted small molecules and targeted proteins and mRNAs using novel photocleavable mass-tagged antibody and oligo probes (MALDI-IHC/ISH).

Result and discussion

This approach was applied to lung cancer tissue microarrays (TMAs) and provided comprehensive spatial multiomic information from the same tissue sections using the same MSI platform. To better understand these high-dimensional results, we also demonstrate a novel computational workflow to integrate multiomic images of the same tissue section, scalable to an entire cohort of samples, and profile them through statistical and machine-learning analyses. This spatially resolved multiomic correlative analysis software, developed using Python/FIJI, is designed to rapidly profile entire cohorts from which multiomic/multimodal measurements were made. We show the applicability of this approach to understanding lung cancer and demonstrate the complex biological interactions between the various classes of tissue biomolecules.

Conclusion

This more complete view of tumor tissue biology is expected to lead to future mechanistic studies and ultimately, improved biomolecular signatures for precision medicine.

EACR25-2398

Establishing Robust In Vitro and In Vivo Models of Mesothelioma for Enhanced Translational Research

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Introduction

Malignant pleural mesothelioma (MPM) is a fatal cancer, and one of the primary risk factors remains asbestos exposure. Developing robust, safe, and efficient therapeutic targets and models that accurately recapitulate the disease is critical for improving MPM therapeutic strategies. This study aims to modulate nucleolin (NCL), a protein ubiquitously expressed in the cell nucleus and specifically present in the cytoplasmic membrane of some cancer malignancies, and develop a robust *in vivo* orthotopic model to explore additional mechanistic insights on tumor drug targeting as was successfully performed by our group previously [1].

Material and method

Murine malignant mesothelioma AB1, AB12, and AB22 cell lines were used to generate NCL-overexpressing models via lentiviral transduction of the pFUGW plasmids encoding NCL tagged to HA and membrane-bound NCL-overexpressing models via plasmid transfection using the pDisplay™ Mammalian Expression Vector. Inducible downregulated NCL models were generated upon DNA transfection of constructs carrying a reporter gene and an shRNA targeting the coding sequence of human NCL gene under a tetracycline-responsive promoter (TRE) and a puromycin resistance cassette under the human CMV promoter. Individual clones were generated via limiting dilution technique and were characterized by FACS, Western Blotting, and q-RT-PCR. Cells were visualized using the Axio Observer Z1 system while controlling temperature (37°C) and humidity. Balb/c mice were implanted intrapleurally with increasing concentrations of luciferase-expressing MPM cells (kindly provided by Dr. Marco Bianchi, San Raffaele Hospital, Milan, Italy), and tumor growth was evaluated by bioluminescence. Tumors and adjacent tissue were collected at the endpoint and included in OCT.

Result and discussion

All MPM cell lines showed positive expression of NCL in the cell membrane in over 50% of the cells. NCL over-expressing MPM cells display stable NCL expression over time, as detected by q-RT-PCR and western blotting. Specific membrane-bound NCL expression is currently being evaluated. Inducible downregulation of NCL was detected in pool transfected cells in the presence of doxycycline over time. Intrapleural administration of 0.5x106 AB1 cells generated tumors that recapitulated the course of disease, up to 13 days. No metastases were observed outside the pleural compartment.

Conclusion

These findings highlight the importance of versatile *in vitro* models that can be effectively manipulated to develop tailored and effective cancer therapies. New treatment strategies can be further evaluated by using this

robust *in vivo* orthotopic immunocompetent mouse model, an invaluable platform in the context of MPM. [1] doi: 10.1016/j.nantod.2021.101095

EACR25-2402

Cytotoxicity of novel multifunctional carbon nanocapsules

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Introduction

Neutron capture therapy (NCT) is a highly precise form of radiotherapy that currently exploits ¹⁰B isotopes to produce high linear energy transfer particles, leading to cancer cell death. Current drugs have substantial limitations for successful clinical translation, including low chemical stability, selectivity, and intracellular persistence during neutron irradiation, causing severe side effects. Multifunctional carbon nanocapsules (CNCs) represent an innovative approach for designing more efficient nanotherapeutic agents by exploring new active nuclides. For this purpose, we will explore CNCs that can accommodate high concentrations of ¹⁰B or Lithium (⁶Li)-active nuclides in their internal cavities.

Material and method

B or Li-filled CNCs were synthesized via solvent-free thermal treatment. The MTT assay was performed to evaluate the cytotoxicity of CNCs. For this study, phenotypically normal cells, the kidney epithelial cell line VERO (CCL-81), and lung fibroblast MRC-5 (CCL-171) cell lines were used. They were treated with 20, 40, 60, 80, and 100 µg/mL of compounds and then incubated for 24h or 72h at 37 °C.

Result and discussion

In MRC-5, higher concentrations (100 µg/mL) of CNCs loaded with Li and B resulted in drops of 20.0 ± 13.63% and 21.6 ± 13.97% after 24H, while at lower concentrations (20 µg/mL), the drops were only 10.80 ± 10.33% and 14.52 ± 12.86%, respectively. With 72 h of exposure, a more pronounced decrease was observed at 100µg/mL with 39.59 ± 5.14% and 42.79 ± 4.48 % Li and B loaded CNCs, respectively.

Conclusion

A reduction in cell viability of more than 30%, according to ISO 10993-5, is considered a cytotoxic effect. Thus, the tested CNCs did not show significant cytotoxicity when the cells were exposed for 24 hours. However, cytotoxicity was observed in Vero cells at high concentrations in a dose-dependent manner, particularly in the case of Li-filled CNCs. The CNCs showed an interesting cytotoxicity profile and potential for use in NTC by exploring new active nuclides.

FCT supports CIBB (10.54499/UIDB/04539/2020; 10.54499/UIDP/04539/2020; 10.54499/LA/P/0058/2020); TEMA (10.54499/UIDB/00481/2020, UIDP/00481/2020 10.54499/UIDP/00481/2020); Project CarboNCT (10.54499/2022.03596.PTDC), and; FCT and European Social Fund support CA-F (10.54499/2022.12228.BD).

EACR25-2404

A systematic review of Cancer spontaneous remission (CSR) and post-radiation abscopal responses (p-rAR)

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Introduction:

Part of radiation's curative power is as a form of "cancer vaccine", by releasing antigenic molecules that contribute to rejection of metastases outside the radiation field (p-r AR). Circulating-lymphocytes are sensitive to radiation, explaining why very-high-dose radiation (more effective at killing cancer cells) that needs 30-35 fractions in 6-7 weeks produces more lymphopenia and worse long-term survival than hypofractionation. Palliative radiation is investigating ultra-hypofractionation i.e. 1-3 fractions in one week rather than in more extensive fractionation schedules as pilot studies have shown increased p-r AR. This abstract compares p-rAR reports to those from reports of CSR

Material and method

This abstract updates systematic CSR and p-r AR reviews, comparing report frequency over 45 years and top tumour types. The p-r AR series reports actuarial survival.

Result and discussion

From 1970-2009, 31 confirmed CSR articles in 40 years (ie 0.78 papers per year). From 2010-2024, 50 confirmed CSR articles in 15 years (ie 3.3 papers per year. 32 lung cancer, 13 colorectal, 9 breast cancer, 29 cases of 8 other cancers). Six systematic reviews of CSR were identified, 3 lung, 1 colon, 1 hepatoma, 1 early cervix cancer (n = 1,481 CIN2, SR: 50.9%, CIN2/3 SR: 36.3%) First reported in 1969, p-r AR has increased from 14 literature reports in 29 years prior to 2010 (1 case in every 2 years) to 41 in 9 years up to 2019 (4.6 cases reported annually), and 33 cases reported in 5 years 2020-2024 (6.6 cases a year). The most recent p-r AR systematic review identified 58 cases up to 2019. On average, it takes 2 months to demonstrate response, the most frequently tumours being lung, renal, lymphoma and melanoma. Actuarial disease controls at 5 years was 39% in this series. There were 2 prospective studies of palliative

radiation, one in non-small cell lung cancer (3/9 PRs) and 2nd in Gastric cancer (4/14 PR)

Conclusion

There is an urgent need to undertake a randomised trial in patients requiring palliative radiation comparing standard of care currently 20 Gy in 5 fractions versus 8Gy in 1 fractions with and without Checkpoint inhibitors

EACR25-2411

3D Tumor Spheroids for Assessing Carbon Nanocapsule-Based Neutron Capture Therapy

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Introduction

Carbon nanocapsules (CNCs) offer a groundbreaking approach to improve the efficacy of multifunctional nanotherapeutic agents in neutron capture therapy (NCT). This study explored the potential of CNCs to encapsulate high concentrations of active nuclides within their cores to maximize therapeutic efficiency. To assess the interactions between CNC-based therapies and the complex tumour microenvironment, 3D tumour models of head and neck cancer were explored, enabling more realistic evaluations and *in situ* monitoring of this novel therapeutic approach.

Material and method

Spheroids of MRC-5 and FaDu cells were generated using an optimized protocol for three-dimensional cultures. Cells were cultured in their respective media and spheroid formation was promoted using the hanging drop technique. Following spheroid formation, CNCs were added at different concentrations (60 µg/mL and 100 µg/mL). The integrity of the spheroids was assessed using fluorescence microscopy, allowing the visualization of the cellular response to treatment and maintenance of the 3D structure.

Result and discussion

Head and neck cancer spheroids had well-defined, compact, and spherical structures with dense cellular packing, providing a favourable study model for new

CNCs. Spheroids with a size of approximately 1–1.5 mm were produced after 5 days of cell plating. Fluorescence microscopy images indicated that the integrity of the spheroids was maintained even in the presence of the test compounds at concentrations of 60 and 100 µg/mL. Minimal surface irregularities were observed compared with the untreated controls, with overall preservation of the 3D architecture.

Conclusion

Head and neck cancer spheroids provide a robust and physiologically relevant model for evaluating CNC-based therapies. The maintenance of spheroid integrity and 3D architecture, even at higher CNC concentrations, suggested good biocompatibility and minimal structural disruption. These results support the potential of CNCs as a promising platform for NCT, warranting further studies to explore their therapeutic efficacy and mechanistic interactions within the tumour microenvironment.

FCT supports CIBB (10.54499/UIDB/04539/2020; 10.54499/UIDP/04539/2020; 10.54499/LA/P/0058/2020); TEMA (10.54499/UIDB/00481/2020, UIDP/00481/2020 10.54499/UIDP/00481/2020); Project CarboNCT (10.54499/2022.03596.PTDC), and; FCT and European Social Fund support CA-F (10.54499/2022.12228.BD).

EACR25-2567

Imaging Mass Cytometry Detects the True Dynamic Range of Low-Abundance Biomarker Expression in Human Tumors

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Introduction

Detecting clinically relevant biomarkers in cancer tissues provides key insights into the unique tumor characteristics of patients, allowing for more personalized and effective therapies. Immunohisto-chemistry (IHC) is the gold-standard technique for biomarker detection and is widely used by pathologists to score low-abundance biomarkers (LABs) in tissues. Limitations related to complexity, quantitation and false signal detection are frequently observed using IHC and day-to-day variability due to multiple steps of signal amplification and pigment mistaken for true signal can misinform pathologists about LAB expression. Imaging Mass Cytometry™ (IMC™) technology is a multiplexed imaging technique that incorporates quantitative assessment of 40-plus biomarkers simultaneously on the same slide. The Hyperion™ XTi Imaging System, in association with an automated slide loader, permits 24/7 data acquisition and provides biological insights critical for assessment of the tumor microenvironment. We strove to determine whether IMC can be used for pathological evaluation of LABs and provides additional key biological insights for clinical evaluation offered through multiplexed analysis.

Material and method

We performed a comparison of IHC and IMC technology to detect clinically relevant LABs (PD-1, PD-L1, CTLA-4 and LAG-3) on human tumor tissue microarray and whole tissue samples. For IMC technology, we detected single cells using the Human Immuno-Oncology IMC Panel, which offers cell phenotyping of tumor and immune cell subtypes and their functional states. We

stained serial sections of tissues using the same antibody clone and generated IHC and IMC data, which was assessed by a board-certified pathologist. We conducted quantitative image analysis to detect LAB expression on single cells.

Result and discussion

Our results demonstrate that while IMC and IHC are similar in detecting LABs, IMC technology can accomplish this without signal amplification, offering an opportunity to evaluate LABs in their true dynamic signal ranges. Quantitative analysis of IHC and IMC data further demonstrated the equivalent performance of both platforms. Multiplexed single-cell analysis using IMC data provided insights about LAB expression on specific immune and tumor cells. While IHC is semi-quantitative and cannot reliably determine the high abundance of a target, IMC technology offers the opportunity of signal quantitation as it displays the complete dynamic range of signal.

Conclusion

Clinical assessment of tissues using IMC technology offers an advantage over traditional IHC methods by providing true biological context due to multiplexing capabilities. The ability of IMC to provide high-dimensional spatially resolved data makes it a powerful tool for clinical and translational applications and shows that it is poised to significantly contribute to biomarker discovery and drug development.

EACR25-2570

A deep-learning approach for tissue mapping and cell phenotyping in imaging mass cytometry data analysis

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Introduction

Imaging Mass Cytometry™ (IMC™) technology is a multiplexed imaging technique that generates high-dimensional spatial data at subcellular resolution without the complications of autofluorescence and cyclic imaging. IMC technology has two distinct whole slide imaging (WSI) modes: Preview Mode (PM) and Tissue Mode (TM). PM rapidly scans stained tissue to provide a comprehensive overview within minutes, while TM provides fast acquisition of the entire tissue at 5-micron resolution, mapping out the distribution of over 40 markers and revealing tissue heterogeneity. Both WSI modes enable researchers to make informed decisions about selecting tissue areas that warrant closer examination at single-cell resolution. Following PM, regions of interest (ROIs) are selected on the same slide for high-resolution imaging using Cell Mode (CM). This facilitates single-cell analysis of the ROIs identified during PM. These imaging modes together with an automated slide loader function support non-stop acquisition of tissue samples all week.

Material and method

Tissue sections of lung cancer were stained with a 40-marker IMC panel by combining the Human Immuno-

Oncology IMC Panel with the Human Immune Cell Expansion IMC Panel to study spatial organization and cellular interactions in the tissue. Images were acquired on the Hyperion™ XTi Imaging System (Standard BioTools), first in PM and then in CM with automatic selection of ROIs using Phenoplex™ software (Visiopharm®). ROIs were automatically selected based on three criteria: 1) tertiary lymphoid structures (TLS) expressing CD20 and CD3; 2) Granzyme B-rich areas and 3) areas with high number of CD68 and Vimentin double-positive cell clusters. An adjacent serial section was acquired in TM for a whole slide morphologic segmentation comparison.

Result and discussion

Tissue segmentation for all modes was performed using Phenoplex by training a deep-learning algorithm to recognize morphological features of the images, including the TLS. Single-cell analysis of the images generated in CM was performed using Phenoplex; cell segmentation was based on iridium DNA channels, and phenotyping was performed using the guided workflow. This data was used to compare the immune contexture through a series of t-SNE plots partitioned by spatial region and clinical variables.

Conclusion

Many mature TLS with proliferating germinal centers were present in the tissue. A high number of granzyme B-expressing neutrophils were found. Vimentin-positive macrophage-like cells were associated with the TLS. This work demonstrates that the Hyperion XTi Imaging System with its three acquisition modes (PM, TM and CM) can greatly advance the ability of IMC users to obtain answers from complex samples. The interactive capabilities of Phenoplex allow the user to quickly identify pertinent cell types, find them within a tissue map, define their spatial relationship and analyze their neighbors, leading to valuable biological insights.

Tumour Biology

EACR25-0012

Heterogenous tumor immune microenvironment in mismatch repair deficient colorectal cancer patients receiving immune checkpoint inhibitor

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Introduction

Mismatch repair deficient (dMMR) colorectal cancers (CRCs) are known to respond well to immune checkpoint inhibitors (ICIs). However, their heterogeneous drug responses and molecular characteristics remain undefined. Here, we conducted a functional transcriptome or spatial proteome analysis to define a heterogeneous immune context reflecting the response to ICIs.

Material and method

Functional transcriptome or spatial proteome analyses of 34 patients with dMMR CRC were conducted to characterize immune subtypes. 11 patients and validation

cohorts ($N = 9$) who received ICIs were further analyzed based on proteomic phenotypes.

Result and discussion

The cohort was dichotomized into “immune-low” group, and “immune-high” group based on transcriptomic data. The percentage of CD8+ TILs was significantly higher in the immune-high group than that in the immune-low group ($p = 0.0256$). The Nearest Neighbor (NN) distances from the CK+PD-L1+ tumor cells to the CD8+ TILs were significantly shorter in the immune-high group when compared to the immune-low group (immune-low vs immune-high, $p = 0.0076$). The NN distances from the CK+PD-L1+ tumor cells to the CD8+ TILs were significantly associated with the response to pembrolizumab in both internal ($p = 0.0114$) and validation cohorts ($p = 0.04762$). CD163+ cells were significantly enriched in the patients with resistance to pembrolizumab.

Conclusion

dMMR CRCs are immunologically heterogeneous, leading to a heterogeneous response rate to ICIs. CD8+ T cells determine the immune subgroups of dMMR CRCs and their response to ICIs. CD163+ macrophages can be considered as potential therapeutic targets. Here, our comprehensive data provide practical insights into predicting ICIs response for heterogeneous dMMR CRC subtypes.

EACR25-0020

The Effects of Palmitic Acid and Insulin as Diabetic Stress Markers in Breast Cancer Associated Fibroblast Model in vitro

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Introduction

Breast cancer progression is strongly influenced by the tumor microenvironment, particularly cancer-associated fibroblasts (CAFs), which play a pivotal role in tumor growth, invasion, and metastasis. Diabetes, a known comorbid condition, is characterized by metabolic stress markers such as palmitic acid and insulin, which may influence CAF activation and tumor progression. This study investigates the effects of palmitic acid and insulin as diabetic stress markers on the induction of the CAF phenotype in fibroblasts *in vitro*.

Material and method

MDA-MB231 and MCF-7 breast cancer cells were treated with palmitic acid in complete media for 48 hours, and the resulting conditioned media was used to treat human dermal fibroblasts at three increasing doses. Simultaneously, the same doses of palmitic acid were directly applied to fibroblasts without cancer-conditioned media. Negative control (vehicle-treated) and positive control (LPS-treated) groups were included for comparison. Fibroblasts were analyzed for gene and protein expression of CAF markers, tumor progression factors (e.g., angiogenic factors, MMPs, TIMPs, drug resistance), signaling pathways, and oxidative stress markers. Cell motility was assessed using a wound-healing assay.

Result and discussion

Results demonstrated a significant increase in the myoepithelial subtype of CAF phenotype in fibroblasts treated with cancer-conditioned media, accompanied by elevated expression of MMP8, GLUT4, and oxidative stress markers. Increased activation of the PI3K/AKT signaling pathway was also observed.

Conclusion

These findings suggest that palmitic acid-induced diabetic stress promotes CAF activation and enhances tumor-supportive functions, highlighting a potential link between diabetes and breast cancer progression. This study provides insights into the molecular mechanisms underlying the interplay between metabolic stress and the tumor microenvironment, offering potential targets for therapeutic intervention.

EACR25-0036

Tumor budding cells express Trop-2 as a driver of colon cancer cell migration and invasion

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Introduction

Trop-2 is a driver of metastatic diffusion of colorectal cancer (CRC) cells upon activation by proteolytic cleavage by ADAM10. Trop-2 activation leads to proteolytic inactivation of E-cadherin, with derangement of β -catenin signaling and disruption of cell-cell junctions. This suggested a direct role of Trop-2 on the acquisition of invasive capacity of CRC, as driven by the loss of E-cadherin and of cell-cell adhesion. Tumor budding was shown to play a role in CRC invasion at the tumor/stroma interface and to impact on disease outcome. We thus investigated whether Trop-2 is expressed by CRC budding cells and drives matrix invasion.

Material and method

We collected 80 primary cases of CRC in a test case series. Outcomes were assessed in a validation case series, that included 73 CRC patients. Histopathology was conducted in all CRC samples. Trop-2 immunohistochemistry (IHC) was performed using anti-framework and anti-activated antibodies. The prognostic impact of the expression of the Trop-2 protein within the tumor, in tumor budding cells (TB) and in poorly differentiated cell clusters (PDC) was determined utilizing Kaplan-Meier curves and Cox regression models. Multiplex Western blotting were performed on cleared cell homogenates, Immunofluorescence confocal

microscopy analysis was conducted in KM12SM cells cultured on glass coverslips. Wound healing assays were performed on transfected MTE4-14 cell monolayers. In vitro invasion assays were conducted on Matrigel matrix. Experimental tumors and metastases were analyzed in CRC xenotransplants in nude mice. Transcriptome profiling was used to reveal Trop-2-driven pro-invasive/pro-metastatic genes. Network analysis of Trop-2-driven pro-invasive/pro-metastatic genes led to the identification of malignant progression models.

Result and discussion

Our findings show that essentially all CRC TB and PDC cells express Trop-2. This associated with tumor stage and disease outcome. In particular, Trop-2-positive tumor TB and PDC associated with metastatic relapse in CRC patients in two independent CRC case series. Trop-2 was then shown to drive cell migration and matrix invasion upon induction of β -catenin signaling and inactivation of E-cadherin. Invasive processes were driven by Trop-2-induced expression of CDC42, phosphorylation/activation of PKC α at Ser657 and of Akt at Thr308. The highest impact on CRC patient prognosis was jointly shown by Trop-2, CDC42 and phosphorylated PKC α , supporting a model of Trop-2-driven CRC progression through a coordinated induction of migration and invasion determinants in tumor TB and PDC cells.

Conclusion

CRC TB and PDC cells were shown to extensively express Trop-2. This associated with tumor stage and disease outcome in independent CRC case series. Our findings candidate Trop-2 as a key determinant of CRC invasion and malignant progression and as a novel diagnostic and prognostic marker in CRC cases.

EACR25-0058

Perinuclear assembly of vimentin intermediate filaments induces cancer cell nuclear dysmorphism

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Introduction

Nuclear dysmorphism, characterized by crumpled or lobulated polymorphic nuclear shapes, has been used as an index for the malignant grades of certain cancers. The expression of vimentin, a type-III intermediate filament protein, is a hallmark of the epithelial-to-mesenchymal transition. However, it remains unclear whether vimentin is involved in cancer cell nuclear dysmorphism.

Material and method

To examine the role of vimentin in nuclear dysmorphism, vimentin gene was knocked out in breast cancer MDA-MB-231 cells and lung cancer A549 cells by the CRISPR/Cas9 system. The nuclear shape was visualized by immunofluorescence staining for the nuclear lamina. RNA-sequencing analysis was carried out to examine whether the transcription of certain DNA repair genes are affected by vimentin gene knockout in MDA-MB-231 cells.

Result and discussion

We found that vimentin intermediate filaments (VIFs) frequently accumulated at the concave of dysmorphic nucleus in breast cancer MDA-MB-231 cells. Depletion

of vimentin apparently restored the nuclear shape of the cells, which was devastated by re-expression of vimentin, but not its assembly-defective Y117D mutant. Depletion of plectin, a cytoskeletal linker, partially prevented the perinuclear accumulation of VIFs and concomitantly restored the nuclear shape of the cells. In addition, depletion of vimentin in lung cancer A549 cells largely prevented nuclear dysmorphism during the epithelial-to-mesenchymal transition induced by TGF β . Moreover, we found that VIF-mediated nuclear dysmorphism led to defects in DNA repair.

Conclusion

Our results unveil a novel role of VIFs in cancer cell nuclear dysmorphism, which is associated with genome instability.

EACR25-0061

Analysis of Perigallbladder Lymphatic Flow as a Pathway of Gallbladder Cancer Progression

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Introduction

Gallbladder cancer remains a disease with a poor prognosis and is often detected as an advanced cancer without specific symptoms in the early stages. In addition to direct invasion, lymphatic progression and hematogenous progression through the gallbladder vein into the portal branches P4 and P5 have been reported. However, there are cases in which peritoneal dissemination is confirmed even in cases without extra-serosal invasion, i.e., metastasis occurs even in sites not in contact with or directly exposed to the tumor. Therefore, in this study, we intraoperatively identified lymphatic flow pathways in cases of gallbladder cancer and cholecystitis/gallbladder stones, and considered the possibility that the pathway of gallbladder cancer progression is closely related to lymphatic flow.

Material and method

Laparoscopic cholecystectomy was performed in 57 cases of cholecystitis and cholezystolithiasis and 3 cases of gallbladder cancer at our hospital. Intraoperatively, indocyanine green (ICG) was injected into the serosa of the gallbladder base and neck, and the lymphatic flow pathways were compared by observing fluorescent staining. Based on the characteristics of the observed lymphatic flow pathways, we then aimed to gain new insights related to cancer progression.

Result and discussion

In cholecystitis and cholezystolithiasis cases, ICG coloration was observed in various lymphatic flow pathways depending on the degree of gallbladder inflammation. In addition, with the passage of time after the injection of ICG, fluorescent staining was also observed under the right diaphragm, suggesting the existence of a pathway for lymphatic flow from the gallbladder to reach the right diaphragm. In a case of gallbladder cancer, peritoneal dissemination was observed. In spite of the absence of extra-serosal invasion of the cancer, and the lymphatic flow in the neck of the gallbladder did not show fluorescent staining in that case, suggesting that lymphatic obstruction in the direction of

the gallbladder neck may promote peritoneal dissemination of cancer to the diaphragmatic side. Lymphatic obstruction in the direction of the gallbladder neck in gallbladder cancer can induce not only the development of lymph node metastases but also an abnormal pathway of tumor progression. The results of this study indicate that cancer progression may obstruct lymphatic flow in the gallbladder neck, resulting in greater allocation to lymph flow in the direction of the right diaphragm and the formation of metastatic pathways such as peritoneal dissemination.

Conclusion

We have shown that lymphatic obstruction in the neck of the gallbladder may alter lymphatic flow distribution, which may contribute to the formation of an abnormal pathway of cancer progression. Further case analyses are needed, and it is hoped that these findings will contribute to treatment strategies for gallbladder cancer.

EACR25-0120

The prostate specific SOCS1-deficient mouse model to study the gut-prostate axis in the pathogenesis of prostate cancer

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Introduction

Recent epidemiological data suggest that chronic inflammatory bowel disease (IBD) is a potential risk factor for prostate cancer by promoting systemic inflammation and microbial translocation. In IBD, dysbiosis promotes the growth of Enterobacteriaceae, which act as “inflammatory allies.” Pathogenic strains such as enteropathogenic *E. coli* (EPEC) contribute to this process by disrupting epithelial integrity and producing genotoxins such as colibactin, leading to DNA damage and oxidative stress in host cells. These inflammatory processes, however, are regulated multiple mechanisms. Suppressor of Cytokine Signaling 1 (SOCS1) is a key regulator of inflammation and is a tumor suppressor. Our preliminary data show that mice lacking SOCS1 in prostate epithelial cells (*Socs1fl/flPbCre* mice) develop hyperplasia and inflammation after puberty. In this study, we investigate the impact of experimental colitis and associated dysbiosis and systemic inflammation on the prostate glands of *Socs1fl/flPbCre* mice.

Material and method

Chronic colitis was induced in 8–12-week-old *Socs1fl/flPbCre* mice using dextran sulfate sodium (DSS). Disease severity was evaluated through disease activity indices. Prostate tissues were studied by histology and immunohistochemistry for oxidative stress, DNA damage, and inflammatory responses. Prostate organoids

were derived from SOCS1-deficient and control mice and exposed to colibactin-producing EPEC and its colibactin-deficient mutant. Oxidative stress, DNA damage, and inflammatory responses were evaluated in prostate organoids. Proteomes of prostate organoids from DSS-treated mice are being evaluated by mass spectrometry.

Result and discussion

DSS-treated *Socs1fl/flPbCre* and control mice exhibited comparable symptoms of chronic colitis, including intestinal epithelial layer erosion, crypt and goblet cell depletion, and inflammatory cell infiltration. However, the prostate glands of DSS-treated *Socs1fl/flPbCre* mice showed significant luminal epithelial shedding, dysplasia, peri-glandular inflammation, and diffuse hyperplasia, accompanied by markedly increased oxidative stress and DNA damage. Furthermore, organoids exposed to colibactin-producing EPEC displayed significantly higher levels of DNA damage and oxidative stress compared to those treated with the colibactin-deficient EPEC strain.

Conclusion

This study shows that DSS-induced colitis and microbial dysbiosis, particularly EPEC and its genotoxins, induce pathological changes in susceptible prostate epithelial cells that could lead to the development and progression of prostate cancer. The prostate-specific SOCS1-deficient mouse is a suitable model to study the gut-prostate axis and gene-environment interactions in prostate cancer pathogenesis, as well as to evaluate dietary interventions such as probiotics and nutraceuticals.

EACR25-0140

LncRNA PRKCQ-AS1 promotes cancer cell proliferation and inhibits etoposide-induced apoptosis via modulation of HOXA5

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Introduction

Long noncoding RNAs (lncRNAs) are major regulators of many cellular processes including cell proliferation, tumorigenesis and chemo-induced cell death.

Material and method

In this study, we report that the expression of lncRNA PRKCQ-AS1 is regulated by transcription factor E2F1 and it plays a role in S phase entry.

Result and discussion

Expression of PRKCQ-AS1 is cell cycle regulated and peaks near the G1/S transition. Inhibition of PRKCQ-AS1 expression in human cancer cells leads to a decrease in the number of cells in S phase and a concomitant increase in the number of cells in G1, suggesting that PRKCQ-AS1 plays a role in S phase entry. In agreement with this notion, prolonged silencing of PRKCQ-AS1 inhibits cells proliferation and, conversely, over-expression of PRKCQ-AS1 induces cell proliferation. Furthermore, PRKCQ-AS1 silencing enhances chemotherapy-induced apoptosis of cancer cells. PRKCQ-AS1 is a nuclear lncRNA and its silencing leads to an increase in the levels of HOXA5 mRNA. Importantly, knockdown of HOXA5 rescues the effect of PRKCQ-AS1 silencing on cell proliferation and etoposide-induced apoptosis.

Conclusion

In conclusions, our data identify PRKCQ-AS1 as a novel E2F-regulated lncRNA that regulates cell-cycle progression, cell proliferation and viability, at least in part via repression of HOXA5.

EACR25-0143

ACSL3-Mediated MUFA Activation Protects Dormant Breast Cancer Cells from Ferroptosis via Fatty Acid Metabolic Reprogramming

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Introduction

Metastatic cancer cells undergo unique metabolic adaptations that support survival in distant organs, differing from those in the primary tumor. Metabolic rewiring, including exogenous lipid uptake and utilization, activates pro-metastatic signaling through mechanisms like protein acetylation, chromatin remodeling, and fatty acid oxidation in metastatic cells. However, the precise metabolic shifts that sustain dormant disseminated tumor cells remain largely unexplored.

Material and method

To investigate metabolic adaptations related to tumor dormancy, we applied state-of-the-art metabolomic technologies to both in vitro and in vivo models of breast cancer metastasis. Stable isotope tracing assessed glucose and fatty acid (FA) metabolism, while gas chromatography-mass spectrometry (GC-MS) analyzed lipid composition. Genetic and pharmacological inhibition of de novo lipogenesis and acyl-coenzyme-A synthetase long-chain family member 3 (ACSL3) was performed to examine their impact on tumor dormancy, ferroptosis resistance, and metastasis. ACSL3 expression in breast cancer patient samples was evaluated for clinical relevance.

Result and discussion

Unlike proliferative disseminated breast cancer cells (DBCs), dormant DBCs are unaffected by FA uptake inhibition. Instead, they upregulate de novo lipogenesis, redirecting glucose-derived carbons toward FA synthesis. This shift enhances the activation and incorporation of

monounsaturated fatty acids (MUFA) into DBCs membranes through ACSL3, protecting them against lipid peroxidation and ferroptosis. Inhibiting lipogenesis or ACSL3 disrupted MUFA incorporation, shifting lipid composition toward polyunsaturated fatty acids (PUFAs), increasing lipid peroxidation, and triggering cell death. In vivo, ACSL3 or lipogenesis inhibition reduced dormant DBC survival and micrometastasis formation. Clinically, ACSL3 was overexpressed in quiescent DBCs in lymph nodes of breast cancer patients and significantly correlated with shorter disease-free and overall survival, validating the pro-survival role of ACSL3 in dormant DBCs.

Conclusion

Our findings highlight a crucial metabolic adaptation in dormant DBCs, where ACSL3-driven MUFA incorporation prevents ferroptosis and enhances survival. This underscores the viability of targeting de novo lipogenesis or ACSL3 as a therapeutic approach to eliminate dormant breast cancer cells and emphasizes the therapeutic potential of targeting the diverse functions of FAs in the metastatic process.

EACR25-0162

Erythrocyte-derived sphingosine-1-phosphate promotes tumor cell survival in the circulation

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Introduction

The majority of tumor cells metastasize through the blood stream. While circulating in the blood, most tumor cells do not survive because of attacks by the immune system or due to mechanical stress exerted by hemodynamic forces. Tumor cell survival during hematogenous dissemination requires particular cell autonomous functions or interactions with other corpuscular components of the blood. Here we explored the possibility that erythrocytes, the most abundant cell type in the blood stream, can promote tumor cell survival. Erythrocytes have various functions besides the exchange and transport of respiratory gases, and e.g. contain G-protein-coupled receptors (GPCRs) or ion channels, which regulate downstream signaling pathways, which can result in the release of different mediators.

Material and method

To study the potential role of erythrocyte GPCR signaling in tumor cell metastasis, mice with erythrocyte-specific deficiency of the G-protein α -subunits $\text{G}\alpha_s$, $\text{G}\alpha_q$ and $\text{G}\alpha_{13}$ were generated, and tumor metastasis was analyzed using B16F10, E0771 and LLC1 syngeneic murine metastasis models. By taking advantage of in vivo bioluminescence imaging, histological techniques, fluorescent microscopy, flow cytometry, mass

spectrometry, and RNA sequencing, we assessed both in vivo phenotypes and molecular mechanism.

Result and discussion

When studying mice with erythrocyte-specific loss of Gs, Gq or G13 in murine metastasis models, we found that mice lacking Gq, but not Gs or G13, in erythrocytes showed reduced metastasis due to increased tumor cell death during hematogenous spreading. Activation of Gq-coupled receptors on erythrocytes led to increased formation and release of various lipids including sphingosine-1-phosphate (S1P). In mice with erythrocyte-specific loss of sphingosine kinase 1, which catalyzes the formation of S1P, tumor cell survival in the circulation and metastasis formation were reduced. Moreover, under in vitro conditions, S1P induced anti-apoptotic effects in tumor cells through activation of the S1P receptor 2 (S1PR2), and tumor cell-specific loss of S1PR2 led to reduced survival and metastasis in vivo. In addition, we confirmed that tumor cells exposed to mechanical stress require the release of ATP through the pannexin 1 channel to promote their survival. Finally, we found that the stimulation of erythrocytes with ATP led to S1P release.

Conclusion

We show that circulating tumor cells utilize erythrocytes to improve their survival in the pro-apoptotic environment of the blood. We propose a model in which circulating tumor cells, that release ATP under the influence of mechanical stress, activate erythrocytes in their immediate vicinity through Gq-coupled purinergic receptors. This leads to the release of S1P, which by activation of the S1PR2 receptor has anti-apoptotic effects on tumor cells and thereby improves their intravascular survival and increases the potential for tumor cell metastasis.

EACR25-0166

Deciphering the Role of TCF21 in Regulating the Cancer-Associated Fibroblast (CAF) State in High Grade Serous Ovarian Cancer

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Introduction

High-grade serous ovarian cancer (HGSOC) is not only the predominant, but the most lethal subtype of ovarian cancer. The roles of CAFs are particularly important in HGSOC as the tumors and metastases have highly complex tumor microenvironments. Our previous study identified CD49e as a novel pan-fibroblast marker in HGSOC. We demonstrated the existence of two distinct CAF states distinguished based on the expression of fibroblast-activation protein (FAP) within the CD49e+ population: FAP-high (FH) and FAP-low (FL) CAFs. Further characterization of the transcriptional profiles of the two CAF subtypes using RNAseq identified transcription factor TCF21 as a master regulator of the CAF state.

Material and method

Here, to elucidate mechanistic insights into how TCF21 transforms FH CAFs to a more FL-like phenotype, we overexpressed TCF21 in FH CAF lines and performed RNAseq and CUT&RUN to characterize the TCF21-regulated transcriptional networks. In addition, BioID proximity ligation assays were carried out in CAFs to identify novel TCF21 binding partners that work together with TCF21 to modulate its target genes.

Result and discussion

Integrated analyses of the RNAseq and CUT&RUN data demonstrated that TCF21 binds to promoters and down-regulates most of its target genes, indicating that TCF21 is a transcriptional repressor. Overlapping targets between TCF21-putative down-regulated genes and the FH gene signature included genes involved in collagen and extracellular matrix organization, and cell adhesion, suggesting that TCF21 inhibits pro-tumorigenic functions of FH CAFs through the down-regulation of highly expressed FH CAF genes. Motif analysis of CUT&RUN data identified TCF21 motifs, as well as motifs for the TEAD family. BioID results indicated that YAP1 (but not TEADs) is a potential TCF21 interactor. Co-IP experiments showed binding of TCF21 to YAP1 when overexpressed in 293T cells. Duolink proximity ligation assay confirmed the competitive binding of TCF21 over TEADs to YAP1 in CAFs. Moreover, YAP1 knockdown in FH CAFs conferred a less aggressive phenotype, and revealed common target genes of YAP1 and TCF21 in regulating the CAF states.

Conclusion

Together, our data suggest that the expression of TCF21 in CAFs may compete with TEADs in binding to YAP1 at the promoters/enhancers of genes related to CAF activation. This would lead to down-regulation of their target genes, and could drive FH CAFs toward a more FL-like phenotype. Further characterizations are being carried out to determine the underlying mechanisms of how TCF21 and YAP1 interaction could potentially reprogram FH CAFs to a more FL-like state. Therefore, targeting TCF21-YAP1 axis in CAFs may represent a potential therapeutic approach to treat HSGOC.

EACR25-0169

Hypoxia tolerance mechanisms in Head and Neck Squamous Cell Carcinoma

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Introduction

Metastasis is a major contributor to poor outcomes in Head and Neck Squamous Cell Carcinoma (HNSCC). This process largely driven by cancer cell plasticity that enables phenotype switching via epithelial mesenchymal transition (EMT). This facilitates migration, alongside drug and radiotherapy resistance. Tumour micro-

environment (TME) stimuli play a pivotal role in regulating cancer stem cell (CSC) plasticity, and the resulting metastasis and therapy resistance. Among these, hypoxia and cancer associated fibroblasts (CAFs) are key prognostic factors for poor patient outcome and radiotherapy resistance in HNSCC. Although the importance of the TME in tumorigenesis is evident, our understanding of CSC-CAF crosstalk under hypoxic conditions remains limited.

Material and method

Herein, we employ two complementary 3D HNSCC models to study the impact of hypoxia and CAFs on CSC dynamics: co-culture spheroids to recapitulate the complexity of tumour biology and microfluidic technologies that enable high-resolution immunofluorescent imaging of individual cells in a 3D environment. Changes in HNSCC CSC phenotype were monitored with flow cytometry and immunofluorescent imaging using established markers CD44 and EPCAM, as well as the novel plasticity marker podoplanin. The oxygen gradients within the 3D models were investigated to mimic physiological conditions and understand cellular responses.

Result and discussion

2D experiments demonstrated that long-term chronic hypoxia promoted EMT in HNSCC cell lines. Similarly, oxygen levels were tailored to influence cellular behaviour in the 3D models. Hypoxic probe immunofluorescent imaging was used to identify hypoxic regions and accordingly 3D models were optimised to ensure control over oxygen levels. 3D spheroid co-culture with CAFs induced EMT and plasticity changes in HNSCC cells. As microfluidic models allow for high-content imaging phenotypic switches and underlying CSC-CAF interactions and hypoxia are being characterised at a single-cell level.

Conclusion

This study highlights the importance of modelling of oxygen gradients and CAFs in 3D models for understanding cellular responses and developing therapeutic strategies. The data suggests long-term hypoxia and CAFs promote a mesenchymal phenotype by modulating CSC plasticity in HNSCC, emphasizing the importance of the TME on tumorigenesis.

EACR25-0184

Defining the metastatic niche for colorectal cancer dissemination

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Introduction

Colorectal cancer (CRC) is one of the most prevalent cancers and is frequently associated with peritoneal metastasis (CRPM), where cancer cells spread from the

primary tumor, enter the peritoneal cavity, and form metastatic nodules at distant sites. However, the factors determining why some CRC patients develop peritoneal metastases while others do not remain unclear.

Mesothelial cells form a protective barrier lining the peritoneum, preventing metastatic invasion. Upon interaction with cancer cells, mesothelial cells undergo mesothelial-to-mesenchymal transition (MMT), losing epithelial characteristics and acquiring a fibroblastic morphology. This transition weakens the protective barrier and contributes to the formation of mesothelial-derived cancer-associated fibroblasts (mesoCAFs), which support tumor progression. We hypothesize that mesothelial cells in patients with peritoneal metastases are more susceptible to MMT, thereby creating a premetastatic niche. The aims of the project are: To elucidate the appearance mesoCAFs in samples from CRC patients with and without metastasis using immunocytochemistry; To define the MMT molecular signature in peritoneal tissue of CRC patients with peritoneal metastasis using multi-omics approaches; To develop a 3D co-culture invasion assay of peritoneal metastasis incorporating primary cells from CRC patients to investigate the spatiotemporal dynamics of colorectal cancer-mesothelial cell crosstalk and induction of MMT.

Material and method

Mesothelial and CAF markers were immunolocalised in peritoneal samples from affected and non-affected sites. A publicly available dataset (ArrayExpress E-MTAB-5998) of purified mesothelial cells was analyzed using enrichment analysis and regulatory networks to identify genes regulating MMT. Induction of MMT by conditioned media from CRC cells (HT-29) was assessed using confocal/phase-contrast imaging, and gene expression of mesothelial and CAF mesenchymal markers was analysed by qRT-PCR, in comparison with Transforming Growth Factor-β1 (TGFβ1) induced MMT.

Result and discussion

MesoCAFs were localised in affected peritoneal samples. TGFβ1-treated mesothelial cells exhibited increased fibroblastic morphology, proliferation, and migration, alongside upregulation of gene expression for Collagen Type I (Col1a1) and downregulation of Cingulin (CGN). Similarly, HT-29 conditioned media-treated mesothelial cells demonstrated enhanced fibroblastic morphology and proliferation. Notably, several MMT-related genes, were upregulated in both treatments or exclusively in CRC-conditioned media.

Conclusion

Soluble factors produced by CRC cells act in a similar fashion to TGFβ1 in the induction of MMT. The long-term goal is to identify mechanisms mediating the generation of mesoCAFs and metastatic niche, as well as possible new targets for therapeutical manipulation to retain a protective peritoneal barrier.

EACR25-0201

Modelling the extracellular matrix to elucidate collagen-mediated alterations in colorectal cancer using 3D patient-derived organoids

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Introduction

Colorectal cancer (CRC) remains a leading cause of cancer-related mortality, with tumour progression heavily influenced by the tumour microenvironment (TME), which consists of fibroblasts, immune cells, and the extracellular matrix (ECM). During cancer progression, the ECM – composed of fibrillar collagens, fibronectin, elastin, and laminins – undergoes aberrant deposition, particularly of collagen I. This disrupts homeostatic control, exposing tumour cells to a collagen-rich stroma that promotes proliferation, invasion, metastasis, and tumour growth.

Material and method

We developed 3D patient-derived CRC organoids (PDCO) cultured in Matrigel® enriched with collagen I to investigate how ECM composition influences cancer behaviour. Microrheology was employed to assess the viscoelastic properties of the model matrix. We integrated transcriptomic and proteomic analyses to identify key molecular pathways modulated by the ECM. Additionally, we utilized atmospheric pressure matrix-assisted laser desorption/ionization mass spectrometry (AP-MALDI-MS) to perform single-cell lipidomic profiling of PDCOs cultured in collagen I-enriched and control matrices. This pioneering study introduces AP-MALDI-MS for single-cell lipidomic profiling in PDCO models, providing a novel approach to studying tumour metabolism in a physiologically relevant microenvironment.

Result and discussion

We successfully optimized a 3D model of a collagen-enriched matrix to better understand CRC progression using PDCOs. Characterizing the viscoelastic properties of the model matrix provided insights into how collagen influences cancer cell behaviour. We found that increased collagen I deposition over time upregulated the cholesterol biosynthesis pathway and activated the Sterol Regulatory Element Binding Protein (SREBP) pathway, a key regulator of cholesterol biosynthesis. This metabolic reprogramming supports cancer cell proliferation. Our multiomics approach offers a comprehensive understanding of how a collagen I-rich TME drives cancer metabolism and progression.

Conclusion

Our findings underscore the critical role of the ECM, particularly collagen I, in driving CRC progression within a 3D *in vitro* model. This research identifies new metabolic vulnerabilities in CRC, potentially leading to novel therapeutic targets and improved precision medicine strategies.

EACR25-0207

Genome-Wide Analysis of Intrinsic Sequence RNAs associated with Invasive Lobular Breast Cancer

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Introduction

Breast cancer is the most prevalent malignancy among women globally, with a lifetime risk of 1 in 8.

Histological examinations of breast cancer can be categorized as ductal or lobular, depending on the location of tumor cells. Invasive lobular breast cancer is the second most prevalent kind, exhibiting a 15% incidence rate. Numerous proteins play a role in the development of breast cancer, as evidenced by extensive research over the years. Research indicates that certain non-coding RNAs may contribute to the development of breast cancer. One of the most fascinating advancements regarding the roles of non-coding RNAs in breast cancer pathology is intronic RNAs, which reside within the introns of protein-coding genes and are synthesized from their own distinct promoters, separate from the promoters of the associated protein-coding genes. This study aims to identify intronic RNAs with differential expression in healthy breast epithelium and invasive lobular breast cancer cell lines.

Material and method

RNA was extracted from the healthy breast epithelial cell line (MCF10A) and the invasive lobular breast cancer cell line (MDA-MB-134), followed by DNase treatment. The purity and integrity of RNAs were assessed using gel electrophoresis and a Nanodrop spectrophotometer. rRNA depletion was conducted on the extracted RNAs, and total RNA, polyA+, and polyA- RNAs were subjected to deep sequencing in accordance with our intronic RNA selection criteria. The nextflow pipeline developed in our laboratory revealed intronic sequences that exhibited differential expression in both cell lines. Potential candidates were visually assessed using Integrated Genome Viewer (IGV). RNAs were transcribed into cDNA, and 3' and 5' RACE was conducted to establish the boundaries of intronic transcripts. Transcripts were inserted into a TA vector and sequenced via the Sanger technique.

Result and discussion

The nextflow pipeline developed in our laboratory identified 367 possible intronic sequence RNAs in the MDA-MB-134 cell line and 276 in the MCF10A cell line. The criteria for selecting intronic RNA were: 1. Presence of a polyA signal, 2. Location within the gene's intron, 3. Absence of any corresponding exon for the candidate transcript, 4. Lack of splicing with adjacent exons. According to these criteria, 16 candidates were identified in MDA-MB-134 cells and 12 candidates in MCF10A cells using visual analysis in IGV. The experiments undertaken thus far have identified the boundaries of one candidate that is highly expressed in MDA-MB-134 and one candidate that is highly expressed in MCF10A.

Conclusion

Intronic RNAs exhibit variable expression in invasive lobular breast cancer. The stability investigations performed in this study will demonstrate the stability of intronic RNAs, followed by their functional characterisation. These investigations may elucidate the regulatory role of intronic RNAs in invasive lobular breast cancer.

EACR25-0211

the MYC dependent lncRNA MB3 inhibits apoptosis in Group 3 Medulloblastoma by regulating the TGF- β pathway

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Introduction

Group 3 (G3) is one of the most common, aggressive and fatal subtypes of the paediatric cerebellar tumour Medulloblastoma (MB), primarily driven by the MYC oncogene. Targeting MYC has long been challenging and this, combined with our incomplete understanding of G3 MB molecular bases, has hindered the development of effective targeted therapies. Long noncoding RNAs (lncRNAs), with their extensive oncogenic roles, cancer-specific expression, and connection to MYC biology, offer opportunities for unravelling this complexity and providing new insights and therapeutic targets.

Material and method

Using genome-wide, molecular and cellular assays, we characterised the activity of the MYC-dependent, anti-apoptotic lncRNA lncMB3 in G3 MB cells. Through transcriptomic and interactomic analyses, we clarified lncMB3 function and mode-of-action and we targeted it with a novel delivery system.

Result and discussion

LncMB3 controls the TGF- β pathway, critically altered in G3 medulloblastomagenesis. This regulation occurs via the direct coding-noncoding RNA interaction between lncMB3 and the mRNA for the epigenetic factor HMGN5, with both sharing targets in the TGF- β cascade. This axis converges on apoptosis through OTX2, another G3 MB driver gene, and photoreceptor lineage genes. Synergistic effects between lncMB3 targeting and cisplatin treatment underscores the relevance of this regulatory network in vitro. Finally, we propose novel ferritin-based nanocarriers as efficient delivery tools for antisense oligonucleotides targeting lncMB3.

Conclusion

LncMB3 emerges as a central node linking MYC amplification to apoptosis inhibition through a circuit involving RNA-based mechanisms, G3 MB key drivers and underexplored factors. This integrated framework deepens our understanding of G3 MB molecular underpinnings and lay the foundation for translating lncRNA research into potential applications.

EACR25-0213

Characterization Of m6A Regulatory Proteins and Identification Of Target Genes In Invasive Lobular Breast Cancer

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Introduction

Invasive Lobular Breast Cancer (ILBC) is the second most common histological subtype of breast cancer and constitutes 15% of breast cancers. It arises in the milk producing lobules and metastasizes to different parts of the body. Although p53 gene mutation and E-cadherin deficiency are known, the regulatory mechanisms of this cancer are not fully understood. Epitranscriptomic mechanisms, such as N6-methyladenosine (m6A) RNA modification, play a role in the regulation of gene expression. m6A is regulated by writer, eraser and reader proteins and affect the fate of RNAs. m6A is known to regulate biological processes such as proliferation, metastasis and cell death in cancer cells. This study aimed to investigate the phenotypic effects and potential target of m6A regulatory proteins on ILBC.

Material and method

Expression levels of m6A regulatory genes were investigated in MCF10A healthy epithelial breast cells and MDA-MB-134 ILBC cells by RT-PCR and western blot analyses. Molecular cloning of FTO eraser protein and overexpression in MDA-MB-134 cells were performed. FTO gene was knocked down in MCF10A cells by siRNA transfection. Then, phenotypic changes such as apoptosis, cell cycle and viability of cells were examined by flow cytometry and WST-8 assay. Based on the phenotypic changes observed in cells, the expression of potential FTO target genes was examined by RT-PCR upon knockdown and overexpression experiments.

Result and discussion

While the expression levels of RBM15 and WTAP genes were similar in MDA-MB-134 and MCF10A cells, the expression levels of FTO and METTL14 genes were decreased in MDA-MB-134 cells compared to MCF10A cells. Overexpression of the FTO gene resulted in increased viability of MDA-MB-134 cells. Additionally, silencing the FTO gene in MCF10A cells reduced cell viability and caused cell cycle arrest.

Conclusion

In this study, m6A regulatory proteins were screened for the first time in ILBC. The difference in the expression levels of FTO and METTL14 m6A regulatory proteins in MDA-MB-134 cells compared to healthy breast cells was revealed. Furthermore, the effect of FTO protein-mediated regulation of m6A modification on the viability of ILBC cells was observed, which could be a potential biomarker for ILBC diagnosis or treatment.

EACR25-0217

Investigation of m1A and m6A Methylation in Metastatic and Non-Metastatic Triple-Negative Breast Cancer Cells

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Introduction

The study of epitranscriptomics covers over 170 RNA modifications including N6-methyladenosine (m6A) and N1-methyladenosine (m1A), which are crucial in a range of diseases such as cancer. The most common type of methylation, m6A, has received significant attention for its potential roles in cancer diagnosis and treatment. On the contrary, although m1A also contains crucial functions, it has not attracted as much attention as m6A until recent studies. Nevertheless, the impact(s) of m6A and m1A RNA methylations remain still unclear in triple-negative breast cancer cells, such as non-metastatic HCC1143 and metastatic MDA-MB-231. This study aims to evaluate the phenotypic effects of m6A and m1A RNA methylation in triple-negative breast cancer cells by considering metastatic feature after knock-down of METTL3 and TRMT61A that has the catalytic activity to add m6A and m1A on mRNA, respectively.

Material and method

The knockdown of METTL3 and TRMT61A was performed by using siRNAs and transfection reagent (DharmaFECT®). Subsequently, cellular phenotypes, such as proliferation, apoptosis and cell cycle were examined to assess the impact of these writers. The viability assay was performed by WST8 colorimetric assay (NutriCulture®). Afterwards, apoptosis rate of cells was measured by staining with annexin V-FITC and 7AAD (Biolegend®) while cell cycle analysis was carried out by propidium iodide (PI) (Biolegend®) staining in flow cytometry. Lastly, RNA sequencing was performed to probe into the molecular pathways affected by knockdown of the writers. The cancer cell lines were also compared with a healthy breast cell line, MCF10A.

Result and discussion

The maximum reduction of METTL3 and TRMT61A was attained at 72h after transfection in MCF10A and HCC1143 cells. The cell viability decreased 40.1 and 27.4% upon METTL3 and TRMT61A knockdown, respectively. To identify the cause of the reduced viability, analyses of apoptosis and the cell cycle were conducted. Although there was no change in the apoptotic rate, 34.27% G2/M phase arrest was noted in HCC1143 cells following the silencing of METTL3 while G1/S phase arrest was obtained in MCF10A cells after TRMT61A silencing. RNA sequencing analysis identified 585 and 687 differentially expressed genes (DEGs) in METTL3- and TRMT61A-silenced cells. Gene Ontology Enrichment Analysis conducted on DEGs with a 1.5-fold change showed that METTL3 silencing significantly impacted pathways related to cell migration, whereas TRMT61A silencing notably affected immune-related pathways.

Conclusion

Our results show that each chemical modification has a different impact on triple-negative cancer cell biology. Further investigation will be performed for the metastatic cell line, MDA-MB-231.

EACR25-0225

Genome-wide profiling of intronic RNAs in triple-negative ductal breast carcinoma

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Introduction

Triple-negative breast cancer (TNBC) comprises 10–20% of all invasive breast cancer cases, with histopathological analyses indicating that most TNBC cases originate from ductal tissue. The heterogeneous nature of TNBC and its limited treatment options, compared to other breast cancer subtypes, underscore the urgent need for targeted therapies. Recent studies have highlighted the critical roles of non-coding RNAs (ncRNAs) in regulating cancer hallmarks and contributing to therapy resistance, underscoring their significance in cancer progression. Long non-coding RNAs (lncRNAs) are among the most extensively studied classes of ncRNAs. However, lncRNAs transcribed from intronic regions of annotated genes remain largely unexplored due to their low expression levels and the historical perception that these regions merely represent transcriptional noise or degradation byproducts. Advances in RNA sequencing technologies have begun to reveal their potential roles in cellular processes and disease. This study investigates novel intronic RNAs in triple-negative ductal breast carcinoma using HCC1143 (triple-negative ductal breast carcinoma) and MCF10A (non-tumorigenic) cell lines.

Material and method

Strand-specific RNA sequencing was performed on ribodepleted total RNA, poly(A)+ RNA, and poly(A)- RNA isolated from HCC1143 and MCF10A cell lines.

Bioinformatic analysis using our custom-developed Nextflow-based pipeline identified intronic RNAs with poly(A) tails. Differentially expressed intronic RNAs were further examined through visual inspection using Cap Analysis of Gene Expression (CAGE) data to map their 5' ends, along with the presence of a polyadenylation signal (PAS) at the hypothesized 3' end. The transcript boundaries of candidate intronic RNAs identified through this process were further validated using rapid amplification of cDNA ends (RACE).

Result and discussion

Bioinformatic analysis identified 270 poly(A)+ intronic RNAs in HCC1143 and 293 poly(A)+ intronic RNAs in MCF10A cells. Interestingly, some of these transcripts exhibited differential expression between HCC1143 and MCF10A cells. The successful identification of transcript boundaries for several selected candidates validates the existence of these differentially expressed intronic RNAs in TNBC; however, further validation by northern blotting is required.

Conclusion

Our findings reveal previously uncharacterized intronic RNAs with distinct expression patterns in TNBC. These results underscore the emerging recognition of intronic RNAs as a novel class of ncRNAs and highlight the need for further investigation into their structural characterization and biological roles in TNBC.

EACR25-0226

Transcriptomics analysis identifies intronic RNAs in ER+/PR+ and HER2-enriched ductal breast carcinoma

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Introduction

Breast cancer is the most prevalent malignancy among women and one of the primary contributors to cancer-related mortality globally, featuring various subtypes. Among these, invasive ductal carcinoma (IDC) represents approximately 80% of breast cancer cases, making it the most diagnosed form. IDC exhibits significant molecular heterogeneity and aggressive behavior. HER2-enriched IDC is characterized by increased tumor proliferation and resistance to targeted therapies, highlighting the urgent need for novel molecular insights. Despite the growing recognition of non-coding RNAs (ncRNAs) in breast cancer development, intronic RNAs have been largely overlooked and remain poorly studied. However, recent advancements in RNA sequencing have uncovered their potential to be functional in various biological processes. In this study, we examine intronic RNAs in ER+/PR+ and HER2-enriched IDC using BT474 cells, with MCF10A serving as a healthy breast cell line.

Material and method

Ribo-depleted total RNA, poly(A)+ RNA, and poly(A)-RNA were extracted from BT474 and MCF10A cell lines and subjected to strand-specific RNA sequencing, followed by the identification of poly(A)+ intronic RNAs using our in-house-developed Nextflow-based pipeline. The Integrative Genomics Viewer (IGV) was used for visual analysis to map the 5' and 3' ends of selected differentially expressed intronic RNA candidates by identifying Cap Analysis of Gene Expression (CAGE) peaks and polyadenylation signals (PAS), respectively. Rapid amplification of cDNA ends (RACE) was performed to validate the 5' and 3' ends of the transcripts.

Result and discussion

A total of 293 poly(A)+ intronic RNAs were identified in MCF10A, and 389 poly(A)+ intronic RNAs were identified in BT474, with some showing differential expression. The identification of boundaries for some of these differentially expressed intronic RNAs suggests their presence and potential role in cancer regulation. However, further studies are needed to validate their presence and confirm their involvement in cancer regulation.

Conclusion

Our findings highlight differentially expressed novel intronic RNAs in ER+/PR+ and HER2-enriched ductal breast carcinoma and underscore the need for functional studies to explore their role in cancer progression.

EACR25-0227

GTF2A1-AS lncRNA orchestrates cisplatin-induced cell death transitions as a stress response regulator

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Introduction

Historically, programmed cell death has been primarily linked to caspase-dependent apoptosis. However, recent findings indicate that caspase-independent cell death (CICD) mechanisms can be activated by genotoxic and oxidative stressors to modulate cell fate. Parthanatos is a type of CICD mediated by PARP1 activation through DNA damage leading to PAR accumulation, and cell collapse. Although several protein-coding genes have been associated with parthanatos, the contribution of long non-coding RNAs (lncRNAs) is not elucidated well. The aim of this study was to uncover the potential role of general transcription factor 2A1 antisense (GTF2A1-AS) lncRNA in modulating cisplatin-mediated parthanatos in HeLa cells.

Material and method

HeLa cells were treated with cisplatin (CP) of 80 µM for 16 h; and DMSO of 0.1% (v/v) as the control group, followed by total RNA isolation and RNA-seq analysis. After selecting GTF2A1-AS as a CP-inducible lncRNA candidate, phenotypic analyses were performed by gain-and loss-of function studies. GapmeR-assisted silenced cells were also subjected to RNA-seq analysis to reveal the possible role of GTF2A1-AS in modulating cell fate-related biological processes. Additionally, higher concentrations of cisplatin were also combined with the overexpression and knockdown experiments to examine the regulatory effect of GTF2A1-AS on drug metabolism.

Result and discussion

GTF2A1-AS lncRNA was a CP-inducible candidate based on the differential expression pattern upon drug treatment. Time-course experiments indicated that GTF2A1-AS silencing elicited apoptosis by 10% whereas overexpression did not induce cell death significantly. RNA-seq analyses of GTF2A1-AS-silenced cells revealed that DNA damage response genes were affected. When GTF2A1-AS silencing and overexpression were combined with 80 and 540 µM cisplatin concentrations, cells showed increased chemosensitivity, as depicted in flow cytometry analysis by shifting to late apoptosis. Notably, 540 µM CP treatment in GTF2A1-AS-silenced cells resulted in a significant increase in PARP-1 and PAR polymer accumulation, as detected in western blot.

Conclusion

This study identifies GTF2A1-AS as a CP-inducible lncRNA, emphasizing its significance in cancer research. Silencing of GTF2A1-AS promoted apoptosis, while its overexpression did not significantly impact cell viability. Additionally, at higher CP concentrations, GTF2A1-AS silencing influenced parthanatos. Given the importance of lncRNAs in cancer progression and drug resistance, it suggests that GTF2A1-AS may serve as a therapeutic target for modulating different cell death modes, enhancing chemotherapy efficacy.

EACR25-0231

Identification of Intronic RNAs Involved in Apoptosis

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Introduction

Apoptosis, a form of programmed cell death, is a cellular process that is crucial for the maintenance of cellular homeostasis. As apoptosis plays a vital role in controlling the balance between cell death and cell proliferation, inappropriate apoptosis can result in various conditions, including cancer. The process of apoptosis is regulated by various proteins and genes within the cell. Also, apoptotic pathways can be induced by certain chemicals, such as the chemotherapeutic drug cisplatin, which activates the intrinsic pathway of the apoptosis. Besides, even if non-coding parts of the genome were referred to as junk regions in the early days, advanced transcriptomics studies have revealed many regulatory non-coding RNAs involved in not only apoptosis but also many other essential cellular processes. Interestingly, recent studies uncovered the presence of intronic RNAs, the long non-coding RNAs that are derived from the introns of other genes. The aim of this study is to characterize intronic RNAs involved in the regulation of apoptosis under cisplatin-mediated apoptotic conditions.

Material and method

Firstly, RNA was isolated from the cisplatin- and DMSO-treated HeLa cells, the cervical carcinoma cell line. After ribosomal RNA depletion, total RNA, polyA⁺ and polyA-eliminated fractions were subjected to deep sequencing. The bioinformatic analysis was performed by using an in-house pipeline to identify intronic transcripts differentially expressed between cisplatin-treated conditions and DMSO-treated control. In order to map the borders of the transcriptional unit of intronic RNAs, 5' and 3' RACE PCRs followed by molecular cloning and Sanger sequencing were performed. The differential expression of candidates was confirmed by quantitative PCR analysis.

Result and discussion

The bioinformatic analysis revealed 598 upregulated and 161 downregulated candidate intronic transcripts upon cisplatin treatment. The deep sequencing profiles of candidate regions were visually examined with Integrated Genome Viewer (IGV). During the candidate intronic RNA selection, the presence of polyadenylation signal near 3' end and the absence of any annotated exon in the candidate region were taken into consideration. This study uncovered candidate intronic RNAs differentially expressed under cisplatin-mediated apoptotic conditions. The transcriptional unit of a candidate intronic RNA was determined. The differential expression of the candidate under cisplatin-treated conditions was confirmed, pointing out its potential to be involved in the regulation of apoptosis.

Conclusion

Intronic RNAs produced from intronic regions of other genes may modulate many cellular processes, including apoptosis which is crucial for cancer development and progression. Understanding the characteristics and functions of these regulatory elements would be valuable to unveil the new insights of the mechanism of cancer.

EACR25-0265

Dose-Dependent Regulatory Effects of Melatonin on miRNA Expression in PANC-1 Pancreatic Cancer Cells

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Introduction

Pancreatic ductal adenocarcinoma (PDAC) is one of the most aggressive and lethal malignancies, largely due to its late diagnosis and resistance to conventional therapies. Recent studies suggest that microRNAs (miRNAs) play a crucial role in cancer progression by regulating gene expression at the post-transcriptional level. Melatonin, a widely conserved neurohormone with antioxidant and anti-tumor properties, has been shown to modulate several oncogenic pathways, including those mediated by miRNAs. This study investigates the dose-dependent effects of melatonin on the expression of miR-27a, miR-148a, miR-203, and miR-196a in PANC-1 pancreatic cancer cells, aiming to identify potential therapeutic implications.

Material and method

PANC-1 pancreatic cancer cells were cultured and treated with 2 mM and 2.5 mM melatonin to determine its inhibitory effects. The cytotoxic effect was assessed using the MTT assay to establish the IC₅₀ value. Total RNA was extracted from untreated and melatonin-treated cells, followed by cDNA synthesis. The expression levels of miR-27a, miR-148a, miR-203, and miR-196a were analyzed using quantitative real-time PCR (qRT-PCR). Statistical analyses determined the significance of expression changes in response to melatonin treatment.

Result and discussion

qRT-PCR analysis revealed significant dose-dependent alterations in miRNA expression following melatonin treatment. At 2 mM melatonin, miR-148a and miR-196a were significantly upregulated, suggesting a tumor-suppressive role, while miR-27a and miR-203 were downregulated, indicating inhibition of proliferation and metastasis-associated pathways. At 2.5 mM melatonin, miR-148a remained upregulated, reinforcing its tumor-suppressive function, while miR-27a, miR-203, and miR-196a showed significant downregulation, suggesting a broader inhibitory effect on oncogenic miRNA signaling. These findings support the hypothesis that melatonin modulates miRNA expression, potentially influencing PDAC progression by targeting key regulatory networks involved in proliferation, apoptosis, and metastasis. The observed miRNA changes align with previous studies highlighting melatonin's anti-proliferative and anti-metastatic effects in various cancer models.

Conclusion

This study provides evidence that melatonin regulates the expression of key miRNAs involved in PDAC progression in a dose-dependent manner. The upregulation of miR-148a and miR-196a, along with the downregulation of miR-27a and miR-203, suggests that melatonin influences miRNA-mediated signaling pathways that control tumor growth and metastasis. These findings highlight the potential of melatonin as a therapeutic agent for pancreatic cancer by modulating miRNA expression. Further studies are needed to validate these results in *in vivo* models and clinical settings, as well as to explore the broader implications of melatonin in miRNA-targeted cancer therapy.

EACR25-0266

Patterns of Bone Marrow Adipogenesis in Response to Chemotherapy

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Introduction

The bone marrow microenvironment is highly saturated with bone marrow adipocytes (BMA), which differentiate from their precursor, mesenchymal stem cells (MSC). Evidence in the literature suggests BMA confer chemotherapeutic resistance to tumour cells, thereby ascribing a tumour-supportive role to BMA. Evidence from patient trials suggests that bone marrow adiposity is increased in patients following some forms of chemotherapy, however it has been suggested this is due to hypertrophy. We hypothesize that chemotherapy actively promotes adipogenesis by altering expression of genes associated with adipocyte differentiation, resulting in increased levels of mature BMA with altered phenotypes that promote tumour growth in bone.

Material and method

Human MSC were treated with adipogenic differentiation media in the presence or absence of doxorubicin.

Differentiation into BMA was confirmed by expression of adipocyte marker genes by RT-qPCR and percentage of lipid-droplet containing cells by BODIPY staining. RNA-seq analyses were performed to identify gene alterations associated with doxorubicin treatment in differentiating MSC. Alterations in the fibroblast growth factor (FGF) pathway were identified and target changes validated by RT-qPCR, ELISA and Western blot.

Contribution of the identified target FGF2 (fibroblast growth factor 2) to adipogenesis was confirmed using siRNA depletion strategies. The effects of conditioned media from doxorubicin or vehicle control treated BMA on PC3 prostate tumour cell growth was also assessed.

Result and discussion

Doxorubicin treatment of differentiating MSC led to increased numbers of BODIPY+ mature adipocytes, which was confirmed by elevated levels of adipocyte markers. RNA-seq analysis identified significant increases in FGF pathway genes, which were validated by RT-qPCR. We confirmed increased mRNA and secreted protein levels of FGF2 with doxorubicin treatment, which may be due to increased levels of the transcription factor EGR1 (early growth response 1), known to bind to the promoter region of FGF2, which was also significantly increased in doxorubicin-treated cells. Furthermore, siRNA targeting FGF2 impeded the doxorubicin enhanced formation of lipid-containing BMA returning it to levels similar to vehicle-treated BMA. As FGF2 is a secreted protein we tested and confirmed that transfer of conditioned media from doxorubicin-treated BMA enhanced proliferation of PC3 prostate tumour cells *in vitro*.

Conclusion

Our findings suggest that doxorubicin treatment of differentiating MSC leads to increased numbers of BMA, which have alterations in the FGF pathway that not only enhance adipogenesis, but also alter prostate tumour cell growth as a result.

EACR25-0269

Hippo signaling pathway predicts dormant phenotype in the GNAQ/11 mutant Uveal Melanoma

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Introduction

Uveal melanoma (UM) is an aggressive intraocular malignancy with limited therapeutic options and high metastatic potential. Mutations in GNAQ or GNA11 are early occurring driver events in UM and induce cellular senescence. These mutations also contribute to the constitutive activation of central mediators YAP/TAZ in the Hippo signaling pathway, thereby promoting tumor proliferation and metastasis. Although, GNAQ and GNA11 mutations are mutually exclusive in UM, studies suggest that GNA11-driven tumors exhibit more aggressive behavior than GNAQ-driven tumors. This study investigates the differential impact of GNAQ and GNA11 mutations on Hippo pathway components to predict tumor dormancy in UM.

Material and method

Whole-exome sequencing was performed on 41 prospective UM cases to determine the mutational status, then cases were segregated into group 1 (GNAQ mutant) and group 2 (GNA11 mutant). Quantitative real-time PCR (qRT-PCR) was performed to assess the mRNA expression level of Hippo pathway components and cell cycle inhibitor in these groups. Western blot was performed to validate the protein expression levels. The

expression profiles were correlated with clinicopathological parameters and patient outcomes.

Result and discussion

Group 1 (GNAQ mutant) and group 2 (GNA11 mutant) includes 25 (60.98%) and 16 (39.02%) cases, respectively. Scleral invasion and optic nerve invasion were found in group 1 (36% & 16%) and group 2 (56% & 6%) cases, respectively. In both groups, the central mediator YAP was upregulated in 16% and 12% cases, respectively. The 14-3-3 retention protein, which stabilizes cytoplasmic YAP, was upregulated in nearly 40% of cases with a mean-fold change of 2.73 (group 1) and 3.30 (group 2). LATS1, a key Hippo kinase, was downregulated in more than 90% of cases in both the groups. However, other kinases were more frequently upregulated in group 2 than in group 1. SAV1, a Hippo pathway scaffold protein, was upregulated in 40% of group 1 cases and downregulated in over 90% of group 2 cases. Protein expression levels corroborated these findings. The cell cycle inhibitor p16 was upregulated in 36% (group 1) and 50% (group 2) cases. Eight patients with mutant GNAQ/11 developed distant metastasis with upregulated p16, and 5 patients died due to the disease. This suggests that p16 upregulation in the presence of oncogenic signals may initially induce dormancy but could later contribute to tumor reactivation and metastasis.

Conclusion

This is the first study to comprehensively analyze the differential impact of GNAQ and GNA11 mutations on Hippo signaling pathway regulation in UM. The upregulation of p16 in the presence of oncogenic signals may support tumor dormancy, potentially delaying metastasis. The observed mutation-specific expression patterns of Hippo pathway regulators could provide insights into novel therapeutic strategies targeting YAP/TAZ and LATS kinases, thereby improving patient outcomes.

EACR25-0272

Reprogramming the immunosuppressive tumor microenvironment to overcome resistance to CAR-T-cell immunotherapy in pancreatic cancer

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Introduction

Pancreatic cancer is rare but highly lethal, with poor survival rates and limited treatment options due to late-stage diagnosis. The most common form, pancreatic ductal adenocarcinoma (PDAC), is highly heterogeneous and resistant to treatment, primarily due to cancer stem cells that drive tumor growth, metastasis, and drug

resistance. Current therapies fail to eradicate these resistant stem cells, leading to disease relapse. While CAR-T cell therapy has shown success in blood cancers, its effectiveness in solid tumors is at least in part hindered by the unique tumor immune microenvironment (TIME), characterized by a distinctive immune composition and complex cellular interactions. Therefore, enhancing the efficacy of CAR-T cell therapy requires transforming TIME from an immunosuppressive to an immunopermissive state.

Material and method

Murine PDAC cells (5,000 cells in 30 µL Matrigel) were injected orthotopically into the pancreas of 6-8-week-old C57BL/6 immunocompetent mice. Tumor growth was monitored by IVIS acquisition, and once tumors were fully established, mice were treated intraperitoneally or intravenously with different TIME modulators. All animal procedures were conducted in accordance with the 3Rs and the regulations for animals in science.

Result and discussion

In vivo studies showed that most of the tested immune modulators at least partially reprogrammed the TIME, e.g. by increasing the presence of T cells and NK cells, or by reducing the presence of myeloid derived suppressor cells (MDSCs). Notably, directly targeting the activity of regulatory T cells showed promising results, inducing a more pronounced remodelling of TIME by increasing the frequency of T cells, NK cells and M1 macrophages, and simultaneously reducing the presence of immunosuppressive populations such as MDSCs and M2 macrophages, which may aid in increasing the efficacy of our CAR-T cells based immunotherapy.

Conclusion

Reprogramming the pancreatic TIME before delivering highly specific CAR-T cell immunotherapy has the potential to slow or even reverse pancreatic cancer progression. Our research identified a key immune population - regulatory T cells - that plays a central role in remodelling the TIME, thereby potentially enhancing the effectiveness of CAR-T cell-based immunotherapy for PDAC.

EACR25-0280

The role of the neuronal-cancer crosstalk in melanoma brain metastases using hPSC-derived Assembloids

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Introduction

Cutaneous melanoma is one of the most aggressive skin cancers due to its high metastatic potential. Over 60% of advanced-stage melanoma patients develop brain metastases, with a 1-year survival rate of only 10-20%. While genetically engineered mouse models have been

useful for studying brain metastasis, much remains unknown about the cellular interactions in the human brain microenvironment. Species-specific differences highlight the need for a human model to study melanoma brain metastasis.

Material and method

In this study, we generated a complex hPSC-derived brain assembloid that recapitulate specific aspects of the human brain microenvironment, including neural progenitor cells, cortical neurons, interneurons, glial populations (astrocytes and microglia), and pericytes. This cerebral environment allows the modelling of the adaptive process of melanoma cells metastasizing to the brain.

Result and discussion

Transcriptional data suggest that melanoma cells exhibit neuronal-like features upon exposure to the human brain assembloid. Specifically, melanoma brain metastases exhibit upregulation of genes associated with calcium signaling, synapse formation, and neuronal plasticity, enabling these cancer cells to adapt and proliferate in the brain environment. Electron microscopy images suggest that melanoma cells may establish synaptic-like connections with surrounding cortical neurons. These aberrant connections appear to alter the brain's electrical network, potentially contributing to the metastatic cells' survival and growth.

Conclusion

With this preliminary data, we aim to investigate the neuron-melanoma crosstalk and its role in the adaptation of melanoma metastatic colonies in the brain. Additionally, we will explore how melanoma metastases influence the brain environment and impact its physiological functions.

EACR25-0290

Immunomodulation of Glioblastoma Tumor Microenvironment by NF1-LRD-Loaded Extracellular Vesicles

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Introduction

Extracellular vesicles (EVs) are potential carriers for cancer therapeutics. In prior studies, we showed that re-expression of the leucine-rich domain (LRD) of neurofibromin (NF1) inhibited glioblastoma (GBM) invasion and inhibited microglia/macrophage recruitment into the tumor microenvironment (TME). Since tumor-associated microglia/macrophages (TAMs) play a critical role in GBM progression, we investigated whether EVs containing NF1-LRD (NF1-LRD-EVs) could modulate the TME and attenuate GBM progression.

Material and method

NF1-LRD-EVs were isolated from glioma cells stably expressing the NF1-LRD domain using ultracentrifugation. Characterization of these EVs followed MISEV2023 guidelines, employing western blotting, Nanosight tracking analysis, and transmission electron microscopy. To assess the immunomodulatory effects of

NF1-LRD-EVs, primary mouse microglia and iPSC-derived macrophages (iMacs) were exposed to EVs for 24h. Morphological and molecular changes were analyzed through immunofluorescence, phagocytosis assays, tumor infiltration assays, and immunoblotting.

Result and discussion

NF1-LRD-EVs significantly reduced TAM recruitment and infiltration by 60-80% in both transwell migration assays, compared to control EVs. These results suggest that NF1-LRD-EVs effectively modulate the TME by inhibiting pro-tumor TAMs infiltration. Furthermore, iMacs and microglia treated with NF1-LRD-EVs exhibited enhanced phagocytic activity, similar to that observed in LPS-stimulated cells, indicating that NF1-LRD-EVs-primed immune cells for a more potent anti-tumor response. Exposure to NF1-LRD-EVs also led to upregulation of pro-inflammatory cytokines, including TNF- α and IL1 β , which are associated with anti-tumor immunity. In contrast, the expression of anti-inflammatory cytokines such as Arginase and TGF β 1 was downregulated, further supporting a shift toward a pro-inflammatory phenotype. These findings, confirmed by qPCR, Luminex assays, and immunofluorescence, suggest that NF1-LRD-EVs reprogram TAMs to promote immune activation while suppressing immunosuppressive pathways within the TME.

Conclusion

Our results demonstrate that NF1-LRD-EVs reprogram TAMs within the GBM TME, enhancing pro-inflammatory immune responses and limiting immunosuppressive pathways. This modulation of the immune environment positions EVs as a promising platform for neuro-immunomodulation, offering a novel therapeutic approach for targeting the TME in GBM. Further studies are needed to evaluate the clinical potential of EV-based therapies to improve outcomes in GBM and other cancers with immune evasion.

EACR25-0300

Combined K8 and Apc loss-derived mouse model for colon-specific tumorigenesis

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Introduction

Loss of the epithelial intermediate filament protein keratin 8 (K8) has been shown to increase susceptibility towards colonocyte hyperproliferation and tumorigenesis. However, most colorectal cancer (CRC) mouse models require carcinogen, develop small intestinal tumors or have long latency period. The aim was to establish a genetic, colon-specific and more human like CRC model driven by loss of K8 and Apc.

Material and method

Colon epithelium specific targeting using CDX2P-CreERT2 mice was used to generate K8flox/flox; CDX2P-CreERT2 and K8flox/flox; CDX2P-CreERT2; Apcflox/+ mice. Body weight and stool consistency were

monitored, and colon was analyzed for tumor burden and histopathology. Keratin expression, inflammation, and proliferation were assessed using immunoblotting and immunofluorescence analysis. This data was compared to K8 expression analysis in patients with CRC using UALCAN database.

Result and discussion

K8 downregulation in adult K8^{flox/flox}; CDX2P-CreERT2 mice triggers mild diarrhea and leads to loss of K8 and reduced partner keratin levels in a mosaic pattern in the colonic epithelium, while ileal K8 protein levels are unchanged. K8-negative colon areas display increased crypt loss and more MPO+ cells predominantly in the proximal colon. Increased colonocyte proliferation is observed as increased percentage of Ki67+ cells and lower IL-22BP protein levels throughout the colon. These results highlight the protective colonocyte-autonomous roles of K8 in maintaining a balance proliferation in the colon, and in protection from inflammation. Mice with combined K8 and monoallelic Apc inactivation show dramatically increased colon tumor formation. The combined loss of the cytoskeletal protein K8 and a tumor suppressor protein Apc in right amount accelerates the process of colon tumorigenesis. Furthermore, this model offers an advantage over Apc mutant models, as tumors are located in the distal colon. This feature makes it a better model for CRC, as patients more frequently develop tumors in the colon and not in the small intestine. The model also recapitulates the here identified downregulated K8 expression in CRC patients, independent of disease type and stage, age or gender, supporting the tumor suppressive contribution of K8 in the colon. Pro-tumorigenic epithelial changes in K8-negative areas of our model adjacent to K8-positive areas also resemble diseased colon epithelium next to the normal epithelium in human CRC.

Conclusion

New genetic and colon-specific mouse model with loss of K8 and Apc adequately resembles human CRC. This study also highlights a role of colonocyte K8 in maintaining colon epithelial integrity and protecting against colon tumorigenesis.

EACR25-0310

Impact of MAG-EPA supplementation on the prostate tumor immune microenvironment

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Introduction

Current treatment options for prostate cancer are often highly invasive and associated with significant adverse effects, which severely compromise patients' quality of life. Chronic tissue inflammation is frequently observed in prostate cancer and contributes to disease progression. Given this, our research team conducted a clinical trial evaluating the effect of a daily supplementation with a long-chain omega-3 fatty acid with known anti-inflammatory properties, called monoacylglyceride-coupled eicosapentaenoic acid (MAG-EPA). Clinical outcomes from this trial demonstrated a significant reduction in cancer aggressiveness (downgrading) between biopsy and surgery, as well as a reduced risk of biochemical recurrence in patients supplemented with MAG-EPA compared to placebo. The present study aims to explore the impact of MAG-EPA supplementation on the tumor immune microenvironment and its potential influence on clinical outcomes.

Material and method

We enrolled 130 patients diagnosed with intermediate- to high-grade prostate cancer, all scheduled for a radical prostatectomy. They were randomized to receive either MAG-EPA or placebo for 6 to 8 weeks prior to surgery and continued for one-year post-surgery. Tissue microarrays were constructed using prostate samples collected at surgery, comprising six cores of 1mm diameter per patient (four from tumoral and two from normal tissue). The main immune cell phenotypes were profiled through immunohistochemistry and multiplex immuno-fluorescence, allowing precise spatial analyses of their density and location.

Result and discussion

Cluster analyses revealed a higher number and larger size of lymphoid aggregates in the microenvironment of MAG-EPA-treated patients who experienced downgrading compared to the placebo group ($p = 0.03$), suggesting a well-organized and functionally engaged immune infiltration. Furthermore, lymphoid aggregates from MAG-EPA-treated patients exhibited a higher density of CD8+FOXP3+ cells ($p < 0.001$). This phenotype may reflect increased immune cell activity or plasticity in the tumor microenvironment of MAG-EPA-treated patients who experienced downgrading. It has been previously observed in prostate cancer, but further analyses are necessary to fully characterize its functional role within the tumor immune microenvironment in this context.

Conclusion

These findings suggest that MAG-EPA induces a reorganization of the immune compartment which is associated with reduced cancer aggressiveness. This underlines the potential of MAG-EPA as a complementary therapeutic approach in prostate cancer management. Further analyses of the immune cell interactions will highlight the anti-tumoral mechanisms implicated.

EACR25-0331

Combined therapy targeting AR and EZH2 restrains the growth of castration resistant prostate cancer by enhancing

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antitumor T cell response

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Introduction

Castration resistant prostate cancer (CRPC) is a fatal disease. Androgen receptor pathway inhibitors, like enzalutamide, are initially effective but resistance eventually occurs, often associated to the emergence of aggressive neuroendocrine variants (NEPC). Even immunotherapy induced limited results in prostate cancer, governed by an immunosuppressive microenvironment.

Material and method

We propose that new approaches to revert castration resistance and to turn the immune milieu from “cold” to “hot” would be synergic against CRPC. To this aim, we investigated in preclinical models the effects of a combination between enzalutamide and the drug GSK-126, which inhibits the epigenetic modulator EZH2.

Result and discussion

We show that enzalutamide and GSK-126 can synergize to restrain the growth of CRPC in vitro and in vivo. Moreover, this therapeutic combination efficiently reduced NEPC differentiation, in both subcutaneous and autochthonous in vivo models, increasing the rate of cured mice with regressed lesions. The antitumor efficacy of the enzalutamide and GSK-126 combination observed in immunocompetent mice bearing subcutaneous syngeneic CRPC tumors was lost in immunodeficient mice. Furthermore, in the TRAMP spontaneous model this combination treatment restored cytotoxic activity and IFNγ production of tumor-specific CD8+ T cells, otherwise tolerant, and increased IL-17 production in CD4+ T cells. The two drugs did not modulate T cell activity in vitro, suggesting the importance of micro-environment accomplices in triggering these effects.

Conclusion

These results promote the combined use of enzalutamide and GSK-126 to restrain CRPC growth and NEPC differentiation, and, simultaneously, to awake antitumor T cell response, opening new possibilities for immunotherapy in prostate cancer.

EACR25-0333

A multitarget drug as potential candidate for antimetastatic adjuvant therapy

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Introduction

Cancer is the second leading cause of death worldwide,

and preventing metastatic dissemination remains a significant challenge. During metastasis, tumor cells migrate and remodel the extracellular matrix. Once in the bloodstream, circulating tumor cells must survive in the circulatory environment, with their interaction with platelets – particularly through the P-selectin/ligand pathway – playing a crucial role. Subsequently, they need to interact with endothelial cells to extravasate from the vessel, a process primarily mediated by the VCAM-1/VLA-4 interaction. Studies have shown that heparin (porcine UFH), a glycosaminoglycan composed of repetitive disaccharide units of uronic acid and glucosamine, can interfere with both P-selectin and VCAM-1 interactions. Our group purified by ion exchange chromatography a fraction from bovine heparin that presents low anticoagulant potential, named LA-hep (~15% porcine UFH) whose major benefit is its reduced risk of bleeding side effects. Our aim with this work was to investigate the LA-hep potential as an antimetastatic agent in vivo and evaluate in vitro its ability to interfere with tumor cell-platelets and tumor cell-endothelium interactions.

Material and method

For this, we challenged 8–12 weeks wild-type and P-selectin KO C57BL/6 mice with 4 mg/kg of porcine UFH or LA-hep followed by intravenous injection of B16F10 cells (murine melanoma). In another assay, we injected 4T1 cells (breast triple negative murine carcinoma) orthotopically in female Balb/c mice and treated them daily from the 7th day to 28th day with 4 mg/kg of porcine UFH or LA-hep. After 21 days (melanoma) or 28 days (breast cancer), lungs were collected, and metastatic foci were counted and analyzed. In vitro, we evaluated the interaction of MV3 cells (human melanoma) with platelets and HUVEC cells in the presence of LA-hep. Moreover, the ability of MV3 cells treated with LA-hep to migrate and invade the matrix was analyzed.

Result and discussion

The number of metastatic foci was strongly reduced when animals were treated with heparins (~70% of reduction in melanoma and ~50% in breast cancer). Even in P-selectin KO mice, which present lower rates of metastases, LA-hep was still able to reduce metastasis burden. In vitro both heparins inhibited platelet adhesion to MV3 cells and MV3 cell adhesion to HUVEC in a dose-response manner. The direct binding of tumor cells to immobilized P-selectin and VCAM-1 was also reduced in the presence of LA-hep. Additionally, LA-hep, but not porcine UFH, seems to reduce MV3 invasion in transwell plates.

Conclusion

In summary, LA-hep, a heparin derivative with low anticoagulant activity, has shown promising multitarget potential in suppressing metastasis across various carcinoma models. Therefore, it could be considered as a potential adjuvant therapy for cancer patients.

EACR25-0355

A whole-genome RNAi screen to identify genes required in Flower-mediated cell competition in *Drosophila melanogaster*

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Introduction

In the initial stages of tumorigenesis, mutated cells escape tissue surveillance mechanisms. The expansion of these pre-malignant cells occurs at the expense of wild-type cells via conserved cell competition mechanisms discovered in fruit flies. These competitive cell-cell interactions rely on the comparison of relative cell fitness. Enhanced metabolic capacity (dMyc over-expression) or resistance to mechanical compression turns mutated cells into supercompetitors. Cells express different isoforms of the transmembrane protein Flower (Fwe), labelling them according to their fitness status. For instance, human tumours display FWE Win fingerprints, while stroma expresses FWE Lose. Despite its significance, this cell selection mechanism remains poorly understood.

Material and method

To overcome the lack of mechanistic understanding of this pathway, we developed the innovative Easy Win Assay in *Drosophila*. It allows to identify genes functionally required in cell competition triggered by the expression of the conserved loser isoform, humanFWE1. We then performed a whole-genome RNAi screen based on phenotypic analysis of results in the eye of the adult fly to identify modifiers of the elimination of hFWE1 loser cells.

Result and discussion

In this blind screen, we categorized candidate genes according to the rescue or enhanced elimination of loser cells. After validating these results, we identified at least 30 genes as being required in this competition scenario. We are going to explore some of the working hypotheses related to the stronger candidate genes and test them in cell competition assays in developing epithelia. Next, through epistasis analysis, we will place these candidates in the pathway, and finally, we will shed light on their requirement in various Fwe-dependent contexts, including development and cancer (dMyc supercompetition).

Conclusion

A better understanding of cell-cell communication between cells with differential fitness will be essential to developing new approaches to optimize tissue fitness and increase healthspan.

EACR25-0358

Local and systemic interactions drive prostatic intraductal carcinoma dissemination

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Introduction

Prostate cancer is a heterogeneous disease with a slow progression and a highly variable clinical outcome. Prostatic intraductal carcinoma (IDC) is a subset of prostate cancer strongly associated with metastatic

spreading and a poor outcome [1]. Thus, unraveling the mechanisms underlying its aggressiveness might lead to the development of personalized therapies. PTEN and TP53 loss are common genetic alterations in IDC [2]. Moreover, recent studies reported that extensive hypoxia is associated with high-risk disease [3], but its contribution to tumor progression is incompletely understood.

Material and method

We characterized tumor progression in genetically engineered mice, in which Pten and Trp53 are inactivated in prostatic epithelial cells (PECs) at adulthood (Pten/Trp53(i)pe/- mice) using single-cell and spatial transcriptomic profiling, as well as by flow cytometry. Hypoxia-inducible factor 1 (HIF1) inhibition was achieved by treatment with PX-478 or by genetic inactivation of Hif1a in PECs.

Result and discussion

Pten/Trp53(i)pe/- mice develop intraductal carcinomas (IDC) that disseminate to liver. We uncovered that the cell plasticity and metastatic potential is driven by a crosstalk between PECs and cancer-associated fibroblasts [4]. Importantly, IDCs of Pten/Trp53(i)pe/- mice are characterized by an hypoxic core and Visium Spatial transcriptomic profiling revealed that the hypoxic regions are enriched in immune system-related pathways. Deeper analyses, including cell type inference and flow cytometry, unraveled that the immune infiltrate is mainly composed of immunosuppressive macrophages and neutrophils. Both genetic inactivation and pharmacological inhibition of HIF1 signaling decrease neutrophil infiltrates in prostatic tumors and resensitize them to androgen deprivation. However, only pharmacological HIF1 targeting impairs circulating neutrophil levels and eradicates hepatic micrometastases, indicating a key role of these immune cells in tumor spreading.

Conclusion

Taken together, our study highlights local and systemic interactions of prostatic epithelial cells that contribute to their metastatic dissemination, and opens novel therapeutic avenues for treatment-resistant and metastatic prostate cancer.

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EACR25-0359

Dichloroacetate modulates tumor cell immunometabolism and inflammation by regulating TNF alpha and nitric oxide in a 4NQO- induced oral carcinoma mouse model

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Introduction

Squamous cell carcinomas of the tongue are among the most common tumors of the upper aerodigestive tract. Despite advances in surgery and radiotherapy, treatment often fails for many high-risk patients, and a 5-year survival rate is achieved in only 30–50% of cases. In an experimental model of oral squamous cell carcinoma (OSCC) generated by 4-nitroquinoline-1-oxide (4NQO) in BALB/c mice, we investigated the ability of arsenite (NaAsO₂), chloroquine (CQ), and dichloroacetate (DCA) to interfere with tumor cell immunometabolism (TNF- α and nitric oxide).

Material and method

Eight-week-old female BALB/c mice were exposed to chemical induction with the carcinogen (100 µg/ml) or treated with placebo (water control) for 16 weeks, then observed for up to 16 additional weeks. At 32 weeks post-treatment, mice were sacrificed, and their tongues were removed. The oral tumor tissues were then cultured for 24 hours in the presence of NaAsO₂, CQ, and DCA. The tissues were analyzed for histology, and culture supernatants were collected to measure nitric oxide (NO) using a modified Griess method and TNF- α by ELISA.

Result and discussion

The histopathological examination of the tongues of 4NQO-treated mice verified the existence of malignancies. Our findings show that inflammatory mediators in the combination-treated invasive tissues were dramatically reduced when combined with DCA compared to untreated OSCC tissues for NO levels (30.28% of reduction) and TNF- α (52.32% of reduction).

Conclusion

Our findings indicate that BALB/c mice are susceptible to developing OSCC after exposure to 4NQO due to the activation of pro-tumoral TNF- α that necessitates nitrosative stress. Our research suggests that DCA in combination with NaAsO₂ and CQ interferes with the inflammatory status of TNF- α /NO in 4NQO tumors. These findings bolster the attractiveness of DCA as an optimal strategy for therapeutic advantage in OSCC.

EACR25-0378

Colorectal cancer hepatic metastasis modelling by advanced 3D bioprinting and demonstration of oncolytic viral chemotherapeutic delivery

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Introduction

Animal replacement in pharmaceutical and advanced chemotherapeutic development requires better in vitro and ex vivo modelling, not just tests that seem useful but

rather models that give results that researchers really need. We advanced our static and bioreactor-created colorectal cancer models with 3D bioprinting modalities creating reproducible screening for solid tumors.

Although organoid / spheroid testing is very useful in the early stages of screening it is not expansive enough to model longer growing tumors, nor for penetration testing. Here, using bioprinting, we tested colorectal cancer cell lines with hepatic donor cells to understand longer term tumor kinetics and demonstrate how oncolytic viral-delivered therapy can penetrate in 3D and evaluate effects over time.

Material and method

JVE-103 and JVE-253 colorectal cancer cell lines were amplified, and primary human hepatocytes freshly prepared and frozen following ethical consent. A 3D computer generated metastatic model was constructed with a central core of colorectal and outer significant covering of hepatocytes and the g-code transferred to a bioprinter. Cells were bioprinted with an alginate-nanofibrillar cellulose loaded bioink into 24 well plates creating multiple reproducible models, growing at 37°C, 5% CO₂, 15–80 days. Evaluation was made for metabolism, Caspase-3 cleavage, Masson-Trichrome histology, Ki67, EPCAM, Pancytokeratin and structural integrity of the resulting tumor formation. Response to infused 5-FU treatment and oncolytic viral-delivered 5-FU was evaluated.

Result and discussion

3D tumor formation, demonstrated with histological evaluation was achieved by 15 days with significant in-situ organoids and cultivation could continue for 80 days allowing for long-term evaluation, formation of necrotic cores and re-metastatic events. 5-FU infusion was dose responsive. Oncolytic virus loaded with the non-toxic chemotherapeutic did not target the hepatocytes, but did penetrate into the area of colorectal tumors. Intracellular enzyme-conversion there into local 5-FU was achieved and had the same effect as a higher 5-FU dose directly infused.

Conclusion

This level of modelling which advances on short-term organoid testing proved not only appropriate for investigation of speed and quantity of tumor formation over time, but also demonstrated defined and effective targeting of the oncolytic viral system as a novel chemotherapeutic delivery system.

EACR25-0381

Complement protein C3 from breast cancer-associated fibroblasts as a regulator of the tumor microenvironment

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Introduction

Cancer-associated fibroblasts (CAFs) are a major component of the breast tumour microenvironment, shaping the behaviour of breast cancer and affecting its progression. Complement protein C3 is a central component of the complement system, essential element of innate immune response. The aim of the current study

was to evaluate the role of C3 in the initiation and progression of breast cancer.

Material and method

Expression data from patients with breast cancer pooled in Kaplan-Meier plotter were analyzed to determine the prognostic role of C3. Single-cell RNA-sequencing data from Single Cell Portal were also used to explore the expression levels of C3 in distinct cell populations within the tumour. The CRISPR/Cas9 system was employed to knock-out C3 in CAF2 cells by deletion of four exons. RNA-sequencing analysis of the obtained CAF2 clones was performed. The Bio-Plex® Pro Human Cytokine Assay was used for simultaneous screening of multiple cytokines, chemokines and growth factors secreted by CAF2 clones. The results were validated by DuoSet® ELISA kits. Finally, a xenograft mouse model was established by co-injecting human MDA-MB-231-Luc cells and control or C3 knock-out CAF2 cells, mixed in a ratio of one to three, orthotopically into NXG immune-deficient mice. Tumour volume was measured twice per week using the digital caliper and metastasis was evaluated using the IVIS® Spectrum 2 in vivo imaging system.

Result and discussion

Database analysis revealed that low C3 expression in breast cancer was associated with decreased overall survival ($p = 3.2e-5$), relapse-free survival ($p = 2.1e-9$) and distant metastasis-free survival ($p = 1.0e-5$) of patients. As C3 is highly expressed in CAFs cells, C3 knock-out in CAF2 cells was achieved by CRISPR/Cas9, as shown by C3-specific ELISA assay and Western blotting. Three control and three C3 knock-out clones were used for the subsequent experiments. The RNA-sequencing data highlighted the role of C3 in cytokine and chemokine activity and binding, cell cycle regulation, angiogenesis, and neutrophil activation. Lack of C3 expression resulted in altered cytokine secretion, IL-8 and MCP-1 in particular, validating the RNA-sequencing analysis data. Finally, the xenograft mouse model revealed that C3 deriving from breast CAFs slows down tumour growth ($p = 0.0096$) and drastically delays metastasis ($p = 0.0052$), as shown by the 2-fold increase of luminance.

Conclusion

Our study indicates that C3 secreted from breast CAFs has a protective effect in tumor progression and metastasis. Nevertheless, the exact mechanism of action of C3 for the regulation of the tumor microenvironment remains to be elucidated.

EACR25-0398

Three-dimensional Microtissues: An In-Vitro Platform to Recapitulate the Heterogeneity and Complexity of Head and Neck Squamous Cell Carcinoma

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Introduction

Head and neck squamous cell carcinoma (HNSCC) is a malignancy with a complex tumour microenvironment

(TME). Conventional in-vitro models, such as two-dimensional (2D) monolayer cultures, fail to capture the intricate cellular and molecular interactions within tumours, limiting their translational and physiological relevance. We have developed an advanced in-vitro model that recapitulates the tumour heterogeneity and the dynamic interplay between cancer cells and the TME as a straightforward and technically simple platform for studying HNSCC biology.

Material and method

Our 3D microtissues integrate three-dimensional (3D) culture techniques based on cells isolated from fresh tumour biopsies, cultured on a collagen type I/hyaluronic acid/ Matrigel® matrix. This allows us to directly culture cell crude suspensions from biopsies, containing patient-derived squamous epithelial cells along with all stromal components (cancer-associated fibroblasts (CAFs), immune cells, and endothelial cells). This system promotes tumour-stroma interactions, resulting in the spontaneous formation of tissue-like structures and matrix remodelling. Various 3D culture conditions differentially stimulate the capacity of the cellular components to mimic the organization of the original tumour. Furthermore, immunofluorescence (IF) staining was performed to validate distinct cell populations in the microtissues, and single-cell RNA sequencing (scRNA-seq) to confirm the preservation of tumour heterogeneity and specific differentiation patterns.

Result and discussion

Our microtissues spontaneously recapitulate key phenotypic and genotypic traits of cancer tissues, with a characteristic cellular heterogeneity that closely mimics the spatial organization of the original tumour. The use of different culture conditions (cell density, media, growth factors, serum etc) significantly modulated the growth, survival, and differentiation of both stromal and epithelial cell types, highlighting the role of the TME in shaping tumour architecture and ECM remodelling. scRNA-seq analysis confirmed that various components of the original tumour heterogeneity were maintained, depending on the conditions used. Strong tumour cell plasticity was also observed, which contributed to rapid phenotypic adaptations to conditions, and differential formation of tissue-like structures. This indicates the model's ability to replicate the architecture of squamous tumours.

Conclusion

By bridging the gap between conventional and in-vivo models, our microtissues provide a robust and translationally relevant platform for investigating HNSCC biology. Its ability to preserve tumour heterogeneity, mimic TME interactions, and model therapeutic responses makes it a valuable tool for preclinical research, personalized medicine, and drug discovery, contributing to improved therapeutic outcomes in HNSCC.

EACR25-0401

Exploring the role of innervation in thyroid cancer and modulation by therapy-induced senescence

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Introduction

Nerves in the tumor microenvironment (TME) actively drive cancer progression, playing a key role in cancer initiation, metastasis, and recurrence across multiple solid tumors. A dynamic crosstalk between cancer cells and nerves creates a feedback loop that accelerates tumor growth, by recruiting neuronal precursors or exploiting locoregional nerves to promote neurogenesis and axogenesis. Despite growing evidence in other malignancies, the impact of innervation in thyroid cancer (TC) remains largely unexplored. Emerging evidence suggests that innervation within the TME may be linked to TC progression, with higher nerve density in TC being associated with extra-thyroidal invasion compared to benign tissue. Cellular senescence, a growth arrest triggered by stressors including anti-cancer treatments (therapy-induced senescence, TIS), can drive cancer aggressiveness via the senescence-associated secretory phenotype (SASP). Notably, senescent cells secrete brain-derived neurotrophic factor (BDNF), a SASP component required for their survival, and axon-guidance molecule Netrin-1. Therefore, our aim is to investigate the role of innervation in TC and examine whether senescent cells, particularly those induced by anti-cancer therapies, contribute to this process through the paracrine effects of the SASP.

Material and method

Five human TC cell lines were used: TPC-1, BCPAP, 8505C, KAT18 and HTCC3. TIS was induced using the CDK4/6 inhibitor Palbociclib and ionizing radiation. Secretion of neurotrophic factors and axon-guidance molecules was assessed in conditioned media using ELISA and western blot analysis, while Trks receptor expression and Sortilin were examined by western blot and immunofluorescence assays.

Result and discussion

Release of neurotrophins and GDNF was observed in all tested TC cell lines, along with the release of axon-guidance molecule Ephrin-B1. Moreover, TC cells activate TrkB, which, along with its ligand BDNF, establishes a tumor-promoting autocrine loop that may further contribute to tumor innervation. In TIS-TC cells the secretion of neurotrophic factors and TrkB expression increases compared to untreated cells. Overall, both TC and TIS-TC cells release factors promoting innervation, with senescent cells potentially playing a greater role in this process.

Conclusion

These results show that TIS-TC cells may enhance processes of innervation in TC, further contributing to cancer progression and aggressiveness. Understanding the mechanisms underlying this interaction could reveal novel therapeutic targets for TC.

EACR25-0406

Tissue-specific fibroblast lipids drive epithelial cancer invasion

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Introduction

Squamous cell carcinomas (SCC) originate from epithelial tissues in various organs across the body and are associated with exposure to environmental carcinogens such as tobacco, alcohol or ultraviolet radiation. Despite the overlapping histopathological features and driver mutations, SCCs from different anatomic sites have different prognoses. Oral and lung SCC are highly invasive and metastatic, whereas cutaneous SCC (cSCC) are seldom fatal and have a metastasis rate of approximately 2%. As fibroblast are key cells in the microenvironment and are critical for epithelial cell development and differentiation, we examined how fibroblasts from different tissues contribute to differences in SCC outcomes.

Material and method

Utilising normal human fibroblast and SCC cell lines from the skin, lung, and head and neck we modelled with in vitro organotypic models how fibroblasts and SCCs interact in a tissue specific manner to drive invasion and proliferation. Mechanisms were explored with lipidomics, RNA sequencing and validated in vivo. We validated our data in patient samples using spatial transcriptomics of clinical samples and analysis of public clinical cohorts.

Result and discussion

We discovered that fibroblasts contribute to SCC aggressiveness in a tissue-specific manner by modulating SCC proliferation and invasion. Oral and lung fibroblasts enhance SCC invasion by inducing epithelial-to-mesenchymal transition, whereas dermal fibroblasts lack this effect, aligning with the low metastatic potential of cSCC. Lipidomic profiling revealed distinct metabolic interactions between fibroblasts and SCCs. Oral fibroblasts transfer sphingomyelins, activating the ceramide/S1P/STAT3 pathway to drive oral SCC invasion, while lung fibroblasts supply triglycerides, fuelling cholesterol synthesis and promoting aggressive lung SCC behaviour. In contrast, dermal fibroblasts exhibit low lipid content, corresponding to the reduced invasiveness of cSCC. Blocking fibroblast lipid metabolism, SCC lipid uptake, or lipid processing suppressed oral and lung SCC invasion, highlighting potential therapeutic targets. Spatial transcriptomics and patient cohort analysis confirmed the presence of these fibroblast-epithelial lipid interactions in clinical samples and their correlation with SCC outcomes. Notably, these

interactions were evident in early-stage lesions, suggesting a role in SCC initiation and progression.

Conclusion

Our study establishes fibroblasts as key regulators of SCC progression through tissue-specific lipid metabolism. By shaping the tumour microenvironment, fibroblasts drive SCC aggressiveness in an anatomic site-dependent manner. These findings open avenues for targeting fibroblast-derived lipid pathways to prevent or mitigate SCC progression, with potential implications for early intervention and precision therapies.

EACR25-0409

Developmental mosaicism underlying EGFR-mutant lung cancer with multiple primary tumors

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Introduction

Patients with non-small cell lung cancer (NSCLC) often present with radiographic findings suggestive of two or more anatomically distinct synchronous lesions. The development of multiple primary tumors in smokers with lung cancer could be attributed to carcinogen-induced field cancerization, while the occurrence of multiple independent early-stage tumors in individuals with EGFR-mutant lung cancer who lack known environmental exposures remains unexplained.

Material and method

We identified ten patients with early stage, resectable, non-small cell lung cancer who presented with multiple, anatomically distinct, EGFR-mutant tumors. We reconstructed the phylogenetic relationships among multiple tumors from each patient using whole-exome sequencing (WES). To quantify the divergence between tumors, we generated poly(G) fingerprints, which measure insertions/deletions (indels) in hypermutable guanine mononucleotide repeats. These mutations occur at high rates during DNA replication as a consequence of polymerase slippage. Therefore, the divergence between the poly(G) genotypes of two somatic cell populations is a reflection of the number of cell divisions that separate them.

Result and discussion

In several patients, developmental mosaicism, assessed by WES and poly(G) lineage tracing, indicates a common non-germline cell of origin. Anatomically distinct primaries contain a number of shared somatic mutations comprising only a small fraction of the total exonic mutational burden and are inconsistent with both unrelated somatic origin nor intrapulmonary metastasis. Our analysis of poly(G) repeats in such tumors suggests that EGFR mutations may occur before cells have undergone half of the divisions on their way to tumor initiation. In two other patients, we identified germline EGFR variants, which confer moderately enhanced signaling when modeled in vitro. Our study suggests that a canonical and fully activating EGFR mutation may arise early during lung development, creating a mosaic of lung epithelial cells harboring this mutation and distributed across the adult organ. The high prevalence of normal lung specimens with rare detectable EGFR-mutant alleles as a result of field cancerization does not explain the occurrence of the multiple EGFR-mutant primary tumors described in the present study, because they would be genetically independent, lacking the multiple shared mutations that define the developmental, mosaically derived primaries.

Conclusion

In summary, patients presenting with multiple EGFR-mutant primary tumors, are likely to harbor either a germline EGFR variant or developmental mosaicism in the lung. These individuals are at risk of developing additional tumors during their lifetime, suggesting the importance of ongoing surveillance and raising consideration of new prophylaxis strategies.

EACR25-0425

Breast Cancer Cell-Adipose Tissue Interaction is Regulated by RET/PDGF-B Axis to Commit Immature Adipocytes in Favor of Tumor Growth

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Introduction

The breast tumor-adjacent adipose tissue harbors modified adipocytes that resemble their precursors, pre-adipocytes (PAs), and exhibit a pro-tumoral function. However, the molecular mechanisms governing the crosstalk between tumor cells and adipose tissue remain largely unknown. In this study, we identify RET, a receptor tyrosine kinase overexpressed in 50% of breast tumors, as a critical mediator of this interaction.

Material and method

To investigate the role of RET in tumor-adipose tissue crosstalk, we employed both in vitro and in vivo approaches. Breast cancer cells either expressing RET (RET-WT) or with RET knockout (RET-KO) were used in co-culture systems with adipocytes and in orthotopic allograft mouse models. Gene expression profiles were analyzed via RNA sequencing, and molecular changes in adipose tissue were assessed through RT-qPCR, Western blot, and histological staining. Additionally, we examined a novel cohort of breast cancer patients, evaluating RET and PDGF-B expression in tumor-adjacent adipose tissue and correlating these findings with clinicopathological features and adipocyte phenotype.

Result and discussion

In the RET⁺ tumor microenvironment, adipocytes exhibit a more immature phenotype, a finding supported by our in vitro results showing that RET-expressing breast cancer cells impair adipocyte differentiation. RNA-sequencing analysis in mouse transgenic RET-expressing glands revealed upregulation of genes involved in maintaining a PA phenotype, including PDGF ligands. In agreement, RET expression positively correlates with PDGF-B levels in human breast cancer biopsies. Functionally, PAs enhance tumor cell proliferation, and in vivo co-injection assays demonstrated that their protumoral effect is specifically dependent on RET expression in cancer cells.

Conclusion

Our findings indicate that the RET/PDGF-B axis mediates tumor-adipose tissue communication, driving a PA-like phenotype that fosters breast cancer progression. The validation of these results in a new patient cohort highlights the potential of targeting this interaction as a novel therapeutic strategy against breast tumors.

EACR25-0446

MCL-1 as a molecular switch between myofibroblastic and pro-angiogenic features of breast cancer-associated fibroblasts

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Introduction

Breast cancer-associated fibroblasts (bCAFs) comprise two pro-tumor populations: inflammatory CAFs (iCAF) producing pro-inflammatory cytokines and myofibroblastic CAFs (myCAF) characterized by their highly contractile and invasive phenotype. We have previously shown that bCAFs promote therapy resistance by overexpressing the anti-apoptotic protein MCL-1 and inducing its expression in luminal breast cancer cells by paracrine effects. This defines MCL-1 as a target in stroma-influenced breast cancers, and advocates for a comprehensive investigation of the effects of its targeting in distinct cellular components, such as myofibroblastic CAFs, of these tumor ecosystems.

Material and method

In this study, we explored the role of MCL-1 in the primary culture of CAFs obtained from treatment-naïve patients with invasive carcinoma after surgical resection by inhibiting MCL-1 pharmacologically with S63845, a BH3 mimetic developed to specifically antagonize its anti-apoptotic function or genetically by silencing MCL-1 gene expression using Crispr-Cas9.

Result and discussion

Single-cell RNA sequencing analysis of bCAFs reveals that MCL-1 knock down induces a phenotypic shift from wound-myCAF to IL-iCAF. Notably, MCL-1 depletion in bCAFs leads to a reduction in the expression of key myofibroblast activation markers, including α -SMA and Myosin IIb, accompanied by a significant loss of contractile, migratory, and pro-invasive properties in a 3D co-culture model with luminal breast cancer cells. Mechanistically, pharmacological inhibition of MCL-1, which stabilizes the protein, enhances its interaction with DRP1, triggering mitochondrial network fragmentation and cytoplasmic retention of the transcription factor YAP1. Concurrently, MCL-1 targeting in bCAFs stimulates VEGFA secretion, which in turn enhances endothelial cell tubulogenesis and drives extensive tumor vascularization in the chicken chorioallantoic membrane (CAM) model. Mechanistically, we identified NF- κ B activation as responsible for the overexpression of VEGF-A and the cytokines/chemokines CXCL1, CXCL8 and IL-1 β leading to the acquisition of an inflammatory phenotype by bCAFs in response to MCL-1 targeting. Importantly, we established that chemotherapeutic treatment promotes inflammatory and pro-angiogenic phenotype of bCAFs too.

Conclusion

These findings highlight a novel role for MCL-1 in regulating the phenotypic plasticity of bCAFs, and provide new insights into the characterization of distinct bCAF subpopulations, particularly post-chemotherapy.

EACR25-0458

Reprogramming of cancer-associated fibroblasts during the stepwise progression of colorectal cancer

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Introduction

Colorectal cancer (CRC) is the second leading cause of cancer-related deaths globally. It evolves a complex tumor microenvironment (TME), where cancer-associated fibroblasts (CAF) stand as the most abundant cell type with major impact on therapeutic responses. High CAF abundance is associated with aggressive cancer type and poor survival, highlighting the need to detailed understanding. Recent advances in single-cell RNA sequencing (scRNA-seq) have revolutionized the research of fibroblasts, leading to the identification of transcriptomic and functional distinctions among subsets. Yet, the exploration of CAFs in the context of CRC remains limited, complicating therapeutic targeting. About 80% of CRCs acquire early mutations in APC gene, followed by sequential accumulation of mutations in KRAS, TP53, and TGFBR2 genes or pathways,

eventually leading to metastasis. Despite extensive research on driver mutations, the impact of accumulating mutations on CAF reprogramming remains unclear. The aim of this study is to characterize how driver mutations activate CAF subsets during CRC tumorigenesis to enable the design of CAF-targeting therapies.

Material and method

We analyzed scRNA-seq and spatial transcriptomic data from human normal and CRC colon to define CRC CAF subtypes, assess their prognostic value and map their distribution. Additionally, we performed 3D mouse primary co-cultures with cancerous organoids modeling CRC progression, and analyzed available organoid-fibroblast co-cultures scRNA-seq data to assess how cancer-inducing mutations in intestinal organoids affect fibroblast activation.

Result and discussion

We identified four CAF subtypes (preCAF1, preCAF2, matrix(m)CAF and inflammatory (i)CAF), and found that they originate from different anatomical sites. Interestingly, the mCAF signature correlated with reduced survival and was significantly enriched in patients with CMS4 tumors and with KRAS mutations, suggesting that KRAS-driven tumor cells creates a TME favouring matrix-producing differentiation of adjacent fibroblasts, leading to more aggressive disease. To explore fibroblasts behavior during the stepwise CRC progression, we analyze data from fibroblasts co-cultured with CRC-modeling organoids and confirmed that epithelial cells induce early CAF expression.

Additionally, fibroblasts influenced the growth of Apc-mutant spheroids, supporting a bidirectional tumor-stroma cross-talk during early CRC steps.

Conclusion

Here, we correlate CRC CAF subtypes with CRC patients survival and clinical features, and investigate how driver mutations in the CRC epithelium reprogram fibroblasts in an ex vivo setting. Identifying specific CAFs signatures correlating with poorer prognostic will contribute to a better knowledge on CAF reprogramming and heterogeneity, which will pave the way to design CAF-targeting therapies.

EACR25-0486

Elucidating the molecular mechanisms involved in pancreatic cancer and liver microenvironment crosstalk facilitating liver metastasis

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Introduction

Pancreatic ductal adenocarcinoma (PDAC) is one of the deadliest cancers, ranking third in cancer-related mortality despite being only the eighth most common cancer. Its poor prognosis is due to late detection, therapy resistance, “cold” and fibrotic microenvironment, and frequent liver metastases, which significantly worsen outcomes compared to extrahepatic metastases. While the liver microenvironment plays a critical role in metastasis,

the molecular mechanisms underpinning these interactions remain unclear. This study aims to uncover the molecular and cellular interactions between PDAC cells and the liver microenvironment, with the goal of identifying therapeutic targets to prevent or slow the progression of liver metastases.

Material and method

We developed in vitro, and ex vivo models to study PDAC-liver TME interactions, including co-culture systems, conditioned media assays, and 3D spheroid invasion models. Cutting-edge techniques such as cytokine arrays, mass spectrometry, multiplex flow cytometry and immunohistochemistry are being used to identify molecules involved in PDAC-liver crosstalk. Promising targets and biological pathways are being analyzed in murine models of spontaneous PDAC liver metastases and validated on human clinical samples. Functional studies will evaluate the effects of target inhibition using antibodies, small molecules, and CRISPR-mediated gene editing.

Result and discussion

Preliminary results demonstrate that hepatocytes enhance PDAC cell proliferation, migration, and invasion through both direct contact and secreted factors. Proteomic analysis revealed candidate biological pathways and several potential key targets mediating these effects. The functional roles of the most promising candidates are being tested in preclinical models.

Conclusion

This study highlights the role of the liver microenvironment in driving PDAC metastasis. Targeting the molecular pathways involved may offer new therapeutic approaches to prevent liver metastases and improve survival outcomes for PDAC patients.

EACR25-0488

A novel approach for the analysis of spatial cell distributions in the tumor microenvironment to predict bevacizumab response

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Introduction

The tumor microenvironment plays a critical role in cancer prognosis and treatment response through complex interactions between cancer cells and their surrounding cells. Spatial analysis methods enable the study of these interactions but often result in highly complex datasets. Several factors may influence how cancer cells are affected by neighboring cells, including the number of cells within signaling distance, their proximity to the cancer cell, and their specific cell type. To address the challenge of analyzing these interactions, we developed a novel approach to characterize and cluster cellular neighborhoods based on the local distribution of neighboring cells. Our goal was to identify neighborhood types associated with treatment response.

Material and method

We profiled breast tumor samples from 13 patients enrolled in the neoadjuvant NeoAva clinical trial (NCT00773695) using cyclic immunofluorescence (cycIF) proteomics of whole tissue biopsies. Analysis was done on the Galaxy platform with manual gating used to classify cell types in pre-treatment samples from patients receiving bevacizumab. The range for each cancer cell neighborhood was chosen to be 200 µm, and any cancer cells too close to sample borders excluded to avoid edge effects. For each neighborhood, radial distribution functions were calculated for each cell type. The bins of the radial distributions are not independent and form a high-dimensional space, thus a downsampling step was required to enable clustering and dimensionality reduction. We performed downsampling using a variational autoencoder and principal component analysis, followed by clustering to identify distinct neighborhood types.

Result and discussion

Following exclusion of cancer cells close to sample boundaries, approximately 2*105 cellular neighborhoods remained for training, testing and validation. Using the autoencoder for downsampling enabled the identification of similar neighborhood types across patient samples. Notably, we found that specific neighborhood types are correlated with treatment response to bevacizumab, suggesting that the spatial organization of the tumor microenvironment influences therapeutic outcomes. Current work is exploring improvements in the down-sampling process with improved autoencoder architectures and tensor decomposition.

Conclusion

Spatial analysis reveals important insights into cancer biology by identifying and quantifying distinct cell interactions. To investigate the added spatial dimension, however, novel methods are required. We believe that the presented approach shows promise for the generalized investigation of cellular interactions in the micro-environment surrounding the tumor cells.

EACR25-0495

RBP and alternative transcriptome characterization in adenoma-to-colorectal cancer progression

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Introduction

Colorectal cancer (CRC), the third most common cancer and the second leading cause of cancer-related deaths globally, presents clinical unmet needs and its molecular complexity remains a key challenge. Advancements in transcriptome analyses have enhanced the understanding of CRC heterogeneity. However, alterations involved in precancerous lesion (adenoma) formation and progression to CRC are unexplored. RNA-binding proteins (RBPs) play a crucial role in post-transcriptional regulation, impacting several processes such as alternative splicing (AS) and polyadenylation (APA). This study systematically explored alterations in RBP expression, AS, and APA patterns in CRC and adenoma patients' matched lesion/adjacent tissue whole transcriptome data.

Material and method

We analyzed total RNA-seq data generated from tissue pairs samples of CRC ($n = 90$) and adenomas patients ($n = 24$, both non-advanced (nAA) and advanced (AA)). We analyzed differential gene expression to evaluate RBP dysregulation from adenomas to advanced CRC. Using STRING, we constructed an RBP protein-protein interaction network to visualize altered RBP functional clusters. Relative isoform expression changes, AS, and APA events were assessed in our data and the RBP depletion experiment collection of the ENCODE Consortium. CLIP data from POSTAR3 and ENCODE were integrated to identify events directly regulated by the altered RBPs. Finally, RNA-seq data from TCGA were analyzed to validate recurrent AS and APA events.

Result and discussion

RBPs exhibited widespread and progressive dysregulation from adenomas to higher tumor stages. In particular, among 1,937 RBPs identified, 668 were consistently upregulated and 107 downregulated across AA and CRC stages. Ten RBPs showed consistently increased (AGO2, BOP1, CCT6A, DDX21, KPNA2, NME1, PCSK9, PUS7, RNASEH2A, WDR43) or decreased (PPARGC1B) expression levels from nAA to CRC stage IV. Functional RBP clusters were significantly affected, including the spliceosome, ribosomal proteins, and initiation factors. Alternative transcriptome analysis identified 624 ASE and 137 isoform switches in CRC and AA. Functional enrichment analysis highlighted alterations in pathways involved in the epithelial-to-mesenchymal transition, cytoskeletal organization, and adherence junctions. APA and splicing/APA/isoform event-RBP pairs are under further investigation using ENCODE and TCGA data.

Conclusion

Our data support cancer-related RBP dysregulation as an early molecular event of carcinogenesis occurring in adenomas, alongside specific isoform expression, AS, and APA events related to dysregulated RBPs. The integration and validation of single RBP perturbation and

CLIP data with those from our cohort offer a comprehensive approach to understand transcriptomic alterations linked to RBPs. Our findings may help to unravel the molecular complexity behind precancerous lesions and CRC progression.

EACR25-0501

A Novel Strategy to Target Pancreatic Ductal Adenocarcinoma Associated Oncogenic circRNAs

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Introduction

By 2030, pancreatic ductal adenocarcinoma (PDAC) is projected to become the second leading cause of cancer-related deaths worldwide. Its high mortality is driven by late diagnosis and ineffective treatments, underscoring the urgent need for the identification of novel targeted therapies. Circular RNAs (circRNAs) are a sub-group of ncRNAs that form a covalently closed continuous circular loop. CircRNAs play a significant role in regulating the transcription of oncogenes and tumor suppressor genes through miRNA sponging and RNA binding protein (RBP) interactions. Studies have shown that circRNA expression patterns can serve as prognostic markers for PDAC. However, whether these circRNAs can be targeted using less toxic treatment strategies, such as natural product derivatives, has not been explored. Here, we evaluated the impact of thymo-quinone (TQ), a derivative of Nigella sativa (black seed), on PDAC-associated circRNAs.

Material and method

We performed circRNA-seq profiling on MiaPaCa-2 and Panc-1 PDAC cell lines treated with TQ. The expression of the top prioritized differentially expressed circRNAs was verified by qRT-PCR using divergent primers. The biological relevance of the selected circRNAs modified by TQ was assessed using the publicly available PDAC circRNA microarray dataset (GSE79634), and pathway analysis was employed to evaluate their biological targets.

Result and discussion

TQ treatment resulted in the downregulation of PDAC gene set enrichment analysis (GSEA) identified oncogenic circRNAs, including hsa_circ_0000567, hsa_circ_0008344, hsa_circ_0058453, hsa_circ_0004405, hsa_circ_0012152, hsa_circ_0008193, hsa_circ_0001900, hsa_circ_0001495, hsa_circ_0006877, hsa_circ_0007643, hsa_circ_0002402, hsa_circ_0000707, and

hsa_circ_0001558. GSEA revealed that the TQ-altered circRNAs were associated with the Wnt and Hedgehog signaling pathways. RT-PCR demonstrated that the identified circRNAs were elevated in PDAC cell lines compared to normal HPNE cells, and their expression was significantly reduced following TQ treatment ($p < 0.05$). Furthermore, circR-0058453 was found to bind to specific miRNAs, miR-1248 and miR-1287, which have been reported to function as tumor suppressors. These findings suggest that circR-0058453 may act as an oncogenic circRNA by sponging tumor-suppressive miRNAs, thereby promoting PDAC growth – a process that can be reversed by TQ. Notably, circR-0058453 contains 22 binding sites for AGO2, and RNA immuno-precipitation assays confirmed that its binding to AGO2 was downregulated by TQ. These results suggest that TQ exerts its inhibitory effects on cell signaling through the regulation of circRNA and RBP interactions.

Conclusion

Conclusions: This study is the first to demonstrate that PDAC associated circRNAs can be targeted by the non-toxic natural product thymoquinone that warrants further clinical investigation.

EACR25-0545

Characterization of colon cancer metastases ecosystem by single nuclei RNA sequencing identifies a fibroblast subset as a biomarker for early relapse and as a potential therapeutic target

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Introduction

The era of single-cell analysis has demonstrated the importance of accurate characterization of all organ-specific cell subtypes. In the context of malignancies, this has greatly improved our understanding of cell interactions and mutual influence. However, metastatic tissues are often forgotten, although they are the real cause of cancer-related mortality, especially in colorectal cancer (CRC), whose prognosis is directly linked to the presence of liver metastases (lm). One reason for this is the inherent difficulties in analyzing lm-CRC tissue at single-cell resolution: first, the liver is composed of cells that are extremely fragile and hardly recoverable after enzymatic digestion; second, most surgical specimens are from patients who have received chemotherapy and are therefore highly necrotic, fibrotic, and fragile. Due to these limitations, most studies have focused on characterizing the immune system, ignoring the role of other components of a rich and complex ecosystem.

Material and method

To address this gap, we characterized the ecosystem of pretreated lm-CRC using single-nuclei RNA sequencing.

The global landscape, subpopulation composition, cell-cell interactions, and tumor cell inference were analyzed across populations and with respect to disease relapse.

Result and discussion

We obtained 31,524 high-quality cells from 9 patients for a total of 30 clusters, including malignant epithelial cells of CRC, immune cells, fibroblasts, and normal liver cells, which together form one of the first comprehensive atlases of lm-CRC. Among all cells, we found a subset of cancer-associated fibroblasts (CAF) that was enriched in patients whose disease has progressed within less than one year (hereafter referred to as “early relapse”). We investigated the specificities of this CAF subset and deciphered its interaction with the microenvironment: it exhibits a myofibroblastic CAF profile and interacts strongly with myeloid immunosuppressive cells and cells in endo-mesenchymal transition. This was also confirmed at the protein level by multiplex imaging and spatial transcriptomic data of lm-CRC. To investigate the function of this subset in early relapse, we examined the secreted factors and inferred their effects on endothelial cells (increased permeability) and epithelial tumor cells (enhanced growth, resistance to chemotherapy and migratory capacity). We are now investigating the enrichment of this CAF subset in lm CRC annotated with clinical data to test their predictive value for early relapse. In parallel, we have identified drugs targeting them by analyzing their gene expression signature.

Conclusion

We have identified a subset of CAFs that can serve as a potential biomarker for early relapse and as a potential target in the context of personalized medicine for metastatic colorectal cancer.

EACR25-0557

Exploring glioblastoma and natural killer cell interactions in a fluidic microphysiological system

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Introduction

Glioblastoma (GBM), a highly aggressive grade IV glioma, is the most common primary tumor of the central nervous system in adults. Despite aggressive conventional treatments, GBM remains incurable.

Efficient treatment is mostly prevented by heterogeneity and therapeutic resistance, latter driven by the complex and dynamic tumor microenvironment (TME). There is a lack of molecular understanding of GBM therapeutic resistance in the context of immune TME and of representative tumor models that mimic GBM-immune cell interactions in humans, especially the dynamic component of immune cell system. The aim of our study was to set up immune-competent glioblastoma ex vivo model to study immune cell tumor infiltration and to explore GBM-immune cell interactions after they enter the TME.

Material and method

To understand the role of GBM-immune cell interactions, we set up microfluidic platform (MIVO® platform, React4life), mimicking the influx of immune cells into the tumors. Tumor cell spheroids, established from patient-derived GBM cells and GBM stem cells, were embedded in Matrigel® and cultured in the tumor chamber above a microcirculation of natural killer (NK) cells for 24h at different effector:target cell ratios. Spheroids from patient-derived GBM cells were established using bioreactors (Clinostar® 2, CelVivo). Fluorescence microscopy, immunofluorescence and flow cytometry were used to determine the infiltration of labelled NK cells into the spheroids and the viability of GBM and NK cells.

Result and discussion

When NK-92 cells were placed in circulation, their infiltration within the GBM spheroids and their viability were investigated. Firstly, flow rate was optimized to ensure NK cell viability and circulation. NK cells interacted with GBM cells since we observed the infiltration of the NK cells into the tumor chamber into the tumor cell spheroids after 24h. Increased effector: target cell ratio did not increase the infiltration of NK cells into the tumor chamber. However, the infiltration of NK cells was increased by serum in the tumor chamber, acting as chemoattractant. We also confirmed that Matrigel is easily removed by washing with cold PBS and that flow-cytometry using non-fixed live cells is a feasible read-out after spheroid dissociation. GBM and NK cells from tumor chamber and circulation remained viable during experiments.

Conclusion

As immunotherapy is a promising approach and its success depends on the immune cells’ interaction with cancer cells, we have established a microfluidic platform that mimics tumor-immune cell interactions in human patients. Several conditions will be optimized such as the use of patient-derived immune cells and organoids and extended times of incubation. With such ex vivo brain tumor models we can explore GBM biology and test combinational anti-cancer approaches, including targeted therapy and immunotherapy.

Acknowledgement: We would like to acknowledge the support and assistance of React4Life.

EACR25-0573

RECQL4 Modulates MHC Class II Expression and Drives an Immune-Evasive Phenotype Associated with Resistance to Immune Checkpoint Therapy in Malignant Melanoma

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Introduction

Immune checkpoint inhibitors (ICIs) have revolutionized metastatic melanoma treatment, yet therapeutic resistance remains a major challenge. RECQL4, a RecQ-like helicase involved in DNA replication and repair through the DNA damage response (DDR), has been implicated in modulating immune signaling pathways. However, its impact on ICI therapy response and tumor immune evasion remains poorly understood.

Material and method

We performed an integrated analysis of whole exome and bulk RNA sequencing data from a pan-cancer cohort ($n = 25,775$) and cutaneous melanoma cohorts (untreated: $n = 471$, anti-PD-1 treated: $n=212$). We examined RECQL4 copy number variations and expression levels in relation to patient outcomes and conducted Gene Set Enrichment Analysis (GSEA) to uncover RECQL4-associated immune pathways. To assess the link between RECQL4 and therapy resistance, we analyzed melanoma cohorts of 95 responders and 85 non-responders before and after ICI therapy. Finally, we used genetically engineered RECQL4 variants and multi-omic profiling – including liquid chromatography coupled with tandem mass spectrometry – to elucidate mechanistic insights.

Result and discussion

Our findings identify RECQL4 as a key driver of poor prognosis and ICI resistance in melanoma. High RECQL4 expression correlates with elevated tumor purity, reduced Tumor Immunogenicity Associated with Response to Anti-PD1 (TIARA-PD1) signatures, and suppression of immune-related pathways.

Mechanistically, RECQL4 downregulates MHC class II expression, limiting antigen presentation and fostering immune evasion. We further identified CIITA as a key mediator of this regulation, linking RECQL4 activity to impaired anti-tumor immune responses.

Conclusion

This study highlights RECQL4 as a critical modulator of tumor immunogenicity and immune escape, demonstrating its potential as both a predictive biomarker and a therapeutic target for overcoming ICI resistance in melanoma and across various cancer entities.

EACR25-0596

The role of the Hippo signaling pathway in gastric cancer initiation and progression

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Introduction

Gastric cancer (GC) is the fifth most common malignant tumor and the fourth leading cause of cancer related deaths worldwide. Due to missing early clinical symptoms, a high number of patients have an incurable cancer at time of initial diagnosis. Based on found mutations GC was classified into four molecular subtypes. Alterations in the EGFR, WNT, TGFβ, NOTCH and NFκB signaling pathways were identified as the main drivers of cancer progression. In addition, the Hippo signaling pathway has been pointed out as an important pathway as it is mutated in about 40 % of all GC patients. However, its role in GC has not yet been adequately investigated and the functional impact of the mutations is unclear. We therefore wanted to figure out the role of the Hippo signaling pathway in GC initiation and progression.

Material and method

To study the role of the Hippo signaling pathway in GC we used our in house generated stomach-specific, inducible Anxa10-CreERT2 mouse line. Since mutations in the Hippo signaling pathway are often associated with alterations in the WNT or EGFR pathway, we mutated the Hippo pathway and one of the above-mentioned signaling pathways in our mouse line. Observed primary tumors and metastases were histologically characterized. A murine tumor organoid biobank of primary tumor tissue was generated and organoids analyzed for their morphology, proliferation and therapy response. RNA sequencing was used to unravel potential altered signaling pathways in tumor organoids.

Result and discussion

The different mouse models allowed the investigation of the gastric epithelium over the time with respect to induced malignant changes. Interestingly, the model combining Hippo+EGFR mutations led to late stage cancer formation with liver and lung metastases while the model with the WNT mutation showed only early stage cancer. Interestingly, the generated tumor organoids of the Hippo+EGFR model showed a resistance to classical chemotherapeutics and small molecules in a medium-scale drug screen. Transcriptome analyses of these organoids revealed alterations in lipid metabolism and interleukin signaling as well as the reorganization of tight junctions.

Conclusion

The combination of a Hippo pathway alteration with other driver mutations led to different tumor stages, morphologies and metastatic pattern. The generated tumor organoids of the different tumor models revealed a divergent therapy response with the Hippo+EGFR model being the most resistant. Changes in lipid metabolism as well as interleukin signaling pathway could be responsible for the observed resistance. All in all, Hippo signaling pathway plays an important role in GC tumorigenesis and the generated tumor organoids can be further used as an excellent laboratory tool to test new therapeutic options with the overall aim to improve the treatment options for GC patients.

EACR25-0602

Neoadjuvant chemotherapy modulates the tumor immune microenvironment in

ovarian cancer based on homologous recombination status

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Introduction

High-grade serous ovarian cancer (HGSOC) remains the most common and lethal subtype among epithelial ovarian tumors. Despite being considered potentially immune-reactive, immunotherapy has yet to demonstrate a significant benefit in HGSOC and chemotherapy stays as the first line standard of care. When administered before surgery, neoadjuvant chemotherapy (NACT) induces a favorable response in most ovarian cancer patients, irrespective of their homologous recombination (HR) status. However, the distinct impact of NACT on the immune response in HR-deficient (HRD) and HR-proficient (HRP) patients remains uncertain. Our study seeks to elucidate the immune response dynamics following NACT in both groups with the goal of identifying novel biomarkers for personalized immunotherapy in HGSOC.

Material and method

Tumor samples were prospectively collected from newly diagnosed advanced HGSOC patients, either at baseline or after 3-4 cycles of NACT. The composition and spatial organization of the tumor immune microenvironment (TIME) were evaluated using flow cytometry (FC), multiplex immunofluorescence (mIF) tissue imaging and coupled single cell RNA sequencing (scRNA-seq) analysis. Dynamics of immune cell proportions, their co-regulation and cellular interactions were comparatively analyzed between HRD and HRP subgroups, both before and post-NACT. ID8p53-/- and ID8p53-/-brca1-/- tumor-bearing mice were respectively used as HRP and HRD NACT models.

Result and discussion

Our longitudinal data show that following NACT, HRD tumors exhibit a significant increase in B cells, cytotoxic T cells (CD8+) and dendritic cells (CD11c+), whereas HRP tumors have no major changes in all immune subpopulations, including macrophages (CD68+). Further spatial mIF tissue imaging revealed that, upon NACT, HRD tumors had prominent immune infiltration (CD11c+ and CD8+) and enhanced mutual cell interactions (CD19+:CD8+ and CD11c+:CD8+) within the stroma, while CD8+:CD68+ were increased within the tumor islets. Importantly, we found that tertiary lymphoid structures (TLS) significantly increased in HRD tumors upon NACT. Consistent with the previous findings, our

HRD model exhibited increased T cell infiltration and TLS-like lymphoid aggregates compared to the HRP model.

Conclusion

NACT differentially reshapes the TIME in HGSOC patients depending on HR status. The distinct immune response dynamics observed between HRD and HRP tumors provide new insights that could inform novel biomarker-driven immunotherapy strategies. Ongoing scRNA-seq analysis and in vivo experiments aim to further characterize the tumor-intrinsic and TIME-dependent mechanisms underlying these differences.

EACR25-0616

Characterization of Dormant Persistor Origins Using Longitudinal Live Imaging

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Introduction

Cancer dormancy poses a significant challenge in oncology, as dormant cells can reactivate years later, resulting in disease recurrence. Our research has shown that adaptation to endocrine therapy in ERα+ breast cancer (BC) involves non-genetic transitions between cell states, contributing to stochastic "awakening" and resistance (D. Rosano et al., 2024). While multi-omics approaches have revealed crucial aspects of dormant cells, these conventional end-point studies fail to capture their dynamic progression. To address this gap, we used longitudinal time-lapse imaging to track single-cell lineages, providing a detailed view of survival mechanisms during therapy. We discovered that the initial cell cycle status of the mother cell influences the fate of its lineage following treatment, suggesting that synchronizing cells could improve their susceptibility to therapy.

Material and method

We engineered MCF7 BC cells with a novel cell cycle reporter (p21-GFP and Geminin-mCherry) and a H2B nuclear marker. The p21-GFP reporter identifies G0/G1 arrest, while Geminin-mCherry labels cycling cells in SG2M. Using the Opera Phenix Plus microscope, we performed 48-hour time-lapse imaging to track cell lineages and survival dynamics. Automated lineage tracing and cell cycle analysis were done using Imaris software. Cells were classified based on their initial cell cycle stage at treatment initiation. For a broader comprehension of persister dynamics and long-term effect of therapy we performed an additional 12-day timelapse experiment.

Result and discussion

Our goal was to trace back to the cell of origin where a transient phenotype arose and inherited across a lineage, unveiling new survival mechanisms. We observed that dormancy led to an increased G0/G1 population, likely linked to prolonged cell cycle durations. Notably, G0/G1 cells at treatment initiation had a higher likelihood of

survival post-treatment compared to SG2M cells. Those results were confirmed by cell synchronization experiments with palbociclib. We conducted a second experiment over a 12-day treatment period to thoroughly disclose persistence dynamics. The volume of data collected necessitated a fully automated approach to evaluate a significantly higher number of single lineages. We developed novel segmentation and tracking algorithms to link nuclei across adjacent frames, thereby connecting mother cells to their daughter cells. This fully automated pipeline greatly improved lineage tracking across multiple fields of view, enhancing the reliability of our findings.

Conclusion

Our study highlights the importance of the initial cell cycle stage in determining cancer cell fate during dormancy and therapy, with implications for synchronizing treatment strategies. This automated pipeline offers a powerful tool for high-throughput screening and could enable further studies on persister cells and therapy resistance.

EACR25-0620

Dysadherin-mediated CA9 expression shapes an acidic tumor microenvironment to accelerate colorectal cancer aggression

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Introduction

The tumor microenvironment (TME) plays a pivotal role in colorectal cancer (CRC) progression by fostering tumor survival and aggressive behavior. A key contributor to this process is the glycoprotein dysadherin, which is highly expressed in aggressive CRC cells and facilitates their adaptation to the TME.

Material and method

In this study, we performed a comprehensive bio-informatics analysis of clinical CRC genomic data, combined with functional studies using dysadherin-knockout CRC cells, patient tissue samples, and a xenograft mouse model.

Result and discussion

Our analysis revealed that increased tumor acidity is a hallmark of CRC progression and correlates positively with dysadherin expression. Functional studies further demonstrated that dysadherin enhances malignant traits, particularly in an acidic TME. Mechanistically, dysadherin activates the integrin/FAK/STAT3 signaling pathway, leading to the upregulation of carbonic anhydrase 9 (CA9). CA9 helps cancer cells thrive in an acidic TME by maintaining intracellular pH homeostasis, thereby promoting adaptability and resilience. Notably, dysadherin deletion in xenograft models reduced tumor growth, while CA9 reintroduction restored malignancy, underscoring the critical role of the dysadherin/CA9 axis.

Conclusion

Overall, these findings establish dysadherin and CA9 as key drivers of CRC adaptation to an acidic TME and

highlight their potential as therapeutic targets for disrupting tumor progression. This study provides new insights into how tumors exploit acidosis to enhance malignancy, offering avenues for novel therapeutic interventions.

EACR25-0624

A novel TROP2 complex affects colon cancer mechanobiology by modulating the cytoskeleton architecture

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Introduction

Although metastasis plays a significant role in the majority of deaths related to colon cancer (CC), its molecular mechanisms have not yet been fully deciphered. Cancer cell mechanobiology has greatly expanded our knowledge on cancer progression by linking the cells' mechanical properties to their metastatic potential. As the cancer driver and cell surface glycoprotein TROP2, known to advance invasion and metastasis, has found its way to the clinics as a novel target for antibody-drug-conjugates, we aimed to evaluate its function on cell mechanical properties in CC.

Material and method

Real-time deformability cytometry (RT-DC) and atomic force microscopy (AFM) were used to determine cell stiffness in CC cell lines and patient-derived organoids, chicken chorioallantoic membrane xenografts and human tumors. We modulated TROP2 expression by over-expression and knockout (KO) experiments via CRISPR/Cas9. A combined TROP2 immunoprecipitation (IP)/mass spectrometry approach and proteomics analysis were performed to assess new TROP2 interaction partners. In silico results were validated by Co-IP and proximity ligation assays (PLA). The intracellular localization of TROP2, its binding partners, YAP/TAZ as well as the cytoskeleton architecture were assessed by confocal and super-resolution microscopy. Gene expression of YAP/TAZ target genes upon RNAi approaches and modifications of the cytoskeleton was assessed by RT-qPCR.

Result and discussion

Results and Discussion High levels of TROP2 in colon cancer cell and organoids lines were associated with an increased cell stiffness, while decreasing TROP2 expression resulted in more compliant cells. By TROP2-IP followed by LC/MS based proteomics, ITGB5 and DIAPH1 were identified as novel interaction partners that were further validated by Co-IP and PLA. The triple

complex TROP2-ITGB5-DIAPH1 was only formed in stiff cells and was enriched at the perinuclear region. TROP2 binding to the cytoskeleton regulator DIAPH1 blocked DIAPH1 function followed by F-actin depolymerization and perinuclear microtubule condensation. The perinuclear localization of both the triple complex and condensed microtubules affected nuclear mechanics and YAP/TAZ gene expression. Thus, stiff cells upregulated YAP/TAZ target genes in 2D and 3D models which could be reversed when perturbing cytoskeleton or protein complex dynamics.

Conclusion

Our mechanistic study identified TROP2 as an essential mediator for cell stiffness in CC. We discovered a novel link between TROP2, ITGB5 and DIAPH1 that affects both the cytoskeleton architecture and gene expression. We suggest that stiffness is linked to an enhanced tumor aggressiveness and higher metastatic potential in CC.

EACR25-0634

Understanding the genetic heterogeneity within clonal spheroids and its interaction with the tumor microenvironment

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Introduction

Head and neck squamous cell carcinomas (HNSCC) exhibit significant inter- and intra-tumor heterogeneity, complicating diagnosis and treatment. Our previous work demonstrated that spheroids developed from patient-derived cells and HNSCC cell lines display distinct proliferative and migratory behaviors. We classified them as hyperproliferative and hypoproliferative spheroids. To uncover the molecular basis of this functional heterogeneity, we utilized single-cell RNA sequencing (scRNA-seq) and advanced computational tools like CellChat. Our analysis revealed key signaling pathways, cellular interactions, and surface markers defining spheroid phenotypes, offering insights into their genetic and functional diversity.

Material and method

scRNA-seq was performed on hyperproliferative and hypoproliferative spheroids derived from the UMSSC-22B cell line. Spheroids were cultured, dissociated into single cells, and subjected to high-throughput scRNA-seq. Bioinformatic analyses identified differentially expressed genes, signaling pathways, and cellular states. CellChat was employed to analyze intercellular communication networks within spheroids. Ligand-receptor interactions were modelled to identify pathways mediating cross-talk between distinct cell clusters.

Result and discussion

scRNA-seq revealed distinct transcriptional profiles between the two spheroids. Hyperproliferative spheroids expressed genes related to cell division and growth, while hypoproliferative spheroids expressed genes linked to migration and invasion. Pathway analysis identified differences in angiogenesis, metastasis, and tumor

microenvironment interactions. Midkine signalling (MDK-SDC) through basal cells was found to be the principal contributor to the proliferative advantage in hyperproliferative spheroids, supporting their role in tumor expansion and progression. While, Desmosomal signaling (DSC2-DSG2) through keratinocytes and fibroblasts was found to play a critical role in maintaining cellular adhesion and promoting migratory capacity in hypoproliferative spheroids. TCGA data linked MDK to advanced tumor stages, poor survival, and therapy resistance, while DSG2 and DSC2 were overexpressed in metastatic tumors. These findings highlight the distinct functional states of spheroids contributing to tumor heterogeneity.

Conclusion

The balance between proliferation and migration in spheroids reflects tumor heterogeneity's dual role in cancer progression. Midkine-driven hyperproliferative spheroids anchor tumor growth, while desmosome-mediated hypoproliferative spheroids drive metastasis. Targeting Midkine or modulating desmosomal remodeling could inhibit growth and prevent dissemination, enabling patient-specific therapies.

EACR25-0635

Insights into tumor-host interactions in high-grade serous ovarian cancer using a 3D model of the tumor ecosystem

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Introduction

Drug resistance in high-grade serous ovarian cancer (HGSC) is strongly influenced by the tumor microenvironment (TME). However, conventional 2D models used for drug testing typically include only one or two cell types and lack the three-dimensional architecture needed to capture essential tumor-stroma and extracellular matrix (ECM) interactions, limiting their predictive value. Patient-derived 3D cultures provide a more physiologically relevant platform to study drug responses and identify predictive biomarkers. Here, we present a novel 3D HGSC model incorporating patient-derived cancer cells, tumor-resident immune cells, and cancer-associated fibroblasts (CAFs), embedded in a human omentum-derived ECM, to better recapitulate tumor complexity and improve drug testing approaches.

Material and method

Tissue samples were obtained from patients enrolled in the ONCOSYS-OVA clinical trial (NCT06117384). To optimize the isolation of cancer, immune, and non-immune stromal cells, we tested three enzymatic dissociation methods: (i) dispase, (ii) a collagenase-hyaluronidase (CH) cocktail, and (iii) sequential dispase treatment followed by the cocktail on the remaining tissue. The optimal method, selected based on cell recovery and viability, yielded two fractions: one enriched in cancer and immune cells, and another containing also non-immune stromal cells. The latter was further purified using magnetic-activated cell sorting.

The purified stromal cells were recombined with the cancer and immune cell fraction in a 1:1 ratio, embedded in an omentum-derived ECM, and cultured under serum-free conditions for up to nine days. Model characterization included flow cytometry for phenotyping and functional assessment, and immunofluorescence to evaluate cellular composition and viability. Further cell-type identification and transcriptional profiles will be assessed by scRNA-seq.

Result and discussion

Optimization of the tissue dissociation allowed efficient recovery of key cellular components. The sequential method effectively separated distinct cell populations into two fractions. Dispase treatment improved the recovery of cancer cells while also yielding immune cells with lower stress-response gene expression compared to those isolated with CH alone. Subsequent CH treatment significantly enhanced the recovery of CAFs. After culturing the cells in the 3D model, flow cytometry confirmed that the cellular composition remained stable over time and retained functional characteristics. Immunofluorescence stainings also demonstrated the persistence of key cell populations within the cultures, supporting the model's structural and phenotypic integrity.

Conclusion

We developed a novel 3D model of HGSC that successfully incorporates key components of the HGSC TME, providing a promising platform to study cell-cell interactions and patient-specific drug responses.

EACR25-0650

MLK4 role in the cross-talk between triple-negative breast cancer cells and tumour-associated macrophages

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Introduction

Triple-negative breast cancer (TNBC) accounts for about 10–15% of all breast cancers (BC) and have poor outcomes, where limited therapies have been proposed so far. Previously, our group reported that MLK4 kinase is highly upregulated in the samples of TNBC patients and is correlated with significantly shorter overall survival. Additionally, we showed that MLK4 can activate the NF-κB signalling, promotes mesenchymal phenotype of TNBC cells and regulates DNA damage response. Cancer progression is driven not only by cancer cells but also by tumour microenvironment (TME) cells. The largest group of immune cells in the TME are tumour-associated macrophages (TAMs), reaching up to 50% of a solid tumour mass. For this moment, there are still no studies investigating the role of MLK4 signalling in TNBC-TAMs communication.

Material and method

In this project, we examined the human monocytic cells (THP1) derived M2 macrophages, human monocyte-

derived macrophages (hMDMs) and two TNBC cell lines (SUM149, HCC1806) with inducible MLK4 knock-down. We performed a set of experiments involving the co-culture, colony-formation, migration and invasion assays, RNA sequencing and cytokine array.

Result and discussion

Our results showed that the co-culture of TNBC cells with TAMs-like M2 macrophages increased the proliferation, migration and invasion of TNBC cells. Importantly, the knock-down of MLK4 in TNBC cells significantly reduces these effects. Furthermore, we explored the MLK4-dependent changes in gene expression of TNBC cells stimulated or not by co-culture with M2 macrophages. Gene set enrichment analysis confirmed that genes elevated by MLK4 kinase and TAMs-like M2 macrophages (e.g., WISP1, ADAM12 and MMP28) are fueling epithelial to mesenchymal transition. Moreover, we marked out CXCL1 chemokine as one of the most significantly elevated factors in the medium from co-culture, high-MLK4 environment compared to the monoculture. Next, we performed the phenotypic assays using CXCL1 chemokine to mimic co-culture conditions. Our data showed that CXCL1 did not increase proliferation but significantly fueled the migration of TNBC cells in MLK4-related manner.

Conclusion

Taken together, TAMs-like M2 macrophages promote the proliferation, invasiveness and migratory potential of TNBC cells, and the knock-down of MLK4 in TNBC cells significantly reduces this effect. Importantly, we found genes potentially involved in these processes. We found that the medium from co-culture experiment is enriched with CXCL1 chemokine. Our phenotypic results showed that CXCL1 enhanced the migration of TNBC cells in an MLK4-dependent manner but was not the factor responsible for the increase in the proliferation rate of TNBC in the co-culture. In the near future, we will precisely describe the cross-talk between TNBC and TAMs, regulated by MLK4.

EACR25-0658

Secretomes of IFN-γ-Activated Colon Endothelial Cells Suppress Colorectal Cancer: Molecular Insights and Cell-Biological Effects

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Introduction

Colorectal cancer (CRC) remains a leading cause of cancer-related deaths worldwide. Its progression is heavily influenced by the tumor microenvironment (TME). While angiogenesis is often associated with tumor growth and metastasis, tumor-associated blood vessels can also exert anti-tumor effects by secreting proteins, such as thrombospondin and SPARCL1, which inhibit tumor progression independently of blood flow. A Th1-dominated, IFN-γ-rich TME correlates with

improved patient prognosis in CRC. Here, we explore the molecular composition and functional impact of secretomes derived from IFN- γ -stimulated human intestinal endothelial cells (HIEC) on CRC cells.

Material and method

HIEC isolated from healthy colon tissues and dermal microvascular endothelial cells (MVEC) were treated with IFN- γ (10 U/mL) for 48 hours. Conditioned media (CM) were collected and analyzed using comparative hyper reaction monitoring mass spectrometry (HRM™ MS). The biological effects of these secretomes on CRC cell migration and proliferation were assessed using IFN- γ -resistant CRC cell lines (DLD-1, SW480) to exclude direct IFN- γ effects. Protein secretion profiles were validated via Western blot, while endothelial expression in CRC tissues was examined through immunohistochemical double staining (CD31/candidate proteins).

Result and discussion

Secretomes from IFN- γ -treated endothelial cells significantly reduced CRC cell proliferation and migration compared to untreated controls. Mass spectrometry analysis identified 1,084 proteins in the HIEC-CM, with 43 proteins showing significantly altered concentrations upon IFN- γ stimulation. Western blot confirmed the presence of the eight major differentially expressed proteins, while immunohistochemistry reconfirmed their endothelial expression in CRC tissues. These findings suggest that IFN- γ -activated endothelial cells secrete factors that may suppress tumor progression.

Conclusion

Endothelial secretomes play a pivotal role in shaping the TME, with IFN- γ -induced factors demonstrating potential anti-tumorigenic properties in CRC. Our study identifies novel angiocrine candidates with potential therapeutic implications, warranting further investigation into their functional roles in CRC suppression.

EACR25-0663

In vitro models to mimic tumor endothelial cell-mediated immune cell reprogramming in lung adenocarcinoma

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Introduction

Responsible for an estimated 1.8 million deaths per year, lung cancer is one of the leading causes of cancer incidence and mortality worldwide, with non-small-cell lung cancers (NSCLC) accounting for 80-85% of cases. Over the past decade, immunotherapies have revolutionized patient outcome but still 60% of the patients remain in therapeutic failure. The tumor micro-environment (TME) is an important parameter mediating such resistance. Particularly, within the TME normal endothelial cells (NECs) are educated into tumor endothelial cells (TECs) and display deregulated immunoregulatory features with therapeutic relevance. However, the precise immuno-regulatory functions of NECs/TECs remain largely unknown and warrant further investigation.

Material and method

In this study we established various in vitro coculture models to unravel how tumor cells could remodel NECs into TECs, and how their remodeling impacts the immune system. As such, we combined multi-omics approaches including single-cell RNA sequencing (scRNA-seq), meta-analysis and various functional assays to dissect the immunoregulatory functions of NECs/TECs.

Result and discussion

We showed that coculturing ECs with various NSCLC cell lines induced profound alterations at the transcriptomic, proteomic and kinomic levels with the induction of pro-inflammatory pathways that reflect their activation state. Despite a limited impact of NSCLC-TECs on CD8 T lymphocytes proliferation and activation, we showed that CD4 T cells differentiate into various immunosuppressive subsets (Treg, Th22, and Th2) when cocultured with NSCLC-TECs. We also discovered that ECs could enhance the expression of M2-like markers on cocultured macrophages. For the first time, we brought to light that OX40L could play a role in the immunoregulatory function of TECs, where it appears downregulated as compared to NECs. In the seek to improve our 2D coculture system, we scrutinized at the single cell resolution, 3D multicellular tumor spheroids (MCTS) encompassing tumor cells, ECs, fibroblasts and monocyte-derived macrophages. We showed that cell heterogeneity was much more complex than 2D cultures, with several cell states identified. Particularly, 3 sub-clusters of MCTS-ECs were distinguished, among which the inflammatory subcluster that was absent from standard 2D culture but present in freshly-isolated ECs from NSCLC biopsies. Finally, MCTS presented various macrophage and fibroblast states that have particular therapeutic importance with regard to their interactions with the vascular compartment.

Conclusion

We established various 2D/3D culture models that allow to better delineate the precise function of TECs within the TME, and how they could modulate tumor immunity.

EACR25-0670

Neuroblastoma and the nervous microenvironment: partners-in-crime

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Introduction

The field of cancer neuroscience has recently highlighted the complex dialogues between cancer and nervous system components, emphasizing the pivotal roles of the nervous system in tumor initiation, growth, and invasion. Specifically, brain tumors have been shown to integrate into bona fide neuron-to-cancer synaptic networks while

also capturing neural activity-dependent factors that shape their tumor properties. This has opened up new therapeutic opportunities, particularly in the pediatric population, where nervous system tumors are among the most frequent. However, knowledge of neuro-cancer crosstalk in the peripheral nervous system (PNS) remains fragmented, especially in malignancies arising within peripheral nervous tissues. Neuroblastoma (NB) is a hallmark and deadly pediatric cancer of the PNS, with primary tumors arising in sympathetic ganglia or the adrenal gland. Despite its anatomical localization and its neural crest origin, it remains completely unknown whether neural activity impacts NB tumor characteristics and metastatic progression.

Material and method

To assess the crosstalk between the developing PNS and NB, we develop innovative methodologies that associate neuroscience paradigms and *in vivo* models of NB within its embryonic environment. We conduct electrophysiology experiments combined with optogenetic and chemogenetic approaches to assess the functionality of the identified interactions and integrate them with omics methodologies at single-cell and spatial scales.

Result and discussion

We have documented the presence of neuro-NB synaptic contacts both in patient biopsies and in our *in vivo* models, along with a reorganization of the innervation pattern in the presence of NB tumors. We have shown that a subset of NB cells in primary tumors depolarize in response to electrical stimulation of tumor-invading nerve fibers, and characterize the pharmacology of this neurotransmission. We investigate the impact of nervous activity on the composition of the tumor microenvironment and its subsequent effects on NB tumor and metastatic properties. Both direct (synaptic) and indirect (paracrine) neural activity-dependent effects on NB are being explored. Together, our data from patient samples and functional experiments suggest that NB tumor and metastatic properties are actively shaped by, and evolve in parallel with, the developing peripheral nervous system, further supporting the idea of specific neural vulnerabilities in early childhood tumors.

Conclusion

Ultimately, our goal is to identify a neuro-responsive class of NB tumors that may benefit from novel therapeutic strategies targeting the neuro-cancer axis.

EACR25-0679

Engineered 3D Tumor Niches to Study the Impact of Stromal Spatial Heterogeneity on Chemosensitivity in Pancreatic Cancer

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Introduction

Pancreatic ductal adenocarcinoma (PDAC) is an aggressive type of pancreatic cancer characterized by a dense stromal compartment and abundant presence of fibroblasts and macrophages. Preclinical studies have shown that these stromal cells play a critical role in tumour progression, including mediating paracrine chemoprotective effects on gemcitabine treatment. Recent clinical data suggest that the spatial arrangement of these stromal cells within the tumour microenvironment impacts their phenotypes, functions, and tumour behaviour, influencing patient outcomes. However, studying the mechanistic contribution of these spatial phenotypes to chemoresistance remains challenging due to a lack of suitable models. To address this, we have developed fully human 3D tumour niches that replicate stromal spatial heterogeneity, enabling the study of paracrine effects on gemcitabine response in PDAC.

Material and method

We utilize pancreatic stellate cells (PSCs) and peripheral blood monocyte-derived macrophages as stromal components. These cells are co-cultured with whole PDAC patient-derived organoids (PDOs) to model direct interactions with cancer cells. Paracrine interactions are modelled by culturing PSC and macrophage monocultures or co-cultures in a PDO-conditioned medium. All cells are embedded in a collagen-based matrix and seeded as microgels in our GLAnCE (Gels for Live Analysis of Compartmentalized Environments) platform. The platform supports imaging assays and single-cell extraction for downstream analysis, enabling cell phenotypic characterization within established niches. We apply the niches for medium conditioning and then screen secreted factors in the presence of gemcitabine to assess the niche impact on PDO viability.

Result and discussion

The established niches reflect key characteristics of PDAC tumours. PDOs show varying growth and epithelial marker expression levels depending on the presence of PSCs and macrophages. Macrophage presence and direct or indirect contact with organoids influence PSC proliferation and activation. Macrophage phenotypes range from inflammatory (CD86+ CD206-) to immunoregulatory (CD206+ CD163+), shaped by their interactions with organoids and fibroblasts, respectively. Screening viability data show that niche-conditioned media protect PDOs from gemcitabine toxicity to varying extents, with the most substantial chemoprotective effects observed for niches maintained in PDO-conditioned medium.

Conclusion

Our novel 3D tumour niches capture spatial stromal-cancer cell interactions and more clinically relevant stromal phenotypes. We anticipate that screening niche-conditioned media with gemcitabine, followed by omic profiling and hit validation, will elucidate how distinct fibroblast and macrophage populations mediate gemcitabine resistance, offering new opportunities for stromal-targeted therapies to overcome chemoresistance in PDAC treatment.

EACR25-0681

Characterization of the tumor microenvironment in pulmonary adenocarcinoma: a new insight into the biology of Spread Through the Alveolar Space

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Introduction

In lung cancer, Spread Through Air Spaces (STAS) corresponds to the presence of cancer cells, either isolated or in small clusters, within the lung airways beyond the boundaries of the tumor. In human lung adenocarcinoma (LUAD), the detection of STAS is associated with local recurrence, poor prognosis and architecture patterns. However, the mechanisms underlying this dissemination remain unknown and controversial. Since STAS may represent a mode of tumor growth and contribute to the formation of secondary tumors, understanding its biology is crucial. We hypothesize that STAS results from an alteration in cellular interactions and signaling within the tumor microenvironment (TME). Our objective is to identify tumor microenvironmental and transcriptomic features associated with STAS.

Material and method

A cohort comprising 246 STAS-negative (STAS(-)) and 152 STAS-positive (STAS(+)) tumors was analyzed. Tissue microarrays were constructed and the TME was characterized using a 35-antibody panel including immune, stromal, and tumor markers, on the Hyperion mass cytometry imaging platform (Fluidigm). Following cell type identification, cellular interaction parameters (cellular neighborhoods (CNs)) were evaluated. We then used regression analysis to compare the frequencies of each cell type, CN, and interaction probabilities between the two groups. In parallel, tumor mRNA from 487 patients (including 261 STAS(-) and 226 STAS(+)) was sequenced. Gene set enrichment analyses (GSEA) were conducted to compare the expression of canonical signaling pathways between the groups.

Result and discussion

We observed an increased frequency of tumor cells in STAS(+) tumors. The CN analysis indicated a higher occurrence of cell clusters involving tumor cells, endothelial cells, and fibroblasts and a decreased frequency of clusters of neutrophils. GSEA indicates alterations in specific signaling pathways suggesting potential extracellular matrix alterations. These results highlight a distinct TME and cellular signaling profile in STAS(+) LUAD, offering new insights into the role of the TME in the biology and clinical progression of this cancer.

Conclusion

Since STAS represents a form of tumor dissemination, identifying the cellular and molecular characteristics

associated with STAS will enhance our understanding of the biological mechanisms underlying tumor spread in LUAD.

EACR25-0705

Deciphering the role of SYNCRIPI protein in modulation of drug sensitivity of persistent cancer cells

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Introduction

Tumor cells are in constant dynamic communication with each other and with their microenvironment. We have previously shown that tumor cells dying during therapy can secrete various spliceosome proteins into the extracellular space, which can be taken up by recipient tumor cells and, thereby, promote their survival, increasing their resistance to subsequent treatment. However, the specific molecular mechanisms of this type of intercellular communication remain unclear. By virtue of their nature, many spliceosome proteins are able to bind to different classes of proteins and nucleic acids. A growing body of evidence suggests that spliceosome proteins can influence a variety of cellular processes in a splicing-independent manner, for example, by binding to chromatin in promoter regions, controlling mRNA export from the nucleus, maintaining genome stability, and participating in DNA repair. A striking example of such multitasking is the SYNCRIPI protein, which in addition to its function in pre-mRNA splicing, is involved in almost all aspects of mRNA metabolism, plays a key role in the biogenesis of RNA-containing extracellular vesicles, and, according to our proteomic data, is an important component of secretomes from dying cancer cells.

Material and method

We conducted a comprehensive analysis of the responses of SKOV3 ovarian adenocarcinoma cells with SYNCRIPI knockdown or overexpression when exposed to cisplatin, which included proteomic and transcriptomic analyses supplemented by investigations of changes in protein-protein (IP-MS) and RNA-protein interactomes (CLIP-seq, poly(A)-tail-seq) of SYNCRIPI under the same experimental conditions.

Result and discussion

Our findings revealed that SYNCRIPI knockdown leads to significant alterations in the polyadenylation pattern of mRNA molecules in SKOV3 cells. Specifically, we observed a shortening of the poly(A) tails of mRNAs associated with DNA repair (PRIMPOL, POLL, PARP3) and an elongation of the poly(A) tails of transcripts that

negatively regulate cell cycle progression (CDK11A, CDKN1A, CDK19). Furthermore, for several SYNCRIP target mRNAs, we demonstrated that their binding to SYNCRIP correlated with an increase in the abundance of the corresponding proteins (e.g., FANCI). We hypothesize that this association may enhance the mRNA stability or efficiency of mRNA translation. Intriguingly, in response to cisplatin, we found an increase in the binding of SYNCRIP to proteins such as RFC5, TRIM25, and USP10 which facilitate translesion DNA synthesis and cell division, as well as MRPL39 involved in mitochondrial translation; and TOP3B, TDRD3 (components of stress granules).

Conclusion

These mechanisms may play a crucial role in enabling tumor cell survival in the presence of chemotherapeutic agents, particularly for cells that receive extracellular vesicles containing the SYNCRIP protein.

This work was supported by the Russian Science Foundation project no. 25-15-00520.

EACR25-0733

BRCA1 mutations in high-grade serous ovarian cancer affect stromal phenotypes in the tumor microenvironment

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Introduction

Tumors are complex ecosystems where cancer cells depend on the recruitment and reprogramming of normal cells to support their survival and progression. Cancer-associated fibroblasts (CAFs) are key players in this process, creating a supportive microenvironment for cancer cells. Recent advances in Single-cell RNA sequencing (scRNAseq) have revealed that CAFs are heterogeneous, arising from multiple mesenchymal origins and exhibiting diverse phenotypes, which could be broadly divided to subsets of myofibroblastic CAFs (myCAF), immune-regulatory CAFs (iCAF), and antigen-presenting CAFs (apCAF). High-grade serous ovarian cancer (HGSOC) is the most lethal gynecological malignancy, with a 5-year survival rate of less than 30% for patients diagnosed at stages III-IV. BRCA1/2 germline mutations increase the risk of ovarian cancer, and over 40% of HGSOC tumors show BRCA1/2 suppression. Studies from our lab and others suggest that BRCA1/2 mutations in pancreatic and breast cancers can alter the tumor microenvironment (TME) by increasing the proportion of iCAF at the expense of myCAF. However, the mechanisms underlying these changes in the TME remain unclear.

Material and method

In this study, we aimed to characterize the TME of BRCA1/2 wild-type (WT) and mutant HGSOC and to explore the mechanisms by which BRCA1/2 mutations induce phenotypic alterations in the TME. To achieve this, we analyzed published scRNAseq data as well as performed multiplex immunofluorescence (MxIF) and histochemical staining on a cohort of HGSOC patient samples. Furthermore, to investigate the mechanisms

underlying these stromal changes, we examined HGSOC BRCA1 mutant and WT cell lines metabolic signaling.

Result and discussion

Analysis of pentachrome and MxIF staining showed that BRCA1/2 WT tumors were enriched in fibrillar extracellular matrix components compared to BRCA1/2 mutant tumors. Furthermore, BRCA1 mutant tumors had higher levels of iCAF and mesothelial cells compared to BRCA1 WT tumors. Similarly, analysis of published scRNAseq data revealed increased levels of mesothelial and steady-state fibroblasts in BRCA1 mutant tumors compared to BRCA1/2 WT. Testing metabolic activity of BRCA1 WT and mutant HGSOC cancer cell lines in-vitro showed higher levels of glycolysis and increased lactate secretion in BRCA1 mutant cells compared to BRCA1 WT cells. scRNAseq data analysis of the non-immune TME of HGSOC showed that mesothelial cells and steady-state fibroblasts – the stromal subpopulations enriched in BRCA1 mutant tumors – upregulated MCT1, a lactate influx transporter, at higher levels than other stromal subpopulations.

Conclusion

Taken together, these findings suggest a possible cross-talk between BRCA1 mutant cancer cells and specific subpopulations of the non-immune TME, leading to distinct TME compositions driven by altered glucose metabolism in BRCA1 mutant cancer cells in HGSOC.

EACR25-0763

FMRP Upregulation in Cancer: Implicating FMRP-expressing Cancer-Associated Fibroblasts in Immune Evasion

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Introduction

Fragile X mental retardation protein (FMRP) has been extensively studied in neuronal development, but its role in cancer biology remains underexplored. Our recent findings (Zeng et al., 2022) demonstrated that elevated FMRP levels in cancer cells promote immune evasion in murine and human tumors. We further investigated whether FMRP plays a role in the stromal compartment. Single-cell RNA sequencing analysis of pancreatic ductal adenocarcinoma (PDAC) tumors identified cancer-associated fibroblasts (CAF) as the primary stromal cell subtype expressing FMRP. Since CAFs are pivotal in tumor progression and therapeutic resistance, we examined whether FMRP expression contributes to CAF-driven immunosuppression in PDAC and breast tumors.

Material and method

To assess FMRP-expressing CAFs, we performed scRNA-seq on murine PDAC tumors and validated

findings in breast tumors. FMRP knockdown and overexpression experiments were conducted in CAFs and naïve fibroblasts to determine phenotypic and functional changes. The impact of FMRP on CAF-mediated immunosuppression was assessed using immune profiling assays and gene expression analyses, focusing on inflammatory CAF (iCAF) and myofibroblastic CAF (myCAF) signatures. To evaluate *in vivo* relevance, we used an orthotopic tumor model in *Fmr1* full-body knockout (KO) mice. Additionally, bulk RNA sequencing was performed to derive an FMRP-associated CAF gene signature, which was applied to human PDAC and breast cancer datasets.

Result and discussion

Our analyses revealed elevated FMRP expression in CAFs from PDAC and breast tumors, whereas it was absent in naïve fibroblasts from healthy tissues. FMRP knockdown in CAFs diminished their immuno-suppressive properties, whereas FMRP overexpression in naïve fibroblasts was sufficient to induce a CAF-like phenotype. Mechanistically, FMRP overexpression led to increased iCAF and myCAF signatures, suggesting its role as a pan-CAF regulator influencing extracellular matrix (ECM) remodeling and the secretory phenotype. *In vivo*, tumors orthotopically implanted in *Fmr1*-KO mice exhibited significantly reduced growth, underscoring the role of FMRP-expressing CAFs in tumor progression. Furthermore, loss of stromal FMRP conferred a substantial survival benefit. Analysis of human datasets revealed that the FMRP-CAF signature was enriched in CAFs from PDAC and breast cancer patients and negatively correlated with immune cell infiltration. Notably, a high FMRP-CAF signature strongly predicted poorer overall prognosis in breast cancer patients, reinforcing its clinical significance.

Conclusion

Our findings establish FMRP as a key regulator of CAF function, linking its expression to tumor progression and immune evasion. Identifying FMRP as a pan-CAF regulator highlights its potential as a novel therapeutic target in cancer treatment. Targeting FMRP in CAFs may enhance immune infiltration and improve patient outcomes in PDAC and breast cancer.

EACR25-0769

Analysis of cellular plasticity of DTPs induced by chemotherapy in triple negative breast cancer

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Introduction

Recent studies have revealed the existence of “Drug Tolerant Persisters” (DTP), cancer cells capable of surviving chemotherapies and reconstituting a sensitive tumor after a period of tumor dormancy. Although initial descriptions of DTP have already been made, the processes of transition into and out of this phenotype are poorly understood. The aims of this study are to elucidate

the transcriptional mechanisms associated with these transitions, identify potential markers and exploitable vulnerabilities, and thus improve the prognosis and therapeutic strategies of patients with triple-negative breast cancer (TNBC).

Material and method

We establish two models of DTP from two TNBC cell lines (MDA-MB-231 and SUM159PT) treated with a high-dose chemotherapy (gemcitabine). To characterize the heterogeneity of the temporalities of interest (entry, maintenance, and exit of the DTP phenotype), we monitor the proliferation, dormancy, and death state of cells from the start of treatment until their return to a control phenotype (20 days), using an ultra-wide field imaging system allowing high throughput at the single-cell scale. In parallel, using scRNA-seq, we analyze the evolution of the transcriptomic profiles over time. We combine the identification of the dynamics of the clusters of interest with timelapse imaging analyses to reveal the transcriptional changes that initiate the conversion of cells to DTP, maintain this phenotype, as well as those that promote the exit of the DTP phenotype with the resumption of growth.

Result and discussion

With our models, while 7.5% of cells survive after one week of treatment, only 0.3% of cells can resume growth and generate colonies. Moreover, if we challenge the cells a 2nd time after one month of drug holiday, the cells are still sensitive to the chemotherapy. Through the scRNA-seq, data are being analyzed to identify specific markers. On the one hand, we observed radical changes in the transcriptomic profiles of the DTP population, and on the other, we observed that the proliferating DTP-derived population at day 20 had transcriptomic profiles similar to those of the untreated population, as predicted by the DTP model. Also, cells under the 1st and 2nd rounds of chemotherapy have the same transcriptomic profiles. Furthermore, in correlation with the literature, we observed that in response to the chemotherapy, the cells express transiently a higher diapause and stem cell phenotype signature, which might be involved in the entry in the DTP phenotype.

Conclusion

These mechanisms will be validated by imaging with expression reporters, complemented by pharmaceutical targeting. This project will characterize the transcriptional mechanisms underlying the phenotypic switches associated with phenotype entry and exit to identify vulnerabilities.

EACR25-0775

Mimicking liver inflammation to identify essential genes in hepatocellular carcinoma: Towards a CRISPR screen based on IL-6/STAT3 signaling

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Introduction

Hepatocellular carcinoma (HCC), the predominant form of liver cancer, is a leading cause of cancer-related mortality worldwide. Inflammation plays a critical role in HCC progression, with the interleukin-6 (IL-6)/STAT3 signaling pathway being hyperactivated in HCC. Loss of heterozygosity (LoH) of the short arm of chromosome 8 (chr8p), is a crucial factor in HCC pathogenesis and chr8p loss correlates with increased mortality in patients possibly due to altered IL-6 signaling. CRISPR screens involving inflammation are just starting to emerge. Here, we aim to establish a screen delineating essential genes in regards to IL-6 in liver cancer.

Material and method

This study performed a genome-wide CRISPR/Cas9 knockout (KO) screen to identify genes that are essential in HCC under inflammatory conditions uncovering vulnerabilities for possible treatment. HCC cell lines were analyzed for their IL-6 responsiveness and STAT3 phosphorylation by Western blot and analysis of target gene expression by qRT-PCR. Cells were infected with the genome-wide Alexandria sgRNA CRISPR library and treated with IL-6 to activate IL-6/STAT3 signaling, mimicking liver inflammation.

Result and discussion

The HCC cell line HuH7 was selected due to its responsiveness to IL-6 and efficient activation of STAT3 by phosphorylation. HuH7 cells which have chr8p LoH were successfully infected with a Cas9-blast vector for stable Cas9 integration. Additionally, an IL-6 over-expressing cell line was established to secrete the cytokine into the medium, which was then used to activate the IL-6/STAT3 pathway in HuH7-Cas9 cells. Furthermore, the CRISPR/Cas9 screen will be performed in isogenic HCC cells with chr8p wildtype or loss of heterozygosity (LoH) of chr8p to dissect potential differences in HCC subgroups with or without chr8p deletion.

Conclusion

This research has the potential to identify clinical markers and drug targets for diagnosing and treating HCC patients, particularly those with inflammation-driven tumors with chr8p LoH. By elucidating gene essentialities in the context of IL-6 signaling, this study may contribute to the development of targeted therapies for HCC.

EACR25-0783

The role of prematurely drug-induced senescent peritoneal cell exosomes in the awakening and progression of dormant ovarian cancer cells

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Introduction

The mechanisms behind the relapse of epithelial ovarian cancer (EOC) remain unclear. In this project, we aim to investigate the hypothesis that EOC relapse may be induced by the presence of peritoneal mesothelial cells

(PMCs) and fibroblasts (PFBs) which entered senescence due to exposure to carboplatin and paclitaxel.

Specifically, we propose that the relapse of EOC is driven by exosomes secreted by senescent PMCs and/or PFBs, which have the ability to activate dormant EOC.

Material and method

Experiments were conducted using PMCs and PFBs isolated from the omentum of non-oncologic patients. The study also utilized EOC cells, including primary high-grade serous EOC and three commercial cell lines: A2780, OVCAR-3, and SKOV-3. To induce senescence, PMCs were exposed to 50 μM carboplatin and 25 nM paclitaxel, while PFBs were treated with 25 μM carboplatin and 10 nM paclitaxel. Exosomes were isolated from the conditioned medium through precipitation and quantified by flow cytometry, based on the expression of specific markers: CD9, CD63, and CD81. The size and morphology of the exosomes were assessed using SEM. The induction and termination of EOC cell dormancy were achieved through sequential serum withdrawal and reintroduction. Additionally, the expression of exosomal microRNA was quantified using qPCR, while the concentration of exosomal proteins was measured with a Human custom array.

Result and discussion

PMCs and PFBs that experienced senescence triggered by the combination of carboplatin and paclitaxel generate considerably more exosomes than untreated cells. Notably, the exosomes released by senescent cells have a greater capacity to activate dormant cancer cells. This activation is evidenced by an increased expression of the proliferation marker Ki67 and a higher proportion of cells entering the S phase of the cell cycle. The ability of exosomes to awaken dormant cancer cells surpasses the effects of soluble proteins secreted to the environment. Analysis of the exosomal cargo, which mediates their biological effects, revealed that exosomes from senescent cells exhibit down-regulation of certain microRNAs: miR-210-3p, miR-409-3p, and miR-421. Additionally, proteomic analysis showed that exosomes derived from senescent PMCs are enriched in proteins such as bFGF, HGF, MMP-1, MMP-3, TIMP-1, and VEGF. In contrast, exosomes from senescent PFBs contain higher levels of amphiregulin, eotaxin-1, MCP-1, CXCL5, SDF-1α, IL-6R, osteopontin, and TGF-β1. Ongoing research aims to further investigate the progression of these awakened cells in both 2D and 3D culture models, along with their molecular and genetic characteristics.

Conclusion

Exosomes produced by drug-inducible senescent normal peritoneal cells may drive the awakening of dormant cancer cells, potentially contributing to cancer relapse.

The study was supported by a grant from the National Science Centre, Poland (2020/37/B/NZ5/00100).

EACR25-0786

Exploring small nucleolar RNAs as predictors of radiotherapy outcome in breast cancer

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Introduction

The discovery of novel biomarkers for earlier diagnosis and better therapy response remains a major focus in cancer research. Small non-coding RNAs such as small nucleolar RNAs (snoRNAs) have recently emerged as promising biomarkers and potential therapeutic targets for various cancer types including breast cancer.

However, additional research with larger sample sizes and high-throughput methods is essential to fully establish their possible role in breast tumorigenesis.

Material and method

We have performed small RNA sequencing in 195 fresh-frozen tumor samples from patients with invasive BC from the Eastern Finnish Kuopio Breast Cancer Project to evaluate the predictive potential of snoRNAs in invasive local breast cancer. Total RNA was extracted from fresh-frozen tissues using the Ambion mirVana miRNA Isolation Kit, followed by processing with the Illumina TruSeq Library Prep Kit and sequencing. The bio-informatic analysis involved assessing read quality, trimming adapters, removing reads aligning to e.g. rRNAs, and aligning the data to the human reference sncRNA transcriptome. The univariate and multivariable survival analyses were conducted using the Cox's proportional hazards model in R. The multivariable analyses calculated hazard ratios and confidence intervals for death (either breast cancer-specific or overall survival) or recurrence. Adjuvant chemotherapy (yes/no), adjuvant endocrine therapy (yes/no), and clinical data were included in the analyses as covariates.

Result and discussion

In invasive local breast cancer cases who underwent radiotherapy, significant associations between 15 snoRNAs and patient outcome were identified, indicating that these snoRNAs may serve as predictors of radiotherapy response. Poorer outcome was observed with the radiotherapy-treated cases with higher expression of eight snoRNAs, whereas the increased expression of seven snoRNAs associated with better outcome. For example, increased SNORD60 expression associated with poorer overall survival and increased SNORD67 level with better breast cancer-specific survival in radiotherapy-treated cases. The identified candidate snoRNAs could help elucidate the mechanisms underlying resistance to RT, and the following further validation of their biological importance may lead to the discovery of novel therapeutic targets.

Conclusion

Our findings suggest that snoRNAs may serve as potential predictive biomarkers for radiotherapy response in invasive local breast cancer, and further validation of these candidates could pave the way for novel therapeutic targets in precision medicine.

EACR25-0813

Identifying transcript variants associated with colon cancer: a comparative analysis of 3D spheroid cultures and patient-derived tissues

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Introduction

Colon cancer remains one of the leading causes of cancer-related deaths worldwide, necessitating a deeper understanding of the molecular factors contributing to its development. By exploring the transcriptomic landscape of colon cancer cell lines and patient-derived tissues, this study aspires to uncover novel biomarkers and potential therapeutic targets for improving colon cancer diagnosis and treatment.

Material and method

The SW620, HCT116 and DLD-1 colon cancer cell lines and immortalized cells derived from healthy gut mucosa HCEC-1CT were cultivated in 3D as spheroids and analyzed by RNA sequencing using Illumina's NovaSeq 6000 platform. Our transcriptomic data were compared with the results of previously published comprehensive transcriptomic study based on 473 patient-derived tumor and 417 non-tumor tissues.

Result and discussion

Comparison of the transcriptomic results obtained for cell lines grown as spheroids with the results of the RNA sequencing data for patient-derived tissues was conducted for 375 transcripts identified by the previously published study as significantly differentially expressed in colon cancer (39 overexpressed and 336 under-expressed). Among these, 32 transcripts were identified with expression patterns in colon cell lines corresponding to the expression patterns observed in patient-derived tissues (4 overexpressed and 28 under expressed). Most prominently deregulated transcripts among those with corresponding deregulated expression patterns in colon cell lines and patient-derived tumors were NTMT1-204 (overexpressed in cancer) and DCTN1-205 (under-expressed in cancer). For other 343 transcripts, matching deregulation pattern observed in patient-derived tissues was not confirmed in colon cell lines and their expression patterns in stromal cell types should be further investigated.

Conclusion

Overall, comparison of RNA sequencing data between colon cell lines grown as spheroids and patient-derived tissues revealed a set of 32 transcripts that may be relevant for colon carcinogenesis and their role in gut epithelial cells should be further explored. Additionally, NTMT1-204 transcript could potentially be used as diagnostic marker for colon cancer, and DCTN1-205 as a potential therapeutical agent.

EACR25-0824**The impact of CFTR impairment on endothelial cell and its role during tumor progression**M. Cotinat¹, M. Le Claire¹, I. Corre¹, L. Trep¹¹CRCI2NA, Nantes, France**Introduction**

Affecting more than 100,000 people worldwide, cystic fibrosis (CF) is caused by pathogenic variants of the CFTR gene. This debilitating disease affects a broad spectrum of organs but thanks to latest therapeutic advances with CFTR modulators, patient life expectancy now exceeds 40 years, hence transforming CF into a chronic disease of adulthood. Unfortunately, while ageing, patients with CF develop comorbidities including cardiovascular diseases, diabetes and cancer. The increased risk of developing cancer is likely multifactorial but could relate to the persistent pro-inflammatory state seen in patients with CF. Indeed, it is estimated that 25% of cancers worldwide are associated with infection and chronic inflammation. And chronic endothelial inflammation is implicated in pathologies leading to the development of cancer (e.g. obesity, diabetes, chronic inflammation of the intestine). However, the precise mechanism linking CFTR defect, the vascular compartment, and cancer onset is not fully understood.

Material and method

In this study we combined in vitro, in vivo and scRNA-seq approaches to study the endothelium features after CFTR impairment. Particularly, we treated a normal model of endothelial cells (ECs) with a specific CFTR inhibitor to unravel, how CFTR loss impacts cancer cell invasion and immune cell trafficking. The effect of CFTR inhibition was also assessed in ovo using the chorioallantoic membrane (CAM) assay in chicken eggs combined with in situ imaging. Moreover, we investigated the vascular compartment of CF lung explant at the single cell level to have a glimpse into disease progression. Finally, we developed and characterize a unique mouse model of conditional and restricted CFTR invalidation in the vascular compartment (CFTR EC KO).

Result and discussion

We showed that CFTR impairment directly dampen ECs and lung cancer cell migration. Interestingly, we present that CFTR-deficiency altered the endothelial barrier property, thereby increasing cancer cell migration and invasion and thus contributes to tumor progression. We have evidence that the endothelial-to-mesenchymal transition (EndMT) could be involved in this phenomenon. Moreover, our scRNA-seq data from CF lung explant revealed an endothelial state characterized by an exacerbated pro-inflammatory phenotype, which was absent in normal lung tissues. Hence, mirroring our recent data obtained in liver explants from CF patients. Finally, our first results in CFTR EC KO mouse model indicate vascular defects compared to control animals, which could have relevance for tumor onset.

Conclusion

The link between CFTR and cancer risk is clear, but the roles of CFTR during tumorigenesis and the involvement

of ECs in this phenomenon are not yet well defined. In this study we have started to explore the mechanism by which endothelial CFTR-impairment could contribute to tumor progression via various mechanisms linked to endothelial plasticity and inflammation.

EACR25-0837**The CMS4 marker HTR2B serotonin receptor is critical in colorectal cancer cell invasion**I. Carmi¹, A. Orosz¹, S. Hajdó¹, A. Zeöld¹, T. Tölgyes², Z. Wiener¹¹Semmelweis University, Genetics, Cell- and Immunobiology, Budapest, Hungary²Uzsoki Teaching Hospital, Oncosurgery, Budapest, Hungary**Introduction**

Colorectal cancer (CRC) is one of the most frequent cancer types. Patient-derived organoids (PDO) maintain the intratumoral heterogeneity, representing a modern tool to study human cancers. The gene expression-based CRC classification identified a cluster of patients (CMS4) with epithelial-mesenchymal transition (EMT), the accumulation of fibroblasts, and a dismal prognosis. Of note, NOTCH3 activation drives cancer progression in a model of mesenchymal CRC. For CRC, the accumulation of collagen-I in the extracellular matrix (ECM) correlates with an adverse prognosis. Although the serotonin receptor HTR2B is one of the epithelial markers of this CMS4 subgroup, its precise role in CRC tumorigenesis is not yet well known.

Material and method

PDOs were isolated from CRC patients, and they were cultured with or without fibroblasts under different conditions. For specific experiments, cells were sorted into high and low-expressing populations for HTR2B and NOTCH3. Cell viability was determined with a luminescence assay, while survival and proliferative potential were determined with flow cytometry (FC). Confocal and light microscopic image analysis were used to quantify changes in invasive potential and EMT. CMS4 and EMT marker expression were additionally quantified using RT-qPCR and FC.

Result and discussion

HTR2B, under the control of the mTOR pathway, is expressed heterogeneously intra- and inter-tumorally. Unfavorable conditions, co-culturing with fibroblasts, and collagen-I accumulation in the ECM increased the number of HTR2B-positive tumor cells. Interestingly, whereas serotonin or an HTR2B agonist did not affect PDOs under normal conditions, they reduced cellular survival when carbohydrates were absent from the medium. In contrast, collagen-I represented a permissive ECM for serotonin-induced invasion, but only in organoids derived from HTR2B+ tumor cells. CRC cells positive for this molecule also expressed NOTCH3 and NOTCH target genes. Similar to HTR2B+ organoids, NOTCH3+ cells had increased levels of EMT markers, and a higher invasion potential in collagen compared to NOTCH3 negative cells. Hence, similar to HTR2B stimulation, inhibition of Notch activity changed EMT marker levels and PDO invasion.

Conclusion

Collectively, we show that the serotonin receptor HTR2B+ marks a CRC cell population with increased proliferative and invasive potential, and serotonin has a dual role under unfavorable conditions and in ECM enriched for collagen. Furthermore, the cell population with active NOTCH signaling overlaps with HTR2B+ cells, and similarly promotes aggressive tumoral behavior.

Funding: OTKA137554 and 2024-1.2.3-HU-RIZONT-2024-00003 (National Research, Development and Innovation Office, Hungary), TKP2021-EGA-24 (Ministry of Innovation and Technology of Hungary). *Ethical permission:* TUKEB 2015, 51323-4/2015/EKU

EACR25-0841

Human immune cell composition of TME depends on tumor cell line-derived xenograft subtype and tumor burden in genO-BRGSF-HIS mice

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Introduction

Emergence of immunotherapies for treating cancer has been an important step towards a more efficient, selective and safe approach for patients. However, only a fraction of patients can respond to treatments currently available, requiring the identification of additional therapeutic targets. The relevance of preclinical models in the identification of new therapeutic targets and assessment of immunotherapies has vastly improved with mice bearing a human immune system. genO-BRGSF (Balb/C Rag2^{-/-}, IL2Rγ^{-/-}, SIRPaNOD and Flt3^{-/-}) is a highly immunodeficient mouse featuring reduced murine myeloid cells. genO-BRGSF mice reconstituted with human cord blood CD34+ cells (genO-BRGSF-HIS) develop functional lymphoid and myeloid compartments. This engraftment is stable for over a year (Labarthe et al., 2019) and mice do not develop GvHD. Human myeloid compartment can be transiently boosted with exogenous human Flt3L injections. In contrast to other models, which overexpress human cytokines to develop human myeloid cells, Flt3L-treated genO-BRGSF-HIS mice do not show side effects. genO-BRGSF-HIS mice are permissive to mouse and human cancer cell lines engraftment and represent a valuable preclinical model to study cancer development and evaluate novel therapeutics. Here, we show that composition of tumor microenvironment (TME) is tumor burden-dependent.

Material and method

For that, genO-BRGSF-HIS mice were inoculated with different tumor types and TME analyses were performed at different timepoints.

Result and discussion

genO-BRGSF-HIS mice grafted with triple negative cancer cell line MDA-MB-231 have a diverse TME, enriched in myeloid cells. The major cell type present in tumors of approximately 200mm³ is CD206+/CD163+ M2-like macrophages, which express high levels of PD-L1 and other immunomodulatory proteins. At this tumor volume, TME is also composed of a small fraction of T cells. However, the frequency of conventional CD4+T cells increase from <5% to 15% of human infiltrate when

tumors reach approximately 500 mm³. Interestingly, NK cells are detected at very low levels in tumors of 200 mm³, but increase to 10% in the TME of tumors of 500 mm³. The infiltration of human immune cells in the TME is also tumor type dependent. Indeed, NK cells are the main cell type present in the TME of A549, an adenocarcinomic human alveolar basal epithelial tumor type, while conventional CD4+ T cells are the major cell type present in the TME (40%) of tumors of 200 mm³, in a colon-derived adenocarcinoma SW480 tumor model. Interestingly, SW480 tumors show an infiltrate of γδ T cells as well, ranging from 6% to 8% of human immune cells in tumors of 200 mm³ and 800 mm³, respectively.

Conclusion

genO-BRGSF-HIS mice hence represent a valuable tool to investigate immune cell infiltration in the TME, enabling a translatable assessment of mechanism of action of immunotherapies.

EACR25-0842

Emerging Subtypes in Ovarian High Grade Serous Carcinoma: Decoding Genomic and Functional Landscapes

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Introduction

Ovarian high-grade serous carcinoma (HGSC) is marked by extensive chromosomal instability (CIN) and heterogeneity. Homologous recombination proficient (HRP) tumors, defined by the absence of homologous recombination deficiency (HRD), are associated with poor prognosis, lack established biomarkers, and have limited treatment options, underscoring the need for improved molecular classification. Our study addresses the heterogeneity driven by CIN and provides a biologically and clinically meaningful stratification of HGSC.

Material and method

We derived CIN signatures from 640 whole-genome sequencing (WGS) HGSC tumors in the DECIDER clinical trial exploiting structural variation and copy number features. Signature activities were used to cluster samples, and the resulting subtypes were further characterized using bulk and single-cell RNA sequencing. Patient-derived organoids were then employed to explore potential targeted treatment options.

Result and discussion

Using CIN signatures from patient WGS data, we identified five distinct HGSC subtypes, each characterized by unique structural variation patterns and biological phenotypes. Notably, three subtypes provided further stratification within the heterogeneous HRP patient group, while the remaining two differentiated BRCA1-like from BRCA2-like HRD tumors. The most prevalent HRP subtype, termed core HRP, was marked by moderate genomic instability, immune evasion in

primary tumors, and high hypoxia in metastatic sites. In contrast, the EMT HRP subtype, which exhibited the lowest genomic instability, was driven by epithelial-to-mesenchymal transition (EMT) phenotype. Lastly, the proliferative HRP had the highest levels of genomic instability and presented high proliferation activity. Furthermore, organoid experiments suggested that CHK1 inhibition may be ineffective for EMT HRP tumors, whereas patients with core and proliferative HRP subtypes could benefit from this treatment. Our data enabled separation of patients with dysfunctional BRCA1 and BRCA2 genes, revealing subtypes with aligned genomic features. BRCA2-like patients showed better demonstrated improved overall survival despite comparable initial treatment responses, implying lower resistance to second-line therapies. Additionally, this approach refined HRD classification by identifying potential HRP patients with poor responses among those classified as HRD based on genomic scars, as well as likely HRD patients with favorable outcomes among scar-defined HRP cases, thereby improving clinical stratification.

Conclusion

In summary, we analyzed the genomic landscape of HGSC in a real-world patient cohort and uncovered clinically and biologically significant subtypes using CIN signature clustering. These findings provide a foundation for developing new therapeutic strategies for patients facing limited treatment options.

EACR25-0857

Genetic Ancestry Influence on the Tumor Microenvironment of Colombian Patients with Triple-Negative Breast Cancer

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Introduction

Triple-negative breast cancer (TNBC) is a highly heterogeneous disease, with a tumor microenvironment shaped by genetic and immune factors. In Latin American populations, genetic ancestry may influence immune infiltration and cytokine activity, potentially impacting tumor progression and response to immunotherapy. Understanding these interactions is crucial for improving personalized treatment strategies. AIM: To evaluate the relationship between genetic ancestry and immune infiltration in Colombian TNBC patients, distinguishing between sporadic and hereditary cases.

Material and method

We analyzed 42 TNBC patients treated at the National Cancer Institute between 2018 and 2023. Genetic ancestry and germline mutations were inferred from NGS data using the TruSight Oncology panel. Immune infiltration was characterized using RNA-Seq data. XCell

was employed to estimate the immune cell composition, while CytoSig was used to assess cytokine and growth factors activity. Statistical analyses were performed in RStudio.

Result and discussion

Heredity TNBC patients exhibited a more active immune response compared to the sporadic group.

- Ancestry and immune cell composition: In the overall cohort, higher European ancestry (EUR) was associated with a lower proportion of naïve CD8+ T cells ($p = 0.048$). In sporadic TNBC, CD8+ ($p = 0.005$), CD4+ ($p = 0.015$), and NK cells ($p = 0.019$) positively correlated with EUR, whereas in hereditary TNBC, naïve CD8+ T cells showed a negative correlation with EUR ($p = 0.021$).

- Ancestry and cytokine activity: Increased African (AFR) and Native American (NAM) ancestry correlated with elevated cytokine activity, including IFNG ($p = 0.003$), IL3 ($p = 0.019$), IFNL ($p = 0.041$), and IL18 ($p = 0.045$). In hereditary TNBC, FGF2 ($p = 0.004$) and IFNG ($p = 0.010$) were negatively associated with EUR and positively with NAM ($p = 0.016$).

Conclusion

Our findings suggest that genetic ancestry influences the tumor immune microenvironment in TNBC, with hereditary cases displaying a more inflammatory profile. These results highlight the importance of integrating ancestry into immuno-oncology research, as it may contribute to immune response heterogeneity and affect treatment outcomes. Understanding these ancestry-driven immune variations could improve precision medicine approaches for TNBC patients.

EACR25-0861

Therapeutic Regimen on A Novel Syngeneic and Cisplatin-resistant HNSCC Cachexia Model

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Introduction

Cancer cachexia (CC), progressed with numerous chronic and end-stage diseases including carcinogenesis, sequentially, with involuntary weight loss via skeletal muscle, fat mass reduction, and imbalance in metabolic regulation, is a multifactorial syndrome and cannot be reversed by nutritional support. CC develops as a secondary disease in 80% of cancer patients and becomes the main cause of death in 22–30% of cancer patients. Approximately, 50–70% of Head and neck squamous cell carcinoma (HNSCC) patients are diagnosed with malnutrition of varying severity, with progressive weight loss causing cachexia. It has also been reported that advanced tumors stage prone to developing CC due to chemotherapy resistance.

Material and method

We established a highly malignant HNSCC cell line, VO4, by sequential rounds of syngeneic transplantation; then a cisplatin-resistant VO4 cell line (VO4cisPtR) was established by stepwise treatment with increased concentrations of cisplatin. The VO4 and VO4cisPtR cells were inoculated subcutaneously into the C57BL/6 mice to observe the progression of cachexia *in vivo*, respectively. Then, the gastrocnemius muscle and adipose tissues were collected and examined for abnormality by immunoblot assays. As *in vitro* progressive cachectic models, the VO4 and VO4cisPtR adipogenic conditioned medium, which collected after cultivation of cancer cells and 3T3-L1 preadipocytes, afterward, treated in C2C12 myoblasts to analyze lipid metabolism and muscle atrophy by immunoblot assays. Further, a compound X was used to assess its anti-cachexia therapeutic regimen both *in vivo* and *in vitro* cachexia models.

Result and discussion

We observed VO4cisPtR tumor-bearing mice showed significant loss of body weight, epididymal white adipose tissue (eWAT), inguinal white adipose tissue (iWAT), and brown adipose tissue (BAT) compared to that of the VO4 tumor-bearing mice. The immunoblot results revealed the expression of the lipolysis marker, ATGL, and beta-oxidation marker, CPT1A, were up-regulated in eWAT; meanwhile, the expression of muscle atrophy marker, artogin-1, was up-regulated in gastrocnemius muscle of the VO4CisPtR-tumor bearing mice. We observed that the differentiation ability of C2C12 was inhibit, and the expression of MyoD and MyHC were significantly downregulated after the treatment with VO4 and VO4cisPtR adipogenic conditioned medium, respectively. Lastly, the compound X treatment ameliorated the loss of body weight, adipose tissues and muscle tissues both *in vivo* and *in vitro*. Further, the molecular mechanisms mediating the above findings are under elucidation.

Conclusion

Overall, we generated a novel syngeneic HNSCCcisPtR cachexia model and characterized the progression and pathophysiology of cancer cachexia. Additionally, compound X would be a potential therapeutic drug for anti-cachexia.

EACR25-0862

Establishing the Immune-Profile of Cholangiocarcinoma and the Utility of Human Precision-Cut Tumour Slices (hPCTS) as a Platform to Assess Immunotherapy Response

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Introduction

Cholangiocarcinoma (CCA) is a rare but aggressive malignancy of the biliary tract, encompassing intra-hepatic (iCCA), perihilar (pCCA), and distal subtypes. Late-stage detection, limited treatment options, and frequent chemoresistance contribute to poor outcomes. Although recent phase III trials (TOPAZ-1, KEYNOTE-966) have investigated immunotherapy-based regimens, many patients derive minimal benefit, highlighting the need for improved insight into the tumour micro-environment (TME). This study combines human precision-cut tumour slices (hPCTS) and mass time-of-flight cytometry (CyTOF) to develop a physiologically relevant *ex vivo* model for exploring CCA biology and refining immunotherapeutic strategies.

Material and method

Patient consented [1] fresh iCCA and pCCA tumour specimens were surgically resected and cut into 250 µm slices (5 mm diameter) using a Krumdieck MD6000 Tissue Microtome. Slices were cultured for up to 15 days. MTS assays assessed metabolic viability, while haematoxylin and eosin staining evaluated tissue architecture. Immunohisto-chemistry (IHC) stained for immune cell subsets, tumour, and apoptosis markers over time (Days 0, 3, 7, 11, 15). SWATH-based proteomic profiling assessed slice proteome stability from day 0 to 15 in culture. A 35-marker CyTOF panel, allowed the identification of major and sub-immune populations and their activation status from single-cell suspensions of resected tumours. CyTOF analyses of day 7 hPCTS was used to determine whether immune phenotypes and activation states are sustained *ex vivo*. Proof-of-concept studies with chemotherapeutic and immunomodulatory agents tested the feasibility of hPCTS for evaluating dose-dependent responses.

Result and discussion

Following an initial dip, the hPCTS proteome was stable from day 3 onwards. There was no significant change in viability over the 15 days in culture. Histological evaluations showed intact tissue architecture, and IHC confirmed the persistence of immune cells. This indicate that hPCTS preserve key TME features over prolonged *ex vivo* culture, including immune components. Preliminary drug treatments revealed dose-dependent responses, suggesting that CCA hPCTS can be used for pharmacological studies. Ongoing CyTOF analysis will characterise immune subsets and activation markers in both primary tumour tissues and hPCTS, providing insight into the functional status of immune populations and informing immunotherapeutic targeting.

Conclusion

Our data establish hPCTS as a robust *ex vivo* model for CCA, retaining architecture, viability, and immune cells for at least 15 days. When integrated with comprehensive CyTOF-based immune profiling, this platform can delineate TME interactions and guide the development of more precise immunotherapeutic interventions.

Ultimately, leveraging hPCTS for mechanistic and drug-response studies may improve clinical outcomes for patients with CCA.

[1] PINCER NW REC 15/NW/0477

EACR25-0878

Secreted luciferases: a scalable, accessible, and 3R-compliant tool for longitudinal monitoring of autochthonous tumor growth in vivo

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Introduction

Preclinical mouse models are indispensable for studying tumor biology and evaluating therapeutic strategies. Especially autochthonous tumor models, developed through somatic gene editing, closely mirror natural disease progression as in patients. However, monitoring tumor growth within internal organs relies on sophisticated imaging techniques that require anesthesia, are time-consuming, and demand substantial resources. To address these challenges, we have established a blood-based monitoring approach for tumor burden using secreted luciferases, offering a minimally invasive, accessible, scalable, and cost-effective alternative to imaging.

Material and method

To enable longitudinal monitoring of autochthonous tumor models, we engineered a Cre-inducible reporter mouse that conditionally expresses the secreted luciferase from *Gaussia princeps* (GLuc). Additionally, we designed a flexible adenoviral (AV) toolkit for delivering Cre recombinase alongside CRISPR nucleases targeting essential tumor suppressor genes.

Result and discussion

Intratracheal infection of GLuc reporter mice with CRISPR-AVs successfully induced GLuc-expressing lung tumors with defined genetic profiles. The secreted GLuc, released by proliferating tumor cells, entered the blood stream, and its circulating levels strongly correlated with viable tumor burden. This allowed for the detection of tumor growth months before animals reached endpoint criteria. Unlike imaging techniques that require specialized equipment and trained personnel, GLuc levels can be rapidly and inexpensively measured from small blood samples without the need for anesthesia, substrates, or contrast agents. This minimally invasive approach minimizes animal distress and permits

frequent, real-time monitoring of tumor dynamics with high temporal resolution in both genetically engineered and transplantable tumor models. While blood GLuc levels integrate signals from the entire body, including small or diffuse metastases, they lack spatial resolution. However, this limitation can be overcome by combining GLuc with classical luciferases, such as Firefly luciferase, enabling simultaneous quantification of tumor burden and spatial tracking of tumor growth, thereby integrating the benefits of blood-based assays and imaging.

Conclusion

Implementing blood-based longitudinal monitoring of tumor burden with secreted luciferases offers a 3R-compliant, sensitive, cost-effective, and scalable strategy to increase the accessibility of autochthonous mouse tumor models for preclinical research and drug development.

EACR25-0892

Understanding the Role of Leukemia Inhibitory Factor (LIF) in the Bone Metastatic Microenvironment

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Introduction

Bone metastasis is predominant in certain cancer types, particularly those originating from the prostate (85%). While Leukemia Inhibitory Factor (LIF) plays a key role in bone remodelling, its involvement in bone metastasis remains insufficiently explored. Data from Stand Up To Cancer (2019) highlight a significant disparity in survival outcomes among prostate cancer patients with bone metastasis based on LIF expression. Patients in the highest quartile of LIF expression exhibit more than a 50% reduction in median overall survival compared to those in the lowest quartile ($p < 0.05$; $n = 12$), alongside an increased expression of osteoclast-related genes, as determined through an unbiased approach ($\log_2\text{-ratio} > 1$). We hypothesise that LIF secreted by tumour cells may induce aberrant bone remodelling, leading to the release of growth factors stored in the bone, thereby facilitating tumour growth.

Material and method

In this study, we investigated the role of LIF within the bone metastatic niche using in vitro, ex vivo, and in vivo methodologies. Also, we compared its effects with those of Receptor Activator of Nuclear Factor Kappa-B Ligand (RANKL), the main target of currently approved therapies. For in vitro, bone marrow cells obtained from the long bones of C57BL/6j mice were treated with recombinant mouse LIF or RANKL (20 ng/mL) for one week. In ex vivo, coculture of RM1 prostate cancer cells with 2 mm calvaria discs from C57BL/6j mice following LIF treatment (20 ng/mL) for 4 weeks was performed. For in vivo, RM1 cells were inoculated into 7-week-old

C57BL/6j male mice and treated over 12 days with anti-LIF (15 mg/kg) or anti-RANKL (10 mg/kg).

Result and discussion

In vitro, both cytokines upregulated the expression of osteoclast-related genes by qPCR. However, the RANKL:OPG mRNA ratio only increased upon treatment with LIF. Flow cytometry analysis revealed a comparable increase in the CD68+CD265+CD11BLow population following both treatments. TRAP staining was consistent with these findings. Moreover, ex vivo co-culture of RM1 prostate cancer cells with 2 mm calvaria discs from C57BL/6j mice revealed a more than two-fold increase in tumour growth following LIF treatment after 4 weeks ($p < 0.05$). These results led us to perform a pilot syngeneic in vivo experiment where RM1 cells were inoculated into 7-week-old C57BL/6j male mice (H&E for tumour area). Upon two doses of treatment with anti-LIF (15 mg/kg) over 12 days, the RANKL:OPG mRNA decreased to less than half ($p < 0.05$). In the same setting, treatment with anti-RANKL (10 mg/kg) decreased mRNA expression of MMP9 ($p = 0.05$), CTSK and ACP5 ($p < 0.001$ both), but increased RANKL:OPG ($p < 0.01$).

Conclusion

In conclusion, these findings unveil the role of LIF in regulating RANKL:OPG dynamics within the bone metastatic niche, offering promising prospects for therapeutically targeting LIF.

EACR25-0911

Proteomic Landscapes of Ewing Sarcoma Unravel Immune Regulation of Tumor Progression and Ferroptosis Inhibition Driving Chemotherapy Resistance

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Introduction

Ewing sarcoma (EWS), a rare pediatric bone tumor defined by EWSR1-ETS fusions, poses unique therapeutic challenges due to its distinct microenvironment and limited molecular understanding. Despite being the sole genetic driver, the oncogenic mechanisms of the fusion protein and its therapeutic potential remain elusive. Genomic and transcriptomic analyses have yet to improve patients' outcomes, prompting the need for a proteomic approach. Our study aims to reveal molecular pathways associated with treatment resistance and the immune landscape related to tumor aggressiveness.

Material and method

We took a cutting-edge mass spectrometry-based proteomic approach to obtain a comprehensive picture of EWS clinical samples. We combined it with multiplexed

imaging to associate the proteomics data with the cancer immune profiles. We assembled the largest cohort of EWS tumors, including primary, treatment-naïve tumors, post-treatment samples, relapsed tumors, and metastases from bones and lungs. We analyzed 170 tumor samples from 74 patients and investigated their association with clinical parameters, including survival, chemotherapy response, and metastatic state.

Result and discussion

We analyzed EWS proteomes at different tumor stages, identifying upregulated ferroptosis inhibitors in post-NACT samples, suggesting a mechanism by which EWS cells evade chemotherapy-induced cell death. Functional assays revealed that chemotherapy-resistant EWS cells exhibit reduced sensitivity to doxorubicin but show enhanced susceptibility to ferroptosis induction via GPX4 inhibition. Treatment with the GPX4 inhibitor RSL3 restored sensitivity to chemotherapy in resistant cells, highlighting ferroptosis as a potential therapeutic vulnerability to overcome drug resistance in EWS. Unsupervised clustering of primary tumors revealed three distinct prognostic groups. Examination of the significantly changing proteins between clusters showed that the good prognosis cluster expresses high antigen presentation proteins and 20S proteasome levels. In contrast, the poor prognosis cluster presented a network of neutrophil proteins, supporting a tumor-promoting role of neutrophils in EWS. Multiplexed immunofluorescence imaging validated the association between poor patient prognosis and tumor neutrophils and the association of macrophages and T-cells with a favorable prognosis. These results suggest that immuno-oncological treatments might be efficacious for a subset of patients.

Conclusion

Our work presents the first EWS clinical proteomics dataset, with a proteome coverage of over 10,000 proteins in 170 samples. Our analysis identified potential combination therapies to enhance chemotherapy responses and immune-related features that could guide better immunotherapeutic approaches. Our database can serve as a resource for the broad cancer biology and EWS communities.

EACR25-0912

Monocyte/macrophage crosstalk with colorectal cancer in co-cultures - in vitro studies

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Introduction

The tumor microenvironment (TME) critically influences colorectal cancer (CRC) progression, either inhibiting or promoting malignancy. Tumor-associated macrophages (TAMs), comprising up to 50% of the tumor mass, exhibit phenotypic plasticity, shifting between pro-inflammatory (M1) and immunosuppressive (M2) states.

While M1 macrophages possess tumoricidal properties, M2-polarized TAMs support tumor growth, angiogenesis, and immune evasion. This study investigated interactions between CRC cells and monocytes/macrophages to better understand their role in modulating the TME.

Material and method

This study examined an *in vitro* co-culture model of human monocytes (THP-1) and stage I colorectal cancer cells (HT-29) to assess the impact of tumor-derived soluble factors. Inserts with 0.4 µm pores allowed the exchange of factors while maintaining physical separation. Models with monocytes/macrophages infiltration at 50% and 20% relative to the cancer cell density were analyzed. Flow cytometry was used to evaluate apoptosis, necrosis, and CD68 expression, a marker of macrophage differentiation.

Result and discussion

The study demonstrated that after 24 hours of incubation with CRC cells, the viability of monocytes/macrophages did not show statistically significant changes. The proportion of live cells, as well as those undergoing different stages of apoptosis and necrosis, remained comparable in co-cultures with 20% and 50% infiltration compared to their respective controls. However, after 48 hours, a significant ($p < 0.01$) reduction in immune cell viability was observed, particularly in the 20% co-culture, suggesting that at lower cell densities, macrophages are more susceptible to tumor-derived soluble factors. Analysis of CD68 expression revealed a slight increase (<1%) in the 20% co-culture compared to the control at both 24 and 48 hours. The small magnitude of this change indicates only a weak differentiation of monocytes into macrophages under the influence of tumor-derived factors. This may be attributed to the activation of monocytes/macrophages under conditions of increased environmental stress and limited direct cell-cell communication. In the 50% co-culture, CD68 expression remained unchanged, suggesting that at higher macrophage densities, exposure to tumor-derived factors does not induce a distinct activation response.

Conclusion

This study demonstrates that prolonged exposure to tumor-derived factors in an *in vitro* model of stage I CRC reduces the viability of monocytes/macrophages, particularly at lower infiltration densities, which suggests increased sensitivity to the tumor microenvironment. The slight increase in CD68 expression in the co-cultures suggests that stage I CRC cells have limited potential to induce macrophage differentiation.

EACR25-0917

Differential Scanning Fluorimetry and compound screening enabled the identification of novel series of microRNA-21 targeting compounds

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Introduction

Aberrant expression of microRNA-21 (miR-21) is recognized as a key determinant in cancer progression through dysregulated post-translational control of several tumor suppressor genes. Hence, identification of compounds directed against miR-21 is representing an emerging opportunity for the development of candidate drugs with innovative modalities, such as riboabs or interferents with pre-miR-21 maturation. Here, we report the identification of compounds targeting pre-miR-21 through screening of small-molecule collections and using differential scanning fluorimetry (DSF) as biophysical binding assay.

Material and method

DSF monitors the ability of small molecules to engage RNA through increased thermal stability. The assay was developed using a hairpin-structured pre-miR-21 and a fluorogenic RNA-binding dye. Compound-induced thermal shift of pre-miR-21 was detected by QuantStudio® RT-qPCR system in 384 well plate format. Reference compounds, as well as single-point pre-miR-21 mutant and equivalent DNA sequences were assessed to validate the assay selectivity. Screening was performed on an unbiased collection of 1300 biologically active compounds and on a focused library of 2200 compounds enriched for RNA binding moieties.

Result and discussion

The DSF assay was optimized to achieve a screening-suited performance while enhancing its sensitivity towards putative RNA-binding molecules. Selectivity was proven by using as positive control dovitinib, a pre-miR-21 binding molecule that inhibits miR-21 biogenesis. The assay displayed very high robustness, with RZ' of 0.83 and CV% of 0.7. Combined screening of unbiased and focused libraries of 3500 compounds in total identified 10 primary hits (0.3% hit rate) triggering a significant Tm shift for pre-miR-21. Primary hits were triaged at increasing concentration, with 8 compounds confirmed to display dose-dependent saturation curves with aKD values in the 1-/2-digit micromolar range. Some chemical scaffolds of confirmed hits displayed similarity with biologically active compounds known to target nucleic acids, while others appeared to be new structures. A subset showed selective binding to pre-miR-21 over other ribonucleotides. In all cases, confirmed hits are novel in the pre-miR-21 binding mechanism, and follow-up studies are currently ongoing with orthogonal biophysical assays, as well as on cancer cell systems to confirm their ability to interfere with pre-miR-21 processing.

Conclusion

The combination of DSF and screening has identified novel series of pharmacophores acting as pre-miR-21 binders, with the potential to serve as building blocks for the development of lead compounds offering an unconventional modality to target cancer progression.

EACR25-0924

Cellular enlargement causes mitotic errors and aneuploidy in cells that evade senescence following chemotherapy

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Introduction

CDK4/6 inhibitors (CDK4/6i) arrest cells in G1 to cause cellular overgrowth and p53-dependent senescence. CDK4/6i are licenced to treat metastatic HR+/HER2+ breast cancer where they have dramatically improved patient outcomes. Sadly, resistance is still common, and this has recently been associated with TP53-loss and senescence evasion. This study examines the fate of enlarged CDK4/6i-treated cells that evade senescence following CDK4/6i-treatment, demonstrating that these enlarged cells are primed to experience mitotic errors and become genetically unstable.

Material and method

Live and fixed single cell analysis is used to examine the fate of cells that escape senescence following CDK4/6i-treatment. A range of mitotic assays are used to characterise key processes that control chromosome segregation and single cell DNA sequencing is used to characterise copy number changes and aneuploidy.

Result and discussion

We show here that enlarged CDK4/6i-treated cells that evade senescence experience chromosome mis-segregations due to defective chromosomal alignment and a weakened mitotic checkpoint. This leads to aneuploidy, as assessed by single cell DNA sequencing, and DNA damage. The chromosome segregation defects are associated with impaired Sgo1 localisation to centromeres and defective sister chromatid cohesion during mitosis. Importantly, all these defects can be rescued by constraining cell size during the CDK4/6i-treatment, and specifically restoring cohesion rescues the chromosome segregation errors. Together, this demonstrates mechanistically how cell enlargement drives genetic and karyotypic changes in cells that re-enter the cell cycle following CDK4/6 inhibition. Similar results are observed with other chemotherapeutics that induce a DNA-damage checkpoint arrest in G1, implying that cell overgrowth could widely contribute to chromosomal instability following chemotherapy. This could help fuel the emergence of chemotherapy-resistant clones, especially in TP53-null cells that evade senescence, perhaps explaining why TP53-loss drives drug-resistance in CDK4/6i-treated breast cancer patients.

Conclusion

In summary, these findings explain how anti-cancer drugs that arrest cells in G1 can have unanticipated consequences later in mitosis. Our data explains how CDK4/6i cause irreversible cell cycle exit, and how they can produce the type of chromosomal aberrations that drive rapid tumour evolution and drug resistance in cells that continue to proliferate, such as those lacking p53.

EACR25-0926

Characterization of hPBMC-ASID and hPBMC-ASID β 2m $^{−/−}$ models for Immune-Oncology Studies

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Introduction

The humanized immune system (HIS) mouse models are the preferred *in vivo* preclinical platforms to evaluate novel immune-oncology (IO) therapeutic candidates.

Among these HIS mice models, human peripheral blood mononuclear cells (hPBMC)-HIS mouse model is one of the most commonly used HIS mouse models due to its rapid engraftment. However, the limitation of hPBMC-HIS model is that mice may suffer from xenogeneic graft-versus-host disease (GvHD), where the human immune cells could attack and damage mouse tissues, leading to the death of mice and a very limited experimental window. Thus, the aim of this study is to identify an optimized protocol for hPBMC-HIS mouse model by using immunodeficient mice developed by NLAC.

Material and method

First, we transplanted 2×10^6 or 5×10^5 hPBMC cells into ASID (NOD.Cg-PrkdcscidIl2rgtm1Wjl/YckNarl) mice pretreated with total body irradiation (TBI), or 1×10^7 or 5×10^6 hPBMC cells into ASID mice without irradiation treatment. Furthermore, it has been reported that β 2m $^{−/−}$ mice can reduce the severity of GvHD due to the lack of MHC class I molecule expression. Thus, we also transplanted 1×10^7 hPBMC cells into ASID β 2m $^{−/−}$ (NOD.Cg-PrkdcscidIl2rgtm1WjlB2M $^{−/−}$ /YckNarl) mice (\varnothing) without irradiation treatment and 2×10^6 hPBMC cells into ASID β 2m $^{−/−}$ mice (\varnothing) with irradiation.

Result and discussion

The higher engraftment rate (>25% hCD45) was found in the following groups: 2×10^6 w/ irradiation (\varnothing), 1×10^7 w/o irradiation (\varnothing) and 5×10^6 (δ) w/o irradiation. However, compared with the lower engraftment groups, mice in these groups showed weight loss, scaly skin, and shorter survival time, implying the occurrence of GvHD. Moreover, compared with ASID mice, the variation of survival periods in ASID β 2m $^{−/−}$ mice was smaller than that of ASID mice, and GvHD score was lower in earlier experimental period, although the engraftment rate of ASID β 2m $^{−/−}$ mice was lower than that of ASID mice.

Conclusion

Thus, using different conditions, both ASID and ASID β 2m $^{−/−}$ mice could serve as the suitable models for hPBMC immune system reconstitution.

EACR25-0928

Unique cellular interactions and enhanced tertiary lymphoid structures in responders to anti-PD-1 therapy in mucosal head and neck cancers

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Introduction

Survival in recurrent/metastatic head and neck mucosal squamous cell carcinoma (HNmSCC) remains poor. Anti-programmed death (PD)-1 therapies have demonstrated improved survival with lower toxicity when compared to standard chemotherapy. However, response to anti-PD-1 therapy remains modest, at 13–17%. Understanding the immunological basis for response to such treatments at mucosal surfaces is critical for improving outcomes.

Material and method

We evaluated the tumour microenvironment (TME) using Imaging Mass Cytometry (IMC) on 27 tumour specimens from 24 advanced HNmSCC patients prior to receiving anti-PD-1 based treatment.

Result and discussion

We provide the first detailed sub-cellular, high-dimensional, *in situ* tissue map of the TME in advanced HNmSCC and identify hallmarks of patients most likely to benefit from anti-PD-1 therapy. Our results showed that the immune landscape largely remained similar irrespective of HNmSCC subsite or p16 status. Quantification of cell densities showed significantly increased central memory T cells and B cells in responders ($n=8$) when compared to non-responders ($n=16$). Spatial mapping identified unique interactions between phenotypically distinct malignant squamous cells, T cells and endothelial cells in responders, and avoidance of these cells in non-responders. Importantly, regional analysis shows responders have more lymphoid aggregates akin to early tertiary lymphoid structures (TLS), with their presence in high proportion (>20%) also associated with longer progression free survival.

Conclusion

Anti-PD-1 therapy response in HNmSCC is associated with specific interactions within the TME and the presence of TLS. This study identifies mechanisms of anti-PD-1 therapy response and resistance in HNmSCC patients, providing a unique opportunity to guide immunotherapy strategies and improve outcomes.

EACR25-0932

Cholesterol Transfer from PI-Activated TREM2+ Macrophages to Tregs Drives Lymph Node Immunosuppressive Pre-Metastatic Niche

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Introduction

The formation of an immunosuppressive pre-metastatic niche in lymph nodes (LNs) is critical for cancer metastasis, yet how tumor-secreted factors reprogram myeloid cells to drive immune evasion remains poorly understood. Here, we investigate the role of lipid metabolic crosstalk between tumor-primed macrophages and regulatory T cells (Tregs) in establishing a metastasis-permissive LN microenvironment.

Material and method

We integrated scRNA-seq from our lab and GEO datasets to map metastasis-associated immune landscapes. Lymph node metastasis was dynamically monitored via immunofluorescence and IVIS bioluminescence. The phosphatidylinositol (PI)-TREM2 axis was mechanistically dissected using promoter-luciferase assays. *In vitro* and *in vivo* functions of Trem2 were evaluated using Trem2 knockout mice, CRISPR-edited cell lines (Raw264.7), and bone marrow-derived macrophages (BMDM) from mouse models. Metastatic lymph node lipid profile and spatial metabolism were used to verify the metabolic crosstalk.

Result and discussion

Tumor-derived IL8 mobilized monocytes to draining LNs prior to metastasis. These monocytes differentiated into TREM2+ macrophages upon sensing high level of PI in lymph node. TREM2 activation triggered the whole cholesterol metabolism in macrophages, leading to cholesterol efflux via extracellular vesicles (EVs). Recipient Tregs exhibited enhanced suppressive function via cholesterol-dependent activation of liver X receptor (LXR) signaling. Genetic ablation of Trem2 and Trem2 antibody suppressed LN metastasis *in vivo*.

Conclusion

Our findings unveil a lipid-centric mechanism by which PI-activated TREM2+ macrophages license metastasis through cholesterol transfer to Tregs. Targeting this macrophage-Treg metabolic axis may reverse immunosuppression in pre-metastatic LNs, offering a therapeutic strategy to impede early metastatic spread.

EACR25-0940

Translational Value of Hepatic Dnaja3-deficient Mice in Preclinical Testing of Novel HCC Therapeutics

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Introduction

Globally, hepatocellular carcinoma (HCC) is the dominant type of liver cancer and accounting for 75% of the total. Nowadays, there are lacking off therapies to cure or reverse the HCC. Therefore; an effective pre-clinical model recapitulate human HCC is crucial to validate and respond to current disease. Dnaja3, a tumor suppressor and mitochondrial co-chaperone protein, is responsible for maintaining mitochondrial polarization and integrity of mitochondrial DNA. Recently, we have demonstrated that hepatic Dnaja3-deficient mice spontaneously developing HCC along with significant mitochondrial dysfunction, sequentially, accompanied by excessive lipid accumulation and inflammatory responses. Hereto, in this study, the hepatic Dnaja3-deficient mice were used as HCC preclinical model to assess the anti-cancerous effect of Sorafenib, a first-line therapy for advanced HCC, and of a promisingly screened compound X.

Material and method

We investigated the in vivo anti-tumor activity of sorafenib on Diethylnitrosamine (DEN)-induced Alb-Dnaja3f/f, and of compound X on spontaneous Alb-Dnaja3f/f mice, respectively. The Alb-Dnaja3f/f mice were randomly divided into two groups: Placebo and Experimental (Sorafenib or compound X). The Placebo group was orally gavage with saline, while the Experimental group was orally administered with 30 mg/kg Sorafenib or 8 mg/kg compound X once a day for 2 weeks, respectively. After the mice were sacrificed, the blood and tissue samples were harvested for biochemical, pathohistological and functional analyses.

Result and discussion

First, the overall median survival of the sorafenib group was significantly longer than that of the Placebo group, however, the sorafenib treatment did not effectively inhibit the tumor growth. Meanwhile, we discovered compound X markedly prevented and suppressed tumor progression. In fact, the biochemistry analyses including AST, ALT, T-CHO and TG levels were ameliorated. We also observed the downregulation of Acc2 protein (an enzyme for converting acetyl-CoA to malonyl-CoA for biosynthesis of fatty acids) in liver tissues of treatment group. Together, these results revealed that the lipogenesis was attenuated. Subsequently, the excessive accumulation of white adipose tissue was suppressed. Strikingly, compound X treatment reversed the remodeling of extracellular matrix.

Conclusion

Overall, we designed a preclinical trial using compound X and to prevent and suppress the tumor progression in hepatic Dnaja3-deficient mice. Apparently, we demonstrated that compound X altering the lipid metabolism homeostasis in liver.

EACR25-0948

Autophagy activation in response to cigarette smoke: Exploring the disparity in laryngeal cancer incidence and outcomes between sexes in South Korea

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Introduction

Laryngeal cancer (LC) presents a significant health challenge globally, with smoking being a major risk factor. Interestingly, LC incidence in females is significantly lower than in males; however, female smokers are more likely to develop Reinke edema (RE) than LC. This study sought to investigate whether autophagy, a major mechanism for RE development, acts as a defense mechanism in laryngeal tissue against cigarette exposure and suppresses LC development in females who smoke.

Material and method

This study analyzed the National Health Insurance Service (NHIS) data of South Korea to explore sex differences in LC incidence and clinical outcomes. Protein expression was compared between tissues from LC and RE patients. The changes in autophagy-related markers were analyzed after exposure to human vocal fold fibroblast (hVFF) and cigarette smoke extract (CSE). In addition, to explore the relationship between the level of autophagy-related gene expression and clinical features, female LC patients were compared with male patients through an analysis of data from The Cancer Genome Atlas (TCGA).

Result and discussion

In the NHIS data analyses, male LC patients had an 11 times higher incidence than female patients, even after adjusting for smoking and age. Additionally, female LC patients had significantly better survival rates. RE tissues exhibited increased autophagy-related protein expression compared with LC tissues. hVFFs after CSE exposure demonstrated elevated autophagy markers along with protein expression similar to RE tissue, suggesting autophagy's role in RE development over LC. The TCGA data analysis did not find a significant difference in autophagy-related gene expression, which would explain the favorable female clinical outcomes, between male and female LC patients.

Conclusion

This study implies autophagy activation by cigarette smoke is a crucial mechanism for lower LC incidence and better outcomes in females, highlighting the potential for autophagy-targeted LC prevention and treatment strategies.

EACR25-0953

Investigate the Anticancer Immunomodulation Mechanism of Fluoxetine Treatment in Oral Squamous Cell Carcinoma

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Introduction

Oral squamous cell carcinoma (OSCC) is one of the most common types of oral cancer. It has been reported that OSCC could achieve metastasis and proliferation by overexpressing galectin-1 (gal-1), even inducing resistance to immune checkpoint inhibitors (ICIs). Meanwhile, due to the dense structure in OSCC, the activation of hypoxia-inducible factor-1 alpha (HIF-1 α) is still the challenge for cancer treatment, and the tumor microenvironment (TME) will transform into an unfavorable situation for immunotherapy. Hypoxia is a common phenomenon in malignant tumors, caused by the fast proliferation of tumor cells and unusual angiogenesis, which can promote tumor growth. When tumor tissues exposure to hypoxic condition, HIF-1 α may reduce or inactivate cytotoxic lymphocytes, including NK cells and CD8+ T cells, leads to the reduction of their anti-tumor efficacy. Fluoxetine is one of the commercially available antidepressants, it has been demonstrated to have the ability to suppress cancer cell growth and increase the sensitivity to antineoplastic agents. However, whether fluoxetine could rescue ICIs' curative effect and mechanism on OSCC is still unclear. Therefore, we aim to the potential pathway of fluoxetine treatment that could regulate the TME of OSCC.

Material and method

CCK-8 assay was used to validate cytotoxicity of fluoxetine. RNA-seq analysis revealed pathways that related to following fluoxetine treatment, and protein levels were showed by western blot. Additionally, transwell migration clarified how fluoxetine change the TME in OSCC. Orthotopic model was established to confirm the therapeutic effect of OSCC *in vivo*, populations of immune cells were analyzed by flow cytometry.

Result and discussion

According to our data, fluoxetine exerted cytotoxic effects on MOC1 cells and reduced the surface expression of programmed cell death 1 ligand 1 (PD-L1). INF- γ pathway was upregulated by fluoxetine treatment, while protein level of HIF-1 α was decreased despite unchanged mRNA expression. Additionally, fluoxetine enhanced the migration capacity of BMDCs in the presence of MOC1 cells. Furthermore, combining fluoxetine with immunotherapy significantly improved therapeutic outcomes and suppressed tumor growth. Cytotoxic lymphocytes were markedly upregulated, whereas immunosuppressive cells such as M2 macrophages, myeloid-derived suppressor cells (MDSCs), and Tregs were downregulated, suggesting that fluoxetine effectively modulates the TME and holds promise as a novel strategy for OSCC treatment.

Conclusion

In summary, our study demonstrates the anticancer effects of fluoxetine in OSCC by alleviating hypoxia through the promotion of HIF-1 α ubiquitination, inhibiting PD-L1 expression, and recruiting cytotoxic lymphocytes while suppressing immunosuppressive cells. These effects, validated in an orthotopic OSCC model and enhanced by combination with immunotherapy, offer a promising new approach for OSCC treatment.

EACR25-0954

Reprogrammed fibroblasts promote melanoma progression and immune evasion in the lymph node niche

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Introduction

Melanoma is an aggressive skin cancer arising from the malignant transformation of melanocytes, the cells responsible for skin pigmentation. When localized in the skin, melanoma is treatable by surgical resection; however, if it is not detected early, it will invariably metastasize, starting by invading the lymph nodes. This lymphatic invasion is a critical step in melanoma progression, as it allows cancer cells to enter the bloodstream and spread to other organs such as the liver, bones, lungs, and brain. Understanding the mechanisms underlying lymph node invasion could lead to earlier and more effective interventions. During the pre-metastatic phase, lymph nodes are reprogrammed by factors secreted by melanoma cells in the skin, creating a niche favorable to tumor invasion and proliferation. During this phase, lymph node fibroblasts, known as Fibroblastic Reticular Cells (FRCs), are reprogrammed. In healthy lymph nodes, FRCs play a key role in organizing the structure of lymph nodes and in regulating T cell recruitment, survival, and activation. In many tissues, fibroblasts in the tumor microenvironment, also known as cancer-associated fibroblasts, are known to promote cancer progression, but little is known about the role of FRCs in the lymph node.

Material and method

To mimic the pre-metastatic reprogramming in the lymph node, healthy human FRCs are incubated with factors secreted by melanoma cells. Reprogrammed FRCs are then cocultured with T cells or tumor cells to understand the dysregulation of their interactions using flow cytometry and real-time microscopy approaches.

Result and discussion

We identified by RNAseq analysis that FRCs are transcriptionally reprogrammed by factors secreted by melanoma cells. Indeed, our previous work demonstrated that IL-1 secreted by dedifferentiated melanoma cells inhibit the contractility of reprogrammed FRCs, thereby facilitating melanoma cell invasion. My research also reveals that reprogrammed FRCs enhance the proliferation of tumor cells, their motility and their resistance to targeted therapies used in the clinic. Additionally, these reprogrammed FRCs disrupt the anti-tumor immune response by altering T cell motility and upregulating immune checkpoint molecules (PD1, LAG3, and CTLA4) on T cells. Future studies will focus on deciphering the precise molecular mechanisms by which FRCs acquire their tumor-promoting phenotype and identifying potential targets for intervention.

Conclusion

These findings highlight the critical role of FRCs in creating a tumor-permissive microenvironment in early melanoma progression, and suggest new therapeutic approaches based on targeting the interactions between reprogrammed FRCs, tumor cells, and immune cells.

EACR25-0966

The NRG1/ERBB axis as a potential target to prevent targeted cancer therapy resistance

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Introduction

Although targeted cancer therapies are widely used in the clinic, their long-term efficacy is compromised by the emergence of acquired drug resistance. One well-documented mechanism contributing to resistance across various oncogene-addicted cancer models is autocrine or paracrine neuregulin 1 (NRG1). NRG1 serves as a ligand for receptor tyrosine kinases ERBB3 and ERBB4, while ERBB2 also participates as a coreceptor in NRG-mediated signaling. The aim of the project is to determine the functional relevance of the NRG1/ERBB signaling axis in the response to targeted therapies and to develop strategies to target this pathway to prevent or delay the onset of drug resistance.

Material and method

Using a panel of oncogene-addicted cancer cell lines, the expression of molecular components of the NRG/ERBB axis was characterized upon long-term treatment with standard-of-care (SOC) targeted therapies. CRISPR-Cas9-mediated ERBB knockouts were used for assessing the functional relevance of the ERBB receptors for drug-tolerant cells. The effect of different therapeutic approaches for targeting the NRG1/ERBB axis, including pan-ERBB inhibitors and antibody-drug conjugates, will be studied *in vitro* using cell viability and apoptosis assays. Most promising drug combinations will be further evaluated in relevant mouse xenograft tumor models.

Result and discussion

Our findings demonstrate that the NRG/ERBB axis is upregulated at both at the transcriptional and protein levels following SOC targeted therapy treatment in a panel of five oncogene-addicted cancer cell lines. This upregulation occurs alongside increased activation of NRG receptors and enhanced downstream signaling. CRISPR-Cas9-mediated knockout of ERBB receptors suggested a dependency of drug-tolerant BRAF-mutant colorectal cancer cells on ERBB2 and ALK-positive non-small cell lung cancer cells on EGFR, ERBB2 and ERBB3. Based on these findings we hypothesize that the drug-tolerant cells upregulate the NRG1/ERBB axis to overcome sensitivity to targeted therapies. Currently we are investigating whether targeting ERBB receptors in combination with the SOC targeted therapy could prevent the emergence of resistance to SOC.

Conclusion

The NRG1/ERBB signaling axis is upregulated upon long-term targeted therapy treatment in multiple oncogene-addicted cancer cell lines. Targeting this axis

in combination to the current SOC targeted therapies could lead to improved treatment efficacy.

EACR25-0969

Tissue-Resident Resting Fibroblasts Function as Tumor Suppressors in the Tumor Microenvironment

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Introduction

Our previous research identified the presence of tissue-resident resting fibroblasts (tr-RFs) within the tumor microenvironment (TME). In contrast to tr-RFs, conventional cancer-associated fibroblasts (CAFs) exhibit hallmark characteristics of mesenchymal stem cells (MSCs). However, the biological role of tr-RFs in the TME remains largely unexplored.

Material and method

To elucidate the functional significance of tr-RFs, we conducted single-cell RNA sequencing (scRNA-seq) analyses of gastric cancer tissues and normal gastric mucosa, identifying distinct CAF subsets. Gene ontology (GO) and gene set enrichment analysis (GSEA) were performed to characterize their molecular signatures. The phenotypic plasticity of tr-RFs and MSCs was assessed via co-culture with gastric cancer cells (NUGC4) using qRT-PCR. Additionally, the impact of tr-RFs and MSCs on tumor progression was evaluated using a mouse xenograft model.

Result and discussion

scRNA-seq analyses delineated seven distinct CAF clusters. Uniform Manifold Approximation and Projection (UMAP) revealed that three of these clusters exhibited phenotypic overlap with normal stomach-resident fibroblasts. Among them, one specific cluster was devoid of mesenchymal stem cell markers (ITGB1, THY1, ENG) and active CAF markers (PRRX1, TNC), thereby identifying this population as tr-RFs. GO analysis of this subset demonstrated significant enrichment in pathways associated with the negative regulation of cellular proliferation, humoral immune activation, and complement system engagement, indicative of a potential immune-mediated tumor-suppressive function. *In vitro*, MSCs co-cultured with NUGC4 exhibited significant upregulation of CAF-associated genes (FAP, TNC, COL1A2, IL1A, IL6, LIF, CXCL12), whereas tr-RFs co-cultured with NUGC4 did not induce such transcriptional changes; instead, select CAF-related genes were downregulated. *In vivo*, MSCs markedly accelerated tumor growth in a nude mouse xenograft model, whereas tr-RFs profoundly suppressed tumor progression. Histological and immunohistochemical analyses further revealed that tumors with tr-RFs displayed significantly reduced MIB-1 labeling indices, and in some cases, elicited a robust immune response, corroborating our scRNA-seq findings.

Conclusion

Our study demonstrates that tr-RFs consist of a subset of CAFs, which remain transcriptionally stable in the presence of cancer cells and function as tumor suppressors, likely by inducing an immune response within the TME. Further mechanistic investigations are

warranted to elucidate the underlying pathways of this tumor-suppressive effect.

EACR25-1003

Impact of L-serine on the pro-carcinogenic effects of colibactin-producing *Escherichia coli* and the microbial community in APCmin/+ mice

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Introduction

Colonic tissues in colorectal cancer (CRC) patients are colonized by colibactin-producing *Escherichia coli* (CoPEC). Colibactin is a genotoxin synthesized by the pks genomic island. Metabolomic studies have revealed that CoPEC infection leads to a reprogramming of intestinal epithelial cell metabolism, resulting in a decrease in L-serine. This study aims to investigate whether L-serine contributes to CoPEC persistence in the gastrointestinal tract and enhances its pro-carcinogenic functions.

Material and method

The serine utilization on adhesion, survival and persistence of CoPEC was assessed using a CoPEC strain unable to metabolize L-serine (11G5-ΔtdcA) in human colorectal carcinoma T84 cells. Additionally, the impact of an L-serine-depleted (SD) diet on CoPEC persistence, genotoxic effects (γ H2Ax immunolabeling) and pro-carcinogenic properties was evaluated in a model predisposed to intestinal adenoma formation (APCmin/+ mice). The composition of the fecal microbial community following the SD diet was studied using 16S DNA gene sequencing at an early stage of cancer development.

Result and discussion

In vitro, exposure to CoPEC in T84 cells led to a decrease in both intracellular and extracellular L-serine levels. Consequently, infection with the 11G5-ΔtdcA strain resulted in reduced adhesion, survival and persistence, underscoring the essential role of L-serine utilization. Furthermore, this amino acid conferred a competitive advantage to CoPEC. To highlight the role of L-serine, APCmin/+ mice were fed an SD diet. This diet induced an early and transient decrease in CoPEC colonization, which was associated with a decrease in DNA damage. The pro-carcinogenic potential of CoPEC was significantly lower in APCmin/+ mice on the SD diet compared to those on the control (S) diet. Concerning the fecal microbial community, the SD diet led to a reduction in microbial richness and a decrease in diversity, specifically in mice that were infected. Additionally, the SD diet increased the abundance of the phylum *Bacillota* while decreasing the abundance of the phylum *Bacteroidota*, a shift associated with reduced carcinogenesis. Consistent with bacterial colonization patterns, the abundance of *E. coli*, belonging to the *Pseudomonadota* phylum, was significantly lower in the feces of mice fed the SD diet compared to those on the S diet.

Conclusion

These findings suggest that L-serine modulates the gut microbiota composition in APCmin/+ mice, promoting the expansion of CoPEC. In addition, this study supports the hypothesis that CoPEC exploit host-derived L-serine to persist in the gut and exert its genotoxic and pro-carcinogenic effects. This work may help identify potential therapeutic targets.

EACR25-1019

New insights into vitamin D receptor signaling in prostate epithelium reveal therapeutic options and biomarkers for cancer progression

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Introduction

Prostate cancer (PCa) is the most common visceral neoplasm and the third leading cause of cancer-related death in men worldwide. With the decline in prostate-specific antigen (PSA) testing – a widely used biomarker for PCa screening – the incidence of advanced cases, including metastatic disease at diagnosis, is rising. Understanding the mechanisms driving PCa progression is therefore of utmost importance for identifying candidate biomarkers and therapeutic targets to limit disease spread.

Material and method

Circulating biomarkers were analysed on 77 patients with newly diagnosed PCa from the HormOS cohort (Paris University Hospitals). Further investigations were conducted with a preclinical mouse model of PCa in which the tumor suppressor gene PTEN is selectively inactivated in prostatic epithelial cells (PECs) at adulthood (Pten(i)pe-/ mice).

Result and discussion

PSA levels and polynuclear neutrophil counts were both negatively associated with circulating vitamin D levels in treatment-naïve PCa patients. To investigate the role of vitamin D signaling in PCa progression, we compared prostatic tumorigenesis in Pten(i)pe-/ and Pten/Vdr(i)pe-/ mice, where PECs are deficient in VDR and/or PTEN. Our results demonstrate that enhanced oxidative stress and increased proliferation during prostatic intraepithelial neoplasia formation in PTEN and VDR-deficient PECs are prevented by treatment with the antioxidant N-acetyl cysteine. Moreover, a combination of single-cell RNA

sequencing and immunophenotyping revealed that the recruitment of tumor-infiltrating immunosuppressive neutrophils is higher in Pten/Vdr(i)pe^{-/-} than in Pten(i)pe^{-/-} mice. Further investigation of prostate tumorigenesis in Pten/Vdr(i)pe^{-/-} mice uncovered the presence of pan-cytokeratin-positive (PanCK+) micro-metastases in the liver, a marker of poor prognosis in patients. These infiltrates contained immune and endothelial cells, and positively correlated with the circulating neutrophil frequency. Moreover, treatment with a CXCR1/CXCR2 inhibitor reduced neutrophil recruitment and decreased the prevalence of micro-metastases in Pten/Vdr(i)pe^{-/-} mice.

Conclusion

Taken together, our findings demonstrate that VDR signaling plays a protective role in PTEN-deficient PECs by limiting oxidative stress, proliferation, and neutrophil recruitment. Furthermore, we provide evidence that reducing neutrophil chemotaxis represents a promising therapeutic target to prevent metastatic spreads in treatment-naïve PCa.

EACR25-1024 Characterizing Neuron-Neuroblastoma circuit development and its role in tumor progression

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Introduction

The influence of the nervous system on cancer progression has been increasingly recognized, yet remains enigmatic in many aspects, especially for cancers like Neuroblastoma (NB), a devastating childhood cancer arising in the developing peripheral nervous system (PNS). NB originates from neural crest cells with primary tumors emerging throughout the sympathetic paravertebral chain and the adrenal medulla. While significant progress has been made in aggressive brain gliomas, where synapses between neurons and tumor cells drive disease progression, it remains unknown whether similar synaptic contacts exist in peripheral nervous tumors such as NB. Our general aims are to determine whether NB cells receive synaptic contacts, to assess their ability to respond and propagate electrical signals, and to evaluate the impact of such dialogs on disease progression.

Material and method

We performed a multidisciplinary approach combining electrophysiology, high resolution imaging and scRNA-Seq analysis. We examined patient samples to study synaptic structures and used a NB-grafted avian embryo model, which recapitulates key aspects of NB pathology, for a comprehensive investigation of synaptic structures and functions.

Result and discussion

In a series of patient biopsies, we could detect synaptic contacts involving NB cells by electronic microscopy. scRNA-Seq in the NB avian embryo model revealed that

NB cells progressively acquire neuronal features during tumorigenesis and dissemination, including the expression of synaptic proteins, neurotransmitter receptors, and ionic channels. In this model, immunohistochemical analyses also evidenced the presence of synaptic structures in NB tumors. These synaptic structures increased in size during embryonic development. To assess synaptic function, we setup and implemented ex vivo whole-cell patch clamp recordings in NB cells. We observed that a fraction of cells exhibited synaptic excitatory currents in response to electric stimulation. We are currently working to identify the involved neurotransmitters using specific blockers. Furthermore, a high fraction of recorded NB cells showed voltage-dependent sodium currents, and this fraction increased during development. Impressively, NB cells were capable of firing action potentials with current injection.

Conclusion

NB cells in primary tumors progressively acquire neuronal features, including the formation of synaptic structures and the ability to respond and propagate electrical stimuli through action potential generation. We propose that these neural characteristics may enhance their capacity for growth and dissemination. Understanding these neuro-cancer mechanisms could uncover critical targets for future drug development, paving the way for innovative treatments.

EACR25-1034 Comprehensive screening of A-to-I editing in colorectal cancer: implications for tumour progression and therapeutic targeting

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Introduction

Adenosine-to-inosine (A-to-I) RNA editing is a post-transcriptional modification that has been shown to influence gene expression, RNA stability, and protein function. In colorectal cancer (CRC), A-to-I editing may contribute to tumor progression by modulating key cellular pathways involved in proliferation, apoptosis, and metastasis. Despite its potential importance, a comprehensive investigation into the role of A-to-I editing in CRC has not yet been fully explored. This study aims to characterise A-to-I RNA editing patterns in CRC and explore its implications for tumor progression.

Material and method

To identify A-to-I editing events, we performed high-throughput small RNA-sequencing on 116 CRC tissue samples and matched 119 normal colorectal tissues. The mapped output file was subjected to identifying reads containing A-to-G changes representing A-to-I editing in mature miRNA using the miRge 3.0 package. The A-to-I editing level was defined as the ratio of the mapped reads with changed nucleotides relative to the total mapped reads for each miRNA. A-to-I editing events were further analysed based on the respective percentage and expression level at the RPM scale. To reveal the biological function of the edited miRNAs, we identified putative target genes using Ingenuity Pathway Analysis (IPA), mirDB and TargetScanHuman 8.0 and performed WikiPathways analysis to investigate cancer-related pathways.

Result and discussion

We identified four unique A-to-I miRNA editing hotspots. Among them, miR-200b-3p ($\log_{2}FC = 1.17$; FDR-adjusted p-value = 1.91×10^{-15}) was overedited, but miR-411-5p ($\log_{2}FC = -1.18$; FDR-adjusted p-value = 1.71×10^{-31}), miR-497-5p ($\log_{2}FC = -1.06$; FDR-adjusted p-value = 6.66×10^{-18}) and miR-99a-5p ($\log_{2}FC = -0.48$; FDR-adjusted p-value = 1.68×10^{-06}) were underedited in tumors relative to normal tissues. All editing events were located in the seed region at the position 1-8 mature miRNAs. The functional analysis of edited miRNA target genes revealed that dysregulated pathways were mainly associated with EMT (Epithelial-Mesenchymal Transition) (FDR-adjusted p-value = 3.44×10^{-10}), PI3K/AKT pathway (FDR-adjusted p-value = 2.48×10^{-11}), mTOR pathway ((FDR-adjusted p-value = 8.50×10^{-09}), and cell cycle control (FDR-adjusted p-value = 4.21×10^{-10}). By ranking the PPI network nodes using the cytoHubba plugin of Cytoscape software, we found that CCND1, IGF1R, AKT1, PTEN, CDK6, CDK4, CDKN2A, SNAI1, GRB2, PRAS40 scores ranked in the top 10 hub genes.

Conclusion

The findings highlight the potentially crucial role of A-to-I editing in the pathogenesis of CRC. Dysregulated A-to-I editing of identified miRNAs could contribute to tumorigenic processes, including metastasis, by altering key regulatory pathways involved in cancer progression.

EACR25-1036

The Role of Hypoxia-Inducible Factor-1 α in shaping Cancer-Associated Fibroblast phenotypes in colorectal cancer

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Introduction

Colorectal cancer (CRC) is a leading cause of cancer-related mortality worldwide. Despite significant advancements in understanding CRC pathogenesis, patient prognosis remains poor due to metastatic dissemination. A key factor in cancer progression is the presence of cancer-associated fibroblasts (CAFs) in the

tumor microenvironment, which regulate processes such as extracellular matrix (ECM) deposition and immune suppression. Additionally, hypoxia and its central regulator, hypoxia-inducible factor-1 α (HIF-1 α), are hallmark features of advanced cancers. HIF-1 α is a transcription factor responsible for adaptive responses to low-oxygen environments, promoting tumor cell survival and resistance to therapy. However, the role of HIF-1 α in CAFs within CRC remains largely unexplored.

Material and method

To unravel the effects of hypoxia on CAF biology, we generated a hypoxia-specific gene signature from patient-derived CAFs exposed to hypoxic conditions. Gene set enrichment analysis (GSEA) was conducted to determine the enrichment of pathways associated with hypoxia in CAFs. Additionally, we employed in vitro functional assays to assess changes in CAF contractility and matrix remodeling capabilities under hypoxic conditions. Furthermore, we will use in vitro co-culture systems using patient-derived CAFs and matched tumor organoids to investigate their reciprocal interactions under hypoxic conditions. A colitis-associated carcinogenesis model in mice with fibroblast-specific deletion of hif-1 α , will allow us to study its role in tumor initiation and progression.

Result and discussion

GSEA revealed that the hypoxic signature in CAFs is enriched in pathways related to epithelial-to-mesenchymal transition (EMT), inflammatory responses, and mTORC signaling, suggesting that hypoxia promotes a tumor-supportive CAF phenotype. Functional assays demonstrated that hypoxic CAFs displayed increased contractility and enhanced matrix remodeling capabilities, highlighting their active role in shaping the tumor microenvironment. Mining in-house scRNAseq data with the hypoxia-associated gene sets will allow us to identify hypoxia-responsive CAF subsets across different stages of CRC progression and metastasis. The in vivo mouse model will further elucidate the role of hif-1 α in CAFs during tumorigenesis.

Conclusion

This project aims to uncover the underappreciated contribution of HIF-1 α in shaping CAF phenotypes during CRC progression. By integrating in vitro and in vivo approaches, we will provide new insights into how hypoxia influences CAF function and identify potential therapeutic targets for disrupting tumor-supportive CAF activity in CRC.

EACR25-1045

Advancing CRISPR Mouse Models – Investigating Single-Nucleotide Substitutions in Lung Cancer with Cytosine Base Editing

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Introduction

CRISPR-based mouse models play a crucial role in lung cancer research, enabling the *in vivo* generation and analysis of cancer mutations. Adenoviral delivery of single guide RNAs (sgRNAs) allows the flexible targeting of various cancer genes and has been previously used to generate an autochthonous model for small cell lung cancer (SCLC). Unfortunately, not all relevant cancer mutations can be investigated, since the canonical CRISPR system predominantly produces knockouts via insertions or deletions, limiting its ability to introduce single-nucleotide substitutions. These mutations, which include nonsense and missense variants, are the most prevalent in cancer patients and frequently affect critical tumor suppressor genes such as TP53.

Material and method

We adopted a novel approach utilizing cytosine base editing (CBE), a CRISPR-based genome editing tool facilitating the conversion of a cytosine to a thymine, enabling the precise induction of point mutations in DNA. Our optimised CBE consists of a human deaminase with increased editing efficiency and a near-PAMless Cas9, allowing for greater flexibility in the choice of the sgRNA and consequently the target mutation. In our SCLC model, adenoviral delivery of the optimised CBE system induces point mutations in the tumor suppressor genes Trp53, Rb1 and Rbl2, resulting in the autochthonous development of lung tumours. Over the course of the experiment, tumour development can be monitored using small blood samples, as specific reporter mice are used in which cancer cells secrete luciferase into the bloodstream.

Result and discussion

For all target mutations, sgRNAs were first validated *in vitro* and then used to generate recombinant adeno-viruses. The first mice developed lung tumours autochthonously 8–9 months after adenoviral delivery of the CBE system, which correlated with an increase in luciferase levels in the blood. The resulting tumours were confirmed to be SCLC by immunohistochemistry as they expressed typical neuroendocrine markers. Moreover, the tumours harbour the specific target mutations – tumours with a Trp53 knockout via a nonsense mutation, as well as the hyperstabilized Trp53-R270C missense variant, were generated and can now be used for further analysis into the role of mutant p53 in SCLC. A major advantage of our CBE-based model is the ability to induce defined nonsense and missense mutations in any gene of interest with great flexibility, allowing for the comparison of various different mutant variants within a defined genetic setting.

Conclusion

This novel CBE-based autochthonous SCLC model successfully generates tumours harbouring either a Trp53 nonsense or the R270C missense mutation, providing a valuable platform for investigating the functional impact of p53 mutations in lung cancer. This approach enhances our ability to study specific cancer-associated mutations, advancing precision cancer research.

EACR25-1062

Large-scale integrative analysis of fecal samples sequencing data from healthy and CRC patients supports novel miRNA-mediated host-microbiota interactions

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Introduction

Dysregulated microRNA (miRNA) profiles were repeatedly reported in studies on Colorectal Cancer (CRC) tissues. More recently, analyses of stool from CRC patients have clearly shown that altered miRNA levels reflect the presence of neoplastic lesions. Moreover, studies in model organisms reported that miRNAs released into the gut lumen, and detectable in stool, can modulate microbial gene expression. However, a comprehensive investigation of miRNA-microbiota interactions in humans is still lacking. Combined profiling of fecal miRNAs and microbial species in a large population of CRC patients and healthy individuals could provide novel insights into host-microbiota interactions dysregulated in this tumor.

Material and method

An integrative analysis of fecal shotgun metagenomic sequencing and small RNA-Seq (sRNA-Seq) data of 1,052 subjects from eight European cohorts was performed. The study included 332 CRC patients, 87 subjects with precancerous lesions, 165 with non-tumoral gastrointestinal diseases, and 468 healthy controls. Microbial profiling was performed using MetaPhlAn 4.1 while a Docker4Seq pipeline was applied for fecal miRNA quantification. Diversity analysis of miRNA and microbial profiles (by Richness, Shannon, Inverse Simpson, Evenness indices) across study groups was performed with an investigation of significant and consistent miRNA-microbial associations across cohorts.

Result and discussion

From the fecal miRNome/microbiome characterization, a significant decrease ($p < 0.05$) of miRNA heterogeneity was observed from healthy to CRC, while the microbial diversity increased in CRC was driven by the oral-to-gut introgression of oral species. 216 detectable miRNAs across cohorts were confirmed to be transcriptionally active in the intestinal tissue and some of them were dysregulated in CRC tissue, as also reported in public datasets. Analysing miRNA levels with respect to abundances of 461 commonly detected microbial species, 2,223 significant and coherent miRNA-microbial associations across cohorts were identified. Despite the observed associations were predominantly positive and detected in CRC ($n = 916$, 47%), 780 associations (35%)

were uniquely detected in controls while only 53 (3%) showed opposite direction among controls and CRC. The associations involved oral species including the previously reported miR-1246 and *Fusobacterium nucleatum* association. The associations changed from healthy to late-stage CRC, with only 3% of them shared among main disease categories.

Conclusion

Our findings indicate that gut microbial composition is associated with fecal miRNA profiles. Most of the associations are specific to CRC and may reflect alterations in both the fecal miRNome and microbiome. To conclude, these data support the role of miRNAs as potential mediators of host-gut microbiota interactions.

EACR25-1063

Reconstructing spatiotemporal dynamics of the tumor and micro-environment in a triple-negative breast cancer murine model

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Introduction

Triple-negative breast cancer (TNBC) is a highly aggressive malignancy with a strong metastatic potential and limited therapeutic options. While tumor-intrinsic factors contribute to disease progression, the tumor microenvironment (TME) undergoes dynamic remodeling, influencing tumor evolution through mechanisms that remain to be fully elucidated. Cellular interactions within the TME promote immune evasion and therapeutic resistance, further driving malignancy. To investigate these processes, we developed a murine TNBC model carrying patient-relevant mutations that spontaneously generate tumors, closely recapitulating human TNBC progression. By applying single-nucleus and high-resolution spatial transcriptomics, we generated a spatiotemporal atlas of TME remodeling, uncovering sequential cellular transitions that shape tumor progression.

Material and method

Four time points – healthy, early, intermediate, and late tumors – with three biological replicates per stage were analyzed. snRNA-seq was performed on all samples, capturing 59,000 cells. OpenST spatial transcriptomics, recently developed by our group (Schott et al., 2024, Cell), was applied to the same timepoints, covering 770,000 7 µm pseudocells. Over 200 mammary ducts were isolated for pseudotemporal alignment. DestVI mapped snRNA-seq clusters onto spatial data, while CellChat inferred cell-cell communication.

Result and discussion

In this project, we developed a TNBC mouse model carrying p53, PIK3CA, and β-Catenin mutations, which spontaneously formed multifocal tumors. Tumor

progression was marked by a progressive trans-differentiation of epithelial cells from a luminal to a basal-like phenotype, giving rise to distinct micro-environmental niches. By calculating pseudotime, we aligned ducts according to tumor advancement, revealing an ordered remodeling process that linked epithelial transitions to stromal reorganization. This transformation of the TME occurred through orchestrated, sequential events driven by TGF-β signaling activation, leading to the emergence of cancer-associated fibroblasts (CAFs) and tumor-associated macrophages (TAMs). Syngeneic transplants in immunocompetent mice demonstrated that a specific myCAF subpopulation strongly enhances tumor growth, establishing its role as a key regulator of TNBC progression.

Conclusion

Our study reveals that TNBC progression is driven by a stepwise remodeling of the TME, where tumor cell transitions are tightly linked to orchestrated stromal reprogramming. We identify myCAFs as key drivers of an aggressive tumor niche, promoting malignancy through extracellular matrix remodeling and TGF-β signaling. These findings provide a framework for targeting early tumor-stroma interactions as a therapeutic strategy in TNBC.

EACR25-1068

Identifying Metastatic Drivers and Biomarkers in Colorectal Cancer – the zAvatar model as a surrogate for patient metastatic potential

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Introduction

Metastasis remains the biggest challenge in cancer treatment, accounting for ~90% of cancer-related deaths. Colorectal cancer (CRC) is no exception, with distant metastasis predominantly affecting the liver and lungs. Despite significant advances, the molecular mechanisms driving CRC metastasis remain controversial, and reliable biomarkers for identifying patients at risk of metastatic disease are still lacking in the clinical practice. Our recent work established the zebrafish xenograft model (zAvatar) as a fast predictive platform for personalized treatment in CRC, forecasting patient progression with 91% accuracy.

Material and method

We observed that zAvatars derived from CRC patients with disease progression exhibited a significantly higher incidence of micrometastases compared to those without progression. Among locoregional tumors, the absence of metastases was sufficient to classify patients as non-progressive, whereas if the zAvatar presented micrometastases, then patient outcome would depend on the efficacy of adjuvant chemotherapy (Costa et al, Nat Commun 2024). This suggests that the model reveals the

inherent biological ability that tumor cells may have or not to metastasize, providing a unique opportunity for patient stratification. To further explore this, we analyzed transcriptional differences between stage III CRC tumors that developed micrometastases in zAvatars those that did not.

Result and discussion

Preliminary RNA sequencing of stage III CRC patients identified a clear metastatic gene signature, consisting of five overexpressed genes associated with micro-metastases in the zAvatars, suggesting their potential role as metastasis drivers. To validate and map these genes within the tumor microenvironment, we will apply spatial transcriptomics to the same tumor samples. The final goal is to perform a functional validation on target genes, by overexpressing them in CRC cells or in the zebrafish host, and assess their role in metastatic progression.

Conclusion

The zAvatar model provides a powerful tool to identify tumors with metastatic potential. With this project we aim to improve patient stratification and expand our understanding of the molecular mechanisms underlying CRC metastasis.

EACR25-1090

miR-27a Targets the Circadian clock circuitry to Drive Lung Adenocarcinoma Progression

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Introduction

Circadian rhythms regulate essential cellular processes, including metabolism, proliferation, and DNA damage response, through transcriptional-translational feedback loops (TTFLs). Core circadian genes, such as ARNTL (Bmal1) and CLOCK, orchestrate these oscillations, maintaining cellular homeostasis. Dysregulation of the circadian machinery has been implicated in tumorigenesis, enhancing cancer aggressiveness and therapy resistance. MicroRNAs (miRNAs) are key post-transcriptional regulators, with miR-27a-3p identified as a modulator of circadian genes. This study investigates the role of miR-27a-3p in lung adenocarcinoma by evaluating its impact on ARNTL and CLOCK expression and associated tumorigenic properties.

Material and method

Lung adenocarcinoma cell lines were genetically modified to overexpress or inhibit miR-27a-3p. The effects on ARNTL and CLOCK expression were assessed at mRNA and protein levels via qPCR and immunoblotting. Functional assays – including proliferation, migration, invasion, and metabolic profiling – were performed to evaluate tumor behavior. Target Site Blockers (TSBs) were used to selectively disrupt miR-27a-3p binding to ARNTL and CLOCK, followed by additional functional assays. To assess in vivo effects,

modified cells were transplanted into immunocompromised mice, and tumor growth was monitored. RNA sequencing (RNA-seq) was conducted to identify downstream pathways regulated by miR-27a-3p.

Result and discussion

miR-27a-3p negatively regulates ARNTL and CLOCK, promoting a more aggressive and invasive phenotype in lung adenocarcinoma cells. Functional assays revealed that miR-27a-3p overexpression enhances migration, metabolic reprogramming, and tumor progression. RNA-seq analysis identified IL-8 as a downstream effector of the miR-27a-3p/ARNTL-CLOCK axis, where its suppression further increased tumor cell invasiveness while reducing proliferative capacity. The in vivo model confirmed that miR-27a-3p promotes an invasive phenotype, reinforcing the role of circadian gene dysregulation in lung cancer progression.

Conclusion

This study demonstrates that miR-27a-3p drives lung adenocarcinoma progression by targeting ARNTL and CLOCK, influencing tumor metabolism, invasion, and proliferation. The identification of IL-8 as a downstream effector highlights a potential link between circadian disruption and oncogenic signaling. Targeting the miR-27a-3p/ARNTL-CLOCK axis may represent a novel therapeutic approach to mitigate lung adenocarcinoma aggressiveness and improve treatment outcomes.

EACR25-1094

Developing Comprehensive In Vitro and In Vivo Models: Patient-Derived Organoids (PDOs) and Xenografts (PDXs) for Morphological and Functional Analysis and Drug Screening in Head and Neck Squamous Cell

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Introduction

About 90% of head and neck cancers originate from the mucosal epithelial cells of the oral cavity, pharynx and larynx referred as HNSCCs. The FDA has approved the use of the anti-epidermal growth factor receptor (EGFR) monoclonal antibody cetuximab (CTX) as a radiosensitizer for recurrent or metastatic HNSCCs. However, despite most of HNSCC exhibit EGFR overexpression, response rate remains limited and nowadays CTX has been replaced by new recently approved immune checkpoint inhibitors, which also show low efficacy. Thus, it is crucial to investigate compensatory mechanisms and new molecular targets that improve CTX sensitivity, identifying subgroups that may benefit the most.

Material and method

Considering the diversity of HNSCCs and lack of representative models, we created a small set of characterized patient-derived xenografts (PDXs), using fresh samples collected during patients' surgery. PDX models were obtained by implanting tumor fragments into the flanks of immunocompromised mice. Palpable

tumors were partially harvested for HNSCC-PDX biobank creation, partially expanded for drug testing and ex vivo analysis, and partially digested in single cells suspension and enclosed into a 3D matrix for patient-derived organoids (PDOs) generation (namely tumoroids). Ex vivo analysis through Immunohistochemistry, RT-qPCR and Western Blot was performed for PDX characterization.

Result and discussion

To date, we have successfully developed three stable HNSCC PDX models achieving a 30% success rate. Through immunohistochemical staining, we demonstrated that PDX samples recapitulate the morphological and functional traits of the patients' tumors. Additionally, HPV genotyping and E6 viral oncogene transcript analysis showed that one out of three samples was HPV-positive while EGFR protein expression varied across PDX samples thus suggesting a differential response to anti-EGFR blockade.

Conclusion

In conclusion, studying molecular mechanisms using well-characterized PDX and PDO models would have a significant impact on therapeutic advances, especially for HNSCCs, where there is an urgent need to identify new potential biomarkers for earlier diagnosis and develop tailored and more personalized treatments to improve survival.

EACR25-1103

Development of a Tumor-On-Chip Model to Predict Drug Response in Breast Cancer

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Introduction

Based on its highly heterogeneous neoplasm with distinct subtypes, breast cancer necessitates innovative strategies for personalized treatment. Here, we present a novel on-chip approach to evaluate the drug response of human breast tumor samples. Previously, we employed this platform with breast cancer cell lines, demonstrating its ability to assess drug response and invasion towards different homing cells. In this study, we extend this platform's versatility by utilizing core needle biopsy samples to gain deeper understanding of drug response and invasion capacity for primary breast tissues.

Material and method

We obtained biopsy samples from breast tumors categorized as BI-RADS 5 during radiological assessment. We used a custom-made slicer to section the tissue into discs of ~400 um. To assess initial viability of the samples for potential exclusion, we used PrestoBlue viability assay and analysed the mean grey value of fluorescence signal detected at 560/590 nm (excitation/emission). Three tissue slices per trial were incubated at 65 °C for one hour to serve as a negative control

representing dead tissue. To mitigate inherent viability variations arising from biopsy sampling, we established a viability index to ensure consistent results across different patient tissues. This index was calculated as the ratio of the mean grey value of the sample to the mean grey value of the negative controls. Samples falling below a predefined threshold were excluded from subsequent drug response experiments.

Result and discussion

To optimize tissue viability, we evaluated various culture media such as DMEM-F12 supplemented with EGF, hydrocortisone, cholera toxin, ITS (Insulin, transferrin, selenium), 3,3',5 Triiodothyronine and adenine. The optimal media was determined as DMEM-F12 supplemented with EGF, hydrocortisone, cholera toxin, and insulin. Subsequently, we assessed the viability of primary breast tissues in the presence and absence of paclitaxel, at a concentration range of (1.6 uM-14 uM), commonly used in clinical settings. Our results showed a significant decrease in tissue viability after 24h of paclitaxel exposure, while control samples were viable for up to 14 days. To investigate invasion capability, tissues were stained with a cell tracker and then embedded in Matrigel on a tumor-on-chip platform consisting of three channels connected to each other. Few cells were migrated towards the channel containing the chemoattractant (20% FBS).

Conclusion

In conclusion, our study demonstrated the applicability of a less-labour-intensive and cost-effective approach without the need for advanced equipment that can be applied for the advancement of precision oncology for breast cancer patients. Additionally, our model allows real-time monitoring of cellular dynamics such as drug responses, providing valuable insights into tumor behavior and potential therapeutic strategies.

EACR25-1106

Peritoneum specific fibroblasts that can arise in response to various local disorders promote colorectal cancer peritoneal metastasis

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Introduction

Peritoneal membrane injury induces the activation of local fibroblasts and tissue remodelling, which ultimately can progress to fibrosis. Metastasis of colorectal cancer (CRC) to the abdominal cavity results in such peritoneal damage. Patients with colorectal cancer peritoneal metastasis (CPM) have a particularly poor prognosis, and CPM tumours are characterised by a high infiltration of

fibroblasts. Here, we characterised the molecular and functional features of these fibroblasts, and investigated their interaction with other cells in the peritoneal microenvironment.

Material and method

Primary fibroblasts were isolated from 89 patients with different malignant and benign disorders of the peritoneum. We performed comprehensive analyses of single cell and transcriptome profiling, secretome characterization, and functional enzymatic activity.

Result and discussion

We were able to identify a peritoneum specific fibroblast population that increases in response to different types of damage inducing peritoneal pathologies, including metastasis. These fibroblasts are characterised by the IGFBP2 dependent expression of CD38, which mediates extracellular non canonical adenosinergic activity and contributes to the suppression of macrophages and T cells. Importantly, peritoneal fibroblasts promoted the growth and invasiveness of tumour cells in a xenograft mouse model of peritoneal metastasis, highlighting their pro tumorigenic role. Their specific gene signature was associated with poor prognosis in a dataset of 51 patients suffering from colorectal peritoneal metastasis. This study revealed that the CPM is infiltrated by a peritoneal fibroblast subtype, which is absent in healthy tissue, but also observed in benign peritoneal diseases.

Conclusion

Given the limited therapeutic options for these patients, these pro tumorigenic peritoneal fibroblasts could represent an attractive target for inhibiting the peritoneal spread of tumour cells.

EACR25-1134

N106S mutation in eIF6 may prevent tumour onset caused by mRNA translation defects in Shwachman-Diamond Syndrome

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Introduction

mRNA translation defects are at the basis of many cancer types. In particular in Shwachman-Diamond Syndrome (SDS) hematopoietic stem cells frequently inactivate p53 to overcome ribosomal stress. Mutations in the SBDS gene are identified in most patients with SDS. The SBDS protein is involved in ribosome biogenesis, and its deficiency leads to impaired ribosomal function that is thought to contribute to bone marrow failure and the predisposition to hematologic malignancies observed in SDS patients. eIF6 is a ribosome anti-association factor that prevents premature joining of the 40S and 60S ribosomal subunits. Overexpression of eIF6 has been linked to increased aggressiveness in certain cancers. However, in the context of SDS, somatic mutations in eIF6, particularly the N106S mutation, have been observed associated with a protective effect against leukemogenesis in SDS patients.

Material and method

HEK293T has been used to install the N106S mutation in eIF6 gene by means of Prime Editing. Cell growth has

been evaluated by metabolism assays and toxicity has been measured by MTT. At molecular level, specific gene patterns have been investigated using both bulk RNAseq and RiboSeq to have a comprehensive view of gene expression

Result and discussion

HEK293T cells were successfully edited to carry the N106S eIF6 mutation in heterozygosis, mirroring the condition observed in SDS patients. The homozygous mutation is not viable, indicating a loss of function that negatively regulates the cell cycle. When SBDS is depleted, wild-type cells – which do not undergo transformation – experience a 20% reduction in protein expression. In contrast, eIF6-mutated cells can tolerate up to an 80% reduction in SBDS levels without undergoing malignant transformation. Puromycin incorporation was used as a proxy for protein synthesis rate. eIF6-mutated cells exhibited a dramatic and significant impairment in protein synthesis. Polysome profiling revealed an accumulation of the 80S ribosomal subunit in eIF6-mutated cells, suggesting a perturbation in mRNA translation that becomes more pronounced when SBDS is reduced.

Conclusion

While SBDS mutations are strongly linked to leukemia risk, somatic mutations in eIF6, such as N106S, may serve as a protective adaptation by restoring translational balance. Understanding this interplay could provide insights into the molecular mechanisms of leukemogenesis in SDS. To further explore the role of eIF6 N106S in SDS and its potential impact on tumor biology, RNA-seq and Ribo-seq data are being analyzed to identify translational programs affected by SBDS loss and determine whether the eIF6 mutation modulates ribosome function to counteract vulnerabilities relevant to malignancy. Targeting eIF6 modulation could represent a potential therapeutic strategy.

EACR25-1139

Chromosomal instability as a driver of cGAS-STING dysfunction

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Introduction

Chromosomal instability (CIN) is defined as the continual gain or loss of chromosome fragments or whole chromosomes and is a feature of high grade serous ovarian cancer (HGSOC). CIN is associated with various aspects of tumour biology, but its the impact of CIN on the tumour immune microenvironment (TME) is less well-defined. One consequence of ongoing CIN is the accumulation of cytoplasmic self-DNA which promotes the activation of innate DNA sensing pathways such as cGAS-STING. Acute cGAS-STING activation is reported to promote CD8+ T cell recruitment and synergize with immunotherapy. Yet, the consequences of chronic cGAS-STING activation, such as in CIN-high tumors, remain undefined. Therefore, we aim to explore the differences in cGAS-STING signaling in CIN-low and CIN-high models, and the implications of such on the wider TME using murine models of HGSOC.

Material and method

Molecular biology techniques including microscopy, RT-qPCR, western blotting, and flow cytometry were utilized to evaluate CIN, and cGAS-STING functionality in human and murine HGSOC-representative cell lines. Bulk RNA sequencing will be used to evaluate differences in response to STING agonists in CIN-low and CIN-high cell lines, and to determine differences in the immune microenvironment of tumors with varying CIN rates.

Result and discussion

CIN-high HSGOC-representative murine cells demonstrated reduced basal STING expression in comparison to matched CIN-low cells. As a consequence, CIN-high cells fail to induce PD-L1 and key cytokines that promote T cell activation and recruitment in response to STING agonism. We mimicked these changes to cGAS-STING signaling in the CIN-low cell line models through repetitive STING agonism using cGAMP. Our current work aims to determine if the changes to cGAS-STING signaling in CIN-high models are reversible. Preliminary data suggests that STING downregulation is maintained when the pressure of CIN is removed, and cells remain unresponsive to STING agonism with regard to PD-L1 and cytokine induction. We are currently investigating if differential responses to STING agonism occur in CIN-low and CIN-high settings using bulk RNA sequencing, with a particular focus on if a differential cytokine profile occurs in CIN-high cells upon STING agonism. In addition, we are evaluating the immune microenvironment in CIN-low vs. CIN-high tumor models both pre- and post-chemotherapy.

Conclusion

cGAS-STING dysfunction is apparent in CIN-high mouse and human cell line models of HGSOC. This may be an irreversible phenotype and can be mimicked by repetitive STING agonism. Current work aims to determine if cGAS-STING downregulation in a CIN-high context results in differential cytokine release upon STING agonism, and how the TME differs in CIN-low and CIN-high *in vivo* models. This work begins to provide insight into how CIN impacts immune-related pathways and may impact the wider TME.

EACR25-1155

Targeting RBP-guided Immune Evasion Enhances Immune Checkpoint Therapy in High-grade Serous Ovarian Cancer

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Introduction

High-grade serous ovarian cancer (HGSC) accounts for the majority (>70%) of ovarian cancer-associated deaths, with only minor therapeutic advancements achieved in recent years. Among the four proposed molecular subtypes of HGSC, the C5 subtype is characterized by high proliferative potential and pronounced immune evasion. However, the molecular drivers underlying the C5 subtype remain unclear.

Material and method

Using single-cell and bulk RNA sequencing, we identified RNA-binding proteins (RBPs) as crucial players of the immune desert phenotype in C5-HGSC tumors. Their role in modulating the immune response was evaluated through an siRNA screen in C5-like cell lines. The molecular mechanisms by which the top candidate RBP, IGF2BP1, governs immune evasion were investigated through RNA sequencing, binding and turnover assays, co-culture experiments, and syngeneic mouse models. Spatial biology by multi-spectral imaging was employed to validate regulatory networks in human HGSC tissue. The small molecule inhibitor BTYNB, alone or in combination with PD-1-targeting antibodies, was assessed *in vitro* and *in vivo* to explore its therapeutic potential.

Result and discussion

We identified eight oncofetal RBPs as molecular drivers of C5-HGSC tumors, contributing to the immune desert phenotype and facilitating immune evasion.

Mechanistically, the lead candidate RBP, IGF2BP1, represses intra-tumoral interferon-gamma signaling by promoting IRF1 protein degradation to diminish MHC-I presentation. Concomitantly, IGF2BP1 uncouples PD-L1 synthesis from IRF1-dependent transcription. This reshapes the intra-tumoral immune receptor landscape to limit immune cell infiltration and activation, while promoting the escape for T cell mediated tumor cell killing. BTYNB-mediated inhibition of IGF2BP1 synergized with immune checkpoint inhibition (ICI) by Nivolumab to counteract IGF2BP1-driven immune evasion.

Conclusion

The inhibition of oncofetal RBPs represents a novel therapeutic strategy to enhance the efficacy of established ICI therapies in the treatment of immunologically cold ovarian tumors.

EACR25-1177

Extracellular Vesicle Subtypes in Mediating Ovarian Cancer Metastasis

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Introduction

Ovarian cancer is the most lethal gynecological cancer due to their extensive peritoneal metastasis and ascites formation. Extracellular vesicles (EVs), a class of nanoparticles released by cells to facilitate communication over short and long distances, plays a significant role in promoting metastasis. Tumor-derived EVs, particularly exosomes (50–190 nm) and exomeres (<50 nm) exhibit distinct cargo profiles compared to those released by normal cells. Little is known about exomeres, aside from

different cargo profiles, they are also smaller and possess different structural properties compared with exosomes. Tumor-derived exosomes have demonstrated to promote metastasis and chemoresistance, while the role of tumor-derived exosomes in promoting metastasis remains unclear. The objective was thus to determine the effect of tumor-derived exosomes in metastasis compared with tumor-derived exosomes as well as to identify potential new candidates and targets in ovarian cancer metastasis.

Material and method

Using sequential differential ultracentrifugation and a three-prong approach validation, the exosomes and exosomes were isolated from metastatic ovarian cancer cell lines. Nude mice were implanted with non-metastatic tumors then treated with metastatic tumor-derived exosomes or exosomes to mimic endogenous EV release in patients.

Result and discussion

Mice bearing non-metastatic tumors treated with tumor-derived exosomes showed increased peritoneal metastasis and mesenteric tumors compared to those treated with tumor-derived exosomes. Treatment of tumor-derived exosomes also increased ascites formation compared to treatments of exosomes.

Conclusion

These findings suggest that tumor-derived exosomes are more potent in promoting pro-metastatic effects than tumor-derived exosomes. Further research on tumor-derived exosomes could lead to the identification of novel targets involved in the peritoneal metastasis of ovarian cancers and ascites formation.

This work is supported by RGC GRF17103523

EACR25-1182

Identify the efficacy and mechanism of engineering UMSC/miR-145-5p-TRAIL and its derived exosome on hepatocellular carcinoma

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Introduction

Hepatocellular carcinoma (HCC) is a highly lethal liver cancer with one of the highest mortality rates worldwide. Standard treatments often lead to low survival rates and severe side effects, underscoring the urgent need for innovative therapies and advanced drug delivery systems. This brings us to exosomes, key mediators of cell communication that have recently emerged as promising carriers for cancer therapy due to their stability, low toxicity, and targeted drug delivery capabilities. Building on this concept, we selected miR-145-5p, a tumor suppressor that is downregulated in HCC and plays a crucial role in regulating oncogenic pathways. Restoring miR-145-5p expression has been shown to inhibit tumor progression. Similarly, TRAIL selectively induces apoptosis in tumor cells but faces clinical challenges, including a short half-life and resistance. Encapsulating TRAIL in exosomes enhances its stability and therapeutic

efficacy. This study aims to utilize exosomes derived from engineered UMSCs as a delivery system for HCC-targeted therapeutics.

Material and method

To evaluate therapeutic efficacy of exosomes, we conducted *in vitro* experiments. Colony formation assay was performed to assess HCC cell proliferation, while a Transwell assay was used to analyze migration and invasion capabilities. Additionally, flow cytometry was conducted to examine cell cycle arrest.

Result and discussion

Our initial findings indicate that the model was successfully constructed. Exosomes loaded with miR-145-5p and TRAIL effectively inhibited cell proliferation, as demonstrated by the colony formation assay. Additionally, they induced cell cycle arrest at the G1 phase. Sphere formation was significantly reduced in the exosome-treated group, accompanied by a marked suppression of HCC migration and invasion.

Conclusion

By leveraging the homing properties of UMSCs and their exosomes, we have achieved precise and targeted delivery of miR-145-5p and TRAIL to HCC tumors. Exosomes secreted by engineered UMSCs effectively regulate tumor migration and invasion, reduce cancer stemness, and induce cell cycle arrest at the G1 phase, ultimately inhibiting tumor growth. Thus, combining miR-145-5p and TRAIL-loaded exosomes represents a promising strategy for improving HCC treatment outcomes. We hope that the findings of this study will pave the way for novel therapeutic approaches for HCC patients, offering them renewed hope.

EACR25-1188

The DNA repair factor NEIL-like DNA glycosylase 3 activates tumor-associated macrophage polarization through regulating genomic stability in HCC

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Introduction

The DNA damage response and repair in tumor cells are closely linked with immune responses in the tumor microenvironment (TME). DNA damage stress triggers generation of the damage-associated molecular patterns (DAMPs), which stimulate specific pattern recognition receptor (PRR) complexes, leading to immune cell activation and infiltration. The DAMP signaling pathway has been proposed as a promising target for anti-tumor immunotherapies. We recently found that the DNA repair factor NEIL3 enhances epithelial-mesenchymal transition (EMT) and worse prognosis in HCC.

Material and method

We aim to characterize the role of NEIL3 in HCC TME and to develop novel DNA damage stress-related TME markers that can serve as biomarkers for the HCC immunotherapy responses.

Result and discussion

We found that NEIL3 blocks DNA damage stress-induced DAMPs activation and the associated tumor-associated macrophages (TAMs) phagocytotic activities.

Furthermore, the NEIL3 glycosylase activity was shown to be required for TAMs phagocytic activities, suggesting a direct functional linkage between tumor DNA repair and macrophage activation in TME.

Conclusion

In conclusion, the DNA repair factor NEIL3 in the HCC affects TAMs and to develop potential novel immunotherapy biomarkers in HCC.

EACR25-1191

Dysregulated m5C RNA Methylation of HDGF Promotes Colorectal Cancer

Growth via De Novo Lipogenesis

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Introduction

Colorectal cancer (CRC) is a leading cause of cancer-related mortality worldwide, driven by genetic and epigenetic alterations. Among epigenetic modifications, 5-methylcytosine (m5C) RNA methylation plays a crucial role in RNA stability and gene expression. Hepatoma-derived growth factor (HDGF) has been implicated in cancer progression, yet its RNA methylation status and functional significance in CRC remain unclear. This study investigated the role of m5C methylation change on 3' UTR of HDGF mRNA in CRC development.

Material and method

Human colorectal cancer tissues and matched adjacent normal colonic tissues ($n = 7$ pairs) were obtained from Kyungpook National University Chilgok Hospital. CRC cell lines were cultured under varying fetal bovine serum (FBS) and glucose-insulin conditions to assess the impact of metabolic changes on HDGF m5C RNA methylation and expression. Cells were treated with 5-aza-2'-deoxycytidine (decitabine) to inhibit m5C methylation. Total RNA was extracted and subjected to quantitative bisulfite pyrosequencing to measure HDGF mRNA methylation levels. Gene expression analysis was performed using qRT-PCR, and protein levels were assessed by Western blotting. Statistical significance was determined using Student's t-test.

Result and discussion

HDGF m5C methylation and expression levels were significantly upregulated in CRC tissues compared to adjacent normal colonic tissues. In CRC cell lines, high FBS and glucose-insulin conditions increased HDGF m5C methylation, HDGF protein expression, and cell proliferation, while serum starvation decreased these effects. Notably, insulin-glucose treatment upregulated DNL-related genes (ACACA, ACLY, FASN), alongside increased HDGF m5C methylation. Conversely, Decitabine treatment suppressed HDGF m5C methylation and downregulated DNL-related gene expression. These findings indicate that HDGF m5C methylation supports CRC growth by promoting de novo lipogenesis,

suggesting RNA methylation as a potential therapeutic target.

Conclusion

Our study identifies HDGF m5C RNA methylation as a key regulator of CRC progression via metabolic reprogramming. Targeting RNA methylation may provide novel therapeutic strategies for CRC.

EACR25-1197

ADAM10 promotes pancreatic cancer progression and modulates the immune microenvironment

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Introduction

Pancreatic ductal adenocarcinoma (PDAC) remains one of the deadliest cancers, with a five-year survival rate below 10%. New treatment options are urgently needed to improve patient outcomes. ADAM10, a membrane-bound metalloprotease, is overexpressed in pancreatic tumour cells and contributes to stromal fibrosis, promoting cancer cell invasiveness and metastasis. It reportedly cleaves a range of substrates involved in tumour progression, including notch, growth factor ligands/receptors, adhesion molecules and immune cell regulators (MICA, PD-L1), highlighting its potential as a therapeutic target. We set out to further investigate the role of ADAM10 in pancreatic cancer with the view to developing novel antibody-drug conjugate (ADC)-based treatments targeting ADAM10.

Material and method

We generated ADAM10 knockout (KO) KPC cell lines using CRISPR-Cas9. KPC ADAM10 KO and control cells were analysed in vitro and as subcutaneous tumours in immunocompetent C57BL/6 mice to evaluate tumour growth kinetics. Quantitative mass spectrometry was used to identify differentially shed proteins in conditioned media (the secretome) from WT and KO KPC cells and differentially expressed proteins in tumour samples. Tumour tissues were also analysed by western blot and immunohistochemistry (IHC) to assess expression of key markers.

Result and discussion

ADAM10 KO in KPC cells showed reduced proliferation and impaired wound healing capacity in vitro, and analysis of the secretome identified changes in proteins involved in adhesion, ECM organisation, notch and other transmembrane receptor signalling. ADAM10 KO tumour growth was significantly slower in vivo, and western blot analysis confirmed reduced expression of ADAM10 and notch in KO tumours. Proteomic analysis of tumours also showed changes in ECM organisation and immune related pathways, including upregulation of the Granzymes (cytotoxic T cell proteases), and Fas, a key regulator of apoptosis and downregulation of EPDR1, which is linked to increased PD-L1 expression and tumour immune evasion. Notably, ADAM10 KO tumours showed increased CD8+ T cell infiltration, and

greater response to the immune-checkpoint inhibitor anti-PD1, suggesting enhanced anti-tumour immunity.

Conclusion

Loss of ADAM10 decreases tumour growth and appears to enhance anti-tumour immunity by modulating diverse pathways, including regulating ECM and immune cell activation within the pancreatic cancer micro-environment. These findings highlight ADAM10 as a promising therapeutic target, with potential to improve immunotherapy responses in PDAC. Disclosures PWJ and AMS are inventors on a patent regarding anti-ADAM10 antibody 8c7.

EACR25-1201

A Novel Exosome Inhibitor Limits Tumor Growth in Mice with Melanoma (B16F10) Allograft

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Introduction

The incidence of melanoma continues to escalate, while the overall therapeutic efficacy of current anti-melanoma strategies remain unsatisfactory. Emerging evidence has implicated exosomes in tumor progression and tumoral immune tolerance. Notably, the melanoma-derived exosomes have been found to contribute to immunosuppression and was associated with anti-PD-1 response. Therefore, it is highly desirable to develop druggable exosome inhibitors for melanoma treatment.

Material and method

The cytotoxicity and exosome inhibitory activity of C1 were tested in murine B16F10 melanoma cells in vitro by MTT assay and a commercialized exosome quantification kit, respectively. The anti-tumor effect of C1 was evaluated in a B16F10 melanoma-bearing mouse model in vivo. The tumor-immune phenotypes were verified by flow cytometry. The classic exosome inhibitor GW4869 was employed as a positive control.

Result and discussion

C1 markedly restricted the exosome secretion in B16F10 melanoma cells in vitro which exhibited no cytotoxicity. In mice with melanoma (B16F10) allograft, C1 could significantly restrict tumor growth. Moreover, C1 increased the proportion of tumor-killing immune cells as determined by flow cytometry, aligning with the effects of the exosome biosynthesis inhibitor GW4869. Future studies will concentrate on the key steps of restricted exosome secretion and the specific target of C1. The prospective utilization of C1 will also be investigated in additional cancer types characterized by poor response to existing therapies attributable to exosome-mediated processes.

Conclusion

C1 effectively limits melanoma growth in vivo, which is probably associated with the inhibition of melanoma exosome release and the restoration of anti-tumor immunity.

EACR25-1213

Small nucleolar RNA host gene 10 promotes pancreatic ductal adenocarcinoma via regulating EGFR/AKT/ERK1/2/mTOR cascade

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Introduction

Pancreatic ductal adenocarcinoma (PDAC) the most lethal cancer due to its late diagnosis, high rate of metastasis and drug resistance. Therefore, the discovery of novel molecular targets is an urgent need. The aim of this study is to delineate the potential of SNHG10 in PDAC.

Material and method

Bioinformatic and qRT-PCR analysis was used to determine the expression of SNHG10. Antisense oligonucleotide and siRNA mediated approach were utilized to unravel SNHG10 role using MTT, clonogenic assay, boyden chamber assay, cell cycle and apoptosis assay. Mechanism of SNHG10 was discovered through western blot analysis. NOD-SCID mice model of PDAC validated the in vitro analysis. Immunohistochemical analysis was used to analyze the altered expression of key oncogenic protein. Furthermore, SNHG10 involvement in gemcitabine resistance was evaluated by developing gemcitabine resistance PDAC cell lines.

Result and discussion

Our analysis displayed a significant upregulation of SNHG10 transcript in 179 PDAC cases and in a panel of PDAC cell lines. Upregulation of SNHG10 showed correlation with clinical stages of PDAC. Depletion of SNHG10 transcript level displayed significant reduction in cell proliferation, clonogenic ability, cell migration, EMT while inducing cell cycle arrest and cell death of PDAC cells. Mechanistically, the depletion of SNHG10 inhibited protein expression of cyclinB1, CDK4, cyclin D1, CDK6, N-cadherin, vimentin, survivin, aurora Kinase A & B. The depletion of SNHG10 led to enhanced expression of p21 and E-cadherin in PDAC cells. Moreover, depletion of SNHG10 significantly suppressed the tumor growth in PDAC xenograft model. Silencing of SNHG10 increased the expression of miR-532-3p and miR-150-5p leading to inhibition of EGFR/AKT/ERK/mTOR/MET signaling in both in vitro and xenograft PDAC mouse model. Noticeably, silencing of SNHG10 transcript increases the gemcitabine sensitivity of PDAC cells resistant to gemcitabine.

Conclusion

Our data indicated the oncogenic role of SNHG10 in PDAC through the EGFR/AKT/ERK1/2/mTOR axis as well as involved in gemcitabine resistance.

EACR25-1215

Development of relevant mouse models for the R&D of therapeutics for Pancreatic Ductal AdenoCarcinoma

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Introduction

Pancreatic ductal adenocarcinoma (PDAC) is one of the deadliest cancers worldwide with a 5-year overall survival of 10-13%. It is defined by a dense fibrotic barrier and an immunosuppressive network, both contributing to therapeutic resistance. Identifying relevant PDAC animal models is critical for R&D given the urgent need to develop PDAC therapeutics to improve patients' quality of life. This project aims to characterize and classify PDAC tumor microenvironment (TME) in current available syngeneic PDAC mouse models including subcutaneous and pancreas tail injections. Second, based on these comparative studies, we have proposed and developed innovative PDAC mouse models mirroring the human TME. In the context of our R&D strategy, this project focuses on two main applications: (i) Early-stage project: Subcutaneous model with rapid tumor growth and easy monitoring (ii) Advanced project: Orthotopic model (head versus tail) with a more robust TME

Material and method

Starting from the transgenic KPC gold standard, the above cited models will be grafted with either cell line or tumor fragment. Cartography of the TME in every model will be evaluated over time and in response to several standard of care. This characterization workflow includes flow cytometry for immune-phenotyping, immunohistochemistry to define TME fibrosis, gene expression associated to PDAC subtyping and disease related monitoring i.e metastasis.

Result and discussion

Based on collagen-I and a-SMA expressions along with fibrosis scoring we observed similar levels of fibrosis between the subcutaneous and orthotopic tail models. However, the orthotopic tail model appears to exhibit a more relevant PDAC-mimicking immune infiltration. Indeed, we observed a majority of M2 macrophage and MDSC infiltrated cells in the orthotopic tail model, representative of human PDAC immune infiltration. Moreover, compared to xenografted subcutaneous models, the orthotopic tail model showed a lower expression of CK19+ cells. To further improve PDAC mouse models, we have developed an orthotopic pancreas head injection. As 80% of PDAC patients developed cancer in the head of the pancreas, developing such model represent a true technical challenge but may constitute a real benefit for drug testing in PDAC therapeutics.

Conclusion

Altogether this project will help to build a full mapping of PDAC TME including immunophenotyping, spatial localization, transcriptomic and response to standard of care. In the effort of upgrading current available models, robust PDAC immunocompetent mouse models are key for PDAC therapeutics drug testing. Therefore, reinforcing Servier's ambition to improve survival rate for these patients.

EACR25-1221

Disruption of the Pentraxin 3/CD44 Interaction can be an Efficient Strategy for Malignant Cancer Therapy

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Introduction

The tumor microenvironment (TME) is characterized by persistent low-grade inflammation, resembling a chronic inflammatory state. Within this environment, tumor-associated stromal cells facilitate tumor cell migration, invasion, stemness, drug resistance, and immune evasion by suppressing antitumor immunity and enhancing immunosuppression. Pentraxin 3 (PTX3), a secretory factor induced by pro-inflammatory cytokines and various stressors, is primarily expressed by fibroblasts and monocytes/macrophages and has been observed to be upregulated in the TME.

Material and method

Loss-of-function assays, including knockdown, genetic knockout, and inhibitor-based approaches, were utilized to investigate PTX3's role in the inflammation-like TME and its association with tumorigenesis. In vitro and in vivo experiments involving conditioned medium and co-injection models were conducted to assess PTX3's protumour functions. Protein-protein interaction assays were performed to elucidate the PTX3/CD44 interaction.

Result and discussion

Our findings indicate that PTX3 not only promotes cancer cell migration, invasion, stemness, and drug resistance but also contributes to immunosuppression by activating M2 macrophages and inactivating cytotoxic CD8+ T cells. WHC-001, a PTX3-specific neutralizing antibody, binds to PTX3 and prevents its interaction with CD44. WHC-001 demonstrated a suppressive effect on colon and triple-negative breast cancer (TNBC) tumor progression by reducing tumor-infiltrating M2 macrophages and increasing cytotoxic CD8+ T cell infiltration.

Conclusion

Our results suggest that the PTX3/CD44 interaction promotes tumor malignancy, and WHC-001 represents a potential therapeutic agent for cancer treatment.

EACR25-1226

Characterization of cysteine peptidase interplay between different cell populations in tumour microenvironment

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Introduction

In cancer many cell types interact with each other in the tumour microenvironment (TME). Among other enzymes cysteine peptidases are known to play a role in the development and spread of tumours. The dynamic and reciprocal connection between different cell populations, especially cancer cells and cancer-associated fibroblasts (CAFs), within the TME has a major impact on tumour

growth, metastasis and resistance to therapy. The aim of our study was to find whether co-cultivation of tumour cells and CAFs affects the expression and activity of cysteine peptidases, in particular cathepsins B, X, V and L.

Material and method

First, to evaluate if secreted cysteine peptidases from one cell population can affect the expression in another cell population breast cancer cell lines were cultured for 3 days, then the cell medium was added to cancer-associated fibroblasts (CAFs) and vice versa. Cell lysates were prepared at different time points, namely after 0, 24 and 72 hours. The protein levels and activity of the observed cathepsins were assessed by western blot and enzyme activity assay. Additionally, indirect and direct co-cultures to evaluate crosstalk between cells that are not in direct contact using transwell inserts were prepared and evaluated for cathepsin expression and activity.

Result and discussion

Our results show that in co-cultures and when the one cell population is supplied with medium from another cell population cathepsin protein levels and activity change. Thus, we demonstrate that cysteine cathepsins are involved in communication between cells in the TME.

Conclusion

In conclusion results of this study show that crosstalk between cell populations does affect expression of peptidases in different cell populations in TME and should be investigated and considered in antitumour therapy.

EACR25-1249

The local microenvironment suppresses the synergy between irradiation and anti-PD1 therapy in breast-to-brain metastasis

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Introduction

Metastatic dissemination to the brain occurs in approximately one-third of patients with breast cancer (BC) and can lead to life-threatening neurological damage. Stereotactic radiosurgery (SRS), a standard-of-care treatment for brain metastasis (BrM), controls over 80% of targeted lesions. However, the emergence of new BrM lesions outside the irradiation field and the progression of extracranial disease limit median overall survival to just over one year. Therefore, there is an urgent need to develop more efficient therapies. To achieve this, it is crucial to understand how the local tumor microenvironment (TME) responds to treatment.

Material and method

To study the changes in the BC-BrM TME, we used various *in vivo* studies, transcriptional analyses, and functional assays to directly compare orthotopic BC tumors with their BC-BrM counterparts.

Result and discussion

Here, we show that while CD8+ T cells can infiltrate breast cancer-brain metastases, their anti-tumor cytotoxicity is locally suppressed in the brain. Conversely, CD8+ T cells exhibited tumoricidal activity

in extracranial mammary lesions originating from the same cancer cells. Consequently, combined high-dose irradiation and anti-PD1 therapy was effective only in extracranial tumors, but not intracranial lesions.

Transcriptional analyses and functional studies identified neutrophils and Trem2-expressing macrophages as key sources for local T cell suppression within the brain, providing rational targets for future therapeutic strategies.

Conclusion

In this study, we show that while CD8+ T cells infiltrate BC-BrM, they lack anti-tumor cytotoxicity even under T cell-stimulating conditions. Single-cell profiling and ex-vivo functional assays identified BrM-infiltrating neutrophils and Trem2+ monocyte-derived macrophages and microglia (collectively termed tumor-associated macrophages, TAMs) as key sources of local T cell suppression.

EACR25-1258

The effects of selected piRNAs on ovarian cancer stem-like cells through piRNA mimic transfection

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Introduction

Ovarian cancer (OC) is the most common cancer among women and has the highest mortality rate among gynecological cancers. Cancer stem cells (CSCs), a subpopulation within the tumor mass, play a critical role in metastasis, chemotherapy resistance, and disease recurrence. CSCs possess tumor-initiating potential and multipotent differentiation ability. Piwi-interacting RNAs (piRNAs), a class of non-coding RNAs involved in epigenetic regulation, have been implicated in CSC maintenance and tumor progression. In this study, we investigated the effects of piR-823, piR-36712, and piR-020326 on ovarian CSCs, employing mimic approaches to assess their regulatory roles.

Material and method

To investigate the effects of piR-823, piR-36712, and piR-020326 on CSC characteristics, the OVSAHO cell line was used. The expression levels of these piRNAs, previously identified as upregulated in ovarian CSCs, were modulated through siRNA mimic transfection. The impact on CSC properties was evaluated by comparing gene expression profiles of key stem cell markers, including ALDH1A1, ALDH1A2, ALDH1A3, SOX2, CD133, and NANOG in between 2D adherent and 3D spheroid cultures using qRT-PCR. The expression differences of piRNAs and CSC-specific genes mRNAs compared to control genes were calculated using the $2^{-\Delta\Delta Ct}$ method based on GraphPad Prism Version 9.2.0 (283).

Result and discussion

In the comparison of gene expression levels in 3D and 2D cell culture groups, significant changes were observed following piRNA mimic transfection. Specifically, piRNA-823 mimic transfection led to a significant reduction in the expression of stem cell markers such as ALDH1A3, ALDH1A1, NANOG, and CD133 in the 3D groups ($p < 0.05$), suggesting a suppressive role of piRNA-823 in cancer stem cell gene expression. In the

case of piRNA-36712, a significant increase in CD133 expression was detected in the 3D group, while ALDH1A1 expression showed a notable reduction, with no significant changes in NANOG, ALDH1A2, or ALDH1A3 expression levels. Similarly, piRNA-20326 overexpression in the 3D group resulted in a decrease in ALDH1A1 and ALDH1A3 expression, whereas ALDH1A2 expression significantly increased. These findings highlight the differential regulatory effects of piRNAs on cancer stem cell-associated gene expression in 3D spheroid cultures.

Conclusion

This study demonstrates that piR-823, piR-36712, and piR-20326 regulate ovarian cancer stem cells (CSCs). piR-36712 supports CSC maintenance, while piR-823 influences pluripotency genes and may have an anti-cancer regulatory effect on CSCs. piR-20326 negatively regulates stem cell markers in adherent cultures but has no significant impact in spheroids. These findings suggest that piRNAs play crucial roles in CSC regulation and may serve as potential therapeutic targets in ovarian cancer.

EACR25-1267

The level of genomic instability in progression of malignant brain cancer

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Introduction

Astrocytoma and glioblastoma are diffuse malignant brain cancers, characterized as the most aggressive and fast-growing types of brain tumors. Glioblastoma IDH wild-type is a primary tumor that develops de novo, while a secondary type is Astrocytoma IDH mutant which progresses from lower grades of brain tumors. Genomic instability is a hallmark of cancer characterized by an increased rate of mutations and chromosomal alterations and plays a critical role in cancer initiation and progression.

Material and method

We analyzed genomic instability in 78 patients with malignant brain cancer, using AP-PCR (arbitrarily primed PCR) a DNA profiling method. Comparing DNA profiles between tumor tissue and normal tissue (blood) of the same patient, we detected qualitative and quantitative differences. Quantitative differences are detected as changes in the intensity of bands and represent chromosomal instability (CIN), while qualitative changes are detected as the presence and absence of bands and are the manifestation of microsatellite instability (MIN). Associations between genomic instability, represented as the frequency of DNA alterations, type and grade of brain cancer were

performed using one-way analysis of variance (ANOVA) and Fisher's exact test.

Result and discussion

The overall mean values of the frequency of DNA changes for MIN, CIN and total genomic instability are 0.175, 0.170 and 0.344, respectively. Based on the frequency distribution of DNA changes we determined the threshold value for each type of instability based on which the samples were divided into two groups, with high and low frequency of DNA changes. The percentage of high total genomic instability, high MIN and high CIN is 53%, 47%, 41%, respectively. The contribution of qualitative and quantitative changes to the level of genomic instability was almost equal. A statistically significant correlation was found between the level of genomic instability and the types of brain cancer ($p = 0.017$). Low genomic instability is characteristic of Glioblastoma IDH wild-type found in 61% of patients, while high genomic instability is characteristic of Astrocytoma IDH mutant grades 4, 3 and 2, found in 81%, 72% and 60% of patients, respectively. There is also a statistically significant difference in the level of high genomic instability between Astrocytoma IDH mutant grades ($p = 0.028$). Astrocytoma IDH mutant grade 2 is characterized by a higher level of high genomic instability (0.58), then grade 3 (0.44) and then grade 4 (0.34).

Conclusion

Our results suggest that MIN and CIN contribute equally to total genomic instability. During the progression of Astrocytoma IDH mutant genomic instability decreases, while low genomic instability is characteristic of Glioblastoma IDH wild-type. This all makes genomic instability a good progressive malignant marker.

EACR25-1272

A single-cell atlas of the clear-cell renal cell carcinoma (ccRCC) tumor microenvironment (TME) identifies an interferon-responsive malignant cell subpopulation associated with lymphocyte-enriched TME

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Introduction

Intratumoral heterogeneity in ccRCC has been widely observed (Turajlic et al., 2018), but its ultimate implications for disease evolution and treatment outcome remain poorly understood. Here, we aimed to explore intratumoral heterogeneity at the single-cell level and

sought to identify potential ways by which this heterogeneity can shape the TME.

Material and method

We retrieved nine publicly available ccRCC single-cell RNA sequencing (scRNA-seq) datasets, adding up to 68 treatment-naïve patients across all stages and grades. Standard scRNA-seq analysis with Seurat and integration with Harmony in R were applied. TME clustering was based on cell-type proportional data, and compositional data was analyzed with sccomp. Characterization of clusters was analyzed with Gene Set Enrichment Analysis (GSEA), and cell-to-cell communication was assessed using LIANA in combination with tensor-c2c.

Result and discussion

Clustering of cell-type proportions revealed four major TME configurations across samples: Lymphoid-, Immune-, Endothelial-, and Malignant-enriched. Tumor-infiltrating lymphocytes within Lymphoid- and Immune-enriched TMEs also exhibited distinct states: the former had more effector/proliferating T-cells, reduced exhaustion, and increased neoantigen-reactive TCRs, while the latter was enriched in regulatory/memory T-cells and had increased exhaustion. Malignant cell reanalysis identified 15 transcriptionally distinct subpopulations. GSEA revealed that subcluster (Sc.) 3 – RBP4 was enriched in interferon- γ response, MHC-II antigen presentation, and protein quality control processes (proteasome, unfolded protein response, and protein maturation). This subpopulation was highly represented in the Lymphoid-enriched TME. Pathway and transcription factor inference showed high involvement of the TNF- α and JAK-STAT signaling pathways, alongside high expression of RFX5 and CIITA, key MHC-II transcription regulators. Cell-to-cell communication pattern analysis revealed factors linked to hypoxia, TGF- β , TNF- α , and JAK-STAT pathways, the former two positively and the latter negatively associated with Lymphoid- and Immune-enriched TMEs.

Significant ligand-receptor interactions included the chemokines CXCL9/10/11, all three overexpressed in Sc. 3 – RBP4, interacting with CXCR3.

Conclusion

Our analysis reveals that the interferon-responsive malignant cell subpopulation Sc. 3 – RBP4 is highly enriched in lymphocyte-infiltrated TME configurations and represents a putative key player in antigen presentation and MHC-II processing pathways. These findings provide insights into malignant-immune interactions, highlighting potential targets for improving immunotherapy response in ccRCC.

EACR25-1286

Rab27a deficiency fuels pancreatic tumor growth via a pro-inflammatory, immune-promoting tumor microenvironment

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Introduction

Pancreatic ductal adenocarcinoma (PDAC) is a lethal malignancy with a 5-year survival rate of 12%. Its resistance to immune modulatory treatments stems from an immunosuppressive tumor microenvironment (TME) and a low mutational burden. Rab27a, a key regulator of exosomes secretion, has been implicated in disease progression and therapy resistance. Targeting Rab27a offers a potential strategy to remodel the TME, enhance immunotherapy efficacy, and improve patient outcomes.

Material and method

Pancreatic ductal adenocarcinoma (PDAC) is a lethal malignancy with a 5-year survival rate of 12%. Its resistance to immune modulatory treatments stems from an immunosuppressive tumor microenvironment (TME) and a low mutational burden. Rab27a, a key regulator of exosomes secretion, has been implicated in disease progression and therapy resistance. Targeting Rab27a offers a potential strategy to remodel the TME, enhance immunotherapy efficacy, and improve patient outcomes.

Result and discussion

Rab27a deficiency impairs exosomes-mediated signaling, leading to earlier tumor onset and reduced survival. Loss of TSP1, which is carried in cancer-derived exosomes, triggered recruitment of CD31+ endothelial cells and MRP8+ pro-inflammatory macrophages, which activated cancer-associated fibroblasts (CAFs). CAFs, in turn, released pro-inflammatory cytokines (i.e. IL-6), driving CD4+ T cell polarization toward a Th17 phenotype, which promoted tumor growth. Targeting this inflammatory axis with dexamethasone or depletion of CD4+ T cells or inhibiting IL-17 significantly impaired tumor growth and improved survival.

Conclusion

Rab27a plays a crucial role in PDAC by shaping the TME and modulating immune and inflammatory responses. This subset of PDAC tumors may be particularly responsive to immunomodulatory and anti-inflammatory therapies targeting the Th17-driven protumorigenic axis, offering a promising avenue for improving patient prognosis.

EACR25-1291

Activity of a platinum(IV)-flurbiprofen conjugate free and immobilized into mesoporous nanostructured silica on colon cancer

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Introduction

Prostaglandin E2 (PGE2), a main mediator of inflammation, is a product of cyclooxygenase-2 (COX-2) activation, whose expression is increased in human colorectal cancer. In addition to its role in inflammation, PGE2 also acts as a mitogenic signaling molecule released by dying tumor cells and is thus involved in the repopulation of tumors. To improve the effect of chemotherapy by elimination of the mitogenic signal mediated by PGE2, a drug with cisplatin core – platinum(IV) complex was synthesized bearing the anti-inflammatory drug flurbiprofen. Additionally, the conjugate was immobilized into mesoporous silica nanostructural material SBA-15 in order to enable improved delivery of the drug into tumor tissue. The cytotoxicity was assessed in 2D and 3D culture system, as well in vivo using a syngeneic mouse model of colon cancer.

Material and method

The potential cytotoxicity of free and immobilized platinum(IV)-flurbiprofen conjugate was determined in murine cell lines 4T1, B16, CT26, and MC38 using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide and crystal violet as viability assays. Organoids were established from MC38-induced tumors in C57BL/6 mice which were then isolated, digested and further propagated. A CellTiter-Glo™ luminescent cell viability assay was employed to evaluate the antitumor effect of the drugs on organoids. The in vivo efficacy of the drugs was investigated in tumor-bearing C57BL/6 mice.

Result and discussion

All tumor cell lines showed a dose-dependent decrease in viability after treatment with the drug conjugate and the corresponding SBA-15 formulation. MC38 cells were selected for further studies as they proved to be very sensitive to the applied treatments. Colon cancer organoids, which better simulate the architecture of tumor tissue than 2D cultures, showed a remarkable decrease in viability after 6 days of treatment with both drug formulations, while IC50 values were approximately several times higher than those obtained in 2D cultures. Finally, administration of both the drug conjugate and the corresponding nanostructured material to tumor-bearing C57BL/6 mice in a therapeutic regimen resulted in a statistically significant reduction in tumor volume compared to the control group.

Conclusion

The strong antitumor potential of the platinum(IV)-flurbiprofen conjugate free and immobilized into SBA-15 in a model of mice colon cancer opens up many opportunities for further research.

EACR25-1292

Apoptosis-induced compensatory proliferation: optimization of colorectal cancer mouse model and establishment of mouse-derived organoids

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Introduction

Recent data suggest a paradoxical link between the dying cells in tumor tissue and accelerated tumor growth. Dying tumor cells trigger a "regeneration" process through a signaling pathway known as "Phoenix rising". To delineate the process of therapy-induced repopulation in advanced colorectal cancer, we developed a mouse model that mimics communication between dying and live cells in a tumor microenvironment (TME). Following the 3R principle in animal experiments, we established mouse-derived organoids from these tumors and used transcriptional analysis to confirm whether this complex 3D model could mirror TME and be used in comprehensive drug screening in the future.

Material and method

The appropriate mouse model representing apoptosis-induced compensatory proliferation was developed by subcutaneous inoculation of the mixture of live and dead MC38 cells into C57BL/6 mice. The dead cells were collected after in vitro treatment with 5-fluorouracil. During the optimization, the number of live cells and the ratio between live and dead cells in the mixture were varied and finally determined. Additional groups representing controls received the same number of only live or only dead MC38 cells. Also, the group receiving the optimal number of MC38 cells to form tumors in the classic animal model was used for comparison. After tumor isolation, one part of each tumor was frozen, and the other part was digested and used to establish mouse-derived organoids. Both tumors and organoids derived from the compensatory proliferation model and classic model were further analysed by RNA sequencing.

Result and discussion

The group that received only live MC38 cells had a low incidence rate and slow tumor growth rate. However, when the same number of live cells is injected with dead cells in a 1:50 ratio, the tumors' earlier onset, fast growth, and 100% incidence rate revealed that this type of inoculation dramatically enhanced tumor aggressiveness. The transcriptional analysis of isolated tumors and their matching organoids from two different models of disease showed 80% of gene expression homology, indicating that organoids represent a valid model system for drug screening.

Conclusion

The suitable mouse model reflecting compensatory proliferation in colorectal cancer was established and compared to the typical form of the disease, showing important features that can be used as targets to prevent tumor repopulation and thus amplify the effect of chemotherapy.

EACR25-1295**Targeting ROCK Pathway in Diffuse Midline Glioma: Implications for Cell Motility and Invasion**

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Introduction

Diffuse Midline Glioma H3K27-altered (DMG) is an aggressive Central Nervous System (CNS) tumor affecting children and adolescents, with no cure and a median survival of 9–12 months. DMG typically arises in the midline regions of the brain such as the pons and thalamus, where pontine lesions are inoperable. A key factor contributing to DMG's dismal prognosis is its highly infiltrative nature that leads to tumor cell spreading in the CNS although the mechanisms associated with this disseminative growth remain not yet well understood. Rho-associated protein kinase (ROCK) is involved in various cellular functions, including cell migration and invasion. While the modulation of ROCK activity has been studied in other tumor types, its role in DMG remains underexplored. Given ROCK's pivotal role in regulating cell motility, targeting this pathway may provide a potential therapeutic option for limiting DMG cells spreading. Our study aims to investigate the role of ROCK in DMG cell motility and explore the implication of targeting this signaling pathway.

Material and method

Three primary patient-derived DMG cell lines (QCTB-R059, OPBG-DIPG002 and HSJD-DIPG007) were used. Cells were cultured as neurospheres and the basal expression levels of Rho, Rac and ROCK were analyzed by Western blot. Immunofluorescence was performed to assess the levels of ROCK substrate MYPT1 and its phosphorylated form (P-MYPT1) in 3D migration and invasion assays. ROCK activator, lysophosphatidic acid (LPA), and three inhibitors (HA1077, Y27632, GSK429286) were used to modulate ROCK activity and their effects on cell viability, migration, invasion and MYPT1 expression and phosphorylation were analyzed.

Result and discussion

Variable basal expression levels of Rho, Rac and ROCK were observed among the three DMG cell lines. QCTB-R059 cells exhibited the lowest levels of P-MYPT1 and the highest levels of MYPT1 in 3D invasion, whereas HSJD-DIPG007 cells showed the highest levels of both forms in 3D migration. Treatment with ROCK inhibitors did not affect cell viability but reduced P-MYPT1 levels, although the effect on P-MYPT1 differed between 3D

migration and invasion in the three DMG cell lines.

While a significant increase in cell invasion was observed for QCTB-R059 and HSJD-DIPG007, an increase in cell migration was observed only for OPBG-DIPG002. However, activating ROCK with LPA, induced an increase in P-MYPT1 levels and decreased cell motility for all the cell lines.

Conclusion

This study provides evidence for a role of ROCK in regulating DMG cell motility. Interestingly, our data suggest that activating ROCK pathway is a potential therapeutic approach to limit DMG cell dissemination, with differences based on the cell motility phenotype.

EACR25-1296**Prognostic and Molecular Features Associated with Tumour Border****Properties in Colorectal Cancer**

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Introduction

Tumour border configuration, characterized by either expansile or infiltrative growth, has been found to be an important prognostic feature in colorectal cancer (CRC), but it has not been widely adopted to current clinical guidelines due to the challenges in reproducibility. Here, we introduce a reproducible, quantitative method for evaluating tumour border configuration based on characteristics of the deepest invasion and assess its value in determining patient survival and association with molecular characteristic of CRC.

Material and method

We developed Tumour Invasive Border Index (TIBI), a novel approach for assessing tumour border configuration, using two CRC cohorts ($N = 1011$ and $N = 776$ patients). TIBI was assessed from HE-stained slides within representative circular regions of 12.6 mm^2 at the deepest area of invasion, where amounts of tumour epithelium and stromal constituents were recorded. Associations between TIBI and clinicopathological features, immune cell infiltration, prognosis, and inter-rater agreement were examined. Additionally, molecular features (mutations, gene expression patterns) associated with tumour border configuration were analysed in a 3rd cohort of 630 CRC cases from The Cancer Genome Atlas (TCGA).

Result and discussion

TIBI proved to be a reproducible method with an inter-rater agreement comparable to or higher than that of tumour budding and Jass classification (mean kappa 0.69). High TIBI, indicative of an infiltrative growth pattern, was associated with advanced stage, lympho-vascular invasion, mismatch repair proficiency, and wild-type BRAF (all $p < 0.001$, except for BRAF in cohort 2

$p=0.007$). High TIBI was also linked to TP53 and KRAS mutations, expression of EMT-associated genes such as L1CAM, and downregulation of MYC signalling.

Additionally, high TIBI was associated with tumour immune cell composition, including lower densities of M1-like macrophages and CD66⁺ granulocytes, but higher densities of mast cells (all $p<0.001$), suggesting that myeloid cells may influence tumour growth pattern. High TIBI was independently associated with higher CRC-specific mortality when adjusted to disease stage, MMR status, lymphovascular invasion, tumor budding, and other conventional prognostic features, with multi-variable hazard ratios of 1.54 (95% CI 1.07–2.21) in cohort 1 and 2.10 (95% CI 1.17–3.75) in cohort 2.

Conclusion

TIBI proved reproducible method for assessing tumour border configuration and demonstrated its independent prognostic value in CRC. Our findings support its clinical integration as a prognostic marker. By analysing two large cohorts and the TCGA cohort, our study also provides deeper insights into the molecular mechanisms underlying infiltrative growth pattern in CRC.

EACR25-1315

Organotypic slice cultures: An advanced preclinical platform for testing pancreatic cancer therapies

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Introduction

Pancreatic ductal adenocarcinoma (PDAC) is a highly aggressive malignancy with a five-year survival rate of only 3% in advanced-stage patients. Its dense tumour microenvironment (TME), composed of stromal and immune cells, contributes to therapeutic resistance. Existing preclinical models, including 2D cultures, organoids, and patient-derived xenografts (PDX), fail to fully recapitulate the native TME. Organotypic slice cultures (OTSCs) preserve tissue architecture and cellular heterogeneity, making them a promising model for precision therapy testing. Here, we established OTSCs from KPC cell line-derived tumour xenografts and patient-derived tumours to assess viability, optimise culture conditions, and evaluate their suitability for therapeutic screening.

Material and method

OTSCs were generated from KPC cell line-derived tumour xenografts, five CryoStor-preserved patient tumour samples, and one freshly resected patient tumour. C57BL/6 mice were orthotopically implanted with 100,000 KPC cells into the pancreas to establish a tumour

model that mimics the pancreatic TME, including stromal components and immune cell infiltration, characteristic of human PDAC. Tumour specimens were excised and embedded in 8% agarose. Sectioning was performed using a Compressionsome® VF-510-0Z. OTSCs were cultured in RPMI 1640 medium supplemented with 10% FBS, 8 ng/mL EGF, 4 µg/mL insulin, 0.3 µg/mL hydrocortisone, and 1% penicillin/streptomycin. Cultures were incubated at 37°C for 3, 5, or 6 days. After cultivation, OTSCs were fixed in formalin and processed for histological and immunohistochemical (IHC) analyses. Hematoxylin and eosin (H&E) staining was performed to assess tissue integrity, and IHC was conducted using antibodies against cleaved-caspase-3 (CC3), Ki-67, granzyme B, CD4, and CD8 to evaluate apoptosis, proliferation, and immune cell infiltration.

Result and discussion

All OTSCs remained viable and structurally intact throughout cultivation. H&E staining confirmed tissue integrity, and CC3 staining determined minimal apoptosis. Ki-67 staining indicated active proliferation, while immune markers revealed immune cell presence within the slices. CryoStor-preserved OTSCs were successfully maintained for up to six days, supporting their feasibility for extended cultures.

Conclusion

OTSCs provide a robust ex vivo model that preserves tumour cytoarchitecture, cellular viability, and immune interactions. Their potential for studying PDAC biology and testing precision therapies makes them a valuable tool in preclinical research. Further studies should explore their use in therapeutic screening and mechanistic investigations of tumour-stroma interactions. This work was financially supported by the projects APPV-21-0197, APPV-20-0143, TRANSCAN2023-1858-117, COST Action grants CA21116, CA21135, E-COST-GRANT-CA21135-59b2e900, and projects No. 0901-03-V04-00073, APD0045 and APP0602.

EACR25-1341

Alveolar macrophages control fibroblast into CAF fate in Non-Small Cell Lung Cancer (NSCLC)

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Introduction

Cancer-associated fibroblasts (CAF) phenotype is associated with poor prognosis in the vast majority of solid tumors, as they contribute to hallmarks of cancer progression, such as inflammation, metastasis, angiogenesis, and critically, immune evasion. Yet, the fundamental cues that control fibroblast into CAF malignant state remain unclear. In the lab, we showed that macrophages from embryonic origin (Alveolar Macrophages or AMs) closely interact with fibroblasts

present in the tissue before the onset of cancer. Interestingly, longitudinal confocal imaging, flow cytometry of lung tumor stroma and macrophage-depleted models, established that AMs play an important role in the malignant conversion from naive fibroblast into CAF in NSCLC.

Material and method

Single-cell RNA sequencing (scRNA-seq) was performed on both CD45+ and CD45- compartments of lung tissue to investigate stromal populations targeted by AMs. A new mouse model, Dpt-CreERT2-LSL-TdTOMO crossed with CD169DTR/+ mouse (AMs-depleted upon Diphtheria toxin administration), was generated to track universal fibroblast to CAF conversion in tumors with/without AMs (CD169+). Flow cytometry and confocal imaging were used to analyze this conversion. CellChat Ligand-receptor analysis was conducted to identify potential AM-secreted factors regulating CAF fate.

Result and discussion

We found that AM-depleted lungs showed an expansion of universal fibroblasts (Pdgfra+Pdpn+Dpt+) but completely lacked CAF-activated phenotypes. Importantly, AM-depleted tumors confirmed significant downregulation of canonical CAF activation genes, as Acta2, Tgfb1, or Fn1 compared with AM-sufficient tumors. Noteworthy, CAF activation profile remained unchanged in mice missing Monocyte-derived macrophages Mo-Macs (CCR2KO/KO), suggesting AM-specificity in the universal to CAF fate conversion. Using flow cytometry and confocal imaging on the fibroblasts tracking model Dpt-CreERT2-LSL-TdTOMO crossed with CD169DTR/+ mouse, we showed that universal fibroblast contributes to CAFs and more importantly, that universal fibroblasts into-CAF conversion is unequivocally controlled by AMs. CellChat Ligand-receptor analysis based on our scRNA-seq dataset identified AM-derived fibronectin (Fn1) and efferocytic Pros1 as macrophage factors interacting with CD44, integrins and AXL in fibroblasts. Finally, as AM-depleted tumors are significantly infiltrated with CD4 and CD8 T cells resulting in a longer survival, we plan to interrogate the impact of early AXL inhibition (Bemcentinib) in KP-tumors to boost T cell surveillance in otherwise T cell desert tumors.

Conclusion

Altogether, our work highlights a novel regulatory axis between AM and CAF that can be therapeutically exploited to promote immune T cell surveillance in NSCLC. We identified AMs as drivers of pathological responses in CAFs via AXL activation.

EACR25-1370

The role of periostin in the adhesion of pancreatic cancer cells

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Introduction:

Pancreatic tumours have a prominent stroma that can

promote tumour progression through various mechanisms including modulating cell adhesion. In addition to anchoring cells to the environment and mediating migration, adhesion facilitates ECM-cell signalling through activation of adhesion molecules such as integrins. Periostin is a matricellular protein over-expressed in the stroma of 90% of pancreatic tumours and high expression correlates with poor patient survival. While periostin is often described as an adhesion molecule, evidence supporting its role in modifying cell adhesion is limited and the role of periostin in pancreatic cancer cell adhesion has yet to be elucidated. This study aims to clarify the role of periostin in pancreatic cancer cell adhesion.

Material and method

Pancreatic cell lines were adhered to a collagen I-coated plate with periostin added either to the coating or in solution during recovery from trypsin. Crystal violet staining was used to quantify cell adhesion after 60 minutes and staining with anti-vinculin and phalloidin was used to image focal adhesions at 24 hours.

Result and discussion

The adhesion of AsPC-1 cell to collagen was increased by exposure to periostin. Although further study is needed, this could suggest that periostin has a role in adhesion receptor trafficking in AsPC-1 cells. However, adhesion of MiaPacA and PANC-1 cells was not affected by the presence of periostin during trypsin recovery. Instead, these cell lines responded to the presence of periostin in the matrix coating, with periostin-coating increasing the adhesion of MiaPacA cells and decreasing the adhesion of PANC1 cells. AsPC-1 cell adhesion was unaffected by the presence of periostin in the matrix coating. Together, this suggests that different pancreatic cancer cell lines have varying capacities to respond to periostin in specific contexts. Interestingly, the adhesion of Capan-1 cells, which are relatively well differentiated, as well as the adhesion of normal pancreatic epithelial Hpde6C7 and PS-1 stromal cells, was unaffected by periostin. Responsiveness to periostin may therefore be associated with an aggressive cancer phenotype. Low numbers of focal adhesions were observed in pancreatic cancer cells and neither focal adhesion number nor area correlated with changes in cell adhesion. This discordance may indicate that alternative types of adhesion such as hemidesmosomes are important for pancreatic cancer cell adhesion.

Conclusion

Overall, this study shows that the role of periostin in pancreatic cancer cell adhesion is context-dependent and varies between cells. Further investigation of the mechanisms by which periostin affects adhesion may reveal novel targets for the therapeutic modulation of adhesion and ECM-cell signalling in pancreatic cancer.

EACR25-1372

Perilipin 2 in Adipocytes of Omental Tumor Microenvironment Promotes Ovarian Cancer Cell Survival and Metastasis Through CXCL12/CXCR4 Axis

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Introduction

High-grade serous ovarian cancer (HGSOC) is the most aggressive subtype of ovarian cancer and exhibits a strong tendency for omental metastasis. Upon interacting with ovarian cancer cells, adipocytes undergo phenotypic changes, acquiring invasive and pro-metastatic characteristics. This highlights the crucial role of adipocytes in driving ovarian cancer metastasis. In this study, we aim to elucidate the crosstalk between ovarian cancer cells and adipocytes within the omental tumor micro-environment and explore an adipocyte-targeted therapeutic strategy.

Material and method

Metastatic lesions were collected from HGSOC patients, followed by paraffin embedding and laser capture microdissection to separate adjacent adipocytes (cancer-associated adipocytes, CAAs) and distant adipocytes (at least 2 mm away from cancer cells and fibrosis areas). Spatial proteomic sequencing was performed to analyze adipocytes protein profiles. For adipocyte differentiation, fully grown 3T3/L1 pre-adipocytes were treated with a differentiation cocktail for six days. Stable Plin2 knockdown (KD) and overexpression (OE) cell lines were generated by infecting 3T3-L1 with lentivirus carrying mouse Plin2 shRNA or Plin2 cDNA, followed by puromycin selection for one week.

Result and discussion

Spatial proteomic profiling revealed a higher perilipin 2 (PLIN2) expression in adjacent adipocytes than distant adipocytes. In co-culture model, Plin2-OE or KD adipocytes could enhance or repress ovarian cancer cells migration and invasion, respectively, compared to controls. Similarly, in chemoinvasion assay, Plin2-OE adipocytes displayed increased chemotactic activity toward ovarian cancer cells, while Plin2-KD adipocytes exhibited reduced chemotaxis. Conditioned media from Plin2-OE adipocytes increased proliferation of ovarian cancer cells, while Plin2-KD attenuated these effects. Mechanistically, Plin2-KD adipocytes exhibited reduced CXCL12 protein secretion in conditioned media, leading to decreased CXCR4 activation in co-cultured ovarian cancer cells. Transcriptomic analysis revealed enhanced oxidative phosphorylation activity in Plin2-deficient adipocytes, suggesting potential metabolic reprogramming.

Conclusion

Our findings indicate that elevated PLIN2 levels in adipocytes promote ovarian cancer cell proliferation, migration, and invasion, highlighting the suppression of PLIN2 in adipocytes as a promising therapeutic target for HGSOC omental metastasis.

EACR25-1379

Unleashing the Power of miR-221 Inhibition perspectives for treatment of melanoma

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Introduction

Malignant Melanoma (MM), an aggressive form of skin cancer, is highly curable in its early stages but still carries a poor prognosis once metastasis occurs. A deeper understanding of the molecular drivers of melanocyte transformation is essential for identifying novel therapeutic targets and predictive biomarkers.

MicroRNAs play a crucial role in tumor progression. Specifically, miR-221 promotes cell proliferation and inhibits apoptosis, and is therefore a compelling target in cancer. Notably, the clinical translation of LNA-i-miR-221, the first microRNA inhibitor to enter a first-in-human study in solid cancer patients, has paved the way for exploring its therapeutic potential in MM.

Material and method

miR-221 expression was quantified via qRT-PCR in a panel of primary and metastatic MM cells, with stromal cells serving as controls. MM cell lines were transfected with 100 nM of the LNA-i-miR-221 or a scrambled control for 72 hours. Cell proliferation was evaluated using the CellTiter-Glo assay, while cytotoxicity was determined by flow cytometry using 7-Amino-Actinomycin D staining. Protein levels of miR-221 canonical targets were assessed by Western blot, and transcriptomic changes were analyzed through microarray profiling.

Result and discussion

MM cells exhibited significantly elevated levels of miR-221 compared to stromal cells. Treatment with LNA-i-miR-221 resulted in a pronounced reduction of cell proliferation and a concomitant increase of cytotoxicity across all cell lines. These effects correlated with a marked decrease in miR-221 expression and restoration of key regulatory miR-221 targeted proteins involved in cell cycle control and apoptosis. Comprehensive gene expression and microRNA profiling further revealed that miR-221 inhibition down-regulated multiple oncogenic pathways associated with MM progression.

Conclusion

Our preclinical findings demonstrate that targeting miR-221 with LNA-i-miR-221 significantly impairs MM cell growth and viability by modulating critical molecular pathways. These promising results underscore the potential of miR-221 inhibition as a transformative miRNA-based therapeutic strategy for MM, further bolstered by its successful translation into a first-in-human study.

EACR25-1394

Analysis of immunoglobulin producing tumour cells in colorectal cancer utilising

highplex immunohistochemistry techniques

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Introduction

Although an integral part of our immune system, in many epithelial cancers an increase in immunoglobulins is linked to worse prognosis. Recent work has shown that in a variety of epithelial cancers tumour cells can produce immunoglobulin G, termed cancer-derived immunoglobulin (cIgG). cIgG's possess a unique sialylation, detected by the RP215 antibody, which allow cIgG to be distinguished from healthy immunoglobulins. RP215 has never been investigated through multiplex immunohistochemistry, which prompted us to develop a highplex panel to analyse RP215+ cells and their environment.

Material and method

Utilising the PhenoCycler-Fusion system, a tissue microarray featuring 43 malignant and 37 healthy cores was stained with 52 oligonucleotide-conjugated antibodies. This panel was designed to identify the lineage, behaviour and structural context of cells.

Following antibody incubation, complementary oligonucleotide-reporters were cyclically added, imaged and removed. Image preprocessing was performed using a custom pipeline and the resulting data analysed through a purpose-built package developed in-house.

Result and discussion

RP215+ cells often formed small epithelial clusters at the invasive front and were more likely to exhibit tumour budding (≤ 4 epithelial cells per bud) than RP215- cells (6.8 vs 3.2%). They showed lower proliferation (12 vs 25.4% Ki67+) but were also less likely to be dormant (2.1 vs 9.4% p27+), suggesting that while most cells are not proliferating, they are still active. This aligns with previous work showing that migratory tumour buds have a lower proliferative index. Significant changes were observed in the immune milieu of RP215+ cells.

Examining the 50 nearest immune cells, the abundance of regulatory T cells rose from 9.5 to 23.7% around RP215+ cells. In contrast, CD8+ T cells decreased from 31.9 to 11.8%. Notably, PD1 expression was higher in CD8+ T cells near RP215+ cells (48.2 vs 23.1%), indicating a more exhausted state and that PD1 inhibitors may be of benefit in these patients. 36.3% of the immune cells surrounding RP215+ cells were macrophages which often express immunoinhibitory SIGLEC receptors that are capable of binding cIgG, fostering a tolerogenic environment. RP215+ cells were often embedded in dense networks of collagen I, collagen VI and fibronectin. Similarly, the number of fibroblasts and myofibroblasts around these cells increased (1 to 5.2% and 1.2 to 9.6% respectively).

Conclusion

Our findings show that RP215+ cells frequently exist as small clusters and tumour buds within an immunosuppressive niche, enriched with regulatory T cells and depleted of functional cytotoxic T cells. Their association with matrix proteins and fibroblast populations suggest a fibrotic environment. We believe that treatments

targeting the sialic-acid-SIGLEC axis in combination with immune checkpoint inhibitors could potentially be a viable treatment option for patients who express RP215 positivity.

EACR25-1399

Translational 3D human cell models to decipher invasion and immunosuppression in the high-grade glioma tumor microenvironment

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Introduction

High-grade gliomas (HGGs) are central nervous system (CNS) tumors characterized by an immunosuppressive tumor microenvironment (TME) that drives invasion and therapy resistance. Crosstalk between CNS-resident cells (e.g., astrocytes, microglia) and HGG cells fosters extracellular matrix (ECM) remodeling and tumor progression. Understanding HGG immunosuppression and invasion mechanisms is critical for improving therapeutic strategies. However, interpatient heterogeneity in invasion and immune modulation remains poorly captured by existing models. This work aims to develop a human cell model that recapitulates key cellular and molecular interactions within HGG TMEs.

Material and method

We employed a CNS-HGG 3D co-culture approach using stirred-tank culture systems. As the CNS component, we resourced to neurospheres (iNSoids) derived from human induced pluripotent stem cells, composed of functional neurons, astrocytes, and oligodendrocytes within their native ECM. As HGG component, we employed enhanced green fluorescent protein (eGFP)-expressing adult and pediatric HGG cell lines (A172, JX6, SF7761).

Result and discussion

Co-cultured CNS and HGG cells remained viable and retained their identity at the phenotypic and gene expression levels. Confocal microscopy imaging revealed that eGFP+ HGG cells infiltrated iNSoids with distinct, cell line-dependent invasion dynamics. Invasion depths exceeded 30 μm , with HGG cells exhibiting diverse morphological adaptations, ranging from rounded amoeboid-like migration to elongated mesenchymal-like processes. Gene expression analysis indicated that each cell line differentially regulated ECM remodeling, with upregulation of matrix metalloproteinases (e.g., MMP2, MMP14) and hyaluronan-associated genes (e.g., HYAL2, HAS2), suggesting distinct invasion mechanisms.

Beyond invasion, HGG cells also influenced the surrounding immune microenvironment. Upregulation of key immunosuppression-associated genes (e.g., TGFB1, CSF-1) suggests the establishment of an anti-inflammatory TME.

Conclusion

Ongoing work is focused on the incorporation of microglia and patient-derived primary HGG cells to

dissect onco-neuroimmune interactions at the single-cell level, increase the clinical translatability of the model, and depict HGG patient cell response to invasion and immunosuppression modulators. Ultimately, our model will provide a platform for preclinical testing that captures the heterogeneity of HGG behavior and better predicts individualized clinical response.

We acknowledge funding from FCT/MCTES (PT): LA/P/0087/2020, UIDB/04462/2020 & UIDP/04462/2020, 2022.0217.PTDC, 2024.02085.BD to I.S.; UI/BD/151253/2021 to C.G., iBETXplore (PI-752).

EACR25-1402

Reactivation of miR-29 Mitigates

Epigenetic Deregulation and Tumor Progression in Advanced Prostate Cancer

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Introduction

Prostate cancer (PCa) is the leading cause of cancer-related mortality among men, largely due to acquired resistance to standard therapies. Comprehensive transcriptional analyses across thousands of PCa specimens revealed a marked upregulation of critical DNA methylation regulators, including DNA methyltransferases and demethylases, particularly during progression to androgen independence. Importantly, these enzymes were identified as direct targets of miR-29a/b/c-3p, whose expression is notably suppressed during PCa progression. We hypothesized that restoring miR-29 expression could reverse aberrant epigenetic regulation, potentially reactivating epigenetically-silenced tumor-suppressive pathways.

Material and method

To test this hypothesis, androgen-independent prostate cancer cell lines (PC3, DU145, LNCaP-abl, 22Rv1) were treated with miR-29 mimics for isoforms a, b, and c. Cells were collected at multiple time points (48–192 hours), followed by transcriptomic analyses, real-time PCR, Annexin V-PI apoptosis and migration assays to evaluate gene expression changes and functional outcomes.

Result and discussion

Transcriptomic analysis following miR-29 mimic in PC3 cells revealed substantial gene expression changes, notably increased pro-apoptotic mediators and decreased expression of genes involved in cell invasion and extracellular matrix remodeling. Functional assays confirmed significant miR-29-induced apoptosis and inhibition of migration capacities. Furthermore, a single miR-29 mimic treatment induced sustained cell-cycle arrest in PC3 cells for up to 10 days. Our expanded analyses demonstrated that miR-29 mimic treatment preferentially induced significant growth inhibition in TP53-deficient prostate cancer cell lines (PC3, DU145), whereas TP53

wild-type cells (LNCaP-abl, 22Rv1) remained largely unaffected. This selective sensitivity highlights a novel context-dependent mechanism, whereby miR-29 restoration compensates for TP53 loss by directly reactivating downstream p53-related apoptotic pathways. Notably, miR-29 itself is a TP53-induced transcript, suggesting that its reactivation may bypass the loss of functional TP53.

Conclusion

Collectively, these findings position miR-29 reactivation as a potential therapeutic strategy to reverse epigenetic dysregulation and overcome treatment resistance in advanced prostate cancer.

EACR25-1410

Development of novel RNA aptamers for precision targeting of cancer-associated fibroblasts in NSCLC

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Introduction

Lung cancer represents one of the most diagnosed and lethal tumours worldwide, with non-small-cell lung cancer (NSCLC) subtypes accounting for the majority of diagnosed cases. NSCLC management is complicated by frequent relapses and the development of resistance to treatment protocols. Over the past few years, a growing body of literature is highlighting how the tumour microenvironment (TME) can influence different mechanisms involved in cancer establishment and evolution, such as proliferation, metastasis formation and therapy resistance acquisition. Cancer-associated fibroblasts (CAFs) represent the most prominent cell population within the TME, being involved in key mechanisms, sustaining the pro-inflammatory milieu. Thus, developing novel compounds targeting NSCLC-CAFs could impair their activity, representing a viable strategy to overcome the current NSCLC-management limitations. Nucleic acid aptamers could represent effective tools for a precision medicine approach. The plasticity of their selection procedure allows for the identification of aptamers which can recognize and bind with high stability a complexity of different targets.

Material and method

In order to identify a novel RNA aptamer able to recognize NSCLC-CAFs phenotype, we performed an internalizing cell-Systematic Evolution of Ligands by EXponential enrichment (SELEX) procedure on functionally characterized NSCLC patient-derived CAFs.

Result and discussion

We identified different sequences which can effectively distinguish CAFs from normal fibroblasts. The most potential aptamer (Apt1) has undergone length optimization and deeper characterization to assess its binding and functional activities *in vitro*.

Conclusion

Our results suggest that Apt1 could represent a potential novel strategy for the therapeutic targeting of CAFs in NSCLC, with the possibility to hinder the CAFs-tumour cells cross-talk, thus hampering cancer progression.

EACR25-1455

Exploring the Crosstalk between Chemoresistant TNBC cells and Tumor-Associated Macrophages: Implications for Tumor Progression

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Introduction

Triple-negative breast cancer (TNBC), accounting for ~15–20% of all breast cancer (BC) diagnoses worldwide, has the poorest prognosis, due to its great heterogeneity and limited treatment options, with a death rate in the metastatic setting disproportionately higher than any other BC subtypes. Analysis of the tumor microenvironment (TME) in TNBC have revealed a marked infiltration of tumor associated macrophages (TAMs), a heterogeneous population with high plasticity. TAMs include both M1 (anti-tumor) and M2 (pro-tumor) phenotypes, with the latter predominating in TNBC. The M2-like TAMs contribute to the establishment of an immunosuppressive milieu, promoting tumor growth, metastasis, and resistance to therapy. During tumorigenesis, there is a complex and dynamic interplay between cancer cells and TAMs. Here, we aim to explore the crosstalk between chemoresistant cancer cells and TAMs within the TNBC microenvironment.

Material and method

Cisplatin-resistant (Cis-Pt-R) and doxorubicin-resistant (Dox-R) cell lines were generated by chronic exposure of TNBC MDA-MB-231 cells to the chemotherapeutics. Human THP-1 monocytes were differentiated into M0 macrophages by incubation with PMA. Once adherent, cells were maintained in PMA-free medium to obtain resting macrophages. Conversely, murine RAW264.7 cells were directly used as M0 macrophages. For polarization experiments, human and murine M0 macrophages were treated with LPS and IFN-γ to induce the M1 phenotype, or IL-4/IL-10 to induce the M2 phenotype. To mimic the TNBC microenvironment in vitro, M0 macrophages were exposed to conditioned medium (CM) from TNBC 4T1 (murine setting) or Cis-Pt-R and Dox-R cells (human setting). In addition, to further investigate tumor-M2 like TAMs crosstalk within the TME, chemoresistant TNBC cells were co-cultured with TAMs. Macrophage phenotype and M1/M2 marker expression were validated by immunoblotting and confocal microscopy. Functional characterization was performed using ELISA assays.

Result and discussion

TAMs generated using CM from chemoresistant TNBC cells showed a significant increase in the expression of M2-like TAMs (e.g. CD206, CD44) while M1-like

TAMs marker levels (e.g. CD86) remained substantially unchanged. Quantification of inflammatory and anti-inflammatory cytokine production revealed M2 polarization. Furthermore, co-culture experiments confirmed that cultured with TAMs, chemoresistant TNBC cells promote the M2-polarization in TAMs. Studies are ongoing to assess the effects of polarized M2 macrophages in increasing EMT, stemness, and chemo-resistance of TNBC cells.

Conclusion

Our findings show that chemoresistant TNBC cells actively modulate the TME by skewing macrophage differentiation toward the M2 phenotype, thus providing an opportune system to investigate therapeutic approaches targeting the crosstalk between tumor cells and TAMs.

EACR25-1462

TGFβ signaling in cancer-associated fibroblasts creates a pro-metastatic niche in colorectal cancer

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Introduction

Colorectal cancer (CRC) frequently metastasizes to the liver, which is the leading cause of CRC-related deaths. The Consensus Molecular Subtypes (CMS) classification suggests a link between transforming growth factor (TGF)-β signaling, cancer-associated fibroblasts (CAFs), and the risk of liver metastasis. However, the key downstream effectors of TGFβ signaling in CAFs and their role in hepatic metastases remain poorly understood.

Material and method

This study investigates whether and how TGFβ signaling in CAFs leads to the formation of a hepatic pre-metastatic niche for CRC metastasis. Primary and liver metastasis-derived CRC CAFs were stimulated with TGFβ1, followed by qPCR arrays and ELISA to identify differentially expressed genes. Results were cross-referenced with RNA sequencing data from CMS-classified CRC samples and fresh patient specimens. Next, hepatocytes were exposed to TGFβ primed CAF-derived cytokines, and their effect on pro-inflammatory gene expression and neutrophil migration was assessed. To determine the underlying molecular mechanism, chemical inhibition and genetic ablation were performed to block specific pathways. Lastly, an orthotopic murine model of CMS4 CRC was used to evaluate these findings *in vivo*.

Result and discussion

TGFβ signaling in primary CRC-derived CAFs increased the expression of different members of IL-6 family of

cytokines, which in turn upregulated Serum Amyloid A1 (SAA1), a key neutrophil chemoattractant, in hepatocytes. This led to increased neutrophil-to-hepatocyte migration in vitro. Chemical inhibition and genetic ablation experiments revealed that gp130, the IL-6 family cytokine co-receptor, plays a critical role in JAK/STAT-mediated induction of neutrophil chemoattractants in hepatocytes, ultimately driving pro-metastatic neutrophil migration to the liver. Furthermore, *in vivo* experiments showed increased neutrophil migration to the liver before overt hepatic metastasis, alongside increased IL-6 family of cytokine-dependent stromal signaling.

Conclusion

TGF β signaling in CAFs promotes a neutrophil-dependent pre-metastatic hepatic niche, potentially driving liver metastasis in CMS4 CRC.

EACR25-1469

Plant-based diet consumption exacerbates colitis-driven tumorigenesis in adolescent mice

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Introduction

The rising incidence of early-onset colorectal cancer (eoCRC) may be a consequence of accelerated colonic biological aging driven by diet-related chronic inflammation. We aimed to study consumption of an optimized plant-based diet (PbD) on the development of eoCRC in adolescent mice.

Material and method

Multi-omics analyses (RNA sequencing, mass-spectrometry based proteomics, NMR metabolomics), immunohistochemistry and *ex vivo* colonic 3D organoid cultures were performed using 6-week-old male and female C57BL/6JRj mice, corresponding to a human age of 12 years. Acute colitis was induced in 16-week-old, corresponding to a human age of 23 years, male and female C57BL/6JRj mice by application of dextran sodium sulphate (DSS) via the drinking water for 7 days. Finally, 6-week-old male or female C57BL/6JRj mice were fed a control or an isocaloric PbD (sucrose-free, high content of fiber and w-3 fatty acids) for 20 weeks. At week 10, colitis-driven CRC was initiated by two cycles of azoxymethane (AOM) i.p. injection followed by DSS treatment. Mice were sacrificed at the end of week 20, corresponding to a human age of 29 years, for further analysis.

Result and discussion

RNA-seq and proteomic analyses of colonic tissue revealed sex-specific clusters. In young male mice, markers for glycolysis and mitochondrial oxidative phosphorylation as well as pro-inflammatory markers such as caspase-1 and NLRP6 were upregulated compared to young female mice. In contrast, adaptive immune marker levels such as IGG2B, IGHM or IGHG were higher in young female mice. Metabolic phenotyping unraveled low serum levels of the poly-unsaturated w-6 fatty acid arachidonic acid (AA) in young male mice compared to young female mice. Furthermore, colonic levels of the lipoxygenase ALOX15 were significantly reduced in young male mice. Multi-omics data were validated in colonic 3D-organoid cultures from analyzed mice, demonstrating significantly increased growth rates of young male mice derived organoids under AA stimulation, pointing to stronger adaptation of intestinal stem cells to AA stimulation in young female mice. Higher energy level and increased basal inflammation in young male mice was reflected by low survival rates during acute DSS-induced colitis experiments. Unexpectedly, PbD consumption for 20 weeks resulted in a significantly higher tumor load compared to control diet in young AOM/DSS treated male mice, with a lower effect in young AOM/DSS treated female mice.

Conclusion

Our findings suggest a higher glucose-driven mitochondrial activity together with low w-6 fatty acid serum level in young male mice compared to young female mice that resulted in exacerbated colitis-driven CRC under plant-based diet.

EACR25-1483

HFE polymorphism affects cancer progression both in human and mouse models of pancreatic cancer

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Introduction

Homeostatic Iron regulator (HFE) is the main altered gene in the Hereditary Hemochromatosis (HH) disease. Of the two main HFE polymorphisms, H63D rarely causes overt HH symptoms, but has been described as a risk factor for the development of cancer. Little is known about H63D impact on Pancreatic Ductal Adeno-carcinoma (PDAC), a devastating disease that still represents an unmet medical need. The few curing progresses achieved through the years compared to other types of cancer still underline the necessity of finding

strategies to better stratify and manage PDAC patients. PDAC patients bearing HFE polymorphisms displayed smaller tumors which were surgically resected in a higher percentage of them compared to HFE wild-type patients. Unfortunately, H63D-mutant patients also displayed a lower overall survival. In the attempt to understand better the tumor progression in these patients, we have generated a spontaneous mouse model of PDAC bearing the Hfe orthologue mutation, namely H67D.

Material and method

KC and KPC mice were crossed with HfeH67D/H67D mutant mice (KCHfeH67D/H67D and KPC/HfeH67D/H67D) and pancreas and organs were collected to histological analyze tumor progression and spreading and characterize the immune infiltrate by immunohistochemistry or flow cytometry. HfeH67D/H67D mutant mice were also immunized with non-proliferating OVA-overexpressing PDAC cells and 3 weeks later with live OVA-PDAC cells to investigate the antigen specific immune response. Antibody and cellular responses were analyzed by ELISA and ELISpot with sera and splenocytes collected two weeks after the challenge with live OVA-PDAC cells.

Result and discussion

Histological analyses of tumors arose in KC/HfeH67D/H67D and KPC/HfeH67D/H67D mice evidenced an increased tumor aggressiveness, with a higher propensity to metastasize and invade neurons into the pancreas. Additionally, H67D-mutant tumors were highly infiltrated by suppressive cells. Functionally, H67D-bearing mice were able to mount an antigen-specific immune response, but in vitro experiments evidenced a tendency towards a suppressive type-II response with a massive production of antibodies, which, however, seem to play a role in the attempt to contrast the tumor growth. Comparable lesion extensions highlighted strong differences in infiltrating immune cells in tumors arose in KC mice bearing or not Hfe mutation.

Conclusion

Overall, the H67D polymorphism seems to worsen PDAC progression and strongly affects immune cell activation, representing a promising prognostic biomarker that deserves further investigations to find better way to manage and treat these patients.

EACR25-1492

Molecular characterization of long non-coding RNA MINCR isoforms and their impact on prostate cancer progression

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Introduction

The gene encoding for MINCR (MYC induced long non-coding RNA) is located on chromosome 8, 15 Mb telomeric to MYC. While the two genes are often coamplified, we identified a substantial proportion of patients with advanced prostate cancer where MINCR is amplified independently of MYC, implying a distinct oncogenic potential for MINCR. While MINCR has emerged as a significant player in many cancers, no efforts have been made to elucidate its impact on prostate

cancer progression and the specific role of MINCR isoforms remains unexplored.

Material and method

MINCR transcript characterization was performed through in silico analyses using publicly available cancer datasets and annotation databases. MINCR silencing was obtained using either blocking or degrading antisense oligonucleotides. For overexpression, isoform-specific sequences were cloned in plasmids. RNA sequencing followed by gene set enrichment analysis (GSEA) was used to provide mechanistic hints on how MINCR impacts prostate cancer tumorigenesis. Quantitative PCR analysis and in vitro cellular assays were performed to validate GSEA results.

Result and discussion

We found that MINCR expression levels positively correlate with tumor grade and negatively with disease-free survival, highlighting that MINCR is highly expressed in more aggressive and advanced cases of prostate cancer. The analysis of PolyA site database, FANTOM CAGE data, and long-read sequencing data revealed the existence of two main isoforms, that we named short and long, localizing in the cytoplasm and in the nucleus, respectively. Modulation of MINCR short in prostate cancer cells resulted in more profound changes in gene expression, as compared to MINCR long.

Overall, silencing of both isoforms impacted on cell growth, with cell cycle related genes (specifically M phase) being downregulated. MINCR involvement in cell proliferation was further confirmed in vitro; indeed, knocked-down DU145 and PC3 prostate cancer cells both showed decreased proliferation, whereas stably overexpressing clones displayed variably increased growth. Moreover, manipulation of both isoforms induced alterations of genes involved in androgen response, immunogenicity and NOTCH-pathway, features typically observed in neuroendocrine, aggressive prostate cancers. On the other hand, metabolism-related pathways, like glycolysis, resulted altered only when the short isoform was overexpressed, while genes involved in cell migration, cytoskeleton and extracellular matrix appeared selectively governed by MINCR long.

Conclusion

Our study reveals that MINCR isoforms exhibit distinct behaviors, yet both play crucial roles in driving prostate cancer progression and enhancing its aggressiveness. Investigating how MINCR influences cancer hallmarks and resistance to treatment could pave the way for novel targeted RNA-based therapeutic strategies.

EACR25-1493

Luminal ER+ breast cancer cells exhibit an enhanced dormant phenotype in softer matrices

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Introduction

Recent *in vitro* 3D models have shown that dormant state of disseminated cancer cells can be induced by physical confinement of the extracellular matrix (ECM), either by its high density and/or low adhesion. In this work, we aim to understand ECM-mediated dormant state of cancer cells by comparing mechanically tuneable stiffness-matched semi-synthetic matrices.

Material and method

MDA-MB-231 cell line as a model for triple-negative breast cancer and MCF7 for the luminal subtype were used. Genetically modified with the FUCCI2 cell cycle reporter, with mCherry as G0/G1 phase indicator and mVenus as S/G2/M phase marker, both cell lines were encapsulated in stiffness-matched hydrogels. Norbornene-modified inert alginate gels were used to mimic dormancy-inducing microenvironments, while adhesive and degradable gelatin methacryloyl (GelMA) gels were chosen as proliferation-permissive microenvironments. Stiffness and swelling values of hydrogels were evaluated by rheology. 3D confocal images of single cells and clusters formed over time were stained with F-actin and segmented using Deep Learning algorithms in ORS Dragonfly. Then, cell morphological features such as volume changes and sphericity were quantified. To determine the role of the matrices in dormancy induction, the cell cycle state and the cyclin-dependent kinase (CDK) inhibitor p27/Kip1 were analysed with FUCCI2 and immunofluorescence, respectively. Currently, further analyses are ongoing connecting dormancy and mechanotransduction.

Result and discussion

We obtained stiffness values of 1 and 10 kPa for soft and stiff matrices, respectively, both for alginate and GelMA gels. First, we observed a higher number of single cells in alginate gels compared to a greater number of clusters in GelMA. Limiting the analysis to single cells, the number of cells in S/G2/M phase decreased while those in G0/G1 increased in all groups, especially in MCF7 cells. Furthermore, cells in G0/G1 phase showed a significantly lower cell volume compared to cells in S/G2/M phase in all groups, regardless of matrix stiffness. Comparing both cell lines, MCF7 cells showed higher volume in both single cells and clusters compared to MDA-MB-231 cells. In terms of sphericity, only MDA-MB-231 cells in soft GelMA gels showed significant morphological elongations, indicative of proliferative behaviour, while MCF7 cells remained rounded. Finally, we validated a dormant phenotype of MCF7 cells by a nuclear translocation of p27/Kip1, which was enhanced in soft matrices.

Conclusion

Inert alginate models restrict breast cancer cluster formation, limiting cell volume increase in growth-arrested cells for both triple-negative and luminal models and independent of matrix stiffness. Interestingly, MCF7 cells express an enhanced dormant phenotype in soft microenvironments.

EACR25-1495

Signaling downstream of tumor-stroma interaction regulates mucinous colorectal adenocarcinoma apicobasal polarity

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Introduction

Mucinous colorectal carcinoma (MUC CRC) dissemination into the tumor stroma and metastasis to multiple organs, including the peritoneum, is associated with poor prognosis. Disseminating MUC CRCs exhibit either a conventional ‘apical-in’ or an inverted ‘apical-out’ polarity phenotype that influence patient outcome. Identifying the mechanisms controlling MUC CRC polarity is critical to understand disease progression.

Material and method

Here, we analyze patient-derived MUC CRC xenografts, with apical-in or apical-out polarity, *ex vivo* or within collagen gels to mimic the peritumoral stroma. Single-cell analyses reveal $\alpha 2\beta 1$ -integrin as a key collagen-binding receptor in these models.

Result and discussion

Collagen- $\alpha 2\beta 1$ -integrin interaction activates Src and ERK/MAPK signaling and upregulates the expression of SorLA, an endosomal sorting receptor. SorLA supports apical-in polarity and carcinoma-stroma interactions by promoting integrin recycling to the plasma membrane and HER2/HER3 expression through a positive feedback mechanism. Accordingly, we observe positive correlation between HER2, HER3 and SorLA in patient samples with the highest HER2 expression in apical-in-presenting tissues. Treatment of tumor spheres with clinically relevant HER2/HER3-targeting antibodies reverts sphere polarity and impedes collagen remodeling and adhesion to mouse peritoneum

Conclusion

This SorLA-integrin-HER2/HER3 signaling axis may represent a basis for MUC CRC-patient stratification and shed light on other carcinomas with similar apical-out phenotypes.

EACR25-1498

The role of RNA Modifiers in Prostate Cancer Bone Metastasis: Insights from m6A RNA Methylation

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Introduction

Prostate cancer (PCa) is one of the most common malignancies among men in industrialized countries and a leading cause of cancer-related mortality. While the five-year survival rate for localized early-stage PCa approaches almost 100%, it declines dramatically to 30–35% upon metastasis, with skeletal bone being the predominant metastatic site (observed in ~80% of cases) and no curative treatments currently available. The interplay between tumor cells and bone cells plays a pivotal role in the establishment and progression of bone metastases. Emerging evidence suggests that RNA-editing and modification processes enable cancer cells to adapt to new microenvironments, facilitating metastatic progression.

Material and method

In this study, we investigated the effects of the bone microenvironment on the expression of almost 100 different RNA-modifying enzymes in PCa cells and validated them in two distinct patient-derived PCa cohorts. Next, we continued with functional studies of the most significant RNA regulators.

Result and discussion

Our findings demonstrate that many RNA modifiers were deregulated in PCa cell lines in our in vitro model for the bone microenvironment compared to normal medium. In silico analyses of patient-derived PCa cohorts support these results, revealing significant upregulation of two key RNA modifiers, RBM15 and RBM15B, in bone metastases compared to primary tumors at both RNA and protein levels. These proteins are components of the m6A methyltransferase complex (MTC), which mediates N6-methyladenosine (m6A) RNA modifications – the most common RNA modification in all eukaryotes. Functional studies employing siRNA knockdown confirmed the essential role of the proteins in regulating m6A RNA methylation and significantly decreased cell growth of PCa cell lines.

Conclusion

In conclusion, these results identify m6A RNA methylation and its regulatory proteins; RBM15 and RBM15B as contributors to PCa progression. Furthermore, the in vitro screening results, as well as patient cohort analyses, suggest a role in bone metastasis and highlight their prognostic potential.

EACR25-1508

A Multiomics Imaging Approach to Characterize lncRNA-Protein Dynamics in Colorectal Cancer Progression

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Introduction

Colorectal cancer (CRC) is one of the deadliest cancers worldwide. Increasing incidence and mortality rates highlight the need for a more in-depth understanding of

its underlying characteristics. Since tumor heterogeneity contributes to therapy resistance and disease progression, it is crucial to identify biomarkers at both the protein and RNA levels. Long non-coding RNAs (lncRNAs) are considered promising biomarkers in CRC, but despite their potential role in cancer prognosis and diagnosis, their spatial distribution is not well understood. We aim to address this knowledge gap by leveraging the COMET™ multiomics platform. Here, immunofluorescence (mIF) has been recently integrated with RNAscope™ (RNA fluorescence in situ hybridization, RNA-FISH) to enable multiplexed imaging of RNA and protein on the same slide. This approach will provide an unprecedented view of lncRNA-protein interactions in CRC.

Material and method

We will select formalin-fixed, paraffin-embedded (FFPE) healthy and CRC specimens covering stages I-IV to construct a tissue microarray (TMA). Using the COMET™ system combined with RNAscope™ technology, we aim to dissect the spatial distribution of selected lncRNAs (MALAT1, ANRIL, NEAT1, and TUG1), which have been shown to play a role in CRC progression. The multiplex immunofluorescence panel consists of EMT markers (E-cadherin, N-cadherin, Vimentin, Fibronectin, ZEB1, ZEB2), proliferation markers (Ki-67, PCNA), stromal markers (CD44, Plexin B2, α-SMA, Desmin, CD31, CD45), and CRC-specific markers (Pan-CK, CDX2, SATB2).

Result and discussion

We assume that multiplex imaging will reveal distinct lncRNA expression patterns across CRC stages. Patients with MALAT1+/ZEB1+/N-cadherin+ profiles have been shown to have poorer outcomes. Our hypothesis is that MALAT1 and NEAT1 may be significantly upregulated in advanced CRC (Stages III-IV) and could correlate with an EMT shift, quantified by high N-cadherin/ZEB1/ZEB2 and low E-cadherin expression. Similarly, ANRIL and TUG1, potentially localized predominantly in stromal regions, might increase in Stage II tumors, suggesting a role in microenvironment remodeling. We anticipate that ANRIL expression could correlate with stromal activation (α-SMA, Desmin, CD31), while high CD45 levels may inversely correlate with MALAT1 expression, potentially indicating immune evasion. Additionally, we hypothesize that the loss of CDX2 and SATB2 could be associated with EMT-driven dedifferentiation.

Conclusion

Acquiring spatial information on lncRNAs associated with poor tumor prognosis and proteins involved in EMT activation across different CRC stages will provide key insights into their interactions. This may help link these lncRNA-protein interactions to both tumor progression and the functional role of the lncRNAs.

EACR25-1526

Stromal-immune interactions shape the bone metastatic niche

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Introduction

Mortality from breast and lung cancers is almost exclusively a result of tumor metastasis, and the most common site for metastatic spread from these cancers is the bone. Advanced metastatic disease is still incurable and therefore, a better understanding of the bone microenvironment as a central niche for breast and lung cancer metastasis is critical. We previously showed that stromal cells in multiple metastatic organs are key players in facilitating metastatic progression. However, the function of osteoblasts in creating a hospitable metastatic niche in the bones remains largely unknown. We hypothesize that the dynamic plasticity of osteoblasts in the bone metastatic niche is a driving force in enabling metastatic relapse by modulating the immune compartment in the bone microenvironment.

Material and method

We utilized a mouse model of spontaneous breast cancer metastasis to bone, following resection of the primary tumor, providing us with a clinically relevant platform to study the changes in the bone metastatic niche and the interactions between stromal and immune cells that facilitate bone metastasis. In addition, utilizing transgenic reporter mice (*Col1a-YFP*) to enable tracking and isolation of osteoblasts at distinct metastatic stages and from defined metastatic niches, we study the functional heterogeneity of osteoblasts during breast and non-small cell lung carcinoma (NSCLC) bone metastasis, at the single-cell level.

Result and discussion

We found that secreted factors from bone-metastasizing breast cancer cells reprogramed primary osteoblasts to an elongated spindle-shaped CAF-like immature phenotype. Cancer-secreted factors also induced upregulation of pro-inflammatory and bone turnover genes indicating increased osteolysis. Following activation, osteoblasts were capable of chemoattracting myeloid-derived suppressor cells and suppressing CD8 T-cell activity and proliferation. Interestingly, cancer-activated osteoblasts also produced less abundant and highly anisotropic cell-derived matrixes, suggesting that they contribute to bone matrix remodeling during the metastatic process.

Conclusion

In summary, a better understanding of the reciprocal interactions of osteoblasts and immune cells in the bone metastatic microenvironment will enable identification of therapeutic targets to treat bone metastasis.

EACR25-1531

Role of mammary adipose tissue-derived versican proteoglycan in breast cancer progression and aggressiveness

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Introduction

In Breast cancer (BC) cell-cell interactions are a prominent mechanism for cancer progression. Mammary

adipose tissue (MAT) represents 90% of breast tissue where epithelial cells and adipose cells constantly interact. In BC, MAT and mesenchymal stem cells (MCSs) have a key role in sustaining tumor development, secreting signaling molecules and proteins. Among them, Extracellular matrix (ECM) proteins secreted by MAT have an essential role in cell-cell communication. Proteoglycans, components of ECM, are involved in the regulation of different pathological processes. Versican (Vcan) proteoglycan sustains cancer progression either by having an impact on the cancer cell or by influencing the tumor microenvironment. Since Vcan is highly expressed in adipose tissue, MAT derived-Vcan might act on BC epithelial cells and vice versa. Therefore, the aim of our work is to study the role of Vcan in BC-MAT bidirectional communication and its potential identification as a novel diagnostic biomarker of BC progression and aggressiveness in MAT.

Material and method

MAT biopsies have been obtained from healthy women (N=16) and from patients with BC (N=17). Total RNA and protein lysates have been collected and analyzed for Vcan expression by Real Time PCR and Western blot analysis respectively. MCSs have been isolated from MAT and co-cultured with MCF7 BC cells in transwell systems and in Ultra low Attachment 96well for spheroids formation. Vcan content was investigated by immunohistochemistry (IHC). Data were analyzed using GraphPad Prism 8.

Result and discussion

In BC-MAT Vcan was detected by IHC at higher extent in the proximity of the tumor, compared to more distant sites. Higher Vcan expression correlates with BC aggressiveness. Vcan expression positively correlates with patients glycemia and cholesterol in MCSs, suggesting that Vcan expression in MAT-MSCs was reminiscent of the physiological patients metabolic status. Moreover, in 2D-co-culture with MCF7 cells, MSC expressing high levels of VCAN (MSC-VCAN+) displayed increased levels of Vascular Endothelial Growth Factor (VEGF), Interleukin-6 (IL-6), Fibroblast Activation Protein (FAP) and ACTA2 genes, and induced Matrix Metalloprotease-9 (MMP-9) expression in MCF7 cells, compared with MSC expressing lower VCAN levels (MSC-VCAN-). These data suggest that MSC-VCAN+, in contact with BC cells, showed an up-regulation of pro angiogenic, inflammatory and fibrotic markers. Finally, spheroids composed of MCF7 cells and MSC-VCAN+ showed higher dimension and number compared to spheroids composed of MCF7 cells and MSC-VCAN-.

Conclusion

All together this evidence pointed out Vcan as a target molecule with a strategical role in the connection between BC and MAT. Characterization of Vcan in MAT surrounding BC may lead to identify a novel biomarker associated to BC progression and aggressiveness.

EACR25-1539

Modeling extracellular matrix-tumor interactions in desmoplastic small round cell tumor: development of a new

organotypic model

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Introduction

Desmoplastic Small Round Cell Tumor (DSRCT) is a rare, aggressive soft tissue sarcoma primarily affecting the peritoneal cavity of children and young adults. It is defined by a specific chromosomal translocation t(11;22) (p13;q12), that leads to the aberrant transcription factor EWSR::WT1, and a rich desmoplastic extracellular matrix (ECM) that supports tumor growth while hindering drug delivery and immune infiltration. Currently, there are no 3D DSRCT in vitro models, which are critical to evaluate treatment strategies. Moreover, it is crucial to identify potential therapeutic targets. Nucleolin (NCL), described by our group as a safe and specific therapeutic target when expressed on the cell surface [1] remains unexplored in DSRCT. Herein, we develop a novel organotypic DSRCT model that closely mimics the ECM-tumor dynamics to study tumor progression and NCL-targeted therapies.

Material and method

JN-DSRCT cells alone/and MRC5 fibroblasts were embedded in a matrix with different ratios of type I and III collagen, with/without fibronectin. Cell growth and spatial distribution within the matrix were analyzed by microscopy. ECM remodeling was assessed by exposure to different compounds: collagenase type I, losartan, a lysyl oxidase-like 2 (LOX2) inhibitor and a fibroblast activation protein (FAP) inhibitor. Cytotoxicity was evaluated using MTT at 72 h in 2D models, prior to 3D model generation. ECM permeability was analyzed via a transwell assay followed by absorbance studies, using calcein and 90 nm NCL-targeted liposomes to mimic therapeutic delivery. NCL expression in DSCRT was analyzed by flow cytometry (FACS) and western blot, compared to a panel of normal/tumoral cell lines.

Result and discussion

The organotypic model successfully mirrors the human DSRCT architecture. Specifically, collagen I concentrations up to 1 mg/ml supported DSCRT cell growth and distribution for at least 9 days. ECM remodeling was observed in response to treatment, leading to a reduced matrix stiffness, observed by microscopy. DSRCT were not affected by collagenase I up to 12U/ml in 2D. However, calcein penetration improved after treatment with collagenase. Furthermore, DSRCT cells expressed NCL on the cell surface highlighting NCL relevance as a potential therapeutic target. NCL modulation studies are currently ongoing.

Conclusion

Our findings suggest that DSRCT ECM can be effectively recapitulated in vitro using an organotypic model, providing a valuable platform for DSRCT research. This study highlights the potential of combining ECM modulation with NCL targeting in tumor cells as a novel therapeutic approach to treating DSRCT.

[1] doi: 10.1016/j.nantod.2021.101095

Funding: CiNTech (PRR-30) C644865576-00000005; Marie-Sklodowska-Curie fellowship (project 101130813); 9th Prémio Rui Osório de Castro / Millennium bcp, 2025.

EACR25-1575

Clinical and molecular characterization of colorectal cancer: is the tumor micro-environment reflected on fecal miRNome and microbial profiles?

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Introduction

Colorectal cancer (CRC) develops within a heterogeneous microenvironment in which immune and stromal cells interact with tumor cells, influencing disease progression. The balance between tumor-promoting and tumor-suppressing mechanisms within this ecosystem determines clinical outcomes. This study explores the relationship between tissue transcriptome data, including deconvolution of tumor microenvironment (TME) composition, and their reflections on fecal molecular markers (miRNA profiles and microbiome composition). The aim is to explore novel insights for an accurate characterization of non-invasive biomarkers for CRC diagnosis and monitoring.

Material and method

RNA-sequencing data from tumor and paired adjacent tissue of 93 CRC patients were analyzed using the MCP-Counter algorithm to estimate TME cell population scores. Clinical and molecular data, including tumor mucinous, immunoscore, microsatellite instability, and consensus molecular subtypes, were integrated to identify specific patterns with respect to the immune infiltrate. Gut microbiome composition obtained by shotgun sequencing and fecal miRNA profiles from small-RNA sequencing were investigated according to previously identified patterns by a multi-omics integration approach. Results were validated on an independent cohort of 159 CRC patients.

Result and discussion

Significant differences in immune cell infiltration were observed between tumor and adjacent tissues, with an increase of cancer associated fibroblasts (CAFs) in tumors and Natural Killer cells in adjacent tissues. Three distinct clusters of stromal and immune cell infiltrate were identified, highlighting reflections across histopathological, molecular and clinical features, also confirmed on the validation cohort. Tumor tissue miRNome profiles showed a pattern of dysregulation when compared with adjacent mucosa that reflected the TME populations. Despite an apparent similar trend of correlations between differentially expressed miRNA

levels and TME scores, either in tumor or adjacent mucosa stronger associations could be appreciated for specific populations such as CAFs and B cells. Putative stool miRNA levels associated with the tumor infiltrate included a positive correlation of macrophage score with miR-342-3p and miR-30a-3p observed also in tissues. Conversely, endothelial cells exhibited a positive correlation with miR-130a-3p tissue levels and a negative correlation with its levels in stool. Analysis of gut microbiome composition in relation to identified TME clusters is ongoing.

Conclusion

These preliminary results highlighted distinct TME patterns and their association with miRNA alterations, in both primary and surrogate tissues. Further analyses will focus on functionally characterizing the associations between TME clusters and fecal molecular signatures, aiming to refine their potential as non-invasive biomarkers for CRC.

EACR25-1576

The role of complement in pancreatic cancer: from complement activation and effector mechanism/s to combination with therapy

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Introduction

The complement system has long been recognized for its role in cancer immunosurveillance, but more recently, it has also been identified as a system that promotes tumor development in several cancer types. Our laboratory previously demonstrated that lectin-dependent complement activation drives sarcoma progression via a C3a/C3aR-dependent mechanism. Although a potential pro-tumoral role of the complement system in pancreatic cancer has already been described, the underlying mechanisms and its potential as a therapeutic target have not yet been fully investigated. This study aimed at investigating the pathway/s involved in complement activation in pancreatic cancer, the potential triggers, and effector mechanisms, and also at evaluating the therapeutic potential of complement inhibition in combination with established treatments, such as radiotherapy (RT).

Material and method

Combining subcutaneous and orthotopic preclinical mouse models of pancreatic cancer (Panc02) with C3 deficiency, we investigated the impact of complement activation on tumor development. Additionally, we examined the role of C3 upstream (C4^{-/-}, MBL1/2^{-/-}, C1q^{-/-}, and fB^{-/-}) and downstream (C3aR^{-/-} and C5aR1^{-/-}) complement molecules. To explore potential trigger/s of complement activation, such as gut micro/mycobiose alterations and tumor cell glycosylation, we employed in vivo antibiotic/antifungal treatments and in vitro C3 deposition assay on tumor cells, upon treatment with glycosylation inhibitor/s. Finally, we assessed the

therapeutic potential of complement inhibition in combination with local RT in vivo.

Result and discussion

Our results in C3^{-/-} (both subcutaneous and orthotopic), C4^{-/-} and MBL1/2^{-/-} mice show reduced susceptibility to Panc02 tumor growth. The use of a specific N-glycosylation inhibitor (Tunicamycin) in vitro resulted in reduced C3 deposition on Panc02 cells, suggesting that alterations in cell membrane glycosylation weaken complement recognition. The downstream effector mechanisms of C3 activation appear to be independent from C3aR and C5aR expression in vivo. Furthermore, we explored the potential benefit of complement deficiency in combination with tumor RT. Notably, MBL deficiency combined with RT led to a more efficient control of tumor growth compared to either RT treatment or MBL1/2 deficiency alone, suggesting an additive effect.

Conclusion

Our work demonstrated that complement activation driven by the lectin pathway upon recognition of altered tumor cell glycocalyx, promotes Panc02 tumor growth. C3-downstream mechanisms need to be clarified. Results obtained with the combination of MBL-deficiency with RT indicate that targeting complement may lead to better response to classical therapies.

EACR25-1582

Exploring the Interactions within the Tumor Microenvironment of pancreatic neuroendocrine tumors (P-NETs)

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Introduction

The tumor microenvironment (TME) plays a crucial role in the progression and clinical behaviour of various cancers. However, its significance in neuroendocrine tumors (NETs) remains largely unexplored. This study aims to elucidate the mechanisms governing the interaction between pancreatic NETs (P-NETs) and their microenvironment, with a focus on identifying factors that influence tumor growth, differentiation, and aggressiveness.

Material and method

Our experimental model is the NT-3 cell line derived from a human well-differentiated P-NET. NT-3 cells were injected into immunodeficient mice to generate xenografts. Subsequent tissue stainings were performed to observe growth patterns and phenotypic characteristics of the tumors. We will also assess the crosstalk between NT-3 cells and several human derived cancer associated fibroblasts (CAFs) as stromal component in co-culturing conditions. Our models were infected with the TurboID system to tag every protein secreted in cell culture media allowing the proteomics analysis of each cellular component in co-culturing conditions.

Result and discussion

Histological analysis of the xenografts revealed two distinct tissue phenotypes. Some tumorgrafts exhibited the conventional morphology of well-differentiated P-

NETs, while others displayed extensive stromal infiltration, deviating from the traditional growth pattern. The presence of these divergent histopathological phenotypes provides a unique experimental setting to study the complex interactions between tumor cells and the TME. We positively validated the stable expression of the TurboID biotin ligase and a good biotinylation efficiency in the infected models, overall confirming the setup of a functional system for the proteomics analyses of the co-cultures' secretome.

Conclusion

Our findings highlight the heterogeneity within the TME of NETs and underscore the importance of understanding these interactions. The distinct growth phenotypes observed in the NT-3 xenografts offer valuable insights into the factors driving tumor behavior and present opportunities for developing novel targeted therapeutic strategies and advance our knowledge of NET biology.

EACR25-1602

Oncofetal-chondroitin sulfate, key to tumor immune surveillance?

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Introduction

Onco-fetal chondroitin sulfate (ofCS) glycosaminoglycan is a cancer-associated modification, initially described in placenta. While absent from healthy tissues, it is extensively expressed in tumors of different origins. We recently developed two antibodies binding with high affinity and specificity to ofCS enabling its depth characterization in cancer. The tumor agnostic expression of ofCS points to a crucial role in tumor development and progression, however the biological role of ofCS remains elusive.

Material and method

In this study we have generated CRISPR-Cas9 ofCS genetic knock-out (KO) cell lines from 4T1 murine breast and A375 human melanoma to study the biological roles of ofCS. The biological implication of the KO was assessed in vitro and in vivo. Differences in the immune cell infiltration were studied with immunofluorescence staining of tissues and flow cytometry analysis of single cell tumor suspensions.

Result and discussion

We observed increased tumor T-cell infiltration in immunocompetent 4T1 ofCS KO allograft compared to wild type (WT) tumors. In line with this, we observed increased immune cell infiltration in A375 tumors in a humanized murine model co-treated with human immune cells. We also observe an opposite spatial correlation between CD3+ cell location and ofCS staining in human colorectal patient biopsies.

Conclusion

Altogether, our studies indicate that ofCS may play a key role in tumor immuno-surveillance.

EACR25-1603

Study of master regulators involved in brain metastasis development in triple-negative breast cancer

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Introduction

Although breast cancer survival rates have improved in recent years, 90% of its mortality is attributed to the development of metastases to vital organs. Among the various subtypes of breast cancer, triple-negative breast cancer (TNBC) is the most aggressive and has the highest metastatic potential. In fact, 50% of patients with TNBC will develop brain metastases, presenting a significant challenge to survival in these patients. Surgical excision is not always feasible, and the lack of specific targets distinguishing TNBC complicates therapeutic targeting, sometimes making it impossible. Consequently, when brain metastases occur, a patient's survival decreases to six months. In this context, it is essential to characterize cerebral metastatic dissemination by identifying cellular factors that may be responsible for subsequent relapses in patients.

Material and method

Therefore, we propose investigating the master regulators (transcription factors, or key regulators of gene) implicated in the early stages of brain metastatic dissemination. To achieve this, we developed an *in vivo* brain metastasis model using intracardiac injections in SCID mice. Next, we established a protocol for recovering tumor cells that have spread to the brain (brain DTCs) to conduct a single-cell omic analysis. Simultaneously, we track brain metastatic dissemination over time utilizing an *in toto* clarification and light-sheet 3D imaging.

Result and discussion

Monitoring cancer cells through bioluminescence after intracardiac injections in SCID mice enabled the establishment of the kinetics of brain metastatic development. A few hours post-injection, most cells are located in the brain. By day 5 after injection the number of disseminated cells decreases drastically, followed by slow growth until day 21, reaching maximum development on day 28. Consequently, brain DTCs were collected at four time-points (D5, D14, D21, and D28) for ongoing transcriptomic analyses following brain dissociation. Simultaneously, an examination of the localization of brain DTCs at the same intervals is conducted through *in toto* clarification of the organ. This highlights the relationship between tumor cells, blood vessels, and the brain microenvironment, including astrocytes, microglia, and neurons.

Conclusion

This project enhances our understanding of early cerebral metastatic dissemination in TNBC. First, we developed a model of brain metastases suitable for scRNA-Seq analysis. Using the results from this model and

leveraging the bioinformatics tool created by our team, we will generate networks of master regulators identified at each stage of early brain metastasis. Additionally, it provides a precise spatio-temporal mapping of the implantation of metastatic cells in the brain, while emphasizing their dynamic interactions with the brain microenvironment.

EACR25-1618

XPO6-Mediated Immune Evasion Limits TCR-T Efficacy in Nasopharyngeal Carcinoma

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Introduction

Nasopharyngeal carcinoma (NPC) is an epithelial malignancy characterized by a complex immuno-suppressive tumor microenvironment (TME) with abundant infiltrating immune cells and cytokines. T cell receptor-engineered T cell (TCR-T) therapy, particularly targeting the NY-ESO-1 antigen, has emerged as a promising adoptive cell immunotherapy for NPC.

Through a genome-wide CRISPR screen, we identified exportin-6 (XPO6), a nuclear exporter, as a critical regulator of immune evasion in NPC cells, enabling resistance to TCR-T-mediated cytotoxicity.

Material and method

We established a genome-wide CRISPR knockout library in the NY-ESO-1-expressing C666-1 cell line and developed a co-culture system with NY-ESO-1-specific TCR-T cells to model tumor cell killing. Following TCR-T-mediated cytotoxicity, surviving tumor cells were collected for genomic DNA extraction and next-generation sequencing (NGS). Model-based Analysis of Genome-wide CRISPR/Cas9 Knockout (MAGECK) analysis identified XPO6 as a key factor regulating immune evasion in NPC, as evidenced by the down-regulated abundance of guide RNA sequences targeting XPO6. Functional validation was performed by knocking out XPO6 in additional NPC cell lines (HK1, NPC43) to assess its roles in tumor progression and immune resistance.

Result and discussion

XPO6 was identified as a potent anti-immune factor, promoting immune evasion by enabling NPC cells to resist TCR-T-mediated killing. Additionally, XPO6 exhibited oncogenic properties, driving tumor progression and enhancing immune tolerance in NPC. Knockout of XPO6 significantly sensitized NPC cells to TCR-T therapy and reduced tumor growth, highlighting its dual role as both an immune evasion regulator and an oncogene.

Conclusion

Our findings reveal that XPO6 plays a critical role in NPC immune evasion and tumor progression. Targeting XPO6 may represent a novel strategy to enhance the efficacy of TCR-T therapy and overcome immune resistance in NPC. These results provide new insights into the mechanisms of immune evasion in NPC and underscore the therapeutic potential of combining XPO6 inhibition with adoptive cell immunotherapy.

EACR25-1619

Oncogenic Molecular Features triggered by the Mechoresponsive Polycystin proteins

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Introduction

Mechanical cues regulate key features of cancer cells. Polycystin-1 (PC1) and polycystin-2 (PC2) are critical proteins that mediate mechanotransduction in cells. The aim was to investigate the impact of PC1 and PC2 on solid tumors.

Material and method

The expression of polycystins was investigated immunohistochemically in archival material of human breast, glioma, and prostate tumors. Functional assays were performed on corresponding neoplastic cell lines. Xenografts of the respective human tumors were developed in immunosuppressed mice.

Result and discussion

There are significant positive correlations between PC1 and PC2 expression in all tumor types, between PC2 expression and glioma grade, PC1 and PD-L1 expression in lung cancer, PC1 with extraprostatic extension and stage in prostate cancer ($p < 0.05$). Functional assays on respective cancer cell lines showed that PC1 gene (PKD1) silencing or PC1 protein's functional inhibition with a specific antibody decreased the clonogenic and migration potential of cancer cells. The A-549 and PC3 cancer cell lines were used to perform xenografts for lung and prostate cancer, respectively, and then the NSG mice were split into control and treated groups with PC-1 inhibitor. After tumor extraction, a 28.09% reduction in volume was observed in treated lung xenografts and a 1.53-fold reduction in tumor weight ($p = 0.071$). Then, immunohistochemistry was performed for markers for epithelial to mesenchymal transition such as E-cadherin, cytokeratins and vimentin. E-cadherin and cytokeratins present increased expression while vimentin was decreased in treated mice ($p = 0.018$, $p = 0.004$ and $p = 0.011$ respectively). In treated xenografts of the prostate cancer there was a reduction in volume by 18.12% and a decrease in weight by 1.39 times ($p = 0.27$). An increase in E-cadherin and cytokeratins was found in treated mice ($p = 0.008$, $p = 0.005$ respectively)

Conclusion

In conclusion, our data suggest that polycystins are involved in the development of solid tumors and influence disease progression, potentially acting as oncogenes.

The research project was supported by the Hellenic Foundation for Research and Innovation (H.F.R.I.) under the "2nd Call for H.F.R.I. Research Projects to support Faculty Members & Researchers" (Project Number: 3226).

EACR25-1652

Role of DDR1 in tumoral progression through matrix extracellular remodeling and discovery of a novel partner of DDR1 in colorectal cancer (CRC)

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Introduction

Colorectal cancer (CRC) is the third most common cancer, with tumor progression influenced by interactions between the tumor microenvironment and the extracellular matrix (ECM). Fibrillar collagen, the main ECM component, is more abundant in tumor tissues than in normal tissues and its remodeling plays a crucial role in CRC tumor progression. Cells interact with collagen through integrins and discoidin-domain receptors (DDR), particularly DDR1. A clinical study carried out by our research team on CRC patients revealed high DDR1 expression in tumors tissues, associated with shorter event free survival. As metastases occur in 40-60% of CRC cases, this study aims to investigate the role of DDR1 in metastatic progression.

Material and method

To investigate DDR1's role in CRC progression, HT-29 and HCT-116 colorectal cell lines were selected. DDR1 inhibition was achieved using the pharmacological tyrosine kinase inhibitors, DDR1-IN-1 and Nilotinib, while DDR1 knockdown was performed using shRNA targeting DDR1. Cell migration and invasion were assessed using wound healing assays, Boyden chamber assay and live-cell imaging. Invasion efficiency was analyzed in spheroid collagen type I invasion assay. DDR1 interaction with its new partner was performed using BRET saturation assays and proximity ligation assays (PLA).

Result and discussion

Our findings reveal that DDR1 overexpression in poorly invasive HT-29 cells enhances cell escape and spheroid growth. Conversely, DDR1 pharmacological inhibition by Nilotinib or DDR1-IN-1 and DDR1 downregulation by shRNA in HCT-116 cells significantly reduce migration (wound healing assay) and invasion (Boyden chamber assay). Time-lapse videomicroscopy study confirms that DDR1 inhibition significantly decreases migration in a 3D collagen matrix, while spheroid models

show a decrease in invasive capacity of colorectal cancer cell lines within a type I collagen rich matrix. As metalloproteinases (MMPs) play a key role in ECM degradation and tumor invasion, we demonstrate that DDR1 promotes matrix remodeling through MMP activation. Moreover, DDR1 can also form heterodimers with other receptors leading to cancer progression. Using a BRET saturation assay, we identified a novel DDR1 partner involved in CRC. This result was confirmed by PLA.

Conclusion

These findings highlight DDR1's key role in CRC cell migration and invasion through ECM remodeling. Furthermore, the discovery of a novel DDR1 partner offers promising insights into CRC molecular mechanisms and potential therapeutic strategies for colorectal cancer.

EACR25-1657

Deciphering lncRNA MTAAT-Mediated PD-L1 Regulation in T-cell Lymphoma: Implications for Immune Checkpoint Therapy

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Introduction

Immune checkpoint blockade has revolutionized cancer treatment, demonstrating significant efficacy in both solid tumors and hematological malignancies. However, the PD-1/PD-L1 axis exhibits variable activity in T-cell lymphomas, with some subtypes responding favorably while others experiencing hyper-progression. While the role of long noncoding RNAs (lncRNAs) in these mechanisms is emerging, predictive biomarkers for response or hyper-progression remain elusive. This study investigates the functional relationship between ALK-anaplastic large cell lymphoma (ALCL)-associated lncRNA MTAAT and PD-L1.

Material and method

Formalin-fixed paraffin-embedded (FFPE) sections from retrospective cohorts of ALCL were analyzed using diagnostic/relapsed primary lymphoma biopsies, with diagnoses based on WHO classification. In vitro experiments integrated omics (RNA-seq) and functional approaches (CRISPR dCas9-KRAB interference system, immunofluorescences and flow cytometry).

Result and discussion

PD-L1 protein expression was evaluated by immunohistochemistry in 20 primary ALK-ALCL cases. PD-L1 positivity was observed in 60% of cases (12/20) with variable expression (25% of cases with >5% PD-L1+ lymphoma cells and 35% of cases with >20%). PD-L1 exhibited predominant membranous staining in 33% of cases (4/12) and combined membranous and para-nuclear/Golgi-like patterns in 66% of cases (8/12). PD-L1 localization, not overall positivity, correlated with lncRNA MTAAT expression. Low lncRNA MTAAT expression was associated with membrane localization of PD-L1 ($p < 0.05$). No other ALK-ALCL-associated lncRNAs ($n = 5$) showed a significant correlation with

PD-L1 localization, candidating MTAAT as a potential regulator of PD-L1 localization in ALK-ALCL. Given this correlation, we explored the lncRNA MTAAT-dependent PD-L1 regulation in ALK-ALCL cells. Depletion of lncRNA MTAAT using a CRISPR dCas9-KRAB interference system led to a time-dependent increase in surface PD-L1 expression in ALK-ALCL cells (10% at 48h and 20% at 72h in MAC2A cells). No changes in PD-L1 transcript level was observed suggesting an indirect regulatory mechanism. RNA-seq data analysis from lncRNA MTAAT knockdown ALK-ALCL cells identified 12 up-regulated pathways, including endocytosis, lysosome, and membrane trafficking. Among these, ADP-Ribosylation factor 6 (ARF6), a small GTPase involved in membrane protein recycling and PD-L1 regulation, was identified as a potential mediator. Immunofluorescence revealed intracellular co-localization of PD-L1 and ARF6 in ALK-ALCL cells with high lncRNA MTAAT expression, which was lost in cells expressing low levels of lncRNA MTAAT, highlighting ARF6 as a potential PD-L1 regulator.

Conclusion

These findings support a role for lncRNA MTAAT in PD-L1 regulation via ARF6 and suggest MTAAT as a predictive biomarker for response to PD-1/PD-L1 inhibitor therapy.

EACR25-1658

miR-30d inhibition activates the cGAS/STING/IFN-I pathway stimulating an innate immune response via LATS2/YAP axis in breast cancer

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Introduction

Lying at the heart of the tumor ecosystem, cancer cells actively shape a dynamic tissue microenvironment that impacts tumor growth, stromal invasion and metastatic evolution. In particular an immune-cold TME, lacking infiltration by antitumor immune populations, allows unrestrained tumor growth and limits the efficacy of both chemo- and immune-therapies. We have previously highlighted that stress- and mutant p53-induced over-expression of the onco-miRNA miR-30d in breast cancer (BC) cells promotes the release of a pro-malignant secretome, which reprograms the TME and enhances tumor progression (Capaci V et al. 2020 Nat Commun).

Material and method

To further explore the cellular pathways regulated by miR-30d in BC cells, we first performed a transcriptomic analysis and further validated the findings with different techniques like Real-time PCR (qPCR), Western blot analysis, ELISA, Immunofluorescence on 2D and 3D system both on cancer and normal cells. We further expanded our findings through in-vivo model and on BC patients' samples. All the statistical analysis was conducted using unpaired Student's t-test in GraphPad Prism software.

Result and discussion

Interestingly, transcriptomic analysis highlighted that miR-30d overexpression inhibits innate immune signaling by attenuating the cGAS/STING/IFN-I cascade. Notably, inhibition of miR-30d both triggered upstream induction of cGAS and enhanced STING/TBK/IFN-I downstream steps of the pathway in BC cells, while not in normal breast epithelial cells. Mechanistically, we found that miR-30d controls the expression of proteins that sustain nuclear envelope integrity via LATS2/YAP axis, thus preventing the release of cGAS-inducing cytosolic DNA in cancer cells. Inhibition of miR-30d led to activation of cGAS/STING/IFN-I response and of antitumor immune surveillance in BC preclinical models. Consistently, analysis of publicly available gene expression datasets revealed a significant inverse correlation between higher miR-30d activity and established gene expression signatures reflecting anti-tumor immunity in BC patients' samples, suggesting that elevated miR-30d activity in breast tumors is associated with an immune-cold, poorly infiltrated TME.

Conclusion

Altogether, our result identifies miR-30d as a key driver of immune evasion in BC and suggests that its inhibition may restore an antitumor immune microenvironment, thus contributing to ameliorate the effectiveness of immune therapies.

EACR25-1663

Cancer Associated Fibroblasts Exert Immunosuppression Through Upregulation of Endoplasmic Reticulum Stress in Pancreatic Cancer

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Introduction

Pancreatic ductal adenocarcinoma (PDAC) is one of the

most lethal cancers, with a dismal 5-year overall survival of 13%. One of the major factors leading to this poor outlook is the presence of a highly fibrotic and immunosuppressive microenvironment, characterized by activation and expansion of fibroblasts and their extracellular matrix (ECM), and immunosuppressive immune cells, representing a challenge that often impedes the success of chemotherapies and immuno-therapies. During PDAC development, cancer-associated fibroblasts (CAFs) produce large amounts of ECM, growth factors, and immunosuppressive cytokines and chemokines, but despite their pivotal pro-tumor roles, the drivers of their activation and pro-tumor functions are still unknown. Paradoxically, elimination of CAFs and their ECM is unfavorable to patients, suggesting that modulation, rather than elimination, of pro-tumor CAFs, is an innovative and attractive approach in developing PDAC therapies. CAFs are highly secretory, which increases the load of misfolded proteins in their endoplasmic reticulum (ER). We postulate that PDAC CAFs are under ER stress, and that ER stress promotes and sustains their pro-tumor functions, catalyzing PDAC tumorigenesis.

Material and method

For *in vitro* functional assays, we used primary fibroblasts from healthy donor pancreata and PDAC tissue. Cells were cultured in a physiologically relevant, 3D culture system, composed by fibroblasts and their secreted ECM. Genes of interest were knocked down using the CRISPRi system. Cytokines were detected in the cell conditioned media by ELISA, and protein levels in cell lysates by western blotting. Naïve CD8 T cells were obtained from healthy donor and their proliferation assessed by flow cytometry. An *in vivo* pancreatic cancer model was developed orthotopically by injecting murine syngeneic pancreatic cancer cells, and treatments with IRE-1 RNase inhibitor were conducted intraperitoneally, five days/week.

Result and discussion

PDAC CAFs upregulated ER stress markers compared to normal fibroblasts, suggesting a role for ER stress in CAF functionality. This was confirmed in human PDAC tissue, using state of the art spatial transcriptomics and single cell RNA sequencing from over 150 PDAC patients. Modulating ER stress in CAFs decreased their secretion of immunosuppressive factors, which in turn allowed CD8+ T cells to proliferate more. Moreover, mice bearing pancreatic tumors treated with an IRE-1 RNase inhibitor (25 mg/kg) had smaller tumors and higher percentage of CD8+ T cells compared to control mice, supporting the notion that ER stress is an important pro-tumor mechanism in PDAC.

Conclusion

Our results suggest that upregulation of ER stress is one of the mechanisms involved in the regulation of tumor supportive functions of fibroblasts in pancreatic cancer, and an important immunosuppressive mechanism in pancreatic cancer.

EACR25-1688 Characterization of extracellular vesicles derived from cancer-associated

fibroblasts in oral squamous cell carcinoma

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Introduction

Oral squamous cell carcinoma (OSCC), the most common histological subtype of oral cancer, accounts for approximately 90% of cases and is a major public health concern, particularly in socioeconomically vulnerable regions. Current therapies often result in significant patient mutilation and are limited by high recurrence and metastasis rates, emphasizing the need for improved treatments and a deeper understanding of this disease. OSCC progression is not solely driven by tumor cells; it involves a complex tumor microenvironment where cancer-associated fibroblasts (CAFs) play a crucial role in promoting tumor growth, metastasis, and resistance to therapy. In this context, CAF-derived extracellular vesicles (EVs) are key mediators of tumor-stroma communication, transferring bioactive molecules that influence tumor behavior. This study aims to characterize EVs derived from CAFs in OSCC.

Material and method

Following cell starvation, the EVs were obtained from a CAF-conditioned medium and subjected to centrifugation and filtration to improve their recovery. Protein extraction was performed using the Total Exosome Isolation Kit (Invitrogen), and protein quantification was done using the QubitTM Protein Assay Kit (Thermo Scientific). Nanoparticle tracking analysis (NTA) was conducted to determine the size and concentration of the EVs using the NanoSight NS300 (Malvern Panalytical), and proteomic analysis was performed to determine the protein composition of the CAF-EVs.

Result and discussion

Preliminary results from NTA showed that the CAF-derived EVs had an average size of 92.9 nm, with a predominant size of 68.9 nm and a concentration of 2.83×10^{10} particles/ml. Protein quantification revealed a concentration of 277.5 µg/ml, suitable for further assays.

Proteomic analysis identified enrichment related to structural molecule activity, protein binding, and cell adhesion molecule binding, suggesting a role in remodeling the extracellular matrix and promoting tumor progression. Additionally, immune modulation pathways were observed, indicating a potential for CAF-EVs to suppress anti-tumor immunity.

Conclusion

These findings underscore the relevance of CAF-derived EVs in OSCC, highlighting their role in tumor progression and potential as therapeutic targets. By mediating tumor-stroma communication, CAF-EVs contribute to the complex biology of OSCC, which has implications for novel therapeutic strategies to improve patient outcomes. To further investigate the role of CAF-derived EVs in OSCC progression, our next steps will include the morphological assessment of EVs using transmission electron microscopy, surface marker labeling of EVs with Annexin-V-FITC (BD Biosciences), CD63-PE (Beckman Coulter), CD81-PE (Invitrogen) and CD90-APC (Invitrogen), and the characterization of EVs obtained from three-dimensional CAF spheroids.

EACR25-1717

CPAP promotes immunosuppression in the tumor microenvironment of Hepatocellular Carcinoma

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Introduction

Hepatocellular carcinoma (HCC) is the third leading cause of cancer-related mortality globally, and the five-year survival rate is below 12% due to late-stage diagnosis and the immunosuppressive tumor immune microenvironment (TIME). Tumor-associated macrophages (TAMs), a predominant immune cell population in the TIME, play a vital role in immune suppression by inhibiting T cell-mediated antitumor responses and macrophage phagocytosis. Cancer cells evade TAM-mediated immune surveillance through "don't eat me" signals, notably involving CD47 and CD24, which bind to SIRP α and Siglec-10 on the cell surface of TAMs, suppress phagocytosis and promote immune evasion. Our previous research identified CPAP is overexpressed in HCC, enhanced tumor growth and liver inflammation. Notably, overexpression of CPAP in human HCC cell lines not only elevated CD24 levels but also impaired macrophage-mediated phagocytosis. Despite these findings, the specific role of CPAP within the TIME remains poorly understood.

Material and method

The mouse liver cancer cell Hep55.1c stably expressing mCherry or mCherry-CPAP were established. Liver orthotopic injection by C57BL/6 was performed to investigate the role of CPAP in HCC TIME in vivo. Immune cell populations were analyzed by flow cytometry and qPCR. Bone marrow-derived macrophages (BMDMs) and splenic T cells obtained from wild-type C57BL/6 mice were co-cultured with conditioned medium collected from mCherry-CPAP-overexpressing Hep55.1c cells to evaluate the effects of

tumor intrinsic CPAP on anti-tumor immunity by flow cytometry analysis, qPCR analysis, trans-well assay, phagocytosis assay, and CCK-8 assay.

Result and discussion

Overexpression of CPAP can increase PD-L1 expression in cancer cells. Tumor intrinsic CPAP stimulates macrophage infiltration, promotes M2 macrophage polarization, increases Siglec-10 expression, and inhibits macrophage phagocytosis and T-cell activation in the TIME. Our results found that CPAP-educated TAMs affect the migration and proliferation of cancer cells. These results suggest that CPAP overexpression contributes to immune suppression and tumor progression within the TIME. Additionally, CPAP may influence other immune cell populations within the TIME, further amplifying its immunosuppressive effects. However, the other roles of CPAP in immune modulation remain unclear.

Conclusion

Our findings indicate that CPAP can regulate the CD24-Siglec-10 axis within the TIME, promoting macrophage-mediated immunosuppression in HCC. This study supports the idea that CPAP may be a potential target for HCC therapy.

EACR25-1729

Unveiling the Influence of Tumor-Derived CCL20 on B Cell-Mediated Immune Responses in Nasopharyngeal Carcinoma

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Introduction

Nasopharyngeal carcinoma (NPC) is characterized by high B cell infiltration and a strong association with Epstein-Barr virus (EBV), which preferentially targets both B cells and epithelial cells. However, the impact of tumor-derived factors on B cell function remains unclear. This study investigates the role of tumor-secreted CCL20 in modulating B cell-mediated immunity and its implications for prognosis.

Material and method

We used multi-center single-cell RNA sequencing data from 12 NPC patients to analyze immune cell dynamics and tumor-immune interactions. Bulk RNA sequencing data from a public dataset ($n = 88$) and an in-house dataset ($n = 82$) were utilized for validation. In vitro experiments included co-cultures of B cells (primary B cells isolated from PBMCs and EBV-immortalized lymphoblastoid B cell lines) with NPC cell lines (C666 and NPC43). RNA sequencing was performed on co-cultured B cells to explore the underlying pathways. Clinical validation was conducted using multiplex immunohistochemistry (mIHC) and spatial transcriptomics on patient samples.

Result and discussion

Our analysis revealed that high infiltration of memory B cells correlated with favorable treatment outcomes, while tumor-derived CCL20 emerged as a prognostic marker for early relapse. We identified increased cell-cell interactions between tumor cells and B cells via the CCL20-CCR6 axis, suggesting a direct role of CCL20 in modulating B cell behavior. Memory B cell abundance was negatively associated with CCL20 expression. In vitro experiments demonstrated that CCL20 significantly reduced the proportion of memory B cells, as evidenced by the downregulation of CD27, a key memory B cell marker. Pathway analysis further revealed that CCL20 impaired B cell activation and BCR signaling, fostering an immune-evasive tumor microenvironment. Co-culture experiments showed that CCL20 suppressed early activation markers (e.g., CD80, MHC-II, CD95) and memory B cell markers (e.g., CD27), while impairing antigen presentation via reduced MHC-I/II expression, which directly impedes T cell activation. Importantly, these effects were reversible upon treatment with an anti-CCL20 neutralizing antibody, highlighting the potential therapeutic value of targeting this axis. Spatial transcriptomics confirmed negative associations between CCL20 expression and B cell markers (CD20, CD27). Multiplex IHC performed on patient samples validated that patients experiencing early relapse exhibited high CCL20 levels and low expression of CD27 and MHC-I on CD20+ B cells, reinforcing the clinical relevance of these findings.

Conclusion

Tumor-derived CCL20 undermines B cell function, promoting immune evasion and treatment failure in NPC. Targeting the CCL20-CCR6 axis may restore anti-tumor immunity, offering a novel therapeutic strategy. Our findings highlight CCL20 as a biomarker for risk stratification and a potential therapeutic target in NPC.

EACR25-1737

Patient-Derived Tumor Tissue Cultures (PDTTCs) to assess immunotherapy responses in Glioblastoma

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Introduction

Glioblastoma (GBM) is the most common and aggressive primary brain tumor. While immunotherapy has shown promise in treating various cancers, it has not significantly improved outcomes for GBM patients. The unique tumor microenvironment (TME) of GBM, coupled with the lack of models that fully recapitulate its complexities, has limited the development of effective immunotherapeutic strategies. For instance, GBM tumors present elevated levels of Leukemia Inhibitory Factor (LIF), a member of the IL-6 cytokine family, which has been shown to modulate tumor-associated macrophages (TAMs) toward an immunosuppressive phenotype.

Material and method

To address these challenges, we developed Patient-Derived Tumor Tissue Cultures (PDTTCs) as a model to evaluate immunotherapy responses in human GBM. PDTTCs consist of tumor tissue slices (350 µm) cultured

in a semi-wet system that allows for the introduction of treatments. Using this platform, we investigated the impact of anti-LIF therapy on the TAM population within GBM tumors.

Result and discussion

After confirming that PDTTCs preserve tumor architecture and TME composition, we utilized this system to analyze transcriptomic responses to immunotherapy. Single-cell RNA sequencing (scRNA-seq) was performed on nine GBM tumors exposed to anti-LIF therapy, with a focus on the myeloid population. This approach revealed target genes regulated by LIF in TAMs that may contribute to the failure of other immunotherapies. These targets were further validated in mouse models, both *in vitro* and *in vivo*.

Conclusion

PDTTCs offer a robust preclinical model that can be used to gain deeper insights into human tumor responses to immunotherapy. By maintaining the native tumor spatial architecture and preserving TME populations, PDTTCs provide a more accurate platform for studying the effectiveness of immunotherapies in GBM.

EACR25-1784

Repurposing Tricyclic Antidepressants for Glioblastoma Treatment

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Introduction

Glioblastoma (GBM) remains a daunting challenge in oncology due to its resistance to existing standard-of-care therapies and inevitable progression to a lethal outcome. Our lab has previously proposed a successful novel combinatorial regimen, including the tricyclic antidepressant (TCA) imipramine, the vascular endothelial growth factor (VEGF) inhibitor bevacizumab, and the immune checkpoint inhibitor anti-PD-L1, which demonstrated significant therapeutic benefit in GBM mouse models (Chryplewicz et al.; *Cancer Cell*, 2022). This triple combination was effective by inducing immunostimulatory autophagy in cancer cells, reprogramming tumor-associated macrophages, and remodeling the tumor vasculature, all contributing to the recruitment and activation of T cells. These promising results have incentivized a proof-of-concept clinical trial, where GBM patients who fail the standard-of-care treatment will receive this triple combination as a second-line therapy. Motivated by these results and encouraged by the clinical support, we aim to further improve this therapeutic regimen by disrupting various capabilities of tumors required for their growth and therapeutic resistance.

Material and method

To optimize this regimen for clinical translation, we screened multiple TCAs - trimipramine, clomipramine, and amitriptyline - alongside imipramine in mouse and human GBM cell lines to evaluate their impact on cell viability. In parallel, we conducted *ex vivo* experiments using bone marrow-derived macrophages polarized toward an M2-like phenotype to assess the reprogramming potential of TCA treatment.

Result and discussion

Among the tested TCAs, trimipramine exhibited the strongest induction of GBM cell death across multiple viability assays. Additionally, all TCAs significantly downregulated M2-like macrophage markers, suggesting their potential to reverse the immunosuppressive tumor microenvironment. These initial findings indicate that TCAs possess anticancer and immunomodulatory properties, prompting further evaluation in preclinical models. Moreover, we are investigating mechanisms of adaptive resistance and relapse following the proposed triplet immunotherapy, with the goal of identifying pharmacological strategies to extend the survival benefit in GBM mouse models.

Conclusion

This study aims to delve deeper into TCA mechanisms of action, assess potential synergies with VEGF pathway inhibitors and immune checkpoint blockade, and ultimately provide critical insights to optimize the proposed triple combination regimen. By leveraging drug repurposing and simultaneously targeting multiple tumor hallmarks, this approach holds significant promise for improving GBM therapeutic outcomes.

EACR25-1787

CircFIRRE and circPROX1 are highly expressed in primary and metastatic colon cancer cells

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Introduction

Circular RNAs (circRNAs) are the class of noncoding RNA associated with physiological and pathological conditions. The circRNAs have been upregulated in diverse types of cancer that are involved in tumorigenesis, proliferation, invasion and metastasis. This study aimed to compare the expression of circRNAs between normal colon epithelial cells (CCD841 CoN), primary colon cancer cells (SW480), and metastatic malignant cells (SW620), while also validating the expression of selected circRNAs across these colon cancer cell lines.

Material and method

RNA sequencing was performed on normal colon epithelial cells (CCD841 CoN), and primary (SW480) and metastatic (SW620) colon cancer cell lines.

Differential expression, gene ontology (GO) and KEGG pathway enrichment analysis were conducted on the RNA-seq data. Selected upregulated circRNAs were also validated by RT-qPCR.

Result and discussion

Differentially expressed circRNA analysis identified 906 and 935 upregulated circRNAs in SW480 and SW620, respectively, compared to CCD 841 CoN. Additionally, 184 circRNAs were upregulated in SW620 compared to SW480 cells. KEGG pathway analysis of differentially expressed circRNA-associated genes between SW480, SW620, and CCD841 CoN highlighted pathways

involved in cancer development and progression. The analysis revealed significant enrichment in pathways related to cellular processes, human diseases, and genetic information processing, with distinct alterations observed in the metastatic SW620 and primary SW480 cells compared to normal colon epithelial cells (CCD841CoN). The circRNAs derived from FIRRE and PROX1 genes showed high-ranking expression in both SW480 and SW620 cell lines. The candidate circFIRRE (hsa_circ_0001944) was dramatically increased by 2789.55 ± 551.44 and 7593.61 ± 818.72 folds in SW480 and SW620, respectively, compared to the normal colon epithelial cells. Additionally, circPROX1 (hsa_circ_0111952) was also highly upregulated by 1810.88 ± 477.11 and 2851.46 ± 498.75 folds in SW480 and SW620, respectively.

Conclusion

This study identifies some predominant levels of circRNAs in primary and metastatic colon cancer cells. The finding reveals high expression of circFIRRE and circPROX1, with even greater levels in the metastatic cancer cell line. The overexpression characteristics of these circRNAs may serve as potential tumor biomarkers for diagnostic, prognostic, or therapeutic applications. However, additional studies are essential to enhance understanding of biological mechanisms and roles in colon cancer progression.

EACR25-1792

Metastatic peritoneal fibroblasts mobilize and respond to TGF- β 1 from the extracellular matrix of colorectal cancer

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Introduction

Colorectal cancer (CRC) peritoneal metastases (PM) are characterized by a high stromal infiltration and activation of the transforming growth factor-beta (TGF β). This multifunctional cytokine mediates crucial interactions between cancer cells, stromal cells and the extracellular matrix (ECM), driving tumour growth and metastatic dissemination. Fibroblasts play a pivotal role in ECM remodelling and promoting cancer cell invasion and metastasis, with TGF- β signalling being a key pathway. TGF- β is secreted by various cell types, including fibroblasts, and is stored in the ECM in an inactive latent form. Among others, proteases activity can release the active TGF- β , enabling downstream activation of the TGF- β receptors (TGFBRs). However, the interplay between patient-derived dECM and fibroblast remains under investigated due to the lack of accurate in vitro models. This study describes the ability of fibroblast from primary CRC (CAFs) and CRC peritoneal metastasis (MAFs) to mobilize TGF β from a patient-derived dECM and characterizes their molecular responses to TGF β .

Material and method

Decellularized CRC- and healthy colon derived ECM (CRC-dECM and HC-dECM) samples were prepared from human biopsies (detergent-enzymatic treatment). Cancer-associated fibroblasts were isolated from primary cancer and from peritoneal metastases, and cultured in the presence of dECM. Secreted proteins were analysed by mass spectrometry (MS) and with a direct antigen-labelling protein array. Gene expression was evaluated by RT-qPCR. The cellular distribution and abundance of TGF β and TIMP1 were investigated using single cell RNA sequencing (scRNA seq) and multiplex IHC in matched CRC and PM patients.

Result and discussion

Exposing peritoneal fibroblasts (MAFs) to CRC-dECM resulted in a substantial increase in free TGF β , growth factors, matrix metalloproteinases (MMPs) and its inhibitors (TIMPs). In vitro exposure of MAFs to exogenous TGF β increased the gene expression of TIMP1, SPARC and SERPINE1, in a dose-dependent manner, which was abolished by TGFBR-I/II blockage. TGFB1 silencing did not affect the CRC-dECM response, indicating TGF β mobilization rather than de novo synthesis and secretion by MAFs. Interestingly, CAFs showed no such activity. Furthermore, PM-dECM contained higher levels of trapped TGF β compared to both CRC- and HC-dECM. To validate our results, we combined scRNA seq and multiplex IHC analysis to demonstrate that MAFs were the stromal cells with increased TIMP1 expression, exhibiting a peritumoral localization in PM.

Conclusion

These results show that peritoneal fibroblasts can mobilize TGF β from the ECM which in turn induces the release of the known pro-tumorigenic modulators TIMP-1/SPARC. Therefore, metastatic-specific peritoneal fibroblasts might be a promising therapeutic target to inhibit this effect in patients with PM.

EACR25-1797

Bioengineering Unitary Lung Tumor-Stroma Organoids in a Biomimetic Human Extracellular Matrix

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Introduction

Lung cancer (LC) remains the leading cause of cancer-related deaths worldwide, highlighting the urgent need for novel therapeutic strategies. However, progress in drug development is hindered by the lack of in vitro models that accurately replicate the complexity of the tumor microenvironment. Three-dimensional patient-derived tumor organoids (3D-PDTOs) have emerged as promising tools due to their ability to mimic patient-specific tumor characteristics. Despite their advantages, conventional 3D-PDTOs often lack stromal components and rely on animal-derived hydrogels, which fail to replicate the biomechanical properties of the human tumor extracellular matrix (ECM). Moreover, conventional culture organoid models tend to generate multiple heterogeneous organoids, limiting

reproducibility and standardization for drug testing. We propose the bioengineering of unitary, stromal-enriched lung tumor organoids embedded in a mechanically tunable human collagen type I matrix. This physiomimetic platform aims to enhance biomimicry and improve preclinical assessment of anti-cancer nanomedicines.

Material and method

The HuLu051421 LC cell line was cultured in suspension to generate tumor organoids, which were subsequently processed to obtain unitary organoids with defined size. To enhance biomimicry, these organoids were co-cultured with stromal cells and embedded in a human collagen type I hydrogel with adjustable stiffness. The generated unitary tumor-stroma organoids were characterized using immunohistochemistry, gene expression profiling, and live-cell imaging to assess cellular composition, ECM deposition, and tumor architecture. To evaluate the model's suitability for drug screening, liposomal nanomedicine formulations were administered, and therapeutic efficacy was assessed using viability assays.

Result and discussion

The engineered unitary tumor-stroma organoids successfully integrated stromal components, ensuring reproducibility for drug screening. The use of a human-derived collagen matrix allowed for precise modulation of ECM mechanical properties, influencing cellular behavior and drug response. Compared to conventional culture strategies, unitary organoids displayed enhanced structural organization, better recapitulation of the native tumor microenvironment, and a more predictable response to treatment. Liposomal chemotherapy formulations exhibited differential efficacy within the engineered organoids, underscoring the importance of ECM stiffness and stromal interactions in therapeutic response.

Conclusion

This study introduces a bioengineered platform for generating unitary tumor-stroma lung organoids in a tunable human collagen type I matrix. By overcoming limitations of existing models, it enhances biomimicry and reproducibility, offering a more physiologically relevant system for preclinical drug testing.

EACR25-1798

Tumor Heterogeneity and Immune Modulation in Oesophageal Squamous Cell Carcinoma Characterised by Single-Cell RNA Sequencing Analysis

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Introduction

Oesophageal squamous cell carcinoma (ESCC), the aggressive subtype of esophageal cancer, has a significant cancer-related mortality rate and incident rate in Asia. The current treatment strategy remains challenging as the prognosis of ESCC patients is dismal.

Moreover, immunotherapy treatments showed selective responses in ESCC patients. This could be caused by the complicated immune cell states within the tumor microenvironment, which differs in ESCC patients due to heterogeneity. Therefore, the communication between tumors with tumor-associated stromal and tumor-infiltrating immune components remains unresolved in the complex immune-suppressive ESCC microenvironment. We aim to decipher the ESCC ecosystem to characterize the evolution of the epithelial cells and identify prognostic immune subtypes that could aid the treatment decision.

Material and method

A total of 55 ESCC patients were included in this study. Single-cell RNA sequencing was utilized to explore the cell subtypes from blood samples, tumor tissues, and adjacent normal tissues. All samples were aggregated in cellranger and integrated by harmony. The R program Seurat was used to perform clustering and marker gene identification. The changes in the inferred copy number were visualized with inferCNV, and gene set analysis was conducted with AUCell. Additionally, monocle was used for trajectory analysis to determine the evolution of ESCC immune subgroups, and cellphoneDB was used to analyze the communication between subtypes.

Result and discussion

Six major cell types were identified: T-cells, B-cells, epithelial cells, fibroblasts, endothelial cells, and myeloid cells. For better classification, we reclustered each of the major cell types. The epithelial cells with copy number changes, visualized by inferCNV, were separated from normal epithelial cells. Gene set analysis was performed to characterize the function of different epithelial cell subgroups, such as inflammatory-related, cell cycle-related, RNA-high, and migratory phenotypes. A fibroblast cluster with matrix metalloproteinase marker 11 (MMP) and high expression of the epithelial-mesenchymal transition gene set was found to correlate to progression-free survival. Immune cells that infiltrated tumors were identified by comparing the proportions in tumor tissues and adjacent normal tissues or blood. The TREM2-high macrophages upregulated MMPs and VEGFA, which could assist the migration process. Additionally, the immunosuppressive T-cell subtype expressing PD-1 was identified using cell markers. The trajectory analysis and receptor-ligand interaction are underway to unravel the biological progression of various cell types and how they communicate within the ESCC microenvironment.

Conclusion

Our study broadened our knowledge of ESCC tumorigenesis and ecosystem by revealing the connection between epithelial, stromal, and immune cell subtypes.

EACR25-1809

Raf Kinase Inhibitor Protein (RKIP) plays a role more than scaffolding protein by modulating cytokines in colorectal Cancer cell lines

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Introduction

As a scaffolding protein, raf kinase inhibitor protein (RKIP/PEBP1) binds lots of proteins important for cellular signaling pathways, which are associated with various cellular processes including proliferation, differentiation and cellular motility. Mostly, the expression of RKIP might be important for regulating signaling pathways through its binding capacity, however, some cancers express RKIP proteins more than enough, implying its function in cancers.

Material and method

To investigate RKIP's role in colorectal cancer, we have established two RKIP knockout (KO) cell lines by CRISPR-Cas9 system using HCT116 a human colon cancer cell line, and validated RKIP KO effect on variety of cellular processes including cell proliferation, motility, and aggregation in suspension culture system.

Additionally, to evaluate cytokine expression, we performed a Cytometric Bead Array (CBA) assay using culture supernatants from parental, MOCK, and RKIP KO HCT116 cells.

Result and discussion

As expected, two RKIP KO cell lines, HCT116 RKIP KO#3 and KO#4 showed reduced cell proliferation up to 20 % (significant but not drastic) and aggregation growth in suspension culture, and increased motility compared to HCT116 parental and MOCK cell lines. Transcriptomic profiling showed enriched gene sets associated with cell cycles, motility, and inflammation. Interestingly, cytokines were ranked highly, which led us to consider the possible role of RKIP on cytokine expression through another binding partners beyond nuclear factor-kappa B-NF- κ B pathway through NIK. Thus, we considered TBK1 as a candidate for modulating cytokines and tumor microenvironment. Flow cytometry-based bead array showed measurable interleukin-8 (IL-8) than the others in HCT116 cells, which was much increased by RKIP KO and reversed by recovering RKIP expression significantly. Thus, we further investigated the relationship between RKIP and TBK1 on IL-8 expression and macrophage polarization.

Conclusion

To further delineate RKIP's role in the tumor microenvironment, we are conducting in vivo studies to explore the therapeutic potential of RKIP as a novel target for metastasis intervention in colorectal cancer.

EACR25-1812

Multiomic profiling of colon cancer sidedness reveals TGF β -driven EMT and Mast cell dynamics as key prognostic factors

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Introduction

Emerging evidence suggests that tumor sidedness is a key prognostic factor in colorectal cancer (CRC). Left- and right-sided tumors differ in embryonic origin, molecular profile and clinical outcome, with left-sided colon tumors (LT) generally having a better prognosis and right-sided tumors (RT) exhibiting more mutations, often linked to greater aggressiveness. Our aim is to perform a multi-omic analysis of LT and RT in order to optimize personalized therapies.

Material and method

A multiomic profiling was conducted on 61 FFPE tissue samples from CRC patients at the Hospital Clínico Universitario of Valencia, Spain. Samples included 32 RT and 29 LT. Genomic and transcriptomic analyses were performed using whole exome sequencing (WES) and RNAseq. Deconvolution analysis was carried out by CIBERSORT and functional enrichment via GSEA utilized a significance threshold of FDR < 0.05.

Result and discussion

Patients with locally advanced RT exhibited a worse disease-free survival (DFS) than those with LT. However, no statistically significant differences were observed in the early stages. Focusing on stage III tumors, 48% of mutated genes were identified exclusively in RT, of which SOX9, MSH3 and NSD1 were related with a lower DFS; while 22% were identified in LT and none of them were associated with a poorer outcome. Moreover, GSEA in stage III RT showed a mutational enrichment in apoptosis, TGFβ signaling and Wnt β-Catenin pathway that was not found in LT. Selecting the genes from the TGFβ signature, an overexpression of TGFB2 and SKIL was observed in stage III RT compared to LT, highlighting the importance of distinctive TGFβ signaling among RT and LT. As TGFβ signaling promotes epithelial-to-mesenchymal transition (EMT) and immune evasion, differential expression of the EMT pathway and tumor immune infiltration between LT and RT were evaluated. 3 genes of the EMT pathway (ID2, SLIT3 and ANPEP) were found upregulated in stage III RT compared to LT. Besides, when focusing on RT an overexpression in 5 EMT genes was observed in stage III in comparison to stage II, suggesting that the poorer prognosis of RT may be due to the TGFβ-driven EMT. Additionally, stage II RT showed more activated mast cells (MC) and increased inflammatory and TNF-α pathway activity compared to stage II LT and stage III RT, suggesting a pro-inflammatory role for MC in stage II RT. Moreover, when dividing primary tumors into TGFβ-high/TGFβ-low by the median expression of TGFβ pathway, most of TGFβ-high were RT and exhibited a significant lower MC infiltration. This suggest a protective role of MC in

early stages, which is lost as the disease progresses in TGFβ-high RT.

Conclusion

Comprehensive multiomic characterization of LT and RT revealed that the poorer prognosis of patients with locally advanced right colon cancer may be due to the TGFβ-driven EMT and the loss of pro-inflammatory MC, enabling the discovery of new therapeutic targets.

EACR25-1816

Oxidative stress promotes Colorectal cancer aggressiveness through transmembrane Chloride Intracellular Channel 1 (tmCLIC1) antioxidant activity

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Introduction

Colorectal cancer (CRC) is the leading cause of cancer-related deaths. The staging of CRC impacts on patient survival, with prognosis closely tied to diagnosis timing. When metastasis is present, treatments are less effective with mostly poor prognosis. In the transition from non-metastatic to metastatic cancer cells, Reactive Oxygen Species (ROS) play a significant role and are involved in multiple processes. Ion channels' activity is closely linked to ROS balance promoting metastasis. Finding a specific target able to tackle oxidative stress or using antioxidants as N-acetylcysteine and glutathione (GSH) could be the key for CRCs therapies, but it was recently seen that metastatic cancer cells rely on GSH to manage oxidative stress and exogenous antioxidants supports cancer cell metastasis. Moreover, many ion channels involved are fundamental in normal physiology. The transmembrane form of Chloride Intracellular Channel 1 (tmCLIC1) protein supports tumoral ROS over-production. In these cells, CLIC1 undergoes a conformational change that release releases GSH into the cytoplasm. Once in the membrane, tmCLIC1 facilitates ROS overproduction, fostering an aggressive cancer phenotype. The peculiar expression of tmCLIC1 in metastatic CRC cells, and its role in ROS balance, make it a promising target.

Material and method

We evaluated CLIC1 level of expression, its activity as a chloride channel, and its involvement in cancer physiology in several human CRC cells chosen at different cancer stages through western blots, patch clamp experiments, 2D and 3D growth curves, migration and invasion assays. Moreover, invitro data were confirmed in vivo on zebrafish and murine models. In addition, we forced non-metastatic CRCs cells to increase tmCLIC1 activity through the addition of N-acetyl-cisteine, and we followed cells behaviour in vitro and in vivo.

Result and discussion

Our findings show that tmCLIC1 protein correlates with cell aggressiveness and that the inhibition of tmCLIC1

activity results in a downregulation of cellular proliferation, migration, and invasion in vitro and in vivo. Moreover, forcing non-metastatic CRC cells to release GSH using the antioxidant N-Acetyl Cysteine (NAC) enriched cytoplasmic GSH and increased tmCLIC1 expression. This effect, however, was absent in cells where CLIC1 was silenced, proving that GSH release depends on tmCLIC1 activity. These cells were converted from non-aggressive cell lines to metastatic ones in vitro and in vivo.

Conclusion

In the present work, we demonstrate that the translocation of CLIC1 in the plasma membrane has a key role in tumor aggressiveness enhancing the transition from non-metastatic to metastatic cells. These findings challenge the traditional view of antioxidants in cancer treatment, suggesting that tmCLIC1 inhibition could serve as a novel therapeutic strategy against metastatic colorectal cancer, addressing a pressing societal health concern.

EACR25-1826

Thrombospondin-1, RANK and OPG interactions in breast cancer osteolytic bone metastasis

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Introduction

Bone is a common site of metastases. Breast cancer commonly develops osteolytic bone metastases, in which excessive bone degradation is due to upregulation of the bone-resorbing osteoclasts. Thrombospondin-1 (TSP-1) is a matricellular protein with a complex modular structure, able to interact with multiple ligands thus playing pleiotropic roles in the microenvironment of primary tumors and metastasis. This study aimed at investigating the role of TSP-1 in bone remodeling associated with breast cancer osteolytic bone metastasis.

Material and method

To assess the effect on osteoclastogenesis, the activity of TSP-1 and its recombinant domains on RANKL-induced differentiation of murine bone marrow precursors and monocyte-like RAW 264.7 cells was tested. Binding of TSP-1 to the RANKL-receptor RANK and to osteoprotegerin (OPG) – key factors in osteoclastogenesis – was analyzed by solid phase assays and Surface Plasmon Resonance. To evaluate the activity on bone metastasis, bone tropic breast cancer 4T1.2 cells, transfected to express and secrete the active TSP-1 fragment, were injected in the caudal artery of mice, and the formation of metastasis-associated osteolytic lesions was monitored by microCT.

Result and discussion

We have identified a C-terminal fragment of TSP-1 active in inhibiting RANKL-induced osteoclast differentiation. Cleavage of TSP-1 by proteases produced by mature osteoclasts released a similar fragment, indicating a possible role as a feedback control mechanism. The fragment bound RANK, the RANKL receptor on osteoclast precursors, and impaired early (the MAPKs p38 and JNK) and late (NFATc1) downstream signaling. The fragment also bound osteoprotegerin (OPG), the decoy receptor of RANKL, in this case further potentiating its inhibitory activity on osteoclastogenesis. In an in vivo model of osteolytic bone metastasis, the expression of the TSP-1 fragment by murine breast cancer cells reduced osteolytic lesions and prolonged mice survival, indicating that the C-terminal TSP-1 fragment is active also in vivo and can protect the bone against metastasis-associated osteolysis.

Conclusion

The release of an active TSP-1 fragment in the bone environment can represent a mechanism to control remodeling and protect the bone against degradation associated with breast cancer osteolytic metastasis.

Supported by grants from AIRC, Fondazione Beppe e Nuccia Angiolini, and MUR under PNRR M4C2II.3 Heal Italia project PE00000019 CUP B43D22000710006 to GT.

EACR25-1855

CRC multi-omics groups reveal distinct tumor microenvironments

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Introduction

Over the past decade, multiple groups have classified colorectal carcinoma (CRC) subtypes based on molecular profiles to explain CRC heterogeneity. Increasing evidence suggests microbial dysbiosis plays a crucial role in CRC development and progression. Our study aimed to identify integrated tumor microenvironmental groups (iTMEg) through multi-omics subtyping based on preselected cancer-related features.

Material and method

Harnessing a unique multi-omics CRC dataset comprising of 6 distinct modalities, we analyzed 138 patients with stage 0–IV CRC (without prior neoadjuvant treatment) using unsupervised consensus clustering of three of the modalities: microbial composition of tumor mucosa, tumor genes expression, and tumor mutational landscape. For further refinement, we studied immune cell fractions inferred from bulk tumor transcriptomics

via xCell, spatial distributions of morphological regions within tumor tissue, and stool-derived microbial and miRNA profiles.

Result and discussion

Through comprehensive multi-omics analysis, we identified 6 distinct iTMEgs, reflecting unique immune, stromal, and microbial compositions. iTMEg 1 (Immune Activated) exhibits an immune-active yet immuno-suppressive microenvironment enriched with oral pathogens such as *Fusobacterium nucleatum*, *Parvimonas micra*; MSI-H status, and BRAF mutations. iTMEg 2 (Immune Desert) represents a CIN-driven, immune-depleted group with low microbial diversity, stromal sparseness, and canonical APC and TP53 mutations. Stromal-enriched tumors divided into two groups: iTMEg 3 (Immune Excluded), showing a desmoplastic phenotype with EMT activity, noncanonical WNT signaling, and NRAS mutations; and iTMEg 6 (Highly Immuno-genic), characterized by active KRAS signaling, *Bacteroides* enrichment, and an immune-activated stromal microenvironment. Mucinous tumors stratified into three groups: iTMEg 1 and iTMEg 4 (Regulated Immunity), both MSI-H, but differing in immune and microbial profiles; and iTMEg 6. iTMEg 5 (Immune Suppressed), in contrast, displays a structured, immune-low profile with complex tubular morphology and PIK3CA mutations. Partial associations of iTMEgs were observed with CMS and iCMS.

Conclusion

Our approach offers a novel, more comprehensive perspective on the tumour microenvironment beyond existing molecular classifications. These groups suggest potential of more targeted therapeutic strategies, including immune checkpoint inhibitors for immune-active groups, stromal modulation for desmoplastic groups, and targeted therapies for CIN-driven tumors.

EACR25-1856

Mouse intraductal model of ER+ breast cancer to study the role of HSF1 in its development

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Introduction

Breast cancer is the most common tumor diagnosed in women. The majority of cases are estrogen receptor α positive (ER+). The lack of relevant *in vivo* models hampers the study of the pathogenesis of ER+ breast cancer cells and the finding of the possible way for drug treatment. The recently developed mouse intraductal (MIND) model, based on the breast cancer cell injection directly into the mouse milk duct system, provides an intraductal microenvironment adequate for ER+ breast cancer cell growth and enables the recapitulation of human disease. We use the MIND model to study the role of HSF1 inhibition in ER+ breast cancer growth. HSF1 plays a key role in the cell response to stress and also supports many aspects of cell metabolism enabling tumor growth. We found that HSF1 is activated by 17 β -estradiol (E2) in ER+ breast cancer cells and gains transcriptional competence to regulate the expression of

several genes associated with E2 signaling. HSF1 deficiency reduces the growth of ER+ cells *in vitro* and may be a potential target for breast cancer treatment.

Material and method

The human breast adenocarcinoma MCF7-Luc2 cells expressing luciferase were knockouted for HSF1 using the CRISPR/Cas9 editing system. The growth of MCF7-Luc2 cells HSF1-deficient or HSF1-proficient was analyzed *in vitro*. Tumor cells were injected into the ductal system of NSG (NOD scid gamma) mice. The tumor growth was monitored through bioluminescence imaging (IVIS system).

Result and discussion

The complete lack of HSF1 led to a substantial loss of heat-inducibility of HSP genes (molecular target of HSF1) and a reduction of the proliferation rate of MCF7-Luc2 cells *in vitro*. After injection of MCF7-Luc2 cells with different HSF1 status into the abdominal mammary gland of adult female NSG mice, they grew without hormonal supplementation for 6 months. *In vivo* monitoring of engrafting mice by luminescence revealed that all cell lines grew exponentially, but the growth of HSF1-deficient MCF7-Luc2 cells was slowed down compared to HSF1-proficient cells.

Conclusion

The MIND model offers the opportunity to study the progression of ER+ breast cancer and the role of HSF1 in its development. It can be used to test new combination therapies targeting HSF1 and estrogen receptors.

The work was supported by the National Science Center, grant no. 2021/43/B/NZ5/01850.

EACR25-1868

Extracellular Matrix-Based 3D Head and Neck Cancer Model: A Biofabricated Platform for Testing Melatonin Therapy

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Introduction

Head and neck squamous cell carcinoma (HNSCC) is an aggressive disease with high recurrence rates, partly attributed to the presence of cancer stem cells (CSCs) within the tumor. Melatonin has demonstrated antitumor properties, although its effects on CSCs remain unclear. This highlights the need for models that reproduce tumor heterogeneity to better understand the effects of this molecule. The aim of this study was to develop a tumor model based on decellularized extracellular matrix (dECM) derived from fibroblasts, replicating the tumor microenvironment (TME) of HNSCC, to analyze the antitumor effects of melatonin in a context closer to the native scenario.

Material and method

CSCs were isolated from the Cal-27 cell line using specific culture conditions to form tumor spheres. Stromal cells were obtained with informed consent from healthy skin through enzymatic digestion. For dECM-

based bioinks, human fibroblasts were cultured and induced to produce ECM. This matrix was characterized and combined with alginate and gelatin to generate a hydrogel that mimicked native tumors and allowed the culture of HNSCC CSCs and TME cells.

Result and discussion

The fibroblast-dECM retained the expression of molecules and growth factors similar to those found in native tumors. The model confirmed that melatonin exerts an antiproliferative effect on HNSCC CSCs, reducing markers of tumor invasion and migration, even in a chemoprotective microenvironment. Additionally, it did not show toxicity toward the healthy cells co-cultured in the model.

Conclusion

This 3D model represents an advanced tool for studying HNSCC, integrating CSCs, stromal components, and a biomimetic matrix, allowing for more representative investigations of tumor reality and facilitating advances in precision oncology.

EACR25-1876

Cellular and molecular features of the aberrant vascularization and mechanisms of TKI resistance in metastatic clear cell renal cell carcinoma

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Introduction

Clear cell renal cell carcinoma (ccRCC) is one of the most aggressive cancers worldwide, primarily due to its high haematogenous metastatic dissemination. This aggressive behaviour stems from mutations in the Von Hippel-Lindau (VHL) gene, present in approximately 70% of cases, resulting to a pseudo-hypoxic context promoting tumor invasion, angiogenesis and extracellular matrix (ECM) remodelling. Accordingly, ccRCC vasculature displays a higher degree of complexity than the vascular network reported in other solid tumors. In primary tumors and metastases, we recently characterized two types of aberrant endothelial structures exhibiting specific features of dimensions and shapes, distinct from tumor capillaries. Since more than 60% of the metastatic ccRCC patients display poor or no response to anti-angiogenic treatments using tyrosine kinase inhibitors (TKI), we suggest that the vascular architecture complexity may influence the ccRCC clinical outcome. The goal of this study is therefore to deeply characterize the involvement of ccRCC vasculature in tumor development, dissemination and response to treatments.

Material and method

This study was conducted on both cohort of metastatic ccRCC patients and an in vitro 3D model of vascularized micrometastases (VMT). Spatial transcriptomic analyses (Visium & Xenium, 10X Genomics) were performed on ccRCC patients for deciphering transcriptomic profiles

specific of aberrant vascular structures and their close microenvironment.

Result and discussion

Analysis of ccRCC patient cohort allowed us to:

- 1) characterize three distinct patient groups based on the predominant architecture of the vascular network;
- 2) identify the involvement of a specific aberrant structure in the metastatic process. Spatial transcriptomic analyses of these aberrant vascular structures revealed specific profiles of genes related to endothelium development, ECM remodelling and TKI resistance. Using the in vitro VMT model that faithfully recapitulates the ccRCC vasculature, we confirmed that the aberrant structures exhibited distinct sensitivities to TKI treatments.

Conclusion

These combined approaches allowed us to characterize the crosstalk between endothelial and tumor cells potentially contributing to TKI resistance and metastatic dissemination in ccRCC. This project opens promising clinical perspectives for ccRCC patients at advanced metastatic stages, by targeting the aberrant vascular structures involved in tumor dissemination.

EACR25-1886

miR-31-3p inhibition as strategy to increase responsiveness to trastuzumab in HER2 positive breast cancer

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Introduction

Trastuzumab is a powerful targeted therapy for HER2 positive breast cancer (HER2+ BC) patients but a consistent percentage of resistant cases are reported. It is well known that miRNAs are involved in a plethora of biological processes, that their dysregulation is a common feature in cancers, and that they can serve both as biomarkers and therapeutic tools. Analyzing tissue samples from NeoALTTO trial, we identified miR-31-3p as negatively associated with pathological complete response upon neoadjuvant trastuzumab. Here we explored the impact of miR-31-3p modulation on HER2+ BC cell response to trastuzumab.

Material and method

MiRNA overexpression and downmodulation were achieved transfecting respectively a miRNA mimic and an LNA-based inhibitor in human HER2+ BC SKBR3 and HCC1954 cells; miRNA and protein levels were assessed by qRT-PCR and Western blot analyses; cell growth was analyzed by 3D assay. In vivo injection of HCC1954 cells was performed in SCID mice and LNA-31-3p was delivered peritumoral 5 times, twice a week; each injection was followed by trastuzumab treatment. RNA-seq analysis was performed in miRNA-over-expressing/inhibited HCC1954 cells treated or not with

trastuzumab. Ingenuity pathway analysis was used to define gene enrichment in RNA-seq dataset. Cytotoxicity ability of natural killer (NK-92) and macrophages (THP1) cell lines, upon their conditioning with miR-31-3p-overexpressing SKBr3 cells supernatant, was assessed by using 51Cr-labelled K562 leukemia cells as target. Correlation analysis between miRNA expression and 60 immune-related metagenes was performed by considering Spearman Corr. coeff. >0.30 in absolute value.

Result and discussion

Overexpression of miR-31-3p upregulates both HER2 levels and activation status in both SKBR3 and HCC1954 cells. These effects are paralleled by increased cell number at baseline and after exposure to trastuzumab. Moreover, miR-31-3p inhibition significantly reduced the in vivo growth of HCC1954 xenografts in trastuzumab-treated mice. Trastuzumab efficacy greatly relies on its ability to trigger an immune response; data from RNAseq analysis of HCC1954 cells revealed that miR-31-3p inhibition induced a significant upmodulation of genes involved in Interferon α/β signaling, regardless of trastuzumab treatment. Moreover, the conditioning of NK-92 and THP1 cells with the supernatant of miR-31-3p-overexpressing SKBr3 cells significantly reduced immune cell cytotoxicity. Accordingly, miR-31-3p expression negatively correlated with the presence of activated immune cell subsets in the NeoALTTO series.

Conclusion

miR-31-3p inhibition holds a promising potential as adjuvant strategy to increase trastuzumab efficacy by simultaneously reducing tumor growth and taming the immune response in HER2+ BC.

EACR25-1910

Adaptation to chromosomal instability in colorectal cancer creates vulnerabilities in epigenetic and growth regulators

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Introduction

Chromosomal instability (CIN) is a hallmark of most solid tumors, including colorectal cancer (CRC). CIN arises from errors in sister chromatid segregation and drives tumor initiation, progression, and therapy resistance. However, there is evidence that excessive CIN exerts an oncosuppressive effect, triggering mitotic catastrophe and immune responses. This suggests that neoplasms evolve mechanisms to keep CIN at tolerable levels during their progression. These adaptations may create specific dependencies in CIN tumors, which could be targeted therapeutically.

Material and method

We used CRC patient-derived spheroids (PDSs) and organoids (PDOs) characterized for their genome, transcriptome, genetic instability status and therapy response. We developed multiple experimental strategies to modulate CIN, including the induction of whole-genome duplication (WGD) with antimitotics, followed by limiting dilution to generate clones with redoubled ploidy. Of note, these models enable the study of cell plasticity, as they are enriched for (PDSs), or derived from (PDOs), stem-like cells. To assess transcriptional changes associated with CIN and evaluate chromatin accessibility, we performed RNA-Seq and ATAC-Seq. CIN cell vulnerabilities were investigated through high-throughput drug screening and proteomics studies.

Result and discussion

Transcriptomic profiling of PDSs and PDOs with low vs. high CIN revealed transcriptional rewiring associated with increased CIN, coupled with the overactivation of multiple epigenetic regulators, including histone post-translational modifiers and chromatin remodelers. These analyses also uncovered heterogeneity among CIN cell lines, with a CIN subtype displaying global mitotic deregulation. ATAC-Seq studies confirmed differences in chromatin accessibility between primary CRC cells with distinct CIN levels. To identify specific vulnerabilities of CIN cells, we first performed a high-throughput immunofluorescence microscopy-based screening in CRC cells with low vs. high CIN. The screening led to the identification of some epigenetic drugs including inhibitors of histone ubiquitination/methylation enzymes and chromatin remodelers, which reduce proliferation and viability by disrupting the DNA replication process. In parallel proteomic and functional studies in low vs. high CIN CRC cells, we revealed a role for the mTOR pathway kinase RPS6KB1 in promoting CIN tolerance in CRC while supporting cell plasticity, growth and survival.

Conclusion

In conclusion, using CRC patient-derived models we demonstrated a complex transcriptional rewiring associated with increased CIN, as we identified epigenetic regulators (histone modifiers and chromatin remodelers) and growth signaling regulators (RPS6KB1) as crucial drivers of CRC adaptation to CIN. These findings could pave the way for the development of therapeutic strategies targeting CIN-associated vulnerabilities in CRC.

EACR25-1914

Harnessing Mechano-Modulation: Enhancing Nano-Immunotherapy Efficacy by Targeting the Tumor Microenvironment in Sarcomas and TNBC

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Introduction

Nano-immunotherapy has shown significant potential for improving outcomes in cancers such as desmoplastic

sarcomas and triple-negative breast cancer (TNBC), but its effectiveness is limited by the tumor micro-environment (TME), which includes factors such as tumor stiffness, vascular dysfunction and poor drug delivery. Mechanotherapy and ultrasound sono-permeation offer potential solutions to modulate the TME and enhance treatment efficacy. Our study explores the synergy of these strategies to optimize nano-immuno-therapy in sarcoma and TNBC models.

Material and method

In vivo experiments were performed using two murine sarcoma and two TNBC models. First, ultrasound sono-permeation was applied using a clinical devise, with parameters optimized in our study. Mice were treated with the mechanotherapeutic ketotifen, sonopermeation, Doxil nanomedicine, and a cocktail of immune checkpoint inhibitors (ICIs). Tumor stiffness and perfusion were assessed using Shear Wave Elastography (SWE) and Contrast Enhanced Ultrasound (CEUS). Flow cytometry and proteomic analysis were used to evaluate immune response, and machine learning identified the most valuable parameters that can predict treatment efficacy.

Result and discussion

Ultrasound sonopermeation with mechanical index MI = 0.6 and number of cycles NoC = 32 significantly optimized improved tumor perfusion and drug delivery in both sarcoma and TNBC models. Ketotifen further enhanced these effects by reducing tumor stiffness and interstitial fluid pressure. The combination of sono-permeation, ketotifen and nano-immunotherapy (Doxil and ICIs) produced the most significant therapeutic outcomes, including tumor regression and improved survival, leading to complete cure. In TNBC models, this combination improved perfusion, increased immune cell infiltration and elevated the levels of chemokines and cytokines at the tumor site. Machine learning identified tumor stiffness and immune cell profiles as key biomarkers of therapeutic success.

Conclusion

The combination of mechanotherapy and ultrasound sono-permeation effectively modulates the TME, enhancing drug delivery, tumor perfusion, and immune responses. This approach significantly boosts nano-immunotherapy efficacy, offering a promising strategy to overcome treatment barriers in desmoplastic cancers and TNBC, with potential to improve survival in resistant tumors.

EACR25-1928

Tunable Biomimetic Hydrogels to Reproduce the Breast Tumor Microenvironment for Advanced 3D Cancer Modeling

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Introduction

Breast cancer is one of the leading causes of cancer-related deaths among women worldwide [1]. Traditional 2D cell cultures fail to replicate the 3D architecture of tumors, while animal models often lack translational accuracy [2]. Advanced 3D models, including hydrogels and bioprinted scaffolds, better mimic the tumor's biomechanical and biochemical properties, providing a more reliable platform for studying cancer progression and drug response [3]. The research activities planned within this project focus on the development and biological characterization of biomimetic scaffolds for breast tumor tissue.

Material and method

Four different concentrations of alginate-gelatine (ALG-GEL) hydrogels were prepared. All groups were weighed at various time points, to assess their swelling capacity and examine their subsequent degradation for long time incubation at 37°C. pH variations were monitored for different time points. A morphological investigation of the prepared hydrogels was also conducted using SEM. To assess if the polysaccharide structure of ALG was preserved after the hydrogel formation and to assess the successful incorporation of gelatin in the system, FTIR was performed. Different rheological analyses were performed on all the prepared samples.

Result and discussion

All samples showed high swelling after 2h incubation and a slight degradation trend after 21 days of incubation. FTIR analysis confirmed successful GEL incorporation within the hydrogels preserving the structure of ALG. SEM imaging revealed a uniform reticulation among all the prepared samples. Rheological analysis showed a G' value around 10 kPa, demonstrating a good fitting for mimic breast cancer tumor's mechanical properties. The different hydrogel formulations are under investigation to assess their capability to support the cell viability of breast cancer cells (the MDA-MB-231 cell line) and allow their distribution inside the hydrogel structure.

Conclusion

The results obtained demonstrated that the incorporation of gelatin effectively modulates the mechanical properties of alginate-based hydrogels, allowing for a more accurate replication of breast tumor tissue stiffness. These hydrogels successfully formed 3D structures capable of mimicking the complexity of the tumor micro-environment, making them valuable models for studying breast cancer progression and response to treatments. Future research will focus on optimizing their bio-printability. By incorporating selected cell lines, these bioprinted models could provide a powerful platform for in vitro cancer research, offering new opportunities to investigate tumor behavior, drug responses, and cell-matrix interactions in a controlled and physiologically relevant setting.

- [1] URLs doi.org/10.1016/j.breast.2022.08.010, doi.org/10.1016/j.intimp.2020.106535
 [2] URL doi.org/10.5114/aoms.2016.63743
 [3] URL doi.org/10.3390/bioengineering10010017

EACR25-1939

In vivo CRISPR/Cas9 screening identifies key genes driving prostate cancer progression and metastatic tropism

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Introduction

Metastatic prostate cancer patients (mPCa) exhibit lower survival rates compared to those with localized tumors. This is mainly due to the complexity of the molecular mechanisms governing malignant cells dissemination, which contributes to the inefficacy of existing treatments. Therefore, to improve the prognosis and diagnosis of mPCa patients, it is essential to elucidate the mechanisms underlying metastases. To address this, our study focuses on the identification of key regulators of mPCa progression using high-throughput CRISPR/Cas9 screenings.

Material and method

To identify novel factors that promote the acquisition of the metastatic phenotype we have developed an in vivo gain of function (GoF) genome-wide screening using orthotopic inoculation of low-metastatic PCa cells (LNCaP) infected with a genome-wide SAM library. Moreover, the temporal and spatial components of metastasis were examined by analysing the detection of gRNAs in 5 tissues (bone marrow, lung, liver, lymph nodes and brain) of the animals at either 4 (early metastasis)- or 6 (late metastasis) weeks post orthotopic inoculation of PCa cells.

Result and discussion

Our findings reveal distinct patterns of key biological processes and pathways involved in dissemination, which vary depending on the target tissue, as well as an enrichment of specific gRNAs in late-stage metastases compared to early metastases. These results highlight the influence of both the microenvironment and time dependent factors on metastatic evolution. The early metastasis analysis identified FGF8 as a common metastatic driver in bone marrow (BM), lymph nodes and liver, while in late metastasis this gene was only significant in BM. FGF8 has been previously described as a metastatic promoter thereby validating our system. BM is the most common metastatic destination in

prostate cancer. By comparing early vs late metastasis BM upregulated gRNAs, we have identified 4 common genes: NELL2, ADAM2, DLG5 and FGF8, of which NELL2, ADAM2 and DLG5 have been exclusively identified in BM. NELL2 and ADAM2 were more enriched in early BM metastasis, suggesting they might be involved in primary metastatic stages like colonization and survival. Conversely, DLG5 and FGF8 were more enriched in late BM metastasis and thus might be regulating proliferation and metastatic outgrowth. Moreover, among the BM exclusive genes we have identified transcriptional repressors ZNF202 and MXI1 as drivers of early and late metastasis, respectively, both with prognostic value in both prostate and breast cancer.

Conclusion

This approach has allowed us to study the whole metastatic cascade in vivo, facilitating the identification of the different tropisms acquired by disseminated tumor cells in response to the host microenvironment, as well as the discovery of new prognostic biomarkers and/or therapeutic targets which could be essential for mPCa treatment.

EACR25-1941

Lung fibroblasts regulate disseminated tumor cells survival, dormancy and progression to metastasis through FGFR2 pathway

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Introduction

Metastasis is a primary cause of mortality among patients with solid tumors. Disseminated tumor cells (DTCs) migrate to secondary organs, where they enter a reversible quiescent state known as dormancy, before contributing to metastasis. The microenvironment within secondary organs is recognized as a crucial regulator of dormancy. Our research has demonstrated that neural derived molecules such as neuropilin 2 (NRP2), regulate DTCs proliferation in the lung. Additionally, our findings indicate that NRP2 upregulation in lung DTCs is modulated by lung stromal cues such as TGFb1 secreted by lung fibroblasts (LF). Within this framework, we are investigating the lung stroma as a regulator of the transition from dormancy to proliferation in head and neck cancer. Moreover, we are focusing on FGFR2 as a potential mechanism underlying this process, a receptor tyrosine kinase that is frequently mutated in head and

neck cancer and has been described to regulate fibroblasts mediated treatment resistance in breast cancer.

Material and method

To investigate the interaction between LF and DTCs, we utilize healthy LF cell lines and four head and neck cancer cell lines as an *in vitro* dormancy model. These include a primary tumor-derived cell line (T-HEp3), a lung DTC-derived cell line (Lu-HEp3), a bone marrow DTC-derived cell line (BM-HEp3), and a dormant cell line (D-HEp3). Conditioned media (CM) are collected from these models and employed in various functional assays. To assess the role of FGFR2 in DTCs regulation, we use pharmacological inhibitors and have generated FGFR2 knockdown cell lines to examine their phenotype and behavior. Additionally, we employ the chorio-allantoic membrane (CAM) assay to evaluate tumor growth under distinct experimental conditions.

Result and discussion

Our results show that treatment with LF CM promotes Lu-HEp3 cells proliferation both *in vitro* and *in vivo* and activates ERK signaling. Furthermore, treatment of LF with CM from proliferative and dormant cell lines revealed that CM from dormant cell lines induces LF activation, suggesting there is a bidirectional crosstalk between dormant DTCs and LF. Additionally, FGFR2 is overexpressed in Lu-HEp3 cells. Pharmacological and genetic inhibition of FGFR2 induces cell cycle arrest in G1 phase, promotes dormancy markers upregulation and reduces proliferation *in vitro* and *in vivo*. Moreover, in dormant cell lines, FGFR2 inhibition induces apoptosis, suggesting FGFR2 plays a critical role in sustaining dormant DTCs survival.

Conclusion

Our findings suggest that LF, upon activation by dormant DTCs, promote DTCs release from dormancy facilitating metastatic progression in the lung. Moreover, our data indicate that the FGFR2 signaling pathway regulates both DTCs proliferation and survival, highlighting its potential as a therapeutic target in metastatic disease.

EACR25-1968

Mapping of human prostate tumour heterogeneity using AtlasPlex immunofluorescence approach

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Introduction

The progression from normal to neoplastic state is characterized by continuous changes in epithelial cell phenotype and in tissue microenvironment. Detailed characterization of this process is crucial for understanding tumour biology, discovery of novel biomarkers and identification of therapeutic targets. Multiplexed immunohistochemistry/immunofluorescence (mIHC/IF) has been successfully used for studies of tumour biology. However, such studies are often limited by the availability of the primary antibodies from different host species, a need for harsh tissue treatment in between the staining cycles or requirement for complex automated

platforms. In the present study, the newly developed mIHC/IF technology ‘AtlasPlex’ was employed for studies of tumour microenvironment in transition from normal to cancer state in human prostate.

Material and method

AtlasPlex was used to perform mIHC/IF for the evaluation of tissue microenvironment in normal (n = 6), benign hyperplasia (n = 6) and prostate cancer (n = 5) FFPE human tissue sections. KRT5 (HPA059479), TP63 (AMAb91224) and AMACR (HPA019527) were used as markers for normal and neoplastic glands. CD3E (HPA043955), CD8A (HPA037756), and CD68 (HPA048982) were utilized as markers of T cells, cytotoxic T cells, and tumour associated macrophages respectively. The AtlasPlex workflow was as follows: primary antibodies were biotinylated and labeled with HRP. A sequential multiplexed TSA-IF assay was then performed using 2 or 3 epithelial and 1 immune cell markers at a time. MetaSystems slide scanner equipped with epifluorescence and appropriate filters was used for image acquisition.

Result and discussion

Normal and benign hyperplasia prostate glands displayed strong KRT5 and TP63 positivity in the basal cell layer, while cancerous glands were negative. In contrast, AMACR positivity was high in the tumour glands, while normal prostate and benign hyperplasia glands showed negative or occasional weak positivity. Analysis of immune cell count indicated significant increase in number of CD3E-positive T cells in cancer samples (normal 71+/-10 vs cancer 176+/-14 cells), CD8A cytotoxic T-cells (normal 204+/-39 vs cancer 559 +/- 108 cells) and CD68 tumour associated macrophages (normal 97+/-21 vs cancer 269+/-27 cells, assessed per 9 FOVs, 1.4 mm²). Both T-cells and macrophages were observed in close proximity to the cancer glands. No significant changes were observed in benign hyperplasia.

Conclusion

The present study demonstrated how AtlasPlex mIHC/IF could be successfully used to analyse normal and tumour tissue microenvironment in prostate cancer. The AtlasPlex approach to combine the StreptaClick-HRP and the TSA-IF technologies allowed multiplex tissue analysis using the same species primary antibodies, with signal quality and assay sensitivity equal to that of gold standard chromogenic IHC.

EACR25-1969

Monitoring Lynch syndrome colonic organoid engraftment in mice cecum

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Introduction

Lynch syndrome (LS) is a hereditary pan-cancer syndrome caused by constitutional pathogenic variants in DNA mismatch repair (MMR) genes (MLH1, MSH2, MSH6, PMS2). Despite intensive surveillance interval

colorectal Cancers (CRC) are common in LS carriers. In vivo modelling of the early events mechanisms leading to MMR-deficient carcinogenesis may lead to its better understanding critical to improve surveillance strategies. The aim of our study is to assess the feasibility of the engraftment of normal tissue-derived colonic organoids from LS patients implanted in the mouse cecum.

Material and method

Colonic tissue-derived organoid lines were generated from biopsies of normal appearing mucosa of 3 LS carriers (1 MLH1, 2 MSH2) and 1 control without Lynch syndrome. Three of the lines were cultured 3 months (25 passages). Organoids were surgically implanted in the caecal submucosa of immunosuppressed mice ($n = 5-7$ mice/organoid line; total 27) at different passages (2, 7, 11, 17 and 25) as described by Fumagalli et al (PMID 29300390). Mice were sacrificed 4 ($n = 14$), 6 ($n = 7$) and 8 ($n = 6$) weeks after the surgery. Histological characterisation was performed by haematoxylin-eosin (H&E) staining. Expression of anti-human Ku80, MMR (MSH6 and PMS2), Ki67 and MUC2 proteins was assessed by Immunohistochemistry (IHC). In selected cases, PCR amplification of human GAPDH was done.

Result and discussion

Engrafted organoids grew in mice for up to 8 weeks after implantation. H&E staining revealed the presence of rounded structures lined with a thin epithelium growing in the submucosal layer in 24 out of 27 mice (3 cases were non-informative). Their human origin was confirmed by anti-human Ku80 homogeneous staining and amplification of human GAPDH. Expression of MMR proteins was conserved in concordance with the expression observed in paired human biopsies and organoids. Ki67 expression was observed in 30-60% of cells of engrafted organoids while MUC2 was expressed after engraftment *in vivo* while absent *in vitro*.

Conclusion

Normal colonic tissue-derived organoids are able to grow for up to 8 weeks in the cecum of immunosuppressed mice. This tool will be used for the characterization of the early events occurring in MMR-deficient colorectal carcinogenesis.

EACR25-1975

P-Selectin as a Key Regulator of Lung Cancer Brain Metastasis: Altering the Immunophenotype of the Tumor

Microenvironment

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Introduction

Our overarching goal is to understand the molecular and cellular mechanisms by which the P-Selectin/P-Selectin ligand-1 (CD62P/CD162) axis mediates lung cancer

brain metastasis (LCBM) progression. Specifically, we investigate how interactions between lung cancer (LC) cells with microglia polarize their immunophenotype into an anti-inflammatory and pro-tumorigenic state, ultimately remodeling the immune landscape.

Material and method

Multiplex immunofluorescence staining was performed on FFPE tissues from LCBM patients to elucidate CD62P and CD162 localization expression across different cell types. To assess microglia's role in promoting LC cell growth, a 3D organoid model embedded in Matrigel was established, incorporating the entire brain tumor micro-environment (btME) to simulate the clinical scenario. The Transwell migration assay was employed to investigate microglia's role in promoting LC cell migration. A cytokine array assay identified factors that mediate migration through P-selectin interaction in a paracrine model. CD62P's contribution was assessed using either a small molecule inhibitor or a neutralizing antibody.

Result and discussion

Spatial analysis revealed that CD62P and CD162 were significantly overexpressed in LCBM tissues compared to healthy controls. Interestingly, CD62P was found to be positive in astrocytes, microglia, CD8+ T cells, endothelial cells, and LC cells. Moreover, the co-expression of the immune markers, PD-L1 and PD-1, with CD62P-positive cells suggested potential pathways for immune modulation. In spheroids embedded in Matrigel with the whole btME, microglia significantly enhanced LC cell proliferation, migration, and invasion, which were markedly reduced by CD62P inhibition. Transwell migration assays confirmed that soluble CD62P promotes the migration of LC cells toward microglia. Additionally, cytokine profiling confirmed that the paracrine interaction between microglia and LC cells upregulated pro-tumorigenic cytokine secretion. Furthermore, inhibition of CD62P using a neutralizing antibody diminished these effects and altered cytokine profiles.

Conclusion

Inhibiting the CD62P/CD162 axis could reprogram microglia and tumor-associated macrophages from a pro-tumorigenic to an anti-inflammatory state, potentially enhancing the adaptive immune response against LCBM. Further exploration of CD62P/CD162 in LCBM may offer novel strategies to reverse brain immuno-suppression and improve patient outcomes.

EACR25-1976

A Novel Transgenic Mouse Model to Assess On-Target Off-Tumor Toxicities of HER2-Targeted Therapies

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Introduction

HER2-targeted therapies have revolutionized the treatment of HER2-positive tumors. However, these therapies can cause severe toxicities due to HER2 expression in healthy tissues, where it is present at low levels. These on-target off-tumor effects can lead to life-threatening complications particularly with novel therapies in clinical

trials. Therefore, there is a need for preclinical mouse models that faithfully recapitulate human HER2 expression patterns in mice to properly evaluate the safety of HER2-directed therapies before use in humans.

Material and method

Our transgenic mouse model was generated using the embryonic stem (ES) cell method combined with CRISPR-Cas technology. Murine ES cells were modified *in vitro*, selected with antibiotic resistance, expanded and analyzed via Southern blot. Genotyping of mice was performed by PCR with primers specific to the human HER2 and mouse Erbb2 genes. The expression of HER2 and Erbb2 mRNA across different organs was assessed by RT-qPCR. Protein expression of human HER2 was analyzed by western blot and immunofluorescence in various mice tissues.

Result and discussion

We successfully developed a novel transgenic mouse model in which the human HER2 gene is knocked-in under the control of the murine Erbb2 promoter. Genomic validation by southern blot confirmed the successful insertion of HER2 in several mouse ES cells, and both heterozygous and homozygous mice bearing the human HER2 gene were obtained. RT-qPCR analyses revealed tissue-specific expression of human HER2 mRNA in organs like the lungs, kidneys or heart, correlating with murine Erbb2 expression patterns in healthy murine tissues and the presence of the human HER2 protein was confirmed in the lungs and kidneys. As proof of concept, the administration of HER2-targeted murine CAR-T cells in transgenic mice resulted in preliminary evidence of toxicity, consistent with the expected on-target off-tumor adverse effects.

Conclusion

In conclusion, this novel transgenic mouse model represents a valuable tool for the preclinical assessment of HER2-targeted therapies, enabling the study of their on-target off-tumor toxicities before clinical translation. This murine model will facilitate the development of safer and more effective HER2-directed strategies for the treatment of HER2-positive tumors.

EACR25-1983

Exploring tumor-suppressing fibroblast population in ovarian adenocarcinoma

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Introduction

Among the various stromal cell types within the tumor microenvironment, cancer-associated fibroblasts (CAFs) are the predominant component, exhibiting diverse oncogenic functions. Despite their potential as therapeutic targets, no effective strategy exists to neutralize CAF activity due to the lack of specific markers and their heterogeneous nature. Current anti-tumor approaches indiscriminately deplete CAFs, overlooking the existence of both pro- and antitumor subpopulations in the tumor, which makes these therapies toxic to the organism. A more promising strategy

involves reprogramming CAFs into a uniform anti-tumor state, necessitating the identification of CAF subsets with exclusively tumor-suppressive properties, which is the objective of this study.

Material and method

To examine the distinctive features of CAF transcriptome, we integrated multiple public datasets, including 11 scRNA-seq datasets of different tumor tissues, mRNA-seq data from 934 cancer cell lines (CCLE), 10,000 patient tumor samples (TCGA), and 10 scRNA-seq datasets of normal tissues (HPA). Public scRNA-seq data of ovarian adenocarcinoma were analyzed to identify CAF subpopulations and their transcriptomic differences. The effect of IFNγ-pretreated dermal fibroblast secretome on SKOV3 cell migration was assessed using a scratch test. Xenium-based spatial transcriptomics analysis was performed to determine the localization of tumor-suppressive CAFs in ovarian adenocarcinoma.

Result and discussion

Developing a custom marker identification algorithm based on comprehensive bioinformatic analysis of large RNA sequencing datasets allowed us to identify a set of genes specific to CAFs across 11 tumor types. In ovarian adenocarcinomas, we identified a CAF population whose higher abundance correlates with a favorable prognosis. This population specifically expressed genes activated in response to IFNγ, which are upregulated in ovarian tumors compared to normal ovarian tissue. Additionally, we demonstrated that the secretome of IFNγ-pretreated fibroblasts reduces SKOV3 ovarian cancer cell migration *in vitro*. Spatial transcriptomics analysis revealed that these fibroblasts are localized within IFNγ aggregates, enriched with epithelial cells, macrophages, and endothelial cells exhibiting enhanced IFNγ response gene expression, as well as CD8+ T cells.

Conclusion

Our findings reveal the existence of a tumor-suppressive CAF population in ovarian adenocarcinoma, characterized by an IFNγ-associated gene signature and its correlation with favorable prognosis. Additionally, our results provide insights into selecting the most appropriate combination of CAF markers across different tumor types. These findings enhance our understanding of CAF heterogeneity and its role in the tumor microenvironment.

This work was carried out with state funding for the "Immunopeptidome" project, state registration number of R&D 124031200004-7.

EACR25-1998

ADAR1-Mediated RNA Editing as an Oncogenic Driver in Prostate Cancer

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Introduction

Prostate cancer (PCa) stands as the second most frequently diagnosed cancer and the second leading cause of cancer-related death in men. The biggest challenge in PCa is treatment resistance and managing metastatic

disease progression. Recent studies have highlighted the role of RNA editing in the pathogenesis of cancer. Adenosine deaminase acting on RNA (ADAR1) binds to double-stranded RNA (dsRNA) and catalyzes the conversion of adenosine to inosine through a process known as A-to-I editing. Previous literature confirmed a role for ADAR1 in multiple cancer hallmarks, but its function in PCa has not been fully elucidated. The aims of this project were to explore the functional role of ADAR1 in PCa as a potential oncogenic driver using *in vitro* assays and the clinical potential of ADAR1 as a biomarker using clinical cohorts.

Material and method

ADAR1 levels were compared among benign prostate tissue, primary tumour, and bone metastatic samples. *In vitro*, the effect of ADAR1 knockdown on proliferation and cell-to-cell adhesion on different PCa cell lines was investigated. Furthermore, tissue microarray cores from radical prostatectomy of 230 patients with PCa were immunostained for ADAR1. ADAR1 levels were compared between cancerous and paired non-cancerous areas, and their association with clinical outcomes was investigated.

Result and discussion

In silico data analysis showed a significant increase of ADAR1 levels in bone metastases compared to the benign and primary group. ADAR1 knockdown significantly reduced A-to-I editing levels and inhibited PCa cell proliferation in different PCa cell lines. On the other hand, ADAR1 knockdown increased cell-to-cell adhesion in different PCa cell lines. Data from the tissue microarray cores showed that ADAR1 expression in the epithelial compartment of cancerous tissue was significantly higher compared to paired non-cancerous areas. Additionally, ROC curve analysis showed that epithelial ADAR1 levels demonstrated a moderate ability to differentiate between benign and cancerous tissues, with an AUC of 0.72. Finally, high ADAR1 expression was associated with an increased risk of biochemical recurrence (BCR).

Conclusion

In conclusion, the findings of this study shed light on the potential role of ADAR1 in PCa progression and its clinical significance, suggesting ADAR1 as a potential target for the development of diagnostic and therapeutic strategies.

EACR25-2010

An mRNA isoform produced by intronic polyadenylation as a new signature in tobacco associated cancers

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Introduction

Tobacco smoking is responsible for approximately 2.5 million cancer deaths annually worldwide, being the primary preventable risk factor for lung, bladder, and head and neck (H&N) cancers. The associated burden on health care systems emphasizes the need for a better understanding on how this carcinogen affects our cells, as well as in new therapeutic strategies for these cancers. Cancer cells tend to express mRNA isoforms with short 3'UTRs, by alternative polyadenylation in the 3'UTR (3'UTR-APA) and intronic polyadenylation (IPA), which may affect the function of genes involved in tumorigenic processes, and lead to the dysregulation of important signaling pathways. Here, we investigated whether lung, bladder and H&N cancers present a specific profile of “mRNA signatures”, which may serve as new biomarkers or therapeutic targets for cancers linked to smoking habits.

Material and method

Bioinformatic analysis was performed using TCGA RNA-seq data from lung, bladder and H&N cancers, as well as in-house 3'mRNA-seq of samples obtained by surgery of heavy-smokers with H&N cancers at Institute of Portuguese Oncology (IPO-Porto). The obtained mRNA isoforms were validated by 3'RACE and RT-qPCR, using samples obtained from IPO-Porto of H&N cancer patients. *In vitro* analysis of protein translation was performed using a coupled transcription/translation cell free assay. mRNA stability was analyzed by Actinomycin D assays.

Result and discussion

The mRNA signatures obtained from the analyses of the TCGA data, that are present in smoker patients while absent from non-smoker patients, were intersected with the ones obtained from our in-house 3'mRNA-seq, identifying 9 common smoking-specific mRNA signatures. We decided to further study one of these signatures, an IPA mRNA isoform from PCGF3, as it may produce a new protein with a still unknown function. PCGF3 is known to be a component of Polycomb group multiprotein complex, a complex involved in epigenetic modifications. We validated PCGF3 expression in samples obtained from IPO-Porto of H&N cancer patients showing that this IPA mRNA isoform is upregulated in tumors when compared to normal tissues. *In vitro*, we showed that the truncated IPA mRNA isoform can lead to the production of a protein, even though it loses all the canonical protein domains, crucial for its function. In fact, after translation, this IPA isoform shares only the first 36 amino acids with the canonical PCGF3 protein. Curiously, the mRNA stability of both the canonical and the intronic isoforms, is very similar, despite their inherent differences.

Conclusion

Overall, we have identified an unknown protein, with new functions to unravel and a possible role in smoking-related cancers. By studying this smoking-specific mRNA signature, common to three different types of cancer, we can open new possibilities for innovative therapeutic targets.

EACR25-2019

Efferocytosis by peritoneal metastasis-associated fibroblasts reprograms the tumour microenvironment to promote cancer progression

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Introduction

Anti-cancer therapies, such as chemotherapeutic drugs, function by inducing apoptosis in cancer cells. The clearance of apoptotic cells (ACs) by efferocytosis is essential for maintaining tissue homeostasis and promoting an anti-inflammatory microenvironment. It has been long thought that efferocytosis is primarily carried out by professional phagocytes, such as neutrophils and macrophages. However, emerging evidence arises that also fibroblasts are able to execute this process. In addition, cancer-associated fibroblasts (CAF), a major component of the tumor stroma, are known to acquire distinct polarization status such as myoCAFs or immunomodulatory CAFs contributing to various aspects of tumor progression. In this study, we investigated the efferocytosis capacity of fibroblasts from normal tissue, primary colorectal cancer (CRC) and peritoneal metastasis and explored whether their phenotype polarization is altered upon the engulfment of ACs.

Material and method

Normal fibroblast (NF), CAFs from CRC and metastasis-associated fibroblasts (MAFs) were isolated from healthy and tumour tissues, as well as from the peritoneal fluid of patients with colorectal peritoneal metastases (CPM).

Efferocytosis capacity was assessed using fluorescence microscopy and flow cytometry. Gene expression analysis was performed on efferocytosing MAFs. To examine possible immunomodulatory effects of efferocytosing fibroblasts, MAFs were co-cultured with monocyte-derived macrophages and macrophage polarization was analyzed by flow cytometry. Moreover, tumor growth was assessed by using conditioned media of efferocytosing MAFs.

Result and discussion

MAFs exhibited remarkable higher efferocytosis capacity compared to normal peritoneal fibroblasts and fibroblasts from early-stage colorectal cancer. Efferocytosing MAFs showed increase in immunomodulatory CAF markers and a decrease in myoCAF markers indicating a switch in fibroblast phenotype. Following efferocytosis, MAFs showed increased expression of epidermal growth factors and cytokines. Correspondingly, tumor cell proliferation assays demonstrated an increased growth rate of colorectal cancer cells when cultured with CM from efferocytosing MAFs.

Conclusion

Our study demonstrates that fibroblasts possess the ability to efferocytose apoptotic tumour cells. Particularly, MAFs from peritoneal metastases exhibit a high efferocytosis capacity. After the uptake of apoptotic cell debris MAFs acquire a specific polarization status,

modifying immune cells and tumor cells in their microenvironment. This may contribute to the progression of peritoneal metastasis in CRC.

EACR25-2021

Intratumoral heterogeneity in microsatellite instability status at single cell resolution

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Introduction

Subclonal diversity within a tumor is highly relevant for tumour evolution and treatment. The cumulative diversity is referred to as intratumoral heterogeneity (ITH) and high ITH can complicate the interpretation of single-test biomarkers. Microsatellite instability (MSI) is one such biomarker, which is used to help guide immune checkpoint inhibitor treatment by classifying samples as either having high microsatellite instability (MSI-H) or as being stable (MSS). Interestingly, one area that has yet to be addressed in depth is whether MSI itself is heterogeneous. To address this question, we collected publicly available single-cell sequencing data that had paired MSI status and developed a computational pipeline to identify MSI-H cells and assess ITH in MSI.

Material and method

We curated a collection of 56 publicly available samples from across 32 individuals that underwent 10X, 3' single-cell RNA sequencing. Data was downloaded in either matrix or BAM format and were then processed with a custom Snakemake pipeline. Briefly, the pipeline identifies clusters of cells using Seurat, distinguishes cancer from normal cells with scATOMIC, and then identifies cells as being MSI-H or MSS with MSIsensor-RNA. Samples are then subset to only cancer cells and are re-clustered. Heterogeneity in a sample is then summarized using the analysis of variance F test statistic based on the MSI scores obtained from each cancer cell cluster. This is complemented by differential gene expression analysis between clusters.

Result and discussion

Our results suggest that heterogeneity in MSI status is more prevalent than has been previously reported. Five of the 32 individuals analyzed showed evidence of divergence in MSI status between distinct clusters of cancer cells ($F > 30$). The individual with the highest level of heterogeneity ($F = 75.2$) had seven distinct cancer cells clusters comprising a total of 779 cells. In contrast, the F statistic of the individual with the lowest level of heterogeneity was 5.1 (based on 6 cancer cell clusters comprising 470 cells). Tukey HSD analysis showed 17 significantly different cluster pairs for the individual with high heterogeneity whereas only five were found for the individual with low heterogeneity. These results are reinforced by differential gene expression between MSI-H and MSS cells. The individual with high heterogeneity had nine differentially expressed genes, whereas the individual with low heterogeneity only had two.

Conclusion

We provide the first single cell resolution analysis of ITB in MSI status. Our results suggest that heterogeneity in MSI is more common than previously reported. Further studies are warranted to determine the frequency of heterogeneity in this biomarker at the population level, and whether the presence of both MSI-H and MSS subclones can have clinical impacts, including the potential to result in more rapid evolution of resistance to treatments for which MSI-H is a biomarker.

EACR25-2035

ERBB Signalling contributes to immune evasion in KRAS-driven lung adenocarcinoma

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Introduction

In lung cancer patients, immunotherapy is now considered a frontline, standard of care therapy. However, the responses seen are varied and often transient, and a deeper mechanistic understanding of tumour:immune interactions and the dynamic immune response to evolving tumours is required. Modelling these processes in GEMMs has proven challenging, primarily due to a low mutation burden and consequent lack of immune visibility of most allelic combinations. APOBEC mutagenesis signatures are prominent in lung cancer and activity is predicted to drive immune visibility through Cytidine deaminase activity and inaccurate DNA-repair response. We have previously shown that initiation and progression of KRAS-driven lung tumours require input from ERBB family receptor tyrosine kinases. Here we utilise the immunogenic KMA model of lung cancer to investigate the immunosuppressive nature of ERBB signalling.

Material and method

Lung tumours were induced in mice carrying conditional alleles *Isl-KRasG12D*, *Rosa26DM.Isl-MYC*, +/- *Rosa26DS.Isl-APOBEC3B*, (KMA or KM respectively) by intranasal inhalation of Adeno-SPC-CRE. Mice were treated with anti-PD1 immune checkpoint blockade and harvested at defined timepoints and analysed by IHC and ScRNA-SEQ. Tumour bearing mice were treated with anti-PD1 +/- a multi-ERBB inhibitor, Afatinib, and examined acutely or monitored for survival benefit.

Result and discussion

Inclusion of the APOBEC allele drove a transiently effective T cell mediated immune response, resulting in extended survival of KMAs compared with KMs. Temporal analysis showed pronounced CD8 tumour infiltration at 8 weeks in KMA lungs but immune exclusion by 12 weeks post induction. PD1 blockade was ineffective, but via a single cell sequencing analysis, we discovered a surprising increase in tumour cell expression of EGFR/ERBB ligands following treatment and present evidence that transient ERBB blockade can restore immune surveillance in KRas mutant lung adeno-

carcinoma and combine effectively with immune checkpoint blockade. We also found additional immune suppressive mechanisms to be upregulated following PD1 blockade, and the potential of these targets will be discussed.

Conclusion

Inhibition of ERBB signalling can combine effectively with immune checkpoint blockade to restore immune infiltration in KRas driven lung adenocarcinoma. We believe the KMA model has the potential to transform our understanding of dynamic anti-cancer immunity and its collapse in lung cancer.

EACR25-2037

PRMT7 as key driver of metastatic process in prostate cancer

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Introduction

Prostate cancer (PCa) patients diagnosed with metastasis have a poor survival rate of less than three years, primarily due to the inefficacy of current strategies to inhibit metastatic progression. Therefore, identifying new therapeutic targets is critical to improving the survival of metastatic PCa (mPCa) patients. Our studies using CRISPR/Cas9 screenings have identified PRMT7, a methyl transferase, as a new essential player in mPCa which depletion significantly impairs the invasive capabilities of metastatic prostate cancer (mPCa) cell lines.

Material and method

In order to better understand the molecular mechanism displayed by PRMT7 we have examined the metastatic dissemination of PCa tumor cell in vivo and the viability of cells in circulation. The molecular basis of this mechanism was further explored through proteomic and chromatin analysis.

Result and discussion

Mechanistically, PRMT7 appears to promote metastasis establishment by inducing an adhesion molecule switch in prostate cancer cells, indirectly through the methylation of transcription factors. We have performed orthotopic injections of mPCa cells into the prostate of murine model and observed that cells expressing PRMT7, form larger tumors and disseminate to key metastatic sites, including the liver, lungs, bone marrow, and brain. On the other hand, tumors derived from PRMT7-depleted cells were smaller and exhibited reduced lesions in these metastatic niches. However, cell survival analysis of cell under suspension conditions and circulation was similar in both cells suggesting a main role of PRMT7 in colonization. The results indicate that PRMT7 plays a crucial role in the regulation of RNA splicing through methylation of splicing factors (SF) like

hnRNPA1. Our analysis showed a significant up-regulation of SF in mPCa cells expressing PRMT7 compared to cells depleted of PRMT7. To further investigate its role in alternative splicing, we conducted MAJIC analysis from RNA sequencing data, identifying metastatic relevant genes regulated by PRMT7. Additionally, chromatin accessibility (ATAQ-seq) analysis showed that PRMT7 is essential for chromatin accessibility and expression of splicing factors and adhesion genes.

Conclusion

PRMT7 may serve as a novel biomarker for poor prognosis in PCa patients through the regulation of splicing of metastatic relevant genes. Furthermore, targeting the inhibition of this protein could potentially improve the life expectancy of patients with mPCa.

EACR25-2068

Functional role of unannotated small RNA cluster 1 (smRC1) during hepatocarcinogenesis and angiogenesis

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Introduction

Despite the revolution in precision oncology, primary liver cancer lacks early detection biomarkers and actionable mechanisms of carcinogenesis are poorly understood. We previously identified unannotated, small non-coding RNAs (smRCs) from circulating extracellular vesicles and developed a signature for early cancer detection (von Felden et al. Gut 2022).

Material and method

Lentiviral CRISPR/Cas9-mediated genome editing was used to generate clonal smRC1 knockout Huh7 cells and validated by RTqPCR. HUVEC cells were treated with either conditioned medium obtained from the Huh7 clones, or EVs isolated from the medium by size-exclusion chromatography. EVs were labelled using ExoGlow EV-labelling kit and EV-positive cells were visualised with a fluorescence microscope. We performed functional assays on both cell types: proliferation curve, MTT assay, morphogenesis assay on Matrigel®, colony formation assay and cell migration on Transwell® supports. Epithelial-to-mesenchymal-transition markers were quantified by RTqPCR, Western Blot, immunocytochemistry. EGFR pathway activation was measured with Human Phospho-Kinase Array Kit and Western Blot. Angiogenesis markers were quantified by RTqPCR and Human Angiogenesis Array Kit. Apoptosis activation by caspase enzymes was measured using Caspase-Glo® 3/7 assay. RNA sequencing was conducted on smRC1-KO and wildtype control clones followed by differential gene expression analysis (DESeq2 package). Spatial transcriptomic analysis (RNAscope™) was performed on primary HCC tissue.

Result and discussion

To investigate smRC1 functional role in carcinogenesis, we established an in vitro CRISPR/Cas9 model to modulate the expression in HCC cell lines. smRC1 expression was associated with increased tumour proliferation, morphogenesis, clonogenic ability and migration, as well as epithelial-mesenchymal transition, apoptosis and EGFR-pathway activation. Interestingly, genes related to angiogenesis were >2-fold upregulated (FDR<0.05) (e.g. FGF2, FGF18, PDGFB, HDAC9 and TMSB4X). Human Umbilical Vein Endothelial Cells (HUVEC) were treated with smRC1 positive and negative EVs, and conditioned medium, respectively, from the wildtype and knockout HCC clones. First, we confirmed the uptake of fluorescently-labelled EVs by HUVEC cells. Spatial transcriptomic analysis on FFPE tumour slides confirmed smRC1 positive expression in the intra-tumoural vessels. Functionally, EVs and conditioned medium from smRC1 expressing HCC cells led to increased proliferation, migration, morphogenesis, as well as increased expression of endothelial activation markers, such as FGFR1, KDR, PECAM and CHD5, in treated HUVEC cells. In order to confirm these findings *in vivo*, chick chorioallantoic membrane (CAM) assay experiments are planned.

Conclusion

Our data suggest a tumourigenic effect of smRC1 expression in HCC cells as well as pro-angiogenic effect of smRC1 on endothelial cells.

EACR25-2070

Bidirectional interaction between Schwann cells and cancer cells of Hepatocellular carcinoma: implications for tumor progression

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Introduction

In cancer research, the role of peripheral nerves has been extensively studied over the past few decades, with increasing attention recently focusing on Schwann cells (SCs), the primary glial cells of the peripheral nervous system. Tumor-activated SCs have been shown to

significantly contribute to cancer progression by modulating the tumor microenvironment (TME) and directly interacting with cancer cells through both paracrine signaling and physical contact. Hepatocellular carcinoma (HCC), the most common primary liver tumor, remains one of the leading causes of cancer-related deaths worldwide. While several studies have highlighted the potential role of autonomic innervation in HCC development and progression, the specific role of SCs in this context remains largely unexplored. To address this gap, we aimed to investigate the biological effects and molecular mechanisms underlying the communication between SCs and cancer cells in the HCC.

Material and method

We decided to focus on the paracrine crosstalk between SCs and HCC cells, employing an *in vitro* approach based on conditioned media (CM), to explore the potential impact of this dialogue on both HCC cells aggressiveness and SCs reprogramming. We used the human hepatoma Hep3B cell line and human SCs, treating each cell type with either control CM (derived from the same cell type) or with CM from the other cell type. The effects of these treatments were assessed through functional assays and analysis of molecular and morphological profiles.

Result and discussion

Our results show that Hep3B cells treated with SCs-CM, compared to those treated with control CM, exhibit more aggressive features associated with tumor spread, including enhanced migration, a doubled capacity for matrigel invasion, changes in the protein levels of epithelial-to-mesenchymal transition markers (N-cadherin, E-cadherin, Vimentin) and structural and topographical alterations evaluated by atomic force microscopy. Concurrently, paracrine signals from Hep3B cells induce a chemotactic response in SCs, promoting processes such as proliferation, migration, matrigel invasion, and the upregulation of repair-related markers (GFAP, N-cadherin), thereby driving their activation.

Conclusion

Overall, our findings demonstrate a bidirectional interaction between SCs and HCC cells, emphasizing the importance of a deeper understanding of the glial component in the TME of HCC, as it could significantly influence tumor progression.

EACR25-2074

Neurosurgery in glioblastoma induces proneural-to-mesenchymal transition, therapy resistance and immunosuppression by ischemic hypoxia

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Introduction

Glioblastoma (GBM) is the most lethal and prevalent malignant brain tumor, with patients facing a median survival of 8 months and only 6.9% reaching five-year survival. The standard of care includes surgical resection of the tumor, radiotherapy, and concomitant chemotherapy with temozolomide. Despite aggressive multimodal therapy, GBM invariably recurs after surgical resection. However, the immediate impact of surgery on residual tumor biology remains poorly understood.

Material and method

To investigate the impact of surgical resection on residual GBM tumor cell plasticity and TME, we developed sophisticated preclinical neurosurgical oncology models. We used two syngeneic GBM mouse models and one patient-derived xerograph and performed fluorescent-guided neurosurgery of sized-matched GBMs. For clinically relevant rodent models of neurosurgical oncology, we replicated each step of the neurosurgical procedure performed in patients with GBM and we used the same materials. In these models, we employed time-resolved bulk- and single-cell transcriptomics, proteomics, epigenomics, digital pathology, and intravital microscopy. Next, to examine the specific effects of surgical intervention on tumor progression in patients, we assembled an international, multicenter cohort of GBM patients who underwent two surgical procedures within a period of less than 10 days. For these particularly rare samples, we conducted a longitudinal investigation comprising neuropathological analysis, single nuclei and spatial transcriptome.

Result and discussion

In both preclinical and clinical samples, we demonstrated that surgical intervention triggered a rapid cascade of events in the residual GBM. Specifically, surgery induces acute vascular disruption and leakage leading to profound hypoxia, which drives proneural-to-mesenchymal transition and extensive chromatin remodeling in tumor cells. This hypoxic environment fosters a robust immunosuppressive tumor microenvironment, characterized by increased infiltration of hypoxic tumor-associated macrophages, Tregs, and neutrophils, along with diminished antigen presentation. Comprehensive multi-omics inter- and intracellular network analyses demonstrated that postsurgical pathways converge on hypoxia-inducible factor (HIF)-1a as a central node. Critically, these postsurgical alterations confer increased resistance to conventional therapies.

Conclusion

These findings revealed a critical window of vulnerability immediately after surgical resection, suggesting that targeted interventions during this period could significantly improve GBM outcomes.

EACR25-2075

CD24-mediated differential platelets-tumor cells binding tunes metastatic progression

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Introduction

While the interaction among platelets and tumor cells in the circulation has been described several decades ago, whether all tumor cells (TCs) are capable of equally bind platelets remains unclear. We recently highlight that different TCs display different abilities to bind platelets, relying on their contribution in distinct stages of the metastatic progression. Yet, the molecular determinant of such, and downstream consequences, remain unidentified. Here, we identified CD24 as a new TC rheostat regulating platelet binding and their subsequent pro-metastatic function.

Material and method

By screening publicly available databases, we assessed CD24 expression across a collection of murine and human TCs and further confirm its expression by flow cytometry. Based on CD24 expression levels, we selected high CD24-expressing cells (4T1) and low ones (B16F10 and 67NR) for the continuation of the study. These cells were testified for their platelets binding ability by immunofluorescence correlating this data with their CD24 expression. To demonstrate its involvement, we silenced CD24 expression by a shRNA approach generating the shCD24 4T1 cell line. After assessing in vitro their platelets binding abilities, we followed in vivo their metastatic progression in an experimental metastasis model. We also dissected the metastatic immune tumor microenvironment and the local secretome. Thanks to a short TCP regimen, we evaluate the early contribution of platelets to metastatic progression in the same in vivo scheme.

Result and discussion

Across both murine and human TCs, CD24 expression correlates with platelets binding ability. Furthermore, high CD24 expression is a poor prognostic factor in triple-negative breast cancer (TNBC) and, indeed, we observed high CD24 expression in metastatic TNBC cells (4T1) while its expression is reduced in non-metastatic isogenic counterpart (67NR). When CD24 expression is reduced by a shRNA approach (shCD24 4T1 cells), platelets binding abilities reaches levels comparable to the ones of low-binding cells. In vivo, we demonstrated that CD24 expression tunes the metastatic progression, rewiring, at the same time, the immune micro-

environment and the local secretome. By shortTCP regimen, we provide a proof-of-concept that this effect is due to early platelets-TCs interaction in a CD24-mediated fashion.

Conclusion

Overall, our work identifies CD24 to be, in part, responsible, for the differential binding of platelets to TCs and the subsequent pro-metastatic function of platelets. In addition, these findings identify CD24 as a potential druggable target to counteract platelet-TC interaction and metastatic progression, avoiding the collateral hemostatic perturbations of classical anti-platelet agents.

EACR25-2078

Development of a microphysiological system reflecting breast cancer lymph node metastasis

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Introduction

A critical step in disease spread involves the crosstalk between the primary tumour and lymph nodes, which drives remodeling of the pre-metastatic niche and facilitates cancer cell invasion. As the number of involved lymph nodes increases, patient survival decreases. Extracellular vesicles (EVs) play a significant role in this intercellular crosstalk. This study employs a 3D dynamic fluidic in vitro model to study crosstalk between the primary breast tumour and lymph node.

Material and method

MDA-MB-231, a triple negative breast cancer cell line, was transduced with CD63-conjugated green fluorescent protein (MDA-GFP), ensuring any released EVs would also carry GFP. Primary tumour stromal cells (TSCs) and lymphatic stromal cells (LSCs) were isolated from patients' tissue. Multicellular breast cancer (MBC) spheroids were generated using MDA-GFP cells and pre-stained Dil labeled-TSCs. Lymphatic spheroids were formed using LSCs. Spheroids were encapsulated in alginate on day 3 and transferred to inserts lined with Matrigel containing human umbilical vein endothelial cells (HUVECs) or lymphatic endothelial cells (LECs) respectively. Interconnected inserts were introduced to the multi-in vitro organ system (MIVO®) with a pump applied for dynamic flow to mimic blood flow. Confocal microscopy was used to image GFP and Dil and for Live/Dead assays. Embedded spheroids were also sectioned for histology.

Result and discussion

MDA and TSC were successfully labeled with GFP and Dil respectively, and confocal microscopy revealed the cells interspersed and integrated throughout the spheroid. Live/Dead assay showed similar viability in the presence or absence of alginate-encapsulation. Cell death in the spheroid core increased over time. Haematoxylin and Eosin (H & E) staining revealed extracellular matrix (ECM) deposition in the spheroid centers while cells around the periphery were polarized and migratory. In the presence of HUVECs which formed branching tubules in matrigel, tumour spheroids developed

branching smaller spheroids which extended beyond the alginate encapsulation. Migrating cells and small spheroids appeared to align with the net of tubules. Transfer of GFP from the tumour to the lymph node spheroid in the interconnected MIVO system was observed, likely originating from MDA-GFP secreted EVs taken up by the lymphatic spheroid.

Conclusion

The MBC spheroid encapsulated in alginate and co-cultured with HUVECs provides a robust approach to model tumour development. The 3D dynamic fluidic model successfully demonstrates crosstalk between MBC and lymphatic spheroids, offering a promising platform for testing novel therapeutic strategies to impede disease progression.

EACR25-2080

miR-574-5p is a regulator of ARV7 expression in a potential allele-specific manner

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Introduction

The Androgen Receptor (AR) is a key oncogenic driver in prostate cancer (PCa). As a result, therapies targeting the AR signaling axis, such as androgen receptor signaling inhibitors (ARSI), remain the gold standard for treating advanced PCa. However, resistance to these therapies inevitably develops. One of the primary mechanisms of ARSI resistance is the upregulation of constitutively active AR variants (AR-Vs), with AR-V7 being the most clinically relevant. AR-V7 mRNA contains a distinct 3' untranslated region (UTR) compared to full-length AR (AR-FL) with different single nucleotide polymorphisms (SNPs) and short tandem repeats (STRs), suggesting differences in post-transcriptional regulation. This finding opens up the possibility that micro RNA (miRNA), known to play a role in post-transcriptional regulation, could potentially be therapeutically exploited in a combination therapy with ARSI.

Material and method

To identify ARV7-specific miRNAs that could be affected by the presence of SNPs in the 3' UTR, a bio-informatic pipeline was used. Expression of differentially binding miRNAs was assessed via publicly available datasets (GSE21036 and GSE60117). Five different miRNA/ SNP combinations were selected for further wet lab validation

Result and discussion

miRNA-133b – rs140988926 G/A, miRNA-133a-3p – rs140982926 G/A, miRNA-140-5p – rs5918762 T/C, miRNA-574-5p – rs7065530 A/T, and miR-324-5p –

rs147764148 G/A were selected for validation. From these five miRNAs, only one, miR-574-5p, was found to bind AR-V7 3' UTR in an allele-specific manner, with rs7065530 alternative T allele as preferred allele, as shown in a dual-luciferase assay. Furthermore, transfection of miRNA-574-5p in AR-V7 positive DuCaP and 22Rv1 cell lines led to a decrease of ARV7 expression, while AR-FL expression remained unchanged. ARV7 downregulation was the strongest in 22Rv1. Currently, the effects of miRNA-574-5p on cellular proliferation are evaluated by live cell imaging.

Conclusion

Our study identifies miR-574-5p as a regulator of AR-V7 in an allele-specific manner, preferentially binding the rs7065530 T allele and reducing AR-V7 expression. Differences in binding efficiency between cell lines suggest a potential influence of STR rs760106489. These findings provide new insights into miRNA-mediated AR-V7 regulation and its possible role in ARSI resistance.

EACR25-2107

Genetically modified M1 macrophages secreting IL-12 as a potential melanoma anticancer therapy

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Introduction

Macrophages are phagocytic cells with remarkable plasticity and heterogeneity that serve a multitude of functions, including tissue homeostasis, regulation of inflammatory responses and have a crucial role in the cancer development. Macrophages undergo different polarization states, pro-inflammatory, anti-cancer M1 phenotype and anti-inflammatory, pro-tumorigenic M2 phenotype. Tumor associated macrophages (TAMs) are responsible for induction of immunosuppression by secreting specific cytokines. Interleukin 12 (IL-12) is a cytokine which activates the effector T-helper 1 cells. The importance of macrophages in the tumor micro-environment (TME) has generated interest in therapeutic approaches. Broadly, the strategies can be divided into two groups: reducing the number of TAMs or altering their functionality within the TME for instance reprogramming TAMs from M2 to M1 phenotype. The aim of the study was development of cell therapy using M1/IL-12/RFP macrophages that destroy tumor cells by secretion of IL-12 protein into TME and transform existing TAM (M2) pro-tumor macrophages into M1 anti-tumor ones. Studies also determined the influence of modified macrophages on the systemic immune response.

Material and method

The experiments were conducted on bone-marrow derived macrophages from C57BL/6NCrl mice. Using adenovirus transduction macrophages were modified to express IL-12 and red fluorescent protein. The efficiency of the transduction was determined by flow cytometry. The amount of IL-12 secreted by modified macrophages was assessed by ELISA. The fluorescence of M1/IL-12/RFP was observed through *in vivo* imaging systems

(IVIS). The M1/IL-12/RFP macrophages were assessed in murine B16-F10 melanoma. Tumor growth was monitored. Parameters of complete blood count in control and M1/IL-12/RFP-treated mice have been analyzed using the hematology analyzer. Additional post-therapeutic analyses were performed to observe changes in the tumor microenvironment.

Result and discussion

Our data show that transduction of primary macrophages depends on multiplicity of infections and cells phenotype. There was noticed that undifferentiated macrophages were more susceptible to transduction than polarized ones. M1/IL-12/RFP macrophages release IL-12 to the medium. In vivo studies showed that M1/IL-12/RFP demonstrate fluorescence after intra dermal injection into mice up to 72h and that second dose prolonged the fluorescence up to 8 days. It has been shown that two doses of M1/IL-12 inhibited B16-F10 tumors growth and increased number of immune cells in mice blood.

Conclusion

The results demonstrate that genetically modified macrophages secrete IL-12 and have influence on tumor growth inhibition and number of the innate and adaptive immune cells. However, further research is needed to investigate their precise effect in the tumor micro-environment.

The work was financed by National Science Center Poland UMO-2021/41/B/NZ5/03245.

EACR25-2113

Cell viscosity influences hematogenous dissemination and metastatic extravasation of tumor cells

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Introduction

Metastases arise from a multi-step process during which tumor cells change their mechanics in response to micro-environmental cues. While such mechanical adaptability could influence metastatic success, how tumor cell mechanics directly impacts intravascular behavior of circulating tumor cells (CTCs) remains poorly understood.

Material and method

In the present study, we demonstrate how the deformability of CTCs affects hematogenous dissemination and identify the mechanical profiles that favor metastatic extravasation. Combining intravital microscopy with CTC-mimicking elastic beads and mechanically-tuned tumor cells, we demonstrate that the inherent properties of circulating objects dictate their ability to enter constraining vessels.

Result and discussion

We identify cellular viscosity as the key property that governs CTC circulation and arrest patterns. We further demonstrate that cellular viscosity is required for efficient extravasation and find that properties that favor extravasation and subsequent metastatic outgrowth can be opposite.

Conclusion

Altogether, we identify CTC viscosity as a key biomechanical parameter that shapes several steps of metastasis.

N.B: This study is available on BiorXiv (doi.org/10.1101/2024.03.28.587171) and currently in revision in Nature Materials.

EACR25-2152

xRNA in Ovo: Target Validation and Efficacy Studies Using Chicken Embryo Model (CAM Assay)

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Introduction

Recently, the chicken ChorioAllantoic Membrane (CAM) assay has been used as an in vivo model for cancer research. In fact, the CAM assay is a highly vascularized extraembryonic membrane surrounding the embryo, which is a good soil for tumors to grow and provides the condition for studying the tumor metastatic invasion in different organs and tissues. Besides, at the Embryonic Development Day (EDD) 9 when tumor cells are xenografted, the embryo is an “adult” embryo and has all organs formed and functional, including an active immune system. Therefore, this model is suited for the development of different types of anti-cancer therapies. Here we used the CAM assay to investigate the therapeutic activity of different RNA-based approaches.

Material and method

On EDD 9, a small window is made in the eggshell and allows easy access to the CAM. Cancer cell lines are then grafted onto the CAM and allowed to form tumors. From EED10 to EDD18 (end of the experiment) tumors can be treated with RNA-based therapeutics. Different therapeutics approaches have been used in ovo:

- (1) either through cell modification in vitro followed by graft in ovo or
- (2) by treating tumors with RNA directly in ovo.

The effect of RNA mutation (1) or RNA therapeutic’s treatment (2) is evaluated through different parameter: the tumor weight, the histopathological analyses of tumors (classical staining or immunohistochemistry), the immune cell infiltration (by RT-qPCR), the detection of metastases in specific target tissues, the analysis of the angiogenic network surrounding the tumor and the analysis of the toxicity (mortality rate and/or abnormal development of the embryo).

Result and discussion

In the first approach, the efficacy of siRNA, miRNA, RNAi and anti-sens RNA in various cancer types was studied:

- On PC9 non-small cell lung cancer model, the expression of a siEx8 reversed the resistance of tumors to gefitinib by blocking a specific variant of the autophagic gene ATG16L1b.
- On LNCaP prostate cancer model, the miR-135a inhibited ROCK1 expression and led to a significant regression in tumor cell invasion ability. In contrast, the inhibition of mTOR by a combination of 2 RNAi induced an increase in metastatic invasion.
- On A375 melanoma cancer model, the suppressive effect of the anti-sens RNA (LADON) in the TGF β pathway inhibited the cancer cell invasion. In the second approach, the efficacy of miRNAs was evaluated: - On

SK-OV-3 ovarian cancer model, the combination of one miRNA with doxorubicin showed an additive effect of both compounds.

- On BT-474 breast cancer model, the anti-miRNA loaded in lipid nanoparticle shows no effect on tumor, while reduced by 50% the metastatic invasion in the lower CAM.

Conclusion

The CAM model is a relevant alternative *in vivo* model that can be used for evaluating the efficacy of RNA-based therapies, in just a few days, with various interpretations on tumor growth, metastasis and as well as deeper analyses, like transcriptomics or proteomics.

EACR25-2155

Dissecting KRAS G12C inhibitor resistance at the transcriptomic level

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Introduction

Oncogenic RAS mutations drive approximately 30% of all carcinomas, with KRAS being the most frequently altered isoform. Despite the historical challenge of targeting KRAS due to its structural and biochemical properties, significant progress has been made with the development of KRAS G12C inhibitors, including sotorasib and adagrasib, which are FDA-approved for treating KRAS G12C-mutated Non-Small Cell Lung Cancer. However, the emergence of adaptive and acquired resistance mechanisms limits the long-term efficacy of these inhibitors. These findings underline the critical need for further research to identify novel therapeutic targets to overcome resistance. Here we uncovered adaptive transcriptomic shifts, spanning gene expression and alternative splicing rewiring, linked to resistance against adagrasib and sotorasib, shedding light on the molecular mechanisms driving therapeutic evasion.

Material and method

RAS-less mouse embryonic fibroblasts (MEFs) and human lung cancer cell lines (LUAD: H23, LUSC: CALU1) were engineered to express the KRAS G12C mutation and exposed to escalating doses of sotorasib and adagrasib until resistance emerged. Total RNA sequencing was employed to map transcriptomic adaptations at the gene and exon level. Differential expression and pathway enrichment analyses were exploited to identify significant gene/exon expression dysregulation and pathway activation.

Result and discussion

RNA-seq analysis uncovered distinct transcriptomic reprogramming in response to prolonged treatment, highlighting key pathways implicated in resistance. Epigenetic regulation, alternative splicing, translational control, and epithelial-to-mesenchymal transition (EMT) emerged as significantly altered processes upon sotorasib and adagrasib. MAPK signaling pathway was

significantly dysregulated in both mouse and human cell lines. Interestingly, sotorasib-resistant mouse and human samples showed higher expression of genes associated with metabolic pathways and cell signaling. Strikingly, splicing factors were dysregulated in all resistant cells, with MBNL1 being the strongest candidate. This impacted on the splicing landscape of resistant cells, with differential inclusion of exons in genes contributing to actin cytoskeleton and cell adhesion and potentially impacting EMT.

Conclusion

Our findings pinpoint that cells dynamically rewire their transcriptome upon sotorasib and adagrasib administration through the dysregulation of splicing factors. Adaptive resistance to these KRAS G12C inhibitors converges on MAPK signaling disruption and isoform remodeling in cell plasticity and adhesion pathways. This regulatory shift is conserved across mouse and human cells, with the strongest effects observed under adagrasib treatment. Our results uncover new therapeutic opportunities to overcome KRAS G12C inhibitor resistance by targeting RNA-based mechanisms.

EACR25-2165

miRNA and circRNA signatures in liquid biopsy for lung cancer early screening

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Introduction

Liquid biopsy-based biomarkers present a promising approach for non-invasive cancer detection. miRNA and circRNA signatures provide advantages such as high stability, easy accessibility, and sensitivity to tumor microenvironment, making them ideal candidates for early cancer detection. To identify a miRNA and circRNA signatures in sputum and plasma samples as a non-invasive biomarker for early detection of non-small cell lung cancer (NSCLC).

Material and method

A case-control study was conducted with individuals enrolled in the Barretos Cancer Hospital Lung Cancer Screening Program (Brazil). Sputum and plasma samples were collected from high-risk controls (based on NLST and/or PLCO criteria; n=61) and non-metastatic NSCLC patients (n=62), matched for age, sex, and smoking

history. The nCounter HumanV3 miRNA panel (Nanostring™) was used to analyze miRNA expression, with the total counts normalized by the top stable miRNAs, followed by differential expression analysis ($p < 0.05$). The miRNA selection was performed using Random Forest, Boruta and LASSO approaches. Machine learning models (ML) were applied (Azure) to determine lung cancer based on differential miRNA expression between cases and controls, with model performance evaluated using the area under the curve (AUC). For circRNA analysis, plasma samples were obtained from controls ($n = 100$) and NSCLC patients ($n = 86$) from Hospital Universitari Quirón Dexeus (Spain). The nCounter Elements custom panel (Nanostring™) was used to analyze the expression of 74 circRNA, and total counts normalized by housekeeping, followed by differential expression analysis using Random Forest, and Recursive Feature Elimination for model adjustment.

Result and discussion

In plasma, 68 miRNAs were identified as differentially expressed, and 6 miRNAs were selected. The best ML model (Voting Ensemble) for the 6-miRNA plasma signature showed high accuracy (AUC = 0.91; CI 95% 0.86-0.97) in distinguishing cases and controls. In sputum, 46 miRNAs were identified as differentially expressed, and 6 miRNAs were selected. The best ML model (SVD, Logistic Regression) for the 6-miRNA sputum signature demonstrated high accuracy (AUC = 0.92; CI 95% 0.86-0.97) in distinguishing cases and controls. The two defined signatures exhibited no overlap in the miRNAs between the two fluids. Regarding circRNAs, 4 circRNAs were identified with high accuracy (AUC = 0.75; CI 95% 0.83-0.97) and a odds ratio greater than 4 (OR = 4.28, range: 2.32-7.88).

Conclusion

This study identified two distinct miRNA fluid-specific signatures for plasma and for sputum, along with a circRNA-based signature in plasma samples. These signatures have the potential to serve as non-invasive biomarkers for the early detection of lung cancer, enhancing precision-based screening and supporting better clinical decision-making in the management of lung nodules.

EACR25-2173

Histamine dihydrochloride induces myeloid cell production of the anti-metastatic protein thrombospondin-1

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Introduction

Metastatic disease accounts for the majority of deaths among cancer patients and new anti-metastatic treatment options are thus highly warranted. Thrombospondin-1 (TSP-1) is a natural endogenous angiogenic inhibitor that may suppress metastasis in cancer patients. In addition to reducing angiogenesis, TSP-1 is reported to modulate the tumor microenvironment via effects on macrophage

polarization. Platelets are the main source of TSP-1, but it may also be produced by other cell types, including myeloid cells. In preclinical studies, myeloid-cell-derived TSP-1 was shown to limit the metastatic burden in mice orthotopically injected with metastatic tumor cells.

Material and method

Primary human monocytes and myelomonoblastic PLB-985 cells were cultured in the presence or absence of histamine dihydrochloride (HDC), and supernatants were analyzed for TSP-1 levels. Additionally, TSP-1 production by human monocytes was analyzed following treatment with different cyclic AMP (cAMP) inducers or HDC in the presence or absence of protein kinase A (PKA) inhibitors. In *in vivo* experiments, wild-type (WT) and TSP-1 knock-out (KO) mice received three bi-daily intranasal injections of lipopolysaccharide (LPS) to induce lung inflammation. HDC or vehicle was administered via intraperitoneal (i.p.) injections prior to each LPS dose. Mice were sacrificed one day after the final LPS/HDC administration, and lungs were analyzed for TSP-1, IL-6, and IL-1 β levels by ELISA or Western blot, as well as for myeloid cell infiltration by flow cytometry. Moreover, WT and TSP-1 KO mice were treated systemically with HDC before and after intravenous injection of B16F10 cells, and the number of lung metastases was enumerated three weeks later.

Result and discussion

HDC was found to be a robust inducer of TSP-1 of human and murine myeloid cells *in vitro* and *in vivo*. The TSP-1-inducing effect of HDC was mediated through histamine type 2 receptors via the cAMP/PKA signaling pathway. Systemic administration of HDC suppressed lung metastases from B16F10 melanoma cells in WT mice, but had no effect on metastasis formation in TSP-1 KO mice. Western blot analysis of lung tissues confirmed increased TSP-1 production in HDC-treated WT mice compared to untreated counterparts. In an *in vivo* inflammation model, we found that HDC reduced LPS-induced production of the pro-inflammatory cytokines IL-1 β and IL-6, in the lungs of WT mice. However, this effect was abolished in TSP-1 KO mice.

Conclusion

Together, our findings suggest that HDC stimulates production of TSP-1 from myeloid cells, which may help limit lung inflammation and reduce metastasis formation. Additional studies are however needed to further define the effects of HDC-induced TSP-1 production on neo-angiogenesis, macrophage polarization, and inflammation within the pre-metastatic niche.

EACR25-2176

Spatial profiling of the colonic immune microenvironment reveals modulation by intestinal estrogen receptor beta and sex

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Introduction

Inflammation and immune cells in the tumor microenvironment play a critical role in colorectal cancer (CRC) progression. Notably, sex differences exist in CRC incidence, with men at higher risk. Estrogen has protective effects against CRC, and intestinal estrogen receptor beta (ER β) has been shown to reduce colitis and CRC development. However, its role in shaping the colonic inflammatory immune landscape remains unclear.

Material and method

Using the COMET multiplex immunofluorescence platform, we analyzed colonic tissues from wild-type (WT) and ER β KO-Vil mice (N=32) to assess immune cell infiltration with full spatial context. Unsupervised clustering, spatial image analysis (SPIAT), and manual quantification were performed to characterize immune cell populations. Additionally, plasma cytokine assays were used to evaluate systemic immune responses. Macrophage polarization assays were conducted to examine the effects of CRC signaling on macrophage differentiation *in vitro*.

Result and discussion

Our findings reveal that intestinal ER β plays a role in modulating the colonic immune microenvironment under both healthy and inflammatory conditions. Loss of ER β significantly alters macrophage infiltration, with a pronounced effect in males. Furthermore, we observed regional differences in immune cell distribution. ER β KOVil mice exhibited an amplified systemic immune response to colitis and CRC, suggesting a broader regulatory role for ER β in inflammation. *In vitro*, macrophage polarization assay did not show ER β -dependent differences.

Conclusion

These findings highlight the sex-dependent effects of intestinal ER β in shaping the colonic immune landscape. ER β deficiency leads to altered macrophage dynamics and heightened systemic inflammation, particularly in males. This provides novel insights into the sex-specific development of CRC and suggests the possibility of using ER β -selective ligands as immune modulators in CRC.

EACR25-2177

Lysosome Exocytosis Inhibition Impairs Breast Cancer Cell Invasion

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Introduction

Breast cancer (BC) is the most frequently diagnosed type of cancer and the second leading cause of cancer-related deaths in women, with metastasis being associated with poor prognosis and a decrease in patient overall survival. Metastasis formation requires the acquisition of migratory and invasive capacities by BC cells, allowing them to colonize adjacent tissues and form secondary tumors in distant sites. Previous studies indicate that lysosome exocytosis is upregulated in BC cells, leading to alterations in the tumor microenvironment and

degradation of the extracellular matrix, therefore creating favorable conditions for tumor progression. In this study, we aimed to understand the effect of modulating lysosome exocytosis in BC cell invasion, which can lead to a novel therapeutic strategy to impair BC progression and prevent metastasis formation.

Material and method

To accomplish our goal, we used two BC cell lines: poorly invasive MCF-7 luminal A and highly invasive MDA-MB-231 triple negative BC (TNBC) cells. To modulate lysosome exocytosis, we silenced known regulators of this process, including RAB10 and RAB7A. Additionally, we treated cells with lysosome exocytosis modulatory compounds. Lysosome exocytosis levels were assessed by measuring the release of the lysosomal enzyme β -hexosaminidase, and invasion was evaluated through a transwell assay.

Result and discussion

Our results show that the silencing of RAB7A, an essential regulator of lysosomal trafficking, increases lysosome exocytosis and invasion of MDA-MB-231 BC cells. Conversely, the silencing of RILP, a RAB7A effector that mediates the retrograde transport of lysosomes, as well as late endosome-lysosome fusion, results in decreased lysosome exocytosis and invasion of MDA-MB-231 cells. Similarly, RAB10 silencing leads to a reduction in lysosome exocytosis and impairment of the invasion capacity of this line of TNBC cells. We also employed a drug repurposing strategy and identified two compounds that significantly decrease lysosome exocytosis and invasion of MDA-MB-231 BC cells. Additionally, treatment of MCF-7 cells with ML-SA1, an agonist of the TRPML family of calcium channels, increases both lysosome exocytosis and cell invasion. Overall, our results indicate a direct causal correlation between lysosome exocytosis and BC cell invasion.

Conclusion

Since lysosome exocytosis and BC cell invasion are directly correlated, our study suggests that the inhibition of lysosome exocytosis is a promising strategy to impair BC cell invasion. Furthermore, we identified two compounds that can be used towards this goal, which can lead to the impairment of metastasis formation and improvement of BC patient prognosis and overall survival.

EACR25-2182

A Vascularized Tumor-on-Chip Model for Patient-Relevant Drug Screening in Non-Small Cell Lung Cancer

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Introduction

The tumor microenvironment (TME) plays a critical role in cancer progression, metastasis, and therapy resistance.

Tumor cells dynamically interact with stromal components and the vasculature, influencing drug responses. While patient-derived xenografts (PDX) are widely used in preclinical research, they lack human immune components and are costly, time-consuming, and poorly scalable. Advanced in vitro microphysiological systems provide an alternative by incorporating patient-derived tumor cells, stromal components, and functional vasculature to better mimic the TME. Here, we developed a vascularized tumor-on-chip model to investigate the influence of microvascular networks on tumor drug response, with a focus on cisplatin treatment in non-small cell lung cancer (NSCLC).

Material and method

A microphysiological system was developed using a microfluidic platform where human endothelial cells and fibroblasts were embedded within a fibrin-based hydrogel to self-assemble into a perfusable microvascular network. NSCLC patient-derived tumor spheroids were placed onto the vascular network to allow integration and vascularization. Cancer-associated fibroblasts (CAFs) were induced from normal fibroblasts using tumor-conditioned medium to enhance stromal interactions. Vascular function was assessed by permeability assays and endothelial marker staining. Tumor responses to cisplatin were evaluated by live-cell imaging, apoptosis assays, and vascular integrity analysis.

Result and discussion

The model successfully established a functional tumor-vascular interface, with tumor spheroids integrating into the microvascular network. VEGF stimulation promoted endothelial sprouting toward tumor spheroids, confirming angiogenic interactions. Chemotherapy response analysis revealed reduced tumor viability and increased apoptosis in vascularized tumors, highlighting the impact of microvascular perfusion on drug delivery. Notably, tumor vascularization influenced cisplatin sensitivity, demonstrating the importance of incorporating functional vasculature in drug testing platforms. The presence of CAFs further modified drug responses, emphasizing the role of stromal interactions in therapeutic outcomes.

Conclusion

This vascularized tumor-on-chip model provides a robust, patient-relevant platform for studying tumor-stroma-vascular interactions and drug responses. By replicating key aspects of the human TME, it offers a scalable and ethical alternative to PDX models, improving the predictive accuracy of preclinical drug screening and paving the way for personalized oncology applications.

EACR25-2191

From genotype to phenotype: how IDH1 mutations alter the landscape of intrahepatic cholangiocarcinoma

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Introduction

Mutations in IDH1 confer a gain-of-function neomorphic activity, leading to the accumulation of the onco-metabolite 2-hydroxyglutarate (2-HG). This metabolite induces epigenetic changes that alter gene expression and cellular differentiation. Although IDH1 mutations are frequently observed in intrahepatic cholangiocarcinoma (iCCA), their role in tumor progression, micro-environment remodeling, and immune modulation remains unclear. This study investigates the impact of IDH1 mutations on tumor-stroma interactions, immune cell infiltration, and potential therapeutic targets, including the chemokine CCL17, which recruits regulatory T cells (Tregs) and may contribute to immune suppression.

Material and method

We used a genetically engineered mouse model allowing in vivo introduction of IDH1 mutations alongside iCCA-associated oncogenic events. Tumor burden, survival, and microenvironmental changes were assessed using histopathology, transcriptomics, and proteomics. To evaluate the effects of 2-HG accumulation on extracellular matrix (ECM) composition and immune modulation, we combined multi-omics analysis with immunohistochemistry to perform immunoprofiling, focusing on stromal and immune cell infiltration. Immunogenic peptide screening was conducted to explore mutation-specific vaccination strategies, and CCL17 was investigated as a potential immunosuppressive factor.

Result and discussion

IDH1 mutations significantly reduced survival in tumor-bearing mice. 2-HG accumulation led to epigenetic dysregulation, promoting tumor differentiation and altering ECM and stromal composition. Notably, IDH1-mutant tumors exhibited a desmoplastic phenotype, characterized by excessive ECM deposition and stromal expansion, which may contribute to immune evasion. Immune profiling revealed shifts in T cell populations, suggesting an immunosuppressive microenvironment. Proteomic and transcriptomic analyses identified increased CCL17 expression, supporting its role in Treg recruitment and tumor-driven immune evasion. Targeting CCL17 could provide a therapeutic strategy for overcoming immune suppression. Additionally, an immunogenic peptide was identified, with potential for mutation-specific immunotherapy.

Conclusion

These findings establish IDH1 as a key driver of tumor microenvironment remodeling, differentiation, and immune modulation in iCCA. The pronounced desmoplastic nature of IDH1-mutant tumors suggests a role for stromal expansion in shaping the immune landscape. The observed stromal and immune changes underscore the impact of 2-HG accumulation, while the identification of CCL17 as a Treg-recruiting chemokine highlights a novel immunosuppressive mechanism. Targeting CCL17 or incorporating mutation-specific immunotherapy may offer new strategies for IDH1-mutant iCCA.

EACR25-2214**Targeting tumor hypoxia and immune blockade to improve breast cancer therapy**

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Introduction

Tumor hypoxia is linked to several negative consequences, such as increased metastatic potential, metabolic reprogramming, and angiogenesis via the activation of the hypoxia-inducible factor 1 (HIF-1). It also contributes to immune suppression which induces programmed death ligand 1 (PD-L1) expression, preventing T cell activation. Immune checkpoint inhibitors (ICIs) have significantly advanced cancer therapy by inhibiting immune blockade-generating molecules. However, HIF-1 promotes aberrant vascularization that impedes immune cell infiltration and increases resistance via ATP-binding cassette (ABC) transporter expression. We hypothesize that combining hypoxia-targeting agents with PD-L1 inhibition may improve ICI efficacy. Therefore, this approach was investigated in breast cancer models and clinical samples.

Material and method

Proteomic analysis was conducted via data-independent acquisition mass spectrometry to evaluate the effects of hypoxia and acriflavine. Pathway analysis was performed using DAVID. TCGA breast cancer datasets were examined through UCSC Xena to assess the relationship between HIF-1α and PD-L1 expression and survival outcomes. Immunohistochemistry tumor microarrays were used to validate these findings.

Result and discussion

Survival analysis showed that while HIF-1α expression did not impact survival, a key HIF-1-target gene correlated with worse outcomes. We did not find a significant relationship between HIF-1α and PD-L1 in breast cancer cell lines. However, the clinical sample analysis proved that HIF-1α levels were associated with elevated PD-L1 expression. Proteomic analysis showed that hypoxic treatment upregulated mitochondrial protein degradation, cholesterol biosynthesis, and fatty acid β-oxidation. Acriflavine counteracted these changes by downregulating mitochondrial translation, protein metabolism, and rRNA processing, impairing hypoxia-driven metabolic shifts.

Conclusion

Our findings highlight that co-targeting HIF-1 and PD-L1 could enhance breast cancer therapy by improving ICI efficacy. The next step is developing *in vivo* models to assess this strategy.

This study was funded by the National Tumor Biology Laboratory (2022-2.1.1-NL-2022-00010), the Hungarian Thematic Excellence Program (TKP2021-EGA-44), and NKFIH-OTKA grants K147410 (JT) and PD142272 (MC).

EACR25-2228**Dissecting the role of retinoic acid signaling in human chronic lymphocytic leukemia using novel 3D lymph node-like microenvironments**

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Introduction

The microenvironment of secondary lymphoid organs is composed of various cell types, including fibroblastic reticular cells (FRCs), which play a crucial role in providing signals to promote activation and survival of normal and malignant B cells such as in chronic lymphocytic leukemia (CLL). We have discovered a retinoic acid (RA)-dependent FRC-leukemia crosstalk during CLL progression in mice and demonstrated that pharmacological inhibition of the RA pathway reduces tissue infiltration and CLL progression in preclinical models. Here we further explored the role(s) of the RA pathway in human FRC-CLL crosstalk using several approaches, including a newly established humanized lymph node (LN)-like microenvironment suitable for mechanistic studies under more physiological conditions.

Material and method

We used 2D and 3D culture systems of human lymph node fibroblasts and primary human CLL cells; RNA-scope, RNA-seq, western blot, flow cytometry and confocal microscopy.

Result and discussion

Using the RNA-scope, we detected upregulation of genes involved in RA synthesis, RA degradation and RA signaling in human CLL-LN biopsies compared to control tissues. Specifically, we found that the transcription factor Retinoid X Receptor (RXR) alpha, an RA nuclear receptor is overexpressed at mRNA and protein levels in hCLL cells compared to control B cells. Using 2D co-cultures and 3D spheroids, we demonstrated that inhibition of RXR alpha reduces CLL adhesion and prevents CLL cell aggregation and spheroid formation. To further investigate the functional role of RXR alpha

inhibition, we used a 3D lymph node-like microenvironment cultured in a bioreactor and found that inhibition of RXR alpha impaired the interaction of hFRCs and hCLL and promoted the mobilization of leukemic cells from the 3D niche. To study the molecular mechanism underlying this phenotype, we performed bulk RNA-seq on hCLL and hFRCs after treatment with an RXR antagonist and found that RXR alpha inhibition caused deregulation of multiple molecular pathways, including cell adhesion, fatty acid metabolism, and cytokine signaling.

Conclusion

Our results show that the retinoic acid signaling pathway is upregulated in hCLL-LN biopsies *in vivo* and in hCLL cells from the peripheral blood. We demonstrated a functional role of RXR alpha and RA signaling in promoting interactions of hCLL and hFRCs, and hCLL retention within the microenvironment. We are currently investigating the underlying mechanism of RXR alpha overexpression and function.

EACR25-2244

Deciphering the involvement of cancer associated fibroblasts on the aberrant vasculature in clear cell renal cell carcinoma and the resistance to targeted treatments

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Introduction

Clear cell renal cell carcinoma (ccRCC) is the most prevalent form of kidney cancer, characterized by a pseudo-hypoxic state that drives tumor invasion, hypervasculization, and the recruitment of stromal components, including cancer-associated fibroblasts (CAFs) in the tumor microenvironment. CAFs play a pivotal role in promoting aberrant blood vessel formation, epithelial-mesenchymal transition, extra-cellular matrix remodeling, immune evasion, and therapy resistance. Our team recently identified aberrant vascular structures within ccRCC that exhibit dramatic dimensions and irregular morphology, surrounded by thin mural cell layers and strongly differing from the tumor capillaries. The presence of these aberrant structures correlates with patient prognosis and is frequently observed in CAF-rich regions, both within the tumor and in the surrounding peritumoral fibroblastic capsule. Notably, a high intratumoral CAF density has been associated with poor clinical outcomes. This doctoral research aims to decipher the role of CAFs in ccRCC pathogenesis, with a particular focus on their involvement in aberrant vascularization and resistance to tyrosine kinase inhibitors and/or immune checkpoint inhibitors.

Material and method

This study investigates the functional interactions between CAFs, tumor cells, and endothelial cells using a

3D *in vitro* model of vascularized microtumors (VMT) developed by our team. Specifically, fibroblast-mediated paracrine regulation of tumor-endothelial cell interactions supports the formation of aberrant vascular structures within the VMT model, recapitulating key features observed in ccRCC patients. Various 3D *in vitro* models are used to assess the impact of patient-derived or *in vitro* engineered CAFs on tumor invasiveness, formation of aberrant structures, and drug sensitivity. In parallel, the contribution of CAFs to aberrant vascularization will be further elucidated in cohorts of ccRCC patients, using spatial transcriptomic profiling (Visium, 10X Genomics).

Result and discussion

Using 3D *in vitro* models, we demonstrated that ccRCC tumor cells activate fibroblasts through both paracrine regulation and direct interactions, enhancing their invasive capacities and ability to acquire CAF-like features. Additionally, the heterotypic spheroid model reveals that leader CAFs drive the invasion of follower tumor cells. Moreover, the alphaSMA⁺ CAFs and/or mural cells exhibit specific intratumoral patterns in proximity to aberrant vascular structures in the ccRCC patient samples.

Conclusion

This ongoing project characterizes paracrine and direct interactions between CAFs, ccRCC tumor cells, and endothelial cells, using both *in vitro* models and patient samples. The study provides insights into the tumor microenvironment, aberrant vascularization, and treatment resistance, potentially leading to therapeutic combinations with CAF-targeted treatments.

EACR25-2264

CSF1R Haploinsufficiency in the Tumor Microenvironment and Its Impact on CLL Progression

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Introduction

Chronic lymphocytic leukemia (CLL) is a malignancy that critically depends on its tumor microenvironment for survival and expansion. In particular, monocytes and macrophages play essential roles by providing survival signals and fostering an immunosuppressive niche. The colony-stimulating factor 1 receptor (CSF1R) is a central regulator of these myeloid cells and has recently emerged as a promising target for therapeutic intervention in CLL.

Material and method

In this study, we used mice with a partial knockout of the *Csf1r* gene to simulate the degree of inhibition that could be achieved with therapeutic antibodies. These heterozygous mice were crossed with the well-established Eμ-TCL1 CLL model. Detailed analyses were performed on the myeloid compartments by assessing CSF1R expression on circulating monocytes and tissue-resident macrophages. Disease progression was meticulously monitored over time through serial *in vivo* observations, and *in vitro* migration assays were employed to evaluate

the functional impact of CSF1R reduction on CLL cell motility.

Result and discussion

Our experiments demonstrated that heterozygous mice had a marked decrease in CSF1R expression on circulating monocytes compared to wild-type controls. Despite this reduction, the absolute numbers of monocytes and macrophages in both blood and lymphoid tissues remained largely unaltered, suggesting compensatory mechanisms that maintain myeloid homeostasis. Importantly, *TCL1tg/wt Csflr+/-* mice exhibited a significant yet transient reduction in leukemia burden at approximately 8 months of age. This initial benefit, however, waned as the disease advanced, with overall survival rates eventually aligning with those of mice expressing normal CSF1R levels. Additionally, our *in vitro* migration assays revealed that diminished CSF1R expression impaired the migratory capacity of CLL cells, indicating that CSF1R may be critical for the effective dissemination of malignant cells within the tumor microenvironment. These results underscore the complex interplay between CSF1R-mediated signaling and the dynamic regulation of immune cell functions in CLL, and they highlight the potential limitations of targeting CSF1R as a monotherapy.

Conclusion

The data presented herein suggest that while CSF1R plays a pivotal role in supporting the CLL tumor microenvironment, partial reduction of its expression only modestly delays disease progression. Our findings support the hypothesis that CSF1R-targeted therapies might yield the best outcomes when combined with additional agents that collectively disrupt the supportive interactions between CLL cells and their microenvironment. Further investigations into combination strategies are warranted to fully exploit the therapeutic potential of CSF1R inhibition in CLL.

EACR25-2272

Decoding Sarcoma Complexity: Harnessing *Xenopus tropicalis* for In Vivo Studies of Liposarcoma, Desmoid Tumors, and Beyond

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Introduction

Sarcomas constitute a heterogeneous group of mesenchymal malignancies with diverse genetic drivers and clinical manifestations—from benign lesions and locally aggressive forms such as desmoid tumor, to malignant entities like liposarcoma. The complexity of these tumors underscores the high need for robust *in vivo* animal models to unravel their underlying biology and to identify novel therapeutic targets.

Material and method

In this study, we leverage the genetic amenability of

Xenopus tropicalis, whose large brood size, external development, and high genomic synteny with humans enable rapid generation of high-penetrance cancer models. Utilizing CRISPR/Cas9-based genome editing tools, we create F0 mosaic mutants (crisprants) to inactivate key tumor suppressor genes and test candidate co-drivers in sarcoma development. Our comprehensive downstream analyses combine targeted amplicon deep sequencing, single-cell RNA sequencing (scRNA-seq), immunohistological profiling, and three-dimensional imaging to thoroughly characterize these cancer models.

Result and discussion

We are investigating two distinct sarcoma models in *Xenopus tropicalis*. Desmoid tumors (DT) were obtained by targeting *apc* with a single guide RNA, creating a genetically straightforward, Wnt-driven model. The clear genetics of our model enables us to focus on tumor behavior; we hypothesize that DTs are immunologically cold—potentially due to diminished immune cell infiltration and/or a physical barrier from extensive collagen deposition. To investigate this, we are spatially mapping the cellular and extracellular tumor microenvironment using cell marker analysis coupled with 3D whole-mount light-sheet imaging. In contrast, our liposarcoma (LPS) model manifests as a paradigm for cancers with more complex genetics and poor therapeutic opportunities. Here, a multiplex CRISPR/Cas9 approach targeting the *p53* and *retinoblastoma* pathways simultaneously generated a mixed well- and dedifferentiated LPS subtype (i.e. WDLPS and DDLPS). Given the poor outcome of this cancer, we employ comprehensive transcriptomic profiling to identify co-driver genes, particularly those involved in the dedifferentiation process toward the more aggressive DDLPS phenotype.

Conclusion

Collectively, these findings illustrate the power of *Xenopus tropicalis* as a versatile *in vivo* platform for modeling sarcoma subtypes. Future investigations are warranted to clarify the molecular mechanisms driving liposarcoma dedifferentiation, refine immunomodulatory strategies in desmoid tumors, and extend these approaches to additional sarcoma subtypes, thereby advancing our understanding and treatment of these challenging malignancies.

EACR25-2274

ETV6::JAK2 fusion promotes central nervous system invasion in murine B-cell acute lymphoblastic leukemia

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Introduction

Acute lymphoblastic leukemia (ALL) is the most frequent pediatric malignancy and remains an important cause of mortality and morbidity in all ages. A subgroup of B-cell precursor ALL (BCP-ALL) patients with bad prognosis carries specific genetic alterations, including JAK2 fusions or mutations and CDKN2A deletions.

Disease relapse in these patients is often associated with central nervous system (CNS) invasion.

Material and method

To study the impact of constitutively active JAK2 kinase signaling in CNS involvement by B-ALL, we generated mice expressing an ETV6::JAK2 fusion in the lymphoid lineage, in a leukemia-prone genetic background (Rag2 and Cdkn2a deficiency).

Result and discussion

Indeed, in comparison to Rag2^{-/-};Cdkn2a^{-/-} littermates, ETV6::JAK2;Rag2^{-/-};Cdkn2a^{-/-} mice exhibited earlier onset of B-ALL and presented more frequent CNS invasion (e.g., paraparesis/paraplegia of hind limbs). Immunohistochemical analyses of diseased ETV6::JAK2;Rag2^{-/-};Cdkn2a^{-/-} and Rag2^{-/-};Cdkn2a^{-/-} mice revealed leptomeningeal invasion by B220+PAX5+ cells expressing phosphorylated STAT5, a JAK kinase substrate. In vitro treatment of primary leukemic cells with AZD1480 (JAK2 inhibitor) significantly impaired the survival of ETV6::JAK2;Rag2^{-/-};Cdkn2a^{-/-} cells but not Rag2^{-/-};Cdkn2a^{-/-} cells. To assess JAK2 signaling dependency in vivo, Rag2^{-/-};Il2rg^{-/-} mice injected intravenously with ETV6::JAK2-driven B-ALL cells were treated for four days with FDA-approved JAK1/JAK2 inhibitor Ruxolitinib (50 mg/kg/daily) upon detection of > 1% peripheral B-ALL blasts. Ruxolitinib-treated recipients exhibited a significant reduction in circulating B-ALL cells and concomitant increased number of cleaved caspase 3-labeled apoptotic cells in the spleen, in comparison to vehicle group. Moreover, to grasp the dynamics of CNS invasion by B-ALL, we infused CD45.2-expressing ETV6::JAK2;Rag2^{-/-};Cdkn2a^{-/-} and Rag2^{-/-};Cdkn2a^{-/-} cells in Rag2^{-/-};CD45.1+ recipients and detected leukemic cells in the cerebrospinal fluid (CSF) at different timepoints, via flow cytometry analysis. Mice injected with ETV6::JAK2-driven B-ALL presented earlier and significant higher invasion of CSF by CD45.2+B220+ cells compared to recipients transplanted with Rag2^{-/-};Cdkn2a^{-/-} leukemia, but similar tumor burden in the bone marrow.

Conclusion

In conclusion, the ETV6::JAK2 fusion promotes leukemia survival, accelerates disease development, and confers increased neurotropism to leukemic cells.

EACR25-2285

Charting the immune landscape and communication axes in metastatic colorectal cancer with spatial transcriptomics

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Introduction

Colorectal cancer (CRC) globally ranks among malignancies with highest incidence and recurrence rates, afflicting almost one million subjects each year. Despite the wealth of studies carried out in CRC correlating the degree and type of immune infiltration with invasive capability, spatial relationships governing the interplay between immune and tumor cells in the CRC tumor microenvironment (TME) have been thus far explored at coarse-grained scale. In this work, we employ spatial profiling technologies to investigate the cellular ecosystem of metastatic and non-metastatic CRC, providing a comprehensive picture of the spatial architecture of immune cell responses at play and elucidating their role in the progression towards an invasive cancer phenotype.

Material and method

To identify links between spatial determinants of the CRC ecosystem and the process of tumor metastasization, we assembled an initial retrospective cohort of primary tumor FFPE samples from 16 CRC patients with either overt or undetectable metastases after surgery. Samples were processed for targeted spatial transcriptomics with the latest version of the CosMx™ SMI platform, profiling the expression of ~6,000 different genes in situ resulting in a comprehensive collection of more than 2.2 million single-cell transcriptomes coupled to their spatial location.

Result and discussion

Leveraging a large-scale scRNA-seq cohort, we uncover the transcriptional diversity of malignant cells across more than 100 CRCs at single-cell resolution. Notably, cancer cells exhibited a state marked by features of TNF signaling and hypoxia response, active in primary tumors prone to form metastases. This transcriptional state also correlated with increased IFN response, inflammation and EMT programs, suggesting an intimate link between the metastasization potential of these cells and immune compartments of the CRC TME. Integrating spatial information with the depth provided by scRNA-seq data facilitated cell type annotation and the identification of malignant cell states directly in situ, revealing how metastatic CRC spatially orchestrates expression of these gene programs. To further connect features of the CRC TME with the development of pro-metastatic cancer cell states, we correlated the abundance of local ligand-receptor interactions in the CRC TME with the activity of transcription factors linked to the emergence of invasive cancer cell states.

Conclusion

By combining large-scale scRNA-seq data and targeted spatial approaches we gathered an unbiased look into the molecular and physical architecture of metastatic and non-metastatic CRC. Cancer cells displayed gene programs which are intimately linked to the surrounding composition of the tumor bed they reside in. Ultimately, this work provides initial mechanistic insights into how TME signals drive metastatic potential in CRC.

EACR25-2292

Development of new in vitro organotypic cultures for the evaluation of anti-tumor drug sensitivity

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Introduction

Gastric cancer represents a poor prognosis disease. Indeed, the 5-year survival rate in Europe is 32%. The heterogeneity of the disease represents a major issue. Molecular profiling studies have revealed the existence of four main different subtypes highlighting different targets for personalized medicine. Currently, chemotherapy is the standard-of-care for this disease, but its high heterogeneity and the discovery of new targets for personalized medicine calls for an urgent need for patient-derived preclinical models aimed at the fast identification of individualized combination therapies to improve clinical outcomes and avoid undesired side effects. The present project is aimed at generating a platform to test drug combinations active in gastric cancer. It is based on the setting-up of Gastric Cancer derived Organotypic Cultures (GC-OC) that retain the main characteristic of the primary tumour.

Material and method

We took advantage of two syngeneic mice models of gastric cancer, YTN3 and YTN16 that differ in drug sensitivity. To generate GC-OC, tumors were subjected to mild dissociation followed by serial filtration. The obtained organotypic spheroids (40-100 µm) were then seeded in a miniaturized microfluidic device to generate 3D-collagene embedded cultures in an appropriate medium. Flow cytometry and immunofluorescence analysis were performed to analyze GC-OC cell composition. Cell viability was evaluated by Acridine Orange (AO)/ Propidium Iodide (PI) staining and confocal imaging of 3D cultures. Cell number was monitored by staining of the nucleus via Hoechst.

Result and discussion

We generated GC-OC as spheroids that keep neoplastic and stromal cells in their original matrisome, maintaining their spatial and functional organization. Immunofluorescence analysis of cell composition confirms the presence of the main components of the tumor microenvironment (e.g. cancer cells, immune cells, endothelial cells and fibroblasts) in the generated GC-OC. Immune cell composition was further investigated in the tumor and spleen of the mouse models leading to the identification of G-MDSC (Granulocyte-Myeloid Derived Suppressor Cell) as the main immune subpopulation that was retained by the GC-OC. Drug effects on cancer cell viability were also assessed, revealing differences in drug sensitivity with respect to the classical 2D culture.

Conclusion

Altogether, the data obtained in our lab has allowed the setting-up of a 3D cell culture system that is expected to result in the generation of tumor in-vitro-twins, a phenocopy of the patient tumor and its microenvironment. These in-vitro-twins could be exploited for therapy identification of effective personalized drug combinations in the clinical setting. Moreover, the eventual application of omics strategies will help to

define the features of the tumors and its microenvironment representing novel drug vulnerabilities.

EACR25-2295

Unveiling the role of Sialyl-Tn in pancreatic cancer progression and therapy

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Introduction

Pancreatic cancer (PC) is a major cause of cancer mortality, with pancreatic ductal adenocarcinoma (PDAC) comprising ~90% of cases. PDAC exhibits extensive desmoplasia, immunosuppression and treatment resistance. While immunotherapy shows promise, current approved therapies for PDAC target PD-1 and are restricted to high microsatellite instability tumors (~2% of cases). Understanding the mechanisms of PDAC pathogenesis is crucial to identify better and more targeted therapeutics. Aberrant glycan expression drives cancer progression. A frequently expressed aberrant glycan is sialyl-Tn (STn), a truncated O-glycan modifying proteins like mucins. STn is linked with immunosuppression, poor survival and metastasis in other cancers[1]. We hypothesize that STn drives PC progression, and its targeting may hinder tumor development. Our study assesses STn and its main carrier protein's role in PC progression, clinical correlation, and immunotherapeutic potential.

Material and method

A cohort of 185 formalin-fixed paraffin-embedded (FFPE) samples, including precursor lesions, PDAC and metastases, was analysed by immunohistochemistry (IHC) for STn expression. Quantification used the H-score, considering luminal and cytoplasmic staining intensities. H-score was calculated based on the intensity of staining (0-3) and the percentage of stained cells. Two independent evaluators, including a pathologist, performed the scoring. Statistical comparisons were made between precursor lesions, PDAC, and metastases.

Result and discussion

STn was absent in healthy tissue and was present in precursor lesions and PDAC samples. STn levels were different across different precursor lesion types. Intestinal and pancreaticobiliary-type IPMNs (Intraductal Papillary Mucinous Neoplasms) had higher STn levels, likely due to differential mucin expression. The intestinal and pancreaticobiliary-type IPMNs express MUC2 and 1, respectively, both reported as STn carriers. STn expression in PDAC was heterogeneous (H-score between 0-260) with a non-significant trend toward reduction in post-chemotherapy. A significant increase in STn expression was found in untreated PDAC and

metastases in relation to low-grade IPMNs, and in metastases against high-grade IPMNs. These findings suggest that STn has a role in PC progression.

Conclusion

The results highlight that STn is tumor-specific and mainly expressed in later PDAC stages, particularly in metastases. These findings suggest STn plays a role in PC progression. Ongoing studies are exploring STn-associated immune phenotypes, STn carriers, immune checkpoints, and inflammation markers in the same cohort, alongside in vitro studies in PDAC cell lines overexpressing STn to further elucidate its role in disease progression.

[1] Ferreira, JA et al. "Overexpression of tumour-associated carbohydrate antigen sialyl-Tn in advanced bladder tumours." *Molecular oncology* (2013) 7(3): 719-731.

EACR25-2296

Impact of Brain-Derived Neurotrophic Factor (BDNF) on Tight Junction (TJ) Proteins and Cell Adhesion and Migration in Breast Cancer: Implications for Brain Metastasis

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Introduction

Brain-Derived Neurotrophic Factor (BDNF) plays a significant role in the modulation of tight junction-(TJ)-proteins and influences cell adhesion and migration, which are essential for both cancer metastasis and blood-brain-barrier (BBB) integrity. This study investigates the effects of BDNF on the expression of TJ proteins and the functional consequences on cell adhesion and migration in breast cancer cells and brain endothelial cells.

Material and method

Following treatment with BDNF, changes in the expression of TJ proteins including Claudins, Occludin, ZOs, JAMs, MAGIs and Nectins were assessed using quantitative PCR (qPCR) at different time points (30 minutes, 60 minutes, 2 hours, 4 hours, and 24 hours) with a baseline control (0-time point) in both MDA-MB-231 (breast) and HMECD3 (endothelial) cell lines. BDNF knockdown models were created using anti-BDNF siRNA to assess the functional consequences of BDNF signalling on cell adhesion and migration using Electric Cell-Substrate Impedance Sensing (ECIS).

Result and discussion

qPCR results indicated dynamic alterations in the expression of multiple TJ proteins, suggesting a regulatory role for BDNF in the maintenance of cell-cell adhesion and barrier integrity in both cell types.

Additionally, BDNF knockdown resulted in a significant decrease in cell adhesion and migration, highlighting the importance of BDNF in promoting the migratory behaviour of cancer cells and in maintaining BBB integrity.

Conclusion

These findings suggest that BDNF is a key regulator of cell adhesion, migration, and TJ proteins expression, implicating its role in cancer metastasis. Targeting BDNF/TrkB could provide a novel therapeutic strategy to

inhibit cancer cell migration and stabilize BBB integrity, preventing brain metastasis from breast cancer.

EACR25-2317

Epitranscriptional interplay of FOXA1 and MYC impact prostate cancer progression

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Introduction

FOXA1 and MYC oncogenes cooperate to drive prostate cancer (PCa) progression by remodeling chromatin and rewiring alternative splicing (AS) landscape. FOXA1 promotes splicing factors' expression to modulate AS, while MYC governs RNA processing machinery. Both regulations impact patient prognosis. While MYC's role in post-transcriptional regulation is well established, FOXA1's contribution remains less understood. Their interplay and overlapping influence on RNA processing is still under investigation, but their convergence in transcriptional control unveils new therapeutic opportunities to target PCa at the RNA level.

Material and method

AR-independent PCa cells (PC3) were depleted of either FOXA1 or MYC using siRNA. Nanopore direct mRNA and Illumina totalRNA libraries of 14 replicates (siFOXA1:4, siMYC:3, siCTRL:7) were sequenced. PC3 transcriptome was reconstructed. Differential analyses of isoform expression, usage, m6A methylation, and polyA tail length were conducted across conditions. To validate FOXA1-mediated m6A methylation, MeRIP-seq was performed on FOXA1 depleted and control samples. To evaluate RNA stability, FOXA1-depleted and control cells were treated with actinomycin D. Total RNA was collected at five time points (0-8 h) and Illumina sequenced. Differential expression and pathway analyses were performed. Finally, 10x-Visium spatial transcriptomic data from five sections of a PCa were analyzed for clustering and transcriptomic signature identification.

Result and discussion

We found that FOXA1 and MYC orchestrate a dynamic regulatory network influencing isoform expression in AS, RNA methylation, and localization. These TFs specifically remodel m6A modifications on isoforms linked to AS and RNA localization. Transcriptional blockade experiments confirmed that FOXA1 enhances mRNA stability of isoforms implicated in RNA processing. Both TFs govern polyA tail selection favouring short tails to increase expression of isoforms. Spatial analyses corroborated FOXA1- and MYC-mediated RNA processing regulation in distinct tumor clones. Network analyses pinpointed candidate splicing and RNA processing factors under FOXA1/MYC control, including SRSF7.

Conclusion

Our data highlight a central role of FOXA1 and MYC interplay in controlling co- and post-transcriptional

programmes of PCa. These TFs fine-tune the production of isoforms involved in AS and RNA localization enhancing their expression, reshaping their m6A methylation, shortening their polyA tails. Our comprehensive catalogue of full-length isoforms, driven by these oncogenic TFs, offers new means to tackle PCa progression.

EACR25-2332

Single-cell transcriptomics identifies vulnerabilities of osimertinib ex-vivo persister cells

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Introduction

Persister cells arise upon chronic treatment and constitute the origin of resistant cells. Understanding the biology and heterogeneity of persister cells is essential for the design of future treatment approaches. However, the study of persister cells has been strongly limited by our capacity to analyse small numbers of cells ex-vivo.

Material and method

PC9 drug-tolerant/persister (DTP) cells were characterised at single cell resolution in vivo. To this end, PC9 cells were implanted in the flank of female mice. Once tumours acquired the desired volume, mice were orally administered osimertinib daily at a dose of 25 mg/kg for 14 days. Vehicle-treated samples were used as a reference. Tumours were then dissociated and processed for single-cell RNA-seq using the 10X Genomics reagents or for single-cell protein analysis using CyTOF.

Result and discussion

Our results showed that the resulting osimertinib DTP population is transcriptionally heterogeneous. The comparison of the different transcriptional states identified a putative target which was confirmed at protein level using CyTOF. Further in vivo studies in PC9 cells using an antibody-drug conjugate (ADCs) against the identified putative target in combination with osimertinib resulted in ablation of a specific subpopulation of DTPs.

Conclusion

Our single-cell RNA-seq analysis of osimertinib-treated tumours highlighted that it is essential to investigate therapeutic response at single-cell resolution. The understanding of cellular heterogeneity is key to better understand combinatorial treatments. In our preclinical model, a specific subpopulation of DTPs was eliminated by combinatorial treatment which resulted in reduction in the heterogeneity of the resulting DTP population and ultimately in delayed re-growth of tumours.

EACR25-2343

Therapeutic Potential of miR-379 through modulating Breast Cancer Stem Cells via HSPA5-TGF β Axis

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Introduction

Breast cancer (BC) remains a leading cause of mortality, with cancer stem cells (CSCs) driving tumour progression, therapy resistance, and metastasis.

MicroRNAs are key regulators of CSC dynamics. This study investigates miR-379, a tumour-suppressive miRNA in breast cancer. Using an *in vivo* proteomic approach, target prediction analysis and functional studies, this study aimed to identify the mechanism of action of miR-379 in BC.

Material and method

A 4T1 murine BC model with stable miR-379 enrichment (4T1-379) or control (4T1-NTC) was established. Tumour tissues were analyzed using liquid chromatography-tandem mass spectrometry (LC-MS/MS) for proteomic profiling. Bioinformatic prediction of miR-379 targets was conducted using TargetScan, miRDB, and miRTarBase. miR-379 enriched HCC1954, MDA-MB-231, and 4T1 BC cells were employed in functional studies. Mammosphere formation assays evaluated tumour initiation and self-renewal capacity. TGF- β 1 secretion in media was quantified by ELISA. Immunohistochemistry (IHC) assessed CD44 expression *in vivo*. Flow cytometry analysis targeting the stemness related panel CD24/CD44/CD90 was also performed.

Result and discussion

Six hundred differentially expressed proteins (DEPs) were identified, with 11 significantly downregulated and 43 significantly upregulated in miR-379 enriched tumours. HSPA5 was a DEP and a predicted target of miR-379 identified through multiple platforms.

Functional annotation using GO and KEGG analysis highlighted that miR-379-HSPA5 interactions regulate TGF- β production and signalling. First-generation mammosphere formation, an indicator of tumour-initiating activity, was significantly reduced in miR-379-enriched cells. This inhibitory effect persisted in second-generation mammospheres, an indicator of self-renewal ability, demonstrating that miR-379 impairs both tumour initiation and self-renewal capacity of BCSCs. This mirrored *in vivo* findings, where miR-379 suppressed tumour growth. ELISA revealed a significant reduction in TGF- β 1 secretion by miR-379-enriched cells. IHC analysis of miR-379-enriched tumours *in vivo* revealed downregulation of CD44, a key BCSC marker and downstream effector of TGF- β signalling. These results demonstrate that miR-379 inhibits stemness of BC by downregulating HSPA5, thereby suppressing the TGF- β 1-CD44 axis. Furthermore, flow cytometry analysis revealed that miR-379 increased the mesenchymal CD90+CD24+CD44+ cell subset, accompanied by a decrease in the less mesenchymal CD90+CD24+CD44- subset. This suggests that miR-379 alters BCSC heterogeneity by shifting cellular states towards a mesenchymal phenotype.

Conclusion

Our study establishes miR-379 as a potent suppressor of BC stemness by targeting the HSPA5-TGF- β 1 axis. By disrupting BCSC-associated pathways, miR-379 emerges as a promising RNA-based therapeutic candidate.

EACR25-2346**A uveal melanoma murine model which faithfully recapitulates organotropism and dormancy**

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Introduction

Uveal melanoma (UM) is the most common intraocular malignancy, affecting more than 2,500 adults yearly in the United States. When confined to the eye, UM is often successfully treated with radiation or surgery. However, nearly half of the patients eventually develop metastatic disease, in some cases decades after primary diagnosis. Metastatic UM is highly resistant to current treatments, resulting in a very poor prognosis, with a mean overall survival of less than two years. The liver is nearly always the initial site of metastases. The preferential dissemination to the liver raises the possibility that hepatic microenvironmental factors contribute to metastatic progression and the resistance of UM to available treatments. However, the identification of such factors is limited by the lack of UM preclinical models that exhibit liver metastasis. We aim to elucidate the mechanisms underlying the characteristic hepatotropism in order to develop strategies to intercept metastatic progression and improve therapeutic options for metastatic UM.

Material and method

Here, we present a metastatic UM model using intraocular, intravenous and subcutaneous injection of patient-derived primary human UM cell lines (MP41 and Mel202) in immunocompromised mice. We performed CycIF (Cyclic Immuno-fluorescence) and scRNASeq on two metastatic sites to evaluate transcriptional differences between the tumor cells deriving from the liver and the lung. Additionally, this model enables in vivo studies of candidate targeted drug treatments such as the PKC inhibitor darovasertib, currently in clinical trial. A parallel approach of in vitro and in vivo CRISPR screens in three cell lines, or mice harboring xenografts respectively, treated with the kinase inhibitor is currently being validated through single gene knockout and mechanistical experiments.

Result and discussion

We describe a novel UM model whose metastasis closely resembles metastatic patterns observed in patients. After a latency of two to four months, we consistently see liver macrometastases irrespective of injection site.

Surprisingly, we observe single cells in non-hepatic sites such as the lung. Using highly multiplexed spatial profiling (CyCIF tissue imaging), we have further

defined the proliferation, dormancy, and oncogenic signaling in the two metastatic sites. Transcriptomic profiling and CRISPR screening of metastatic cells is currently being analyzed.

Conclusion

This model faithfully represents a unique opportunity to dissect the pathways that lead to UM hepatotropism and an extrahepatic dormancy-like phenotype as well as the opportunity of developing more effective therapeutic options in order to improve patient outcomes.

EACR25-2356**Genetic deconstruction of prostate cancer heterogeneity through a unique preclinical mouse modelling platform (ProMPt)**

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Introduction

Prostate cancer is among the leading causes of cancer related death in men. An unmet need is the availability of genetically complex preclinical models to study disease development and response to therapy. The Prostate Cancer Preclinical Mouse Modelling Platform (ProMPt) utilizes a biobank of prostate cancer organoids that covers 150 genetically unique combinations as seen in patients. An exploratory characterization of 20 models is presented to showcase this platform and investigate the genetic basis of therapeutic response to androgen deprivation therapy (ADT).

Material and method

Mice harbouring inducible alterations for PTEN, TP53, MYC, TMPRSS2/ERG, RB1, and APC were bred to achieve 20 genetically unique models. Prostatic organoids were generated from these models and this syngeneic biobank has been characterized through bulk RNA sequencing and mass cytometry by time of flight (CyTOF) for phenotypic alterations in response to androgen deprivation therapy. Permutation of organoid culture media in combination with ADT revealed upregulation of druggable pathways that have been assessed through targeted drug screens in vitro and ProMPt mouse models.

Result and discussion

Viability assessment revealed varied response to long term ADT exposure across 20 genetically unique organoid models, with resistant lines harbouring 3 or more genetic alterations. To investigate the influence of culture media on response to therapy, organoids were deprived of EGF, Noggin, R-Spondin1, and A83-01 in combination with ADT prior to CyTOF data collection. A striking response was seen between EGF removal and increased sensitivity to ADT in a genotype dependant manner when focused on alterations in a Pten and P53 deleted background. Overexpression of MYC rescued this sensitivity and CyTOF analysis revealed Pten-P53-MYC organoids maintain upregulation of phosphorylated 4E-BP1 in EGF null and ADT conditions, indicating a cell autonomous mode of survival. These results were

translated to a targeted drug screen where ErbB, AKT, MAPK and translation inhibitors were assessed in multiple combinations in conjunction with ADT. Homoharringtonine, an FDA approved translation inhibitor, showed exceptional results in reducing viability when used in combination with ErbB inhibitors. This effect is currently being assessed *in vivo* against a Pten-MYC and Pten-P53-MYC model of prostate cancer.

Conclusion

ProMPt allows for rapid *in vitro* and *in vivo* modelling that can be used to investigate many aspects of prostate cancer biology. To demonstrate the platform, the therapeutic response of 20 unique prostate cancer organoids were assessed at the proteomic level. Vulnerabilities were further highlighted through permutation experiments which have been translated to active preclinical *in vivo* trials. ProMPt will soon be publicly available, providing a novel catalogue of prostate cancer material.

EACR25-2377

Microfluidic System for Tumor Microenvironment Investigation

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Introduction

Microfluidics offers a transformative approach to biological experimentation by providing precise fluid control and high throughput. The miniaturized scale of these devices makes them especially suitable for investigating the tumor microenvironment (TME). Integrating microfluidic technologies with hydrogels, that can mimic extracellular matrix enables more accurate simulation of *in vivo* condition.

Material and method

A microfluidic device was designed and fabricated using a combination of materials, primarily PMMA, a specialized membrane, and PDMS. The system comprises several distinct elements joined by an adhesive tape: a glass base serves as the support, overlaid by a main culture chamber with an upper membrane. Above this chamber, a medium channel is integrated with a PDMS barrier that permits gas exchange with the external environment. Thermoresponsive microgels based on poly(N-isopropylacrylamide) were used to create an artificial extracellular matrix. At 25 °C, these microgels form a stable colloid in the culture medium, allowing for the cell suspension and introduction to microchip. Upon heating to physiological temperature, microgel colloid undergoes a phase transition, gelling to form macroscopic physical hydrogel with an interconnected porous structure.

Result and discussion

Experiments with cancer cell lines demonstrated that the microfluidic platform supports long-term cell culture while enabling continuous microscopic observation. The device maintained cell viability and proliferation under dynamic conditions, highlighting its capability to replicate essential TME characteristics. The controlled modulation of matrix properties and flow dynamics further allowed for detailed investigations into cell-matrix interactions and potential therapeutic strategies.

Conclusion

The developed microfluidic system provides a robust and versatile platform for TME studies. The integration of multi-material microchip and thermogelling hydrogel enables a close simulation of *in vivo* microenvironment, offering significant potential to advance cancer biology research and therapeutic development.

EACR25-2378

Engineered 3D osteosarcoma microenvironment model: Bridging *in vitro*- *in vivo* gap in cancer research and anticancer drug screening

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Introduction

Current treatments for osteosarcoma typically include surgical excision followed by neoadjuvant and adjuvant chemotherapy. Research attempts to streamline and improve these treatments, but progress is slow mostly due to limited translation of *in vitro* to *in vivo* studies. The aim of this work was to develop and validate an engineered three-dimensional (3D) osteosarcoma model based on macroporous composite scaffolds, as cell carriers, and biomimetic perfusion bioreactor for osteosarcoma research and anticancer drug screening.

Material and method

The scaffolds (4 mm thick discs, 9 mm in diameter) were produced by controlled gelation of hydroxyapatite (HAP) suspension in Na-alginate solution (2 wt.% alginate and 2 wt.% HAP) followed by freeze-drying and rehydration in the culture medium. Murine osteosarcoma K7M2-wt cells were seeded onto the scaffolds (15x106 cells cm⁻³ scaffold volume) and cultivated for 7 days in "3D Perfusion" bioreactors under continuous medium superficial velocity of 40 µm s⁻¹, while static cultures served as a control. To evaluate this model for anticancer drug screening, bioreactor cultures were treated with doxorubicin (1 µg cm⁻³), on day 1 (first study) or on day 7 (second study) and lasted for 1 day, while untreated bioreactor culture served as a control. The scaffolds were assessed regarding the cell metabolic activity by MTT, morphology and distribution by histological and scanning electron microscopy analyses. Masson-trichrome and reticulin staining were used for extracellular matrix (ECM) analysis, while quantitative real-time PCR (qRT-PCR) assessed osteosarcoma marker expression.

Result and discussion

After short-term cultures, biological assessment showed that the cells stayed viable and metabolically active, produced ECM, expressed osteosarcoma markers and spontaneously formed aggregates under both culture conditions. However, cells in the bioreactor culture exhibited higher metabolic activity, while the cell aggregates were slightly larger (~1.2-fold), more compact with higher amounts of reticular fibers, more numerous and more uniformly distributed throughout the scaffold compared to the static culture. These results could be explained by positive effects of flow on cells due to enhanced mass transport and adequate hydrodynamic shear stresses. Evaluation of the model for anticancer drug screening has shown a negligible effect of doxorubicin on individual cells as well as cell aggregates implying that the developed model more closely mimics *in vivo* drug responses than 2D cultures.

Conclusion

This study has shown potentials of engineered 3D osteosarcoma microenvironment model based on macroporous composite scaffolds, and perfusion bioreactor for relevant and reliable osteosarcoma research and anticancer drug screening.

Acknowledgement: Science Fund of the Republic of Serbia, grant no. 7503.

EACR25-2403

Doxorubicin-induced Cardiotoxicity represents a Risk Factor for Lung Metastasis in Osteosarcoma

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Introduction

Osteosarcoma is the most common primary malignant bone tumor in children and adolescents, with lung metastasis remaining the leading cause of mortality. Doxorubicin (DOX), a cornerstone chemotherapeutic agent, is widely used due to its efficient antitumor activity; however, its cumulative dose-dependent cardiotoxicity significantly limits its therapeutic potential. Moreover, emerging evidence suggests that chemotherapy may inadvertently promote metastasis, raising concerns about its long-term impact on disease progression. Herein, we investigate whether DOX-induced cardiotoxicity, with a focus on cardiac fibroblasts, promotes a pro-metastatic lung microenvironment in osteosarcoma.

Material and method

Cardiac fibroblasts were first exposed to DOX for 24 hours and then maintained in a DOX-free medium for 48 hours. Fibroblast activation status and fibrotic and inflammatory profiles were assessed by RT-qPCR, western blot and immunofluorescence. Senescence was evaluated by β-galactosidase immunostaining, p38/NF-κB signaling and SASP factors. A co-culture model was used to mimic the paracrine signaling of DOX-treated cardiac fibroblasts on lung fibroblasts, which are the most abundant stromal cells in the lung. Swiss nude mice

were pre-treated with DOX or PBS (control), followed by intravenous injection of 1×10^6 143B-luciferase osteosarcoma cells and monitored for lung metastasis formation by optical imaging. Heart and lung tissue were collected for analysis.

Result and discussion

Upon exposure to DOX, cardiac fibroblasts acquire a myofibroblast-like phenotype, with a marked expression of α-SMA, FAP, and PDGFR, along with upregulation of fibrotic (LOXL2, SPARC, CTGF, and Galectin-3), ECM remodeling markers (periostin and MMPs) and of pro-inflammatory cytokines TNF-α, IL-6, and IL-1β. Phalloidin staining revealed an increase in cell size, indicative of senescence, confirmed by the activation of p38, NF-κB, and the upregulation of SASP-related genes. Paracrine signaling from DOX-treated cardiac fibroblasts in co-cultures induced a pro-fibrotic and inflammatory phenotype in lung fibroblasts, resembling cancer-associated fibroblasts. *In vivo*, pretreatment with DOX, induced cardiac fibrosis and accelerated the formation of lung metastasis.

Conclusion

Our findings identify DOX-induced cardiotoxicity as a potential risk factor for lung metastasis and unveiled the role of cardiac fibroblasts in inducing a profibrotic premetastatic niche favorable to tumor cell seeding and outgrowth. Mitigating DOX-induced cardiac remodeling may represent a preventive strategy to reduce metastatic progression in osteosarcoma.

EACR25-2406

Extracellular Vesicles from Melanoma-Associated Fibroblasts Promote Pro-Tumourigenic Changes in Brain Endothelial Cells

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Introduction

Melanoma frequently metastasizes to the brain, where it is associated with poor survival and limited treatment options. While cancer-associated fibroblasts (CAFs) are known to support melanoma progression, their role in brain metastasis remains poorly understood. Small extracellular vesicles (sEVs) are key mediators of cell-cell communication within the tumour microenvironment, and CAF-derived sEVs may contribute to changes in brain endothelial cells that support metastasis.

Material and method

A melanoma-educated CAF model was developed *in vitro*, and sEVs were isolated and characterised using nanoparticle tracking analysis, electron microscopy, and western blotting. Proteomic and RNA sequencing were performed to profile sEV cargo. Functional assays assessed the effects of CAF sEVs on melanoma cell behaviour and brain endothelial remodelling, including invasion, migration, and vascular permeability.

Result and discussion

CAF-derived sEVs were taken up by melanoma cells and brain endothelial cells, promoting invasive behaviour in melanoma and altering endothelial cell morphology and function. Proteomic and RNA analyses identified sEV cargo linked to TGF β signalling and endothelial-mesenchymal transition. Blocking sEV secretion reduced these effects, suggesting a role for CAF sEVs in shaping the pre-metastatic niche.

Conclusion

These findings indicate that CAF-derived sEVs influence both melanoma cells and brain endothelial cells, potentially contributing to metastasis. Targeting CAF sEV pathways may offer new therapeutic approaches for patients with melanoma brain metastases.

EACR25-2417

Self-assembling peptide hydrogels as animal-free models of the tumour microenvironment

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Introduction

Current 3D in vitro models for studying development and disease often rely on animal-derived matrices, which suffer from ill-defined composition, batch-to-batch variability, and poor representation of human biology. These limitations contribute to the high attrition rate of new drugs transitioning from the lab to the clinic, particularly in oncology. Consequently, there is growing interest in synthetic alternatives that offer greater control over mechanical and biological properties to better replicate human tissues.

Material and method

We have developed a novel, animal-free 3D cell culture platform using a simple self-assembling peptide hydrogel (SAPH) to overcome the limitations of existing in vitro models. This SAPH is chemically defined, exhibits minimal batch variability, and can be tailored to mimic the mechanical and biological characteristics of specific tissues more accurately than traditional animal-derived matrices.

Result and discussion

Our platform has successfully modeled various tissue types, including breast cancer, glioblastoma, colorectal cancer, hepatocellular carcinoma, and acute myeloid leukemia. By tuning the SAPH's mechanical and biological properties, we have enhanced cell viability and in vivo-like behaviors, demonstrating its potential for both mechanistic studies and drug screening.

Conclusion

This chemically defined, animal-free 3D culture platform presents a viable alternative to Matrigel® and other animal-derived matrices. However, further research is needed to optimize matrix properties for specific cell lines and applications, ensuring robust and reproducible in vitro models for drug development and disease modeling.

EACR25-2429

Pioneering the study of the RNA-binding protein TDP-43 in ER+ Breast Cancer

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Introduction

RNA-binding proteins (RBPs) regulate gene expression post-transcriptionally, and their dysregulation can amplify cancer driver effects, stimulate tumor progression, and increase aggressiveness. TDP-43, an RBP involved in mRNA metabolism, is known for its role in neurodegenerative diseases (NDs), such as frontotemporal dementia and amyotrophic lateral sclerosis, but recent evidence implicates it in cancer. In triple-negative breast cancer, TDP-43 upregulation drives alternative splicing promoting tumor progression. Here, we investigated TDP-43 expression and function in estrogen receptor-positive (ER+) breast cancer.

Material and method

TDP-43 expression was assessed via Western blot in soluble and insoluble fractions of MCF-7 cells. Cells were treated with IC50 doses of 17 β -estradiol (E2), 4OH-tamoxifen (TAM), or fulvestrant (FUL). After 24 hours, confocal microscopy determined the subcellular localization of TDP-43 and ER α . TDP-43 was knocked down with a siRNA cocktail; after 24 hs, cells were processed for RNA sequencing or exposed to E2/anti-estrogens for 3 days before quantifying proliferation. Additionally, TDP-43 expression was examined in a small cohort of paired ER+ breast cancer samples ($n = 16$, pre- and post-neoadjuvant antiestrogen treatment) from the Portuguese Oncology Institute (CES IPO: 369/2017). Expression associations in the luminal TCGA and CPTAC datasets were analyzed using cBioPortal.

Result and discussion

RNA sequencing identified 17 upregulated and 14 downregulated genes (\log_2 fold change >2 or <-2 , FDR < 0.01). Upregulated genes related to cell cycle arrest, mitotic disruption, apoptosis, and altered cell adhesion/cytoskeletal integrity, while downregulated genes were involved in cell adhesion, extracellular matrix interactions and cytoskeletal organization. In the TCGA and CPTAC datasets, low TDP-43 expression associated with genes in innate immunity, cell adhesion, and lipid metabolism, whereas high TDP-43 expression correlated with genes related to transcription, spliceosome function, and DNA repair. Following TDP-43 knockdown, a 40% reduction in cell number and Ki67 expression were observed after 3 days. Moreover, TDP-43 knockdown abrogated E2's mitogenic effects and amplified growth

inhibition by TAM and FUL. Notably, ER inactivation with TAM or FUL increased TDP-43 insolubility promoted its nuclear translocation and reduced co-localization with ER α . In breast cancer tissues, TDP-43 showed cytoplasmic mislocalization, normalized to nuclear localization after endocrine therapy. CPTAC protein expression correlation analysis further indicated a significant association between TDP-43 and key ER-regulated genes.

Conclusion

This study is the first to demonstrate TDP-43's impact in ER+ breast cancer, suggesting a functional crosstalk of relevance to endocrine therapy and the need for further mechanistic research.

EACR25-2433

3D Patient-Derived Tumor Microtissues: Next Level In Vitro Model for Scalable and Reliable Preclinical Drug Testing

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Introduction

Recent advancements in tumor biology have significantly deepened our understanding of the tumor microenvironment, enabling the development of innovative therapies. However, the majority of oncology drug candidates still fail during clinical trials due to inadequate preclinical models that do not sufficiently replicate tumor complexity and heterogeneity. These shortcomings limit the ability to predict therapeutic efficacy and resistance mechanisms. To address this issue, we have developed a refined preclinical model based on 3D patient-derived tumor microtissues.

Material and method

Our 3D InSight™ Primary Tumor platform preserves essential components of the patient tumor microenvironment while offering scalability for high-throughput screening and validation of therapeutics. Unlike other 3D in vitro and ex vivo models, including patient-derived organoids, PDX-derived organoids, and organotypic slice cultures, our platform uniquely retains the structural integrity and heterogeneity of the original tumor. Cellular composition was assessed using flow cytometry following tissue dissociation, while treatment responses to standard-of-care chemotherapeutics were evaluated

Result and discussion

Our findings confirm that 3D patient-derived tumor microtissues maintain key physiological traits of their original tumors. Flow cytometry analysis demonstrated that crucial tumor and stromal cell populations were preserved, highlighting the model's ability to reflect patient-specific tumor heterogeneity. Using fresh colorectal and breast cancer tissue samples, we successfully established uniform 3D microtissues with a 70% success rate. Additionally, exposure to chemotherapeutic agents produced responses consistent with those observed in clinical settings, underscoring the platform's translational relevance. By providing a model that mirrors tumor complexity, this system enables a

more accurate assessment of therapeutic responses and resistance mechanisms.

Conclusion

The 3D InSight™ Primary Tumor platform presents a robust and scalable approach for preclinical drug development. By maintaining the structural and cellular diversity of tumors, it offers an effective system for evaluating novel therapeutics in a clinically relevant manner. This high-throughput model facilitates the development of personalized oncology treatments, ultimately contributing to improved patient outcomes and advancing precision medicine.

EACR25-2438

Androgen Receptor Loss Drives Nuclear Instability in Cancer-Associated Fibroblasts: A Novel Mechanism of Tumor-Stroma Remodeling

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Introduction

Cancer not only mutates its own cells but also hijacks its surrounding microenvironment to support its growth. Cancer-associated fibroblasts (CAFs), the dominant stromal players, actively remodel the tumor niche. While nuclear abnormalities are well-documented in cancer cells, their role in CAFs remains largely unexplored. Here, we uncover a striking discovery: the loss of androgen receptor (AR), an early event in CAF activation, triggers nuclear instability – disrupting nuclear architecture, increasing micronuclei formation, and promoting nuclear envelope ruptures.

Material and method

Using primary human dermal fibroblasts (HDFs) and patient-derived CAFs from skin squamous cell carcinoma (SCC), we examined the effects of AR depletion on nuclear integrity. Genetic AR overexpression and pharmacological activation with the synthetic AR agonist ostarine were used to rescue nuclear abnormalities. AR silencing in melanoma cells was performed to assess whether this mechanism extends beyond fibroblasts. Chromatin immunoprecipitation (ChIP) and protein interaction studies were conducted to uncover lamin-associated transcriptional regulation and nuclear structural changes.

Result and discussion

Loss of AR in fibroblasts resulted in severe nuclear abnormalities, a phenotype mirrored in SCC-derived CAFs, which naturally exhibit reduced AR expression. Restoration of AR – either genetically or pharmacologically – rescued these defects, directly linking AR function to nuclear stability. Similar nuclear abnormalities were observed in AR-silenced melanoma cells, suggesting a broader role beyond fibroblasts. Mechanistically, AR loss disrupted the interaction between lamin A/C and the nuclear protein phosphatase PPP1, leading to increased lamin A/C phosphorylation at Ser 301 – a defining characteristic of CAFs. Strikingly,

phosphorylated lamin A/C at Ser 301 bound to the regulatory regions of CAF effector genes associated with the myofibroblast subtype, amplifying their activation. ChIP analysis revealed increased lamin A/C association with chromatin, directly influencing CAF gene expression. Furthermore, AR was identified as a key lamin A/C interactor, and its depletion led to persistent lamin A/C phosphorylation, depolymerization, and nuclear instability.

Conclusion

This study uncovers a previously unrecognized AR-lamin axis that safeguards nuclear integrity in the tumor microenvironment. The accumulation of phosphorylated lamin A/C and nuclear abnormalities in SCC and melanoma stroma suggests that AR loss drives CAF activation through lamin-mediated chromatin remodeling. By weakening stromal resilience and promoting tumor-supportive CAFs, AR depletion fuels tumor progression. Targeting AR signaling could offer a novel therapeutic strategy to restore nuclear stability and disrupt CAF-driven tumor support, potentially dismantling cancer's protective fortress.

EACR25-2449

Spatial mapping of breast cancer highlights discrete tumour microenvironments and differences in spatial tumour architecture between Black British and White British women

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Introduction

Breast cancer presents with earlier onset and more aggressive progression in certain ethnic populations, yet these groups remain underrepresented in clinical trials. Standard therapies are largely based on responses observed in Caucasian populations, often failing to adequately address the unique biological characteristics of breast cancer in diverse ethnic backgrounds women. Understanding the ethnic-specific biology of breast cancer by investigating the spatial architecture of the breast tumour microenvironment (TME) is crucial for developing more effective, tailored therapeutic approaches that improve outcomes across all populations.

Material and method

We set to investigate the biological drivers for ethnicity associated early onset of breast cancer by using a British patient cohort comprised of 45 Black British and 45 White British patients. These groups were matched for age, breast cancer subtype and disease stage, with all patients being treatment naïve. To capture spatial tumor heterogeneity, two tumor cores were obtained per patient: one from the tumor centre and one from the tumor leading edge. GeoMx spatial transcriptomics and Cell DIVE hi-plex immunostaining were employed to identify and validate differentially expressed genes and proteins within the tumor and TME.

Result and discussion

Through spatial mapping of the TME, our findings indicate significant transcriptional differences arise mainly in the immune and αSMA-positive stromal compartments of Black British and White British women. Interestingly, we identified significant alterations of hallmark pathways including ECM and immune related pathways across distinct tumour areas (centre vs edge) from these different ethnic groups. Additionally, Cell DIVE analysis reveals cellular phenotypic architectural and difference according to ethnicity.

Conclusion

Overall we show that breast cancer biology is differentially regulated in various ethnic backgrounds and lays the foundation for a deeper understanding of potentially identifying ethnically relevant treatment strategies for breast cancer.

EACR25-2454

Targeting RNA splicing as a novel therapeutic strategy in ovarian high-grade serous carcinoma

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Introduction

Ovarian High-grade Serous Carcinoma (HGSC) is the predominant ovarian cancer subtype and a leading cause of cancer mortality in women. Although new therapies such as PARP inhibitors have improved treatment options for some homologous recombination deficient (HRD) patients, disease progression is characterised by cycles of relapse, and inevitably, resistance to therapy. Thus, there is a clinical unmet need to address the lack of therapies available for these patients. Alternative splicing of RNA is a key mechanism for phenotypic diversity and splicing variants, and important in tumour progression and drug-resistance. Aryl-sulfonamides such as indisulam are molecular glue degraders that inhibit RNA splicing via protein degradation of RNA splicing factor RBM39. This leads to genome-wide RNA splicing defects such as intron retention and exon skipping that we hypothesise generate opportunities for combination therapy.

Material and method

We use comprehensive transcriptomic and proteomic analyses including long-read sequencing to elucidate functional consequences of RNA splicing interference. We employ 2D, 3D, and in-vivo models of ovarian HGSC for experimental validation and conduct drug library and CRISPR/Cas9 screens to identify new drug combinations and genetic dependencies for PARP inhibitors.

Result and discussion

Exposure to indisulam leads to large scale RNA splicing defects that are not random and enriched for cell cycle and DNA damage repair pathways. Consequently, indisulam increases the sensitivity to platinum and PARP inhibitors drug-resistant models in-vitro and in-vivo. Combination drug library and CRISPR/Cas9 knockout screens in therapy-resistant models have identified new combination therapies and additional RNA splicing modulators as dependencies for PARP inhibitors. Finally, proteogenomic approaches combining long-read

sequencing with mass-spec have demonstrated that indisulam produces aberrant transcripts that are translated into detectable peptides that may be targetable with immunotherapy.

Conclusion

RNA splicing interference with molecular protein degraders offers new strategies to sensitise to therapies in resistant ovarian HGSC through a dual action of targeting DNA damage repair and splicing-derived neoantigens.

EACR25-2464

ADAMTS1 orchestrates cell state dynamics in muscle regeneration and rhabdomyosarcoma

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Introduction

Rhabdomyosarcoma (RMS) is the most frequent form of pediatric soft-tissue sarcoma and remains a medical challenge. RMS shares histological features with cells of the muscle lineage and this cancer is thought to arise from malignant transformation of myogenic precursors. It has been proposed that some early steps of myogenic differentiation are blocked in RMS, and that understanding how the normal process has gone awry could help to decipher the biological underpinnings of tumorigenesis and tumor escape.

Material and method

Here, we combine the use of murine transgenic and xenograft models, in vitro tools including organoids, and integrative multi-omic bulk and single-cell data analyses, to define the function of ADAMTS1 in muscle pathophysiology.

Result and discussion

Our results show that a matrix protein with hitherto unknown function, ADAMTS1, is involved in skeletal muscle regeneration, via modulation of focal adhesion pathway activity. We demonstrate that ADAMTS1 is a good prognosis factor in Fusion Positive-RMS (FP-RMS). In FP-RMS, ADAMTS1 drives a phenotypic switch that modifies invasion potential of tumor cells, suggesting that it may constitute a new therapeutic target.

Conclusion

Our results identify ADAMTS1 as a new prognostic marker in FP-RMS and underlies the need to characterize in depth the crosstalk between matrix and tumor cells, as a new putative lever to impact tumor aggressive cell state.

EACR25-2472

m6A epitranscriptome landscape in bladder tumors: translational impact in patients' prognosis and treatment outcome

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Introduction

Bladder cancer (BlCa) exhibits a highly heterogeneous molecular landscape, resulting in non-personalized prognosis and treatment decisions. N6-methyladenosine (m6A) represents the most prevalent RNA modification, regulating RNA biology/metabolism and cellular homeostasis. Herein, we have profiled mutations/copy number variations (CNVs) and expression of m6A RNA machinery genes as well as m6A epitranscriptome in bladder tumors and healthy urothelium, and assessed their clinical relevance in BlCa patients.

Material and method

DNA-seq (MGI Tech) was performed in 96 bladder specimens (tumors: n = 87; normal urothelium: n = 9) using a custom-designed panel of enrichment probes (Agilent) for m6A writers (METTL3, METTL14, METTL16, VIRMA, WTAP, RBM15, RBM15B, ZC3H13), erasers (FTO, ALKBH5) and readers (YTHDF1, YTHDF2, YTHDF3, YTHDC1, YTHDC2, IGF2BP1, IGF2BP2, IGF2BP3). m6A-seq/MeRIP-seq (MGI Tech) was performed in 55 bladder specimens (tumors: n = 50; normal urothelium: n = 5) following m6A RNA immunoprecipitation. m6A writers and erasers expression quantified by RT-qPCR in a screening cohort of 213 patients. The UROMOL (n = 535) was analyzed as validation cohort for non-muscle-invasive BlCa (NMIBC), while the TCGA-BLCA (n = 412) and Mariathasan et al. (n = 348) cohorts were analyzed for muscle-invasive BlCa (MIBC). Colorimetric ELISA-like assay using m6A Abs performed for %m6A quantification. Disease relapse/progression for NMIBC and patients' mortality for MIBC were used as clinical endpoints for survival analysis.

Result and discussion

Deleterious mutations in m6A writers were highlighted in ~30% of tumors (25/87), compared to m6A erasers (3.2%) and readers (5.3%). Moreover, CNVs were detected in ~60% of the patients (52/87), with ~22% gain/amp of VIRMA in NMIBC. MIBC patients with deleterious mutations in m6A writer's complex presented higher progression risk ($p = 0.036$) and worse survival ($p = 0.010$). Moreover, VIRMA gain/amp was associated with higher %m6A levels and short-term relapse ($p = 0.019$) in NMIBC. Finally, reduced METTL3 expression correlated with higher risk for short-term relapse ($p = 0.004$) and progression ($p = 0.013$) of superficial (TaT1) to muscle-invasive (T2-T4) tumors, as well as with worse survival of MIBC patients ($p = 0.027$). Consistently, validation cohorts confirmed the poor treatment outcome of the patients with METTL3 loss, while METTL3-fitted multivariate models improved risk-stratification compared to clinical established markers.

Conclusion

Overall, genomic variations in m6A machinery and METTL3 expression regulation emerge as modern molecular markers to address BlCa clinical heterogeneity and guide personalized prognosis and treatment.

Acknowledgements: The research project was supported by the Hellenic Foundation for Research and Innovation (H.F.R.I.) under the "2nd Call for H.F.R.I. Research Projects to support Faculty Members & Researchers" (Project Number: HFRI-FM20-3765).

EACR25-2483

Unveiling the influence of Citrate on Prostate Carcinoma Tropism to Bone by a microfluidic model of bone metastasis

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Introduction

Prostate cancer (PCa) exhibits distinct metabolic traits – including unique citrate metabolism, high lactate production – and a pronounced affinity for bone. While bone's mineralised matrix, rich in citrate, mirrors healthy prostate tissue, PCa's reliance on bone-trapped citrate and its interplay with lactate in the metastatic niche remain poorly understood. Conventional 2D models inadequately replicate the 3D stromal-metabolic crosstalk critical to tumour progression.

Material and method

To dissect citrate's role in PCa bone tropism, we engineered a 3D microfluidic bone-on-a-chip model. This system integrates Osteocytes (citrate-secreting MLO-Y4 cells), a collagen type I matrix mineralised with hydroxyapatite nanoparticles (HA-NPs) synthesised with or w/o citrate, and physiologically tunable citrate gradients. Metastatic (PC3) and less metastatic (LNCaP) PCa cells were tested for chemoattraction toward citrate-enriched HA-NPs versus HA-NPs alone. Post-seeding, we quantified spheroid formation, viability, clonogenicity, metabolic adaptations (e.g., lactate production), cytotoxic effects on osteocytes, and osteogenic activity.

Result and discussion

The model replicated osteocytes' 3D dendritic network and bone metabolic dynamics. Citrate-enriched HA enhanced PCa chemoattraction, particularly in bone-metastatic PC3 cells, and increased spheroid formation without compromising viability. While tumour cell metabolic activity remained stable, citrate and HA buffered microenvironmental acidification during proliferation of highly glycolytic PCa cells, correlating with elevated clonogenicity. Notably, PCa-derived lactate triggered osteocyte apoptosis and stimulated citrate release, suggesting a feedforward loop where tumour-driven bone remodelling enriches citrate deposits, potentially fuelling metastatic growth.

Conclusion

Our 3D model uncovers a bidirectional interplay between PCa and bone: citrate enhances tumour aggressiveness, while tumour-derived lactate promotes osteocyte death and citrate-enriched bone formation, creating a vicious cycle. These findings advance physiologically relevant metastasis models and identify citrate-bound hydroxyapatite as a therapeutic target to disrupt PCa-bone crosstalk.

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EACR25-2484

miR-214 Modulates Pigmentation and Therapy Resistance in Cutaneous Melanoma

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Introduction

Despite significant advances in cutaneous melanoma (CM) treatment, particularly with immune checkpoint inhibitors (ICI), resistance remains a major challenge, necessitating innovative therapeutic strategies. Melanin plays a crucial role in CM progression, with hyperpigmentation correlating with therapy resistance, including resistance to ICI. While melanin primarily protects against light-induced damage by scavenging reactive oxygen species (ROS), it can also act as a photosensitizer and pro-oxidative agent, depending on its type and intracellular redox state. Its ROS-scavenging activity largely depends on its ability to chelate metal ions, particularly iron, which paradoxically induces melanogenesis due to its high ROS-generating activity. Experimental evidence suggests that miR-214 is a key player in melanoma hyperpigmentation and therapy resistance. This study aims to elucidate the interplay between miR-214 and resistance in CM and to identify potential molecular targets for restoring therapy sensitivity.

Material and method

Melanoma cells were stably transfected to overexpress miR-214 (miR-214+) using the PiggyBac transposon system. Intracellular melanin content was quantified by spectrophotometry, melanosomes were visualized via transmission electron microscopy, and key melanogenic proteins were analyzed through western blot. ROS levels were assessed by flow cytometry, and intracellular iron content was measured using a colorimetric assay.

Therapy response was evaluated in bi- and three-dimensional experimental settings, prevalently using MTT assay, live/dead flow cytometry dye, and colony formation assay. Melanogenesis inhibitors (e.g., the tyrosinase inhibitor deoxyarbutin and the iron chelator pyridoxal isonicotinoyl hydrazone) were used to confirm the role of pigmentation in the acquisition of resistance. Similarly, miR-214-3p/5p mimics were used to ensure the role of miR-214 in pigmentation and therapy resistance of melanoma cells. Serum levels of miR-214

were quantified by droplet digital PCR in CM patients treated with ICI.

Result and discussion

miR-214+ melanoma cells exhibited increased pigmentation, deregulated iron metabolism, and elevated ROS levels accompanied by reduced antioxidant response. These hyperpigmented cells displayed reduced responsiveness to chemo-, targeted-, radio-, and immunotherapy *in vitro*, but sensitivity was restored upon inhibition of miR-214 signaling and melanogenesis. Significantly higher levels of miR-214 were found in the serum of metastatic patients, and within the metastatic setting under ICI treatment, an increasing expression trend was observed in non-responders compared to responders.

Conclusion

miR-214 drives the development of hyperpigmented, resistant melanoma phenotypes. A deeper understanding of its molecular network is crucial for identifying novel therapeutic targets to overcome resistance in non-responsive CM patients.

EACR25-2491

Impact of obesity on the interaction between stem cells and cancer cells in ovarian cancer

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Introduction

With obesity considered a global pandemic and one of the major health concerns, it is important to understand the impact of body fat levels on other diseases, such as cancer. Adipose tissue is an active secretory organ influencing a number of metabolic processes. It comprises various cell types, including stem cells, which may play a role in cancer development, progression, and metastasis. In ovarian cancer obesity is considered as both a risk factor and negative prognostic factor. As our earlier study on the interaction between omental derived adipose stem cells (O-ASC) and ovarian cancer cell lines suggested some BMI-dependent differences in gene expression, we aimed to further investigate this relation.

Material and method

We used three ovarian cancer cell lines (OVCA433, OVCA429, and SKOV3) and three O-ASC lines derived from patients with varying body mass index (BMI) (O-ASC1, O-ASC4, and O-ASC15; in the increasing donors BMI order). Cancer and O-ASC cells were cultured in Boyden chambers (cell culture inserts with 0.4 µm pores) to enable paracrine communication without direct contact

(co-culture), or alone (control). Total RNA was isolated and used for gene expression profiling with Affymetrix Human Gene 1.0 oligonucleotide microarrays. After initial pre-processing and exploratory analysis, interaction models were applied to identify genes whose expression changes between control and co-culture differed according to BMI. For O-ASCs, additional filtering was performed to ensure monotonic changes with increasing BMI.

Result and discussion

Gene expression profiles of both the ovarian cancer cells and O-ASCs were affected by inter-cellular interactions. For cancer cell lines, we identified 504 genes that differ in expression level between co-culture and control and depending on the O-ASC cell line (22 of them with $|\log_{2}FC| > 0.5$). In O-ASCs, principal component analysis revealed correlation between gene expression levels and BMI of the donor. Additionally, the effect of interaction with cancer cells varied depending on BMI – we found a signature of 36 genes whose expression in co-culture vs control consistently increased (in case of some genes) or decreased (in case of other genes) along with increasing BMI.

Conclusion

Obesity may influence the gene expression profile of O-ASC and ovarian cancer cells interacting with one another, as well as to modulate the strength of these interactions, particularly in genes related to proliferation and migration. These results, encourage to further study these interactions on the proteome and metabolome level. They also suggest O-ASC – ovarian cancer cell interactions as potential therapeutic target, and highlight the importance of weight management in patient care.

EACR25-2492

Simple and cost-effective cancer diagnosis in liquid biopsy through native tRNA sequencing

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Introduction

Every year, 20 million people are diagnosed with cancer, and 10 million people die due to this complex and heterogeneous disease. Early cancer diagnosis is highly correlated with good prognosis and higher survival rates. For example, patients diagnosed with stage IV lung cancer have 1-year survival rates of only 15–19 %, whereas stage I patients show 81–85 % 1-year survival rates. Screening liquid biopsy (LB) samples has been proposed as a promising approach to diagnose cancer in earlier stages, since it is a minimally invasive approach that can be applied to the whole population. The discovery of relatively stable, extracellular RNAs in blood plasma has generated much interest in their potential use as non-invasive biomarkers. Most efforts have so far focused on miRNAs or lncRNAs as potential

biomarkers. By contrast, tRNAs remain unexplored as potential biomarkers, despite their information-rich nature – abundances and modifications – and their well-documented dysregulation in cancer samples. This is mainly due to the lack of a simple, fast and cost-effective approach to quantify them. Indeed, current approaches to study tRNAs require steps that introduce bias in the process of quantification, are lengthy and laborious, and have very high costs. Thus, the high costs of existing diagnostic tests, the lack of adequate biomarkers and the inability to capture RNA modification information using next-generation sequencing (NGS) technologies have hindered the development of tRNA-based screening tests for early cancer detection, and the use of tRNA molecules as biomarkers.

Material and method

Here we propose to take advantage of a recently developed sequencing technology, native RNA nanopore sequencing, to quantify small RNA abundances and modifications in liquid biopsy samples. More specifically, we will employ our recently established Nano-tRNAseq method, which we have patented, to sequence information-rich circulating tRNA molecules to profile their abundance and modification dynamics. We will then couple this method with artificial intelligence to predict:

(i) whether the sample is “normal”, “cancerous”, or “metastatic” based on the profiles we build from training samples, as well as
(ii) the cancer tissue of origin.

Result and discussion

Our preliminary experiments demonstrate that Nano-tRNAseq captures human tRNA dynamics across cell lines, tissues and nutrient statuses, confirming their potential as biomarkers. Moreover, using tRNA abundance and modification data from paired lung normal and tumor tissues, we were able to distinguish their disease state. Notably, we have also been able to isolate and quantify small RNAs from human plasma samples with high quality.

Conclusion

In this study, we aim to fully validate our approach for the differential analysis of low-input RNA amounts in liquid biopsy samples.

EACR25-2505

Studying glioblastoma-associated blood vessel changes across tumor progression to improve targeted therapies

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Introduction

Glioblastoma is the most common and aggressive primary brain tumour and despite current treatments that combine chemo- and radiotherapy with surgery, recurrence is overwhelmingly inevitable for most of the patients. Importantly, treating glioblastoma is particularly challenging as it requires crossing the blood-brain barrier and reaching the tumor cells within the heterogeneous brain microenvironment. In addition, tumour micro-

environment shifts as the tumour progresses hindering therapy efficacy. Thus, understanding the biological influence of the tumor on the blood brain barrier and its response to therapies as glioblastomas develop is key to engineer the most effective and personalized targeted therapies. In this work, we investigated the changes in tumor-associated endothelial cells (ECs), the first component of the blood brain barrier, at different stages of a mouse model of glioblastoma.

Material and method

To decipher changes in ECs across tumor progression, single-nucleus RNA sequencing of GL261 tumour-bearing mice was performed at early and late stages of tumour development and upon treatment with temozolamide (TMZ), the standard of care for glioblastoma, and with a promising cell-penetrating peptide that inhibits the oncoprotein Src, TAT-Cx43266-283.

Result and discussion

By comparing the vascular landscape from tumors at their final stages with early stages, we observed that ECs acquired a more activated phenotype, as seen by the increase in the adaptive immune response and antigen presentation; and a relevant boost in translation by the increase in ribosome assembly or protein folding that may underlie cell growth; and metabolic pathways that may fuel these processes, such as fatty acid metabolism and regulation of lipid transport. TMZ treatment, however, reduced the inflammatory response, and increased the regulation of calcium and ion transmembrane transport, both essential for barrier function. Interestingly, ECs from early tumor stages treated with TAT-Cx43266-283, compared with TMZ, showed a decrease in pro-migratory pathways, such as cell adhesion mediated by integrins; and important pro-angiogenic pathways such as WNT, suggesting a delay in the onset of the glioblastoma-associated proangiogenic vascular phenotype.

Conclusion

In conclusion, this work shows that glioblastoma triggers a more complex blood vessel machinery than just an angiogenic response, and that a deeper insight into the phenotypic changes induced by both tumors and therapies is needed. Understanding the tumor and therapy-associated microenvironment changes will improve the current knowledge on the vascular response that may influence the prospects of the disease.

EACR25-2507

High-throughput Bioprinting of Physiomimetic Tumor-Stroma Microenvironment 3D Models for Screening Therapeutics

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Introduction

Engineering tumor microenvironments (TMEs) with cancer-associated fibroblasts (CAFs) has emerged as a powerful tool for screening chemotherapeutics and nano-

therapeutics. The TME plays a crucial role in tumor progression, drug resistance, and therapeutic efficacy, yet conventional two-dimensional (2D) models fail to replicate its complexity. Herein we bioengineered three-dimensional (3D) TME model incorporating CAFs to improve drug screening and enhance translational relevance. The models are generated as high-throughput platforms using superhydrophobic surfaces and our automated technique - FLORE (Flow-On-Repellent) bioprinting. This approach enables the rapid, automated fabrication of cancer-on-a-bead models, providing a scalable and reproducible system for preclinical drug evaluation. Additionally, the models are embedded within tissue-specific decellularized extracellular matrix (dECM) to better mimic the native tumor microenvironment and enhance physiomimetic relevance.

Material and method

We developed a 3D tumor-CAF co-culture system using patient-derived cancer cells and CAFs within a biomimetic, tissue-specific dECM scaffold. The system was fabricated using FLORE bioprinting on superhydrophobic surfaces to enable high-throughput generation and ensure reproducibility and scalability. The dECM was derived from decellularized tissue to retain key biochemical and structural cues. High-content imaging and viability assays were conducted to assess cancer-stromal cells spatial distribution and drug responses.

Result and discussion

Our engineered TMEs demonstrated enhanced recapitulation of *in vivo* tumor-CAF interactions compared to traditional models. CAFs influenced drug responses by modulating extracellular matrix composition and secreting key cytokines. The use of tissue-specific dECM provided a more physiomimetic environment, supporting cellular organization and enhancing drug response predictability. The integration of FLORE bioprinting and superhydrophobic surfaces ensured high-throughput and reproducible screening, highlighting the necessity of incorporating CAFs and dECM in preclinical drug testing to improve the predictive accuracy of such tumor microenvironment surrogates.

Conclusion

The engineered 3D tumor-CAF model represents a significant advancement in preclinical drug screening by providing a more physiologically relevant, high-throughput platform to assess chemotherapeutics performance. By leveraging FLORE bioprinting and tissue-specific dECM, this approach enhances biomimicry and predictive accuracy, paving the way for more effective drug development and personalized treatment strategies. *This work was developed within the framework of EU INSPIRE project (grant ID: 101057777 a Cluster 1 - Health).*

EACR25-2508

Impact of preoperative treatment on the tumor microenvironment of liver metastases from colorectal cancer

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Introduction

Colorectal cancer (CRC) often spreads to the liver (LM-CRC). While surgery can be curative for some patients, most receive preoperative chemotherapy. Chemotherapy alters the tumor microenvironment (TME). Understanding this could uncover therapeutic targets, resistance mechanisms, and new treatment vulnerabilities. This study aims to investigate the influence of FOLFOX-based chemotherapy on the TME of LM-CRC.

Material and method

The human HSC line LX-2 was treated with FOLFOX for six cycles, mimicking clinical protocols. Conditioned media and cell lysates were collected, and proteomic analysis was performed. Functional assays, including immune cell recruitment, tumor proliferation, and migration, were done using treated and untreated LX-2 cells. RNA sequencing was performed on co-cultures of LX-2 cells, THP-1, and SW620 tumor cells. Tissue microarrays from chemotherapy-naïve and FOLFOX-treated patients were used for validation.

Result and discussion

Proteomic analysis revealed carbonic anhydrase 9 (CA9) as the most significantly upregulated protein in treated LX-2 cells compared to untreated controls. CA9 overexpression was confirmed by Western blot, immunofluorescence, and immunohistochemistry in TMA. IPA analyses reported a metabolic shift from oxidative phosphorylation to glycolysis, being HIF-1α and STAT3 upstream regulators of the enriched pathways in treated HSC. Western blot revealed HIF-1α overexpression in treated cells. Control cells displayed a more HSC-quiescent state, involving the metabolism of fatty acids, lower proliferative rate, and organization of the actin filament bundle. The proinflammatory profile of treated-LX-2 was also observed in the analyses of the analytes in the conditioned media where IL-4, IL-1β, CCL2, CCL4, CCL20 and M-CSF were overexpressed in treated-LX-2 in comparison to non-treated ones. Also, other factors such as HGF, VEGF, PAI-1, COL1A1, and COL4A1 were upregulated. RT-PCR and protein levels showed an overexpression of FAP and Periostin in treated LX-2 compared to chemonaive. Analyses of 3D cocultures displayed enrichment of interferon pathways activation in LX-2 T cells, as well as in co-cultured SW620 and THP-1 cells, relative to untreated controls. THP-1 cells co-cultured with LX-2 T cells exhibited enrichment in proliferation-related pathways. Untreated LX-2 cells displayed enrichment in cholesterol and oxidative phosphorylation metabolism pathways, and co-cultured tumor cells showed enrichment in proliferation pathways. Functional assays demonstrated differential effects of the treatment on immune cell recruitment, tumor growth modulation, and tumor cell migration. Galectin 9 is tested as a surrogate marker of IFN response between chemonaive and FOLFOX-treated samples.

Conclusion

FOLFOX-activated HSC displayed transcriptomic, proteomic, and metabolic changes that impact in the TME, including tumor cell responses and immune modulation.

EACR25-2517

Neuroendocrine plasticity diversity in prostate cancer: role of redox state

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Introduction

Neuroendocrine (NE) plasticity contributes to prostate cancer progression affecting both tumour cell and its microenvironment, however, NE phenotypes display strong variability. Some factors involved in prostate cancer risk such as inflammation or therapy, such as androgen deprivation strategies are able to initiate NE plasticity. In these work we study diversity of NE plasticity to clarify what aspects truly contribute to disease progression.

Material and method

In vitro NE plasticity is induced in androgen-dependent human adenocarcinoma LNCaP Cells by androgen deprivation (Ad), increase levels of cAMP, Interleukine-6 and the redox-hormone Melatonin (MEL). Conditioned media were used to evaluate pro-angiogenic activity (HUVEC matrigel assay and VEGF secretion), pro-fibrotic activity (Collagen production in 3T3 cell culture) and growth proliferation (PC-3 cell culture). Survival to Chemotherapy (Etoposide and Docetaxel) and Prostate Specific Antigen (PSA) secretion was also studied. Androgen Receptor (AR) protein levels were measured by IHC and Mitochondrial Superoxide Dismutase (MnSOD/SOD2) levels were studied by western blot and enzymatic activity gel. DFC, MitoSOX and H2O2 secretion by amplex red were used to monitorize redox state. The following animal models of prostate cancer were used: Transgenic Prostate Cancer (TRAMP)-WT and triploid for SOD2 allele. Xenograft of LNCaP alone or in combination with NE-LNCaP.

Plasticity of NE in human prostate cancer was inquired by using Hyperion Spatial Proteomics System (EACR-Fluidigm Grant).

Result and discussion

Substantial differences in all the progression features studied were observed in the different NE groups, AR expression levels, survival to Chemotherapy treatment, PSA production, angiogenesis or fibrosis. NE plasticity induced by Ad and MEL showed similar features with increase of paracrine effect on PC-3 growth or decrease in VEGF secretion. All the NE-stimuli however, showed a significant increase in MnSOD levels and activity, together with a general decrease in redox state especially in Ad group. Consistently, TRAMP triploid for SOD2 showed significant less VEGF in the prostate tissue and higher rates of NE plasticity in response to surgical castration. Antioxidant treatment is able to change in vitro fibrosis and VEGF production in cAMP, IL-6 and MEL but the effect in Ad were the redox levels were already very low is modest. In xenograft models when LNCaP cells are administered together with NE-LNCaP cells, tumours are much less haemorrhagic and exhibit a non-collagenous extracellular matrix. Antioxidants prevent partially the NE-characteristics in the TME and delays tumour onset. In patient tissue, NE-plasticity only shows

impact in TME in those tissue with substantial levels of MnSOD.

Conclusion

NE plasticity caused by Ad has the strongest impact in TME. NE plasticity occurs together with a rearrangement in redox state which contributes to TME effects.

EACR25-2524

3D Cell-derived ECM Production for Humanized Lung Cancer in vitro Model Development

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Introduction

Despite significant advances in the development of 3D in vitro tumor models, the majority still fails to recapitulate the complexity of the lung tumor microenvironment (TME) extracellular matrix (ECM). Instead of applying microenvironment rich in tissue-specific ECM components, most common approaches rely on the application of singularly isolated ECM components, or complex non-tissue specific hydrogels, that do not recapitulate the specificity of lung-tumor ECM. Cell-Derived Matrixes (cdECM) offer an alternative pathway to address the need for tissue and tumor specific ECM biomaterial production. However, conventional methodologies of cdECM production are recognizably associated with small biomaterial yields, making the application of these matrixes in the context of high-throughput model production challenging.

Material and method

To address this limitation, we developed a bioreactor-based approach for large-scale production of human lung cancer ECM by assembling and maturing 3D tumor microtissues composed of A549 lung adenocarcinoma cells and MRC-5 lung fibroblasts in dynamic stirring conditions. Moreover, MRC-5 cells were stimulated with different biochemical cues (i.e., TGF-β1 and Ascorbic acid) to induce their activation into cancer-associated fibroblast (CAF)-like phenotypes. Obtained matrixes were fully decellularized, and posteriorly characterized with recourse to previously established approaches, and proteomic characterization.

Result and discussion

Structural SEM and biochemical quantification analyses of ECM main components confirm the development of a tumor-relevant ECM composition, providing a biomimetic scaffold that could then be processed into workable hydrogels. These lung specific cdECM are expected to enhance the physiological relevance of in vitro lung cancer models. This stimulated ECM deposition and remodeling, mimicking the stromal adaptations observed in lung tumors.

Conclusion

Our study systematically optimizes key culture parameters, including cell ratios, biochemical stimulation, and maturation time, to maximize ECM production while preserving tissue-specific characteristics. Structural SEM and biochemical quantification analyses of ECM main components confirm the

development of a tumor-relevant ECM composition, providing a biomimetic scaffold that could then be processed into workable hydrogels. These lung specific cdECM are expected to enhance the physio-logical relevance of in vitro lung cancer models. By establishing guidelines for ECM-enriched microtissue production, this work presents a robust framework for generating humanized 3D tumor models with improved fidelity to in vivo conditions, advancing their applicability in preclinical research, drug screening, and personalized medicine.

EACR25-2532

Pancreatic Ductal Adenocarcinoma on Chip for Therapy Screening

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Introduction

Pancreatic Ductal Adenocarcinoma (PDAC) is one of the most aggressive cancers, hallmark by an immuno-suppressive tumor microenvironment (TME) enriched with cancer-associated fibroblasts (CAF), pro-tumoral macrophages (TAM), and abnormal vasculature. This complexity hinders therapeutic efficacy, highlighting the urgent need for advanced preclinical models. Here, we developed a PDAC-on-chip model incorporating pancreatic cancer cells, immune cells, and stromal components, enabling a more accurate high-throughput characterization of tumor-immune cell interactions.

Material and method

A polymethylmethacrylate (PMMA)-based chip was designed with media and tissue channels separated by a semi-permeable membrane. PANC-1 spheroids were loaded in the chip containing a hydrogel with fibroblasts and lymphatic endothelial cells (LECs). CD14+ monocytes from peripheral blood mononuclear cells (PBMCs) were differentiated into macrophages and integrated into the hydrogel. Labeled T cells were perfused through the chip to assess infiltration under different treatments. Microvascular endothelial cells (MVECs) were seeded onto the chip's membrane to simulate vascular perfusion [1]

Result and discussion

The PDAC-on-chip sustained long-term cell culture for over a week, enabling continuous monitoring of tumor spheroid growth, drug response and immune cell dynamics. To validate the model, we confirmed:

- (i) CAF marker expression, including αFAP, Podoplanin, and Vimentin;
- (ii) the formation of a stable MVECs monolayer

facilitating functional vasculature-like perfusion;

- (iii) integration of LECs in the stroma, forming vessel-like structures;
- (iv) incorporation of macrophages expressing pro-tumoral markers; and
- (v) perfusion of monocytes and T-cells through flow, allowing a semi-quantitative assessment of tissue penetration in response to pharmacological treatment.

Conclusion

The PDAC-on-chip successfully replicates tumor-stroma-immune cell interactions, offering a physiologically relevant model for immunotherapy screening [5]. Additionally, this platform enables the testing of nanotherapies targeting fibroblasts, macrophages, or vascular components, providing new insights into overcoming PDAC's immunosuppressive TME. These findings contribute to cancer research, paving the way for innovative strategies to improve patient outcomes.

[1] The use of human-derived cells was approved by the Ethical Committee of the Eberhard Karls University Tübingen.

The authors would like to thank FCT-MCTES for the PhD fellowship 2021.07349.BD, and the projects UIDB/04138/2020, and PTDC/BTM-SAL/4350/2021; Bayer Foundation for the Otto-Bayer Fellowship OB-2022-044; and "la Caixa" Foundation under the framework of the Healthcare Research (LCF/PR/HR19/52160021, NanoPanther; LCF/PR/HR22/52420016, MultiNano@BBM and LCF/HR24/52440018, PINT).

EACR25-2533

Spatial Profiling of Exhausted T cells Using High-Plex Imaging Mass Cytometry

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Introduction

The spatial organization and cellular composition of the tumor microenvironment (TME) has the potential to inform clinical and translational researchers about mechanisms of disease progression and therapeutic success. Of particular interest are immune cells, especially T cells, which can become exhausted due to chronic stimulation. This poses a significant challenge in cancer therapy as exhausted T cells have reduced effector functions and sustained expression of inhibitory receptors such as PD-1 and CTLA-4, resulting in failure to effectively eliminate tumor cells. Imaging Mass Cytometry™ (IMC™) technology is a high-plex imaging technique that enables deep characterization of the heterogeneity and complexity of the TME. The Hyperion™ XTi Imaging System utilizes IMC technology to provide signal intensities over a wide dynamic range and entails one-step detection of 40-plus markers without issues of tissue autofluorescence, making it ideally suited for spatial biology applications. Whole slide imaging modes and an automated slide loader function enable a streamlined, versatile scalable workflow for high-throughput analysis.

Material and method

We used IMC technology to characterize immune cell populations and the spatial distribution of T cell exhaustion markers across various cancer types. We applied a 40-plus-marker IMC panel by integrating the Human Immuno-Oncology IMC Panel with the Human T

Cell Exhaustion IMC Panel to study the TME of multiple human tissues.

Result and discussion

IMC analysis revealed striking heterogeneity with distinct tumor and immune-rich niches in the TME. In colon adenocarcinoma, we detected functional effector T cells and multiple tertiary lymphoid structures (TLSs). In contrast, we observed fewer TLSs and more T cells expressing PD-1 and TIM-3 at the tumor periphery in the urothelial carcinoma tissue, suggesting that they are in advanced exhaustion stages. We also observed expression of PD-L1, IDO and VISTA in the tumor cells. t-SNE and PhenoGraph clustering analysis of Cell Mode data segregated and spatially resolved the T cells into effector and exhausted T cell subpopulations, which were mapped back to the segmented cell masks. Unsupervised pixel clustering and analyses of Tissue Mode data further delineated distinct tumor areas based on the presence of infiltrating immune cells, tumor replicative activity and spatial proximity to stromal components.

Conclusion

This work characterizing T cell exhaustion markers in multiple cancers showcases the capabilities of IMC technology and establishes it as a reliable highplex, high-throughput spatial biology imaging platform. IMC technology is ideally suited for developing future translational and clinical applications and has the potential to help guide personalized therapeutic strategies for cancer treatment. For Research Use Only. Not for use in diagnostic procedures.

EACR25-2534

Standardization of a Murine Model of Colorectal Cancer Induced by AOM/DSS and Evaluation of the Chemopreventive Capacity of the 5-Fluorouracil-Curcumin Hybrid

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Introduction

Colorectal cancer (CRC) is a prevalent malignancy, often diagnosed at advanced stages. 5-fluorouracil (5FU) is a common chemotherapeutic agent for CRC but is limited by toxicity, variable responses, and resistance. Thus, alternative preventive and therapeutic strategies with proven in vivo antineoplastic activity are essential. Hybrid molecules, such as the 5FU-curcumin conjugate, combine the 5FU pharmacophore with curcumin, an antioxidant pigment, to enhance efficacy and reduce adverse effects.

Material and method

A colorectal cancer (CRC) model induced by azoxy-methane (AOM) and dextran sodium sulfate (DSS) was standardized in Balb/c mice, evaluating two induction protocols: protocol 1 (AOM + 1 cycle of DSS) and protocol 2 (AOM + 2 cycles of DSS), selecting the optimal one through histopathological analysis. Then, treatment was administered using 5-fluorouracil (control) and the 5-fluorouracil-curcumin hybrid in two periods (weeks 3 to 7 and weeks 5 to 10). Positive (CRC-AOM/DSS) and negative controls were included. After euthanasia, the colon was extracted for methylene blue staining and histopathological analysis. The study was approved by the bioethics committee (Act No. 114/2017, UdeA).

Result and discussion

A single DSS cycle (protocol 1) did not induce tumors in all animals, whereas two cycles (protocol 2) did after week 10. Wilcoxon test analysis showed no significant sex differences, confirming that the AOM/DSS CRC model in BALB/c mice replicates the adenocarcinomatous process and is applicable to both sexes with one AOM dose and two DSS cycles. Preneoplastic lesions appeared in week 3, progressing to tumors by week 12. Dysplasia was observed from week 2, preceding neoplastic proliferation and infiltration. Fibrosis developed in response to inflammation detected in week 2. Both 5FU and 5FU-curcumin delayed lesion progression, with mild to moderate histopathological alterations compared to the positive control. However, tumor growth resumed by week 12. Mice treated with 5FU experienced up to 40% mortality and weight loss, whereas those receiving 5FU-curcumin did not, indicating lower toxicity. Histopathological analysis confirmed reduced tissue damage, as ulceration levels were lower in the 5FU-curcumin group than in 5-FU alone.

Conclusion

The AOM/DSS system effectively replicates colorectal cancer progression in humans and is useful for evaluating chemopreventive strategies.

- The model can be applied to both male and female mice.
- The 5FU-Curcumin hybrid slightly delays tumor progression, but its effect is insufficient after treatment discontinuation.
- Unlike 5FU alone, the 5FU-Curcumin hybrid showed no toxicity and slightly reduced tissue damage.
- Hybrids like 5FU-Curcumin could be a promising strategy for colorectal cancer chemoprevention.

EACR25-2539

Cryobioprinted human tumor models for off-the-shelf preclinical drug screening

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Introduction

Human tumors are difficult to treat owing to their unique tumor microenvironment (TME) in which stromal cells foster drug resistance, leading to the inefficacy of current treatments. In this focus, the urgent need for predictive and accurate preclinical models has motivated researchers to explore new strategies for recapitulating the native TME for preclinical drug screening. Cryobioprinting, which integrates 3D bioprinting with cryopreservation methods, offers a promising approach for generating on-demand 3D tumor models at a relevant scale. Aiming to fabricate storable tumor-stroma models, that capture native TME elements and key tumor signatures, with potential for predictive screening of new therapies, here we take advantage of 3D cryobioprinting technique. The optimization of a tissue-specific de-cellularized extracellular matrix (dECM)-based bioink, comprising tumor-associated cells and optimal cryoprotective agents (CPAs) was conducted aiming to generate a cryoprotective bioink that protects cells during the cooling, storage and thawing process. The ability of the cryopreserved tumor platforms to reproduce key hallmarks as found *in vivo* was evaluated followed by its suitability to screen anti-cancer therapeutics.

Material and method

Two distinct bioinks were formulated:

- (i) the tumor bioink comprising lung cancer or pancreatic cancer cells, lung or pancreas dECM precursor hydrogel solution, and a combination of CPAs (i.e., melezitose + glycerol or DMSO); and
- (ii) the stroma bioink composed of cancer-associated fibroblasts (CAFs), dECM-HA-Tyr precursor hydrogels, and a combination of CPAs (melezitose + glycerol or DMSO).

The emulation of key biomarkers including tumor fibrosis was then characterized so as to address the potential of the cryobioprinted systems. Additionally, the biofabricated models were used for screening the anti-tumoral agents, in order to demonstrate the screening functionality of storable platforms.

Result and discussion

The formulated bioink-cryoprotectant combinations using tissue-specific dECM has enabled the cryofabrication of tumor models and the recapitulation of its TME. The discovered CPA combination of melezitose-glycerol ensured cellular viability after 3D tumor models cryobioprinting and cryopreservation, in comparison to the conventionally used CPAs (i.e., DMSO- melezitose), demonstrating the potential of this cryoprotective formulation to establish off-the-shelf tumor models. The cryopreserved 3D models also showed preclinical drug screening functionality, exhibiting no major differences in terms of response to standard-of-care chemotherapeutics, when compared to non-cryopreserved controls.

Conclusion

Overall, the herein cryobioprinted tumor-stroma models represent a significant advancement in cancer research and drug screening, offering the ability to fabricate shelf-ready biomimetic tumor models with screening functionality.

EACR25-2542

Biogenesis of miR-155 under oxidative stress in lymphoma cells

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Introduction

MicroRNAs (miRNAs) are short single-stranded RNA molecules that play a key role in gene expression regulation. MiRNAs can be located in introns and exons of protein-coding or noncoding genes as well as in intergenic regions. Biogenesis of miRNAs is a multi-step process that starts in the nucleus, where the primary miRNAs (pri-miRNAs) are transcribed, processed to precursor miRNAs (pre-miRNAs), which are transported to the cytoplasm for further processing to mature miRNAs. MiR-155 is crucial for B-cell lymphoma development. It is also a well-known oncogenic miRNA considered a therapeutic strategy for miR-155-expressing leukemias. miR-155 originates from the MIR155HG gene. The MIR155HG gene can also generate other functional products: long non-coding RNA 155 (lncRNA-155, also known as BIC) and the micropeptide miPEP155.

Material and method

Our aim was to develop a mathematical model for the biogenesis of miR-155 and other MIR155HG products under oxidative stress conditions. First, we examined the impact of ionizing radiation (IR) on miR-155 biogenesis in Burkitt's lymphoma (BL) cell lines: ST486, and DG75.

Result and discussion

Small RNA-seq performed 4- and 12-hours (h) post 4Gy of IR revealed that miR-155 levels increased at least 1.5-fold in DG75 and ST486 cell lines. By qRT-PCR, we showed that pri-miRNA-155 levels increased within 1–4 h post-IR preceding the increase in miR-155 levels at 12 h post-IR, suggesting that IR transcriptionally activated MIR155HG. Additionally, we showed that expression of spliced transcript/lncRNA155, and not unspliced BIC was upregulated after IR. We used the experimentally obtained data to develop a mathematical model describing interactions between unspliced and spliced MIR155HG transcripts, miR-155, and miPEP155. Our preliminary model defines five variables representing MIR155HG products and identifies parameters of biogenesis from MIR155HG.

Conclusion

Our findings indicate that IR affects miR-155 biogenesis by transcriptional activation of MIR155HG in BL cells. The mathematical model we developed provides a

framework for understanding the complex interactions between MIR155HG-derived products and their potential role in lymphoma pathogenesis. Model improvement and experimental validation are ongoing.

Funding: Project supported by the Excellence Initiative - Research University programme implemented at the Silesian University of Technology, year 2024: IDUB programme for co-financing of breakthrough research (to I.S.-P) and 12th funding competition for Project-Based Learning (PBL).

EACR25-2543

Tracking neuroblastoma-derived small extracellular vesicles: uptake and functional impact in the tumor microenvironment

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Introduction

Neuroblastoma (NB) is a rare, yet aggressive pediatric cancer often resulting in poor prognosis. Small extracellular vesicles (sEVs) have gained scientific interest due to their ability to transfer genetic cargo between cancerous and non-cancerous cells. Understanding how sEVs influence tumor progression is critical for elucidating their role within the tumor microenvironment (TME). Notably, sEVs have the ability to induce pre-metastatic niche formation through oncogenic cargo transfer. This study utilizes a 2D co-culture model to visualize the transfer of sEVs from NB to non-NB cells, further providing insights into their potential impact on recipient cells.

Material and method

Human NB cell lines Kelly and SKNAS, fluorescently labeled with green fluorescent protein (GFP), were co-cultured with a human non-NB cell line, HS-5, using a hanging well insert model. The insert contained a porous membrane with seeded NB cells, preventing direct cell contact and allowing sEV transfer. Following a 7-day incubation period, fluorescence microscopy confirmed the uptake of sEVs in HS-5 cells. Downstream analysis, including cell proliferation using a PicoGreen assay, cell viability using Cell Titer-Glo assay and confocal microscopy, elucidated the influence of sEV interactions.

Result and discussion

HS-5 cells had successful sEV uptake from NB cells based on their exhibited GFP fluorescence. During the first 4 days of incubation, there was minimal fluorescence observed. By the final incubation day, 80% of the HS-5 cell population was fluorescent. These results demonstrate the ability of NB-derived sEVs to transfer cargo to non-NB recipient cells without direct cell contact. Preliminary observations of cell proliferation and cell viability suggest NB sEV transfer affects HS-5 cell behavior, resulting in increased cell proliferation, without negative impacts on cell viability. Ongoing work includes characterizing the impact of sEV interactions on HS-5 cells before and after exposure to NB-derived sEVs through gene expression profiling using RT-qPCR.

Conclusion

This study provides direct visualization of sEV-mediated communication between NB and non-NB recipient cells. Further reinforcing the hypothesis that sEVs contribute to

modification of the TME. Future work will focus on translating the 2D co-culture model to the Rastrum™ 3D bioprinter to study NB-derived sEV transfer in a physiologically relevant model that mimics the TME. A new understanding of sEV influence in the TME may improve our knowledge of NB progression as well as ultimately reveal new therapeutic targets.

EACR25-2560

Extracellular matrix profiling reveals human colon cancer-specific features related to tumor sidedness

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Introduction

For many years, colon cancer has been studied and treated as a unique entity, however, it is now well-known that left and right colon tumors have distinct embryologic origins, mutational signatures, microbiota, immune infiltrates and exhibit different responses to therapy, with impact on patients' prognosis. Although many cell studies have been performed to dissect the basis of these differences, the potential role of the Extracellular matrix remains unexplored.

Material and method

In this study, using paired tumor and normal matrices, derived from colorectal cancer patients surgical resections, we unveiled the major biomechanical and biochemical differences between right and of left tumors and appointed sidedness unique proteins that may constitute targets of future therapeutic strategies.

Result and discussion

Microscopic and rheological analysis revealed that both ECM fiber density and stiffness were enhanced in tumors compared to adjacent normal tissues. These differences were side-dependent being observed only on left-sided tumors. Analysis of the ECM protein signature revealed that tumor matrices exhibited increased ECM collagens, glycoproteins, secreted factors, and inflammation-associated proteins and lower expression of proteoglycans. Despite similar number of differentially abundant proteins were observed, between tumor and normal decellularized tissues in both right and left sides, altered signaling pathways differed. Proteomic analysis further showed that left-sided tumors are enriched in proteins related to elastic fiber formation and TGF-β signaling. Specifically, ADAMTS1, PLXDC2, and WNT11 were identified as tumor-specific proteins across both sides, while SPARC and TIMP1 were elevated exclusively in left-sided tumors, potentially associated to its fibrotic and stiffer properties.

Conclusion

These findings suggest that ECM composition differs significantly between tumor and normal tissues in a side-dependent manner, with left-sided tumors displaying unique characteristics that may contribute to their distinct disease profiles and clinical outcomes. To our

knowledge, this is the first study to identify a sidedness signature in the ECM of colon tumors, and rising the enthusiasm for its biomarker and therapeutic significance.

EACR25-2569

characterization of murine bone marrow derived macrophages (BMDM) following Interleukin 6 treatment in two mice

strains: C57BL/6 and Balb/c

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Introduction

M2 macrophages facilitate wound healing, tissue remodeling, and resolution of inflammation. The M2 phenotype is subdivided into several subsets: M2a, M2b, M2c, M2d, each with distinct functions in immune regulation. M2d macrophages, also known as tumor-associated macrophages (TAMs), are induced by the TLR antagonists or IL-6. The activity of IL-6 in the stimulation of M2d macrophages has been confirmed by *in vitro* studies. However, the characterization of macrophages after IL-6 treatment is still poorly understood. The aim of these work was to characterize murine bone marrow derived macrophages (BMDM) following Interleukin 6 treatment in two mice strains: C57BL/6 and Balb/c.

Material and method

Bone marrow was collected from femurs and tibias of 6–10 weeks of age C57BL/6 or Balb/c mice. Isolated cells were differentiated into macrophages using M-CSF for 7 days. After one week, BMDM were incubated for 48h with IL-6, IL-4 (to obtain control M2a macrophages), IL-10 (to obtain control M2c macrophages) and IFN- α (to obtain control M1 macrophages). Additionally, the combination of IL-6 with IL-4 or IL-10 were tested. Unstimulated cells were control macrophages - M0. Characterization of the obtained macrophages were performed by flow cytometry and western blot analysis. Cell migration was determined by wound healing assay and Boyden chambers. The amount of cytokines released by macrophages was assessed using the bead-based multiplex assay.

Result and discussion

IL-6 enhances the stimulation of isolated macrophages to the M2 phenotype (CD206+) in Balb/c mice strain, as well as IL-4. Combination of IL-4 and IL-6 increased the expression of CD206 antigen and the viability of BMDM in both mouse strains. IL-4 and the combination of IL-6 with IL-4 increased the level of arginase protein. Only IL-4 increased the phagocytic properties of BMDM. The combination of IL-6 with IL-4 increased the migration of macrophages in wound healing assay in both mice strains compared to cytokines used individually. The combination of IL-6 with IL-4, as well as cytokines used individually increased the migration of macrophages towards the media containing chemotactic factors

secreted by endothelial cells in Balb/c mouse strain. The greatest activation of the STAT3-6R pathway was observed in BMDM following treatment with IL-6 and IL-6 with IL-10. The main cytokine secreted by macrophages stimulated with IL-6, IL-10 and combination of IL-6 with IL-4 was Interleukin 6.

Conclusion

The obtained results provide new information on the properties of M2 macrophages cultured with the addition of IL-6 and its combination with IL-4 or IL-10. Effective reprogramming of macrophages using the tested cytokines may contribute to achieving better efficacy of anticancer therapies by the tumor microenvironment repolarization.

This study was financed by National Science Centre, Poland, grant No. UMO-2018/29/N/NZ4/01689.

EACR25-2571

High-Throughput Biofabrication of Unitary Lung Tumor Organoids in Superhydrophobic Surfaces

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Introduction

Lung cancer remains one of the most aggressive and lethal malignancies, necessitating the development of advanced *in vitro* tumor models that better recapitulate the *in vivo* tumor microenvironment, in order to effectively understand tumor biology and test new nanomedicines. Conventional 2D culture models fail to mimic the spatial complexity and cellular interactions of native tumors. Tumor organoids offer a promising alternative, however, existing methods often lack reproducibility and high-throughput capabilities. Herein, a novel biofabrication approach for generating physiomimetic unitary lung tumor organoids using superhydrophobic surfaces is presented. The technology is leveraged to generate new Organoid-on-hydrogel bead models that provide biological cues that are relevant in the tumor microenvironment.

Material and method

The HuLu051421 lung cancer cell line was cultured in suspension to generate tumor organoids, which were subsequently processed to obtain unitary organoids with defined size. To enhance biomimicry, these organoids were co-cultured with cancer associated fibroblasts and embedded in a gelatin/hyaluronan photocrosslinkable hydrogel, with a bioprinting-assisted deposition. The generated unitary tumor-stroma organoids were characterized through immunohistochemistry, proteomics, and live-cell imaging to assess cellular composition, ECM deposition, and tumor-stroma architecture. To evaluate the model's suitability for drug screening, different standard-of-care chemotherapeutics were administered, and therapeutic efficacy was assessed using viability assays and bioimaging analysis.

Result and discussion

The biofabricated lung tumor organoids successfully recapitulated key aspects of the native tumor microenvironment, including the presence of heterogeneous cell populations and ECM. The integration of cancer-associated fibroblasts within the hydrogel matrix led to enhanced stromal remodeling and the secretion of tumor-promoting factors (i.e., PDGFB, FGF and TGF-B1). Immunohistochemical analysis revealed distinct cellular organization patterns and proteomic analysis confirmed the upregulation of lung cancer protein signatures, supporting the model's physiomimetic relevance. Drug screening assays demonstrated differential responses to chemotherapeutics, with tumor-stroma organoids displaying increased resistance compared to standalone models.

Conclusion

Overall, the generated organoid-on-a-bead models demonstrate that the inclusion of stromal components and ECM mimetic hydrogels contributed to a higher resistance profile, reinforcing the necessity of tumor-stroma interactions and of ECM components inclusion in preclinical drug response studies.

Acknowledgments: This work was developed within the framework of EU INSPIRE project (grant ID: 101057777 a Cluster 1 - Health).

EACR25-2580

circRNAs-miRNAs-mRNAs regulatory axis of genes associated with hereditary prostate cancer: in silico analyses for screening potential biomarkers

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Introduction

Given that prostate tumors often develop asymptotically, the search for new biomarkers for early detection or improved prognosis evaluation of this type of tumor is essential. However, to date, a limited number of studies have explored circular RNAs (circRNAs), a class of long noncoding RNAs that are covalently closed loops and may function as microRNAs' (miRNAs) sponges, as potential biomarkers of prostate cancer (PCa), especially in the hereditary context (HPCa). This study aimed to analyze the set of miRNAs and circRNAs that regulate the expression of main genes associated with the occurrence of HPCa (HPCa-associated genes) through in silico analyses.

Material and method

The set of HPCa-associated genes was selected from a commercial genetic testing panel (Invitae) previously validated from the literature and widely used in clinical practice. To search comprehensively regulation of these HPCa-associated genes by miRNAs and circRNAs, three tools were used to gather experimentally validated miRNA-target (mRNA) and circRNA-miRNA interaction data: MirTarBase Release 9.0 beta, Tarbase-v9.0, and circRNADisease v2.0.

Result and discussion

Among the remarkable findings, 11 genes were reported as strongly associated with HPCa: ATM, CHEK2, BRCA1, BRCA2, MLH1, MSH2, MSH6, PMS2, EPCAM, HOXB13, and TP53. From the bioinformatics approach, TOP13 miRNAs over-represented as regulators of the genes of interest were identified: hsa-miR-18a-5p, hsa-miR-26a-5p, hsa-miR-19b-3p, hsa-miR-484, hsa-miR-15a-5p, hsa-miR-16-5p, hsa-miR-21-5p, hsa-miR-26b-5p, hsa-miR-19a-3p, hsa-miR-27a-3p, hsa-miR-454-3p, and hsa-miR-454-3p. Furthermore, four (4) specific circRNAs (hsa_circ_SMARCA5, hsa_circ_LARP4, hsa_circAGO2, and hsa_circ_PRKCI) were identified with significantly deregulated expression in biological samples (tumor tissues, cell lines, and xenograft models) related to prostate tumors and which regulate several of the TOP13 miRNAs found in the previous analysis. Importantly, upregulation of hsa_circ_PRKCI (also named circ_0081234) has been previously reported to promote migration, invasion, and epithelial-mesenchymal transition of PCa cells, through regulation of the miR-1/MAP3K1 axis.

Conclusion

Taken together, these in silico findings highlight 13 miRNAs and 4 circRNAs as important modulators of expression of HPCa-associated genes, which represent promising candidates for future experimental validation of their role as a putative set of biomarkers for early diagnosis and prognostic assessment in the CaPH context.

EACR25-2582

Drugable oncofetal RBP Musashi-1 provides a novel Approach for Therapy in immunologically cold High-grade Serous Ovarian Cancer Tumors

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Introduction

High-grade serous ovarian cancer (HGSC) remains one of the most devastating female gynecological cancerous diseases. Characterization of gene expression patterns gave rise to four molecular subtypes (C1, C2, C4 and C5) with subtypes C1 (mesenchymal) and C5 (proliferative, immune evasive) being associated with the worst prospects of therapy. Despite minor advancements, effective therapy remains a challenge.

Material and method

In earlier work from our group using bulk RNA sequencing and siRNA screening of suitable C5-like cell lines, MSI1 emerged as a significant factor in modulating the landscape of immune-regulating tumor cell surface receptors, notably the expression of PD L1. Employing molecular in vitro binding studies, reporter and turnover assays, FACS and live cell imaging, we investigated the mechanisms through which MSI1 post-transcriptionally influences the fate of the PD-L1 mRNA and unfolds other oncogenic functions in suitable ovarian cancer cell models. Furthermore, known small molecule MSI1-Inhibitors Gossypol, Luteolin and R08-2750 were used

for in vitro compound testing to assess their therapeutic potential in HGSC.

Result and discussion

Here we report compelling evidence that MSI1 acts as a molecular oncogenic driver in the context of ovarian cancer, influencing proliferation and invasiveness. Additionally, high expression of MSI1 leads to an increase of PD-L1 presentation on the surface of C5-like HGSC tumor cells, unbalancing the delicate composition of activatory and inhibitory immune checkpoint receptor. This perturbation possibly substantially contributes to the immune desert phenotype of C5 HGSC tumors, diminishing T-cell infiltration and activation. The mechanism of this regulation is dependent on the 3'UTR of the PD-L1 mRNA, which contains six potential binding sites for MSI1. Whether or not this post-transcriptional control is achieved via enhancing translation, increasing transcript stability or other means remains elusive. MSI1-Inhibition using Gossypol, Luteolin and R08-2750 revealed promising micro-molecular effective concentrations in vitro, hopefully paving a road to novel therapy approaches.

Conclusion

Targeting oncofetal RBP MSI1 with small molecule inhibitors could complement established therapeutic regimes for treatment of HGSC tumors, possibly even enhancing efficacy of immune checkpoint therapies.

EACR25-2600

Cellular Model of Giant Cell Tumor of Bone: Insights from In Vitro and In Vivo Studies

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Introduction

Giant cell tumor of bone (GCTB) is a disease mainly affecting epiphyses and metaphyses of long bones. Although GCTB is primarily benign, the choice of the correct treatment prevents recurrence and transformation into malignant disease. GCTB is specific for the presence of two cell types, neoplastic stromal cells and osteoclast-like giant cells. Although a cell model composed of such distinct populations is not easy to establish, its existence is important for studying the efficacy of combination treatment approaches.

Material and method

Tumor models were established by co-culturing GCTB-derived cell lines with macrophages. GCTB-derived cell lines were used as a source of stromal cells. The THP-1 human monocyte cell line was used as a source for the formation of macrophages. Both in vitro and in vivo mouse co-cultivation models were established.

Expression of selected markers was analyzed in cells of these models by immunocytochemistry and immunohistochemistry.

Result and discussion

The ability to co-culture both cell types under 2D and 3D conditions was demonstrated. In addition to the detection of specific markers (H3.3G34W, CD68, and CD163) lines tagged with GFP and RFP were also used to correctly distinguish the origin of the cell populations. Only 3D aggregates formed from both cell line types showed tumorigenic potential in the mouse model. However, the in vivo model showed limited efficiency of the originally expected differentiation of monocytes into osteoclast-like cells. The tumor mass from the in vivo model showed neither osteoclasts nor stromal cells but was composed mainly of "histiocyte-like" cells.

Conclusion

To date, efforts to establish a cellular model of GCTB have explored several possibilities. The integration of osteoclasts, respectively the full differentiation of monocytes into osteoclasts, still represents a current challenge. Focusing on the properties of the extracellular matrix and its resemblance to bone represents a promising strategy.

This study was supported by project no. NU22-10-00054 from the Ministry of Healthcare of the Czech Republic and by the project National Institute for Cancer Research (Programme EXCELES, ID Project No. LX22NPO5102)—Funded by the European Union—Next Generation EU.

Tumour Evolution and Heterogeneity

EACR25-0147

Hijacking cerebral blood vessels: therapy-induced transcriptomic reprogramming of vessel co-opting glioma cells

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Introduction

Glioblastoma (GBM) patients are suffering from poor prognoses and therapeutic responses to the only few treatment options. After initial remission in response to the standard treatment of newly-diagnosed GBM, the tumor recurs in 90% of patients within two years. For recurrent GBM, anti-angiogenic combination therapy is considered the most promising intervention. Although anti-angiogenic therapies (AATs) may achieve progression-free survival temporarily, most patients die within one year after tumor recurrence. Cellular mechanisms underlying this initial therapy responsiveness followed by an aggravated lethal progression remain unclear. Vessel co-option (VCO), the hijacking of cerebral blood vessels (BVs) by tumor cells, which escape the central tumor mass, forms a potential mechanism of GBM to escape AAT. However, a lack of knowledge concerning the drivers and cellular mechanisms of AAT-driven VCO prevents specific targeting of therapy escape mutants and thus, addressing the aggravated lethal progressions in patients.

Material and method

We performed lightsheet fluorescence microscopy (LSFM) of intact murine brain hemispheres carrying

hypoxia-reporter (HRE-dUnaG3ALFA) expressing human GBM xenografts, which enabled the 3D-visualization of tumoral BV networks and oxygenation patterns in their entirety. Here we observed hypoxia-inducible transcription factor (HIF)-stabilizing vessel co-opting GBM cells that hijacked cerebral BVs in response to AAT with Bevacizumab. To identify drivers of this GBM sub-population, we isolated and RNA bulk sequenced these tumor cells to screen for differentially expressed genes.

Result and discussion

LSFM of GBM xenografts revealed a tumor cell sub-population that vessel co-opted cerebral and apparently well-perfused BVs in response to AAT. These cells activated a hypoxia-responsive element (HRE)-dependent hypoxia-reporter, potentially by non-canonical HIF stabilization. Consistent with our observation, hypoxia and HIF-signaling have been proposed to fuel VCO in various tumor types. Our RNA bulk sequencing approach revealed a differential transcriptomic profile of this perivascular/HRE-active sub-population in comparison to cells isolated from the central tumor of AAT-treated mice or vehicle-treated tumors. In particular, candidate genes associated with cancer cell stemness and epithelial-to-mesenchymal transition (EMT) were significantly upregulated and might present as potential drivers of VCO.

Conclusion

VCO as an escape mechanism to AAT might contribute to the aggravated lethal progressions in recurrent GBM patients. So far, there is no therapeutic strategy available to target vessel co-opting therapy escape mutants. Here, we identified differentially expressed genes that were specifically upregulated in vessel-co opting GBM cells during AAT and might act as drivers of VCO in AAT-induced therapeutic escape.

EACR25-0383

New simple 3D model to study tumour-adipocyte communication and cancer progression

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Introduction

Cellular heterogeneity is the major obstacle to cancer cure. Despite the development of available anti-tumour therapies, resistance and recurrence are common, reflecting their ineffectiveness against all tumour cell phenotypes. The dynamic interactions between the tumour microenvironment and cancer cell plasticity drives intratumor phenotypic heterogeneity that promotes disease progression and underpins therapy resistance, representing an attractive therapeutic target. It has recently emerged that bi-directional interactions between cancer cells and adipocytes drive fatty acid uptake from

the microenvironment and represent a major factor in disease progression in cancer. Previous data of the group reveal the capacity of melanoma and colorectal cancer cells to induce lipolysis and fatty acid release from human adipose tissue in a phenotype-specific manner, the fatty acid uptake by tumoral cells, and the subsequent impact of fatty acids in invasiveness and metastatic dissemination in 2D models. It is essential to develop new in vitro three-dimensional models that more accurately mimic tumour structure and extracellular matrix (ECM) to study the role of the environment in tumour progression and to dissect the implicated molecular mechanisms. The aim of this work is to develop new in vitro three-dimensional models to study tumour-stroma signalling and therapeutic efficacy.

Material and method

Primary and metastatic melanoma and colorectal cancer cells and human preadipocytes were used to generate 3D multicell type spheroids. Standard cell biology techniques were used to characterise the spheroids.

Result and discussion

We have developed a simple scaffold-free protocol to generate human tumour/adipocyte 3D models. This method allows full differentiation of adipocytes in vitro, resulting in larger lipid droplets and increased lipid content of melanoma cells. We have optimised the culture conditions to avoid dedifferentiation of adipocytes, one of the main problems of in vitro adipocyte culture. Our model presents a complex extracellular matrix that closely resembles that of in vivo adipose tissue.

Conclusion

Our mixed spheroid model provides an improved culture system to study the molecular mechanisms involved in the contribution of adipose tissue to melanoma metastasis and acquisition of therapeutic resistance. This model could be used to study multiple tumours, as we demonstrate with colorectal cancer.

EACR25-0555

Pan-cancer analysis of successive whole genome duplication in the 100,000 Genomes Project

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Introduction

Whole genome duplication (WGD), involving the doubling of a complete set of chromosomes, is a pivotal evolutionary event in cancer that contributes to accelerated cancer genome evolution, increased tumour cell diversity, and poorer prognosis as well as exhibiting tissue-specific patterns. While the impact of a single WGD event is well-documented, the role of successive WGD (WGDX2) in driving cancer evolution remains underexplored. This study develops a consensus method for identifying WGDX2 events, integrating multi-faceted

genomic analyses to elucidate their impact on tumour progression and clinical outcomes.

Material and method

Whole-genome sequencing data from the Genomics England 100,000 Genomes Project, encompassing 9,420 tumour samples spanning 60 histologies, were analysed. Five distinct methods of WGDX2 detection were implemented: loss of heterozygosity and ploidy regression, allele-specific counts, average chromosome number calculation, phylogenetic tree reconstruction (MEDICC2) and subclonal mutational timing analysis (ParallelGDDetect). Samples were categorised as WGDX2 based on the agreement of three or more methods. Mutation timings were determined using MutationTimeR and evolutionary trajectories of WGDX2 samples were reconstructed using PlackettLuce Timer.

Result and discussion

WGDX2 was observed predominantly in specific tumour types, including breast, lung, colorectal, hepatopancreatobiliary, and upper-gastrointestinal cancers. Survival analysis revealed significantly poorer outcomes for WGDX2 tumours compared to WGD and diploid groups, with a higher prevalence of metastatic and recurrent disease in WGDX2 cases.

Conclusion

This study establishes a robust consensus framework for WGD+ detection, highlighting its association with aggressive tumour phenotypes and worse clinical outcomes. The findings underscore the importance of WGDX2 in shaping tumour evolution and progression. Understanding WGDX2 may reveal new therapeutic vulnerabilities and improve prognostic stratification in cancer.

EACR25-0643

Barcode-Based Approach Reveals Clonal Heterogeneity in Response to Combinatorial Treatment

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Introduction

Combination therapy has emerged as a promising strategy for treating pancreatic ductal adenocarcinoma (PDAC). Our previous findings show that combination therapy can reprogram immunosuppressive mechanisms, particularly in the mesenchymal PDAC subtype. Specifically, the novel combination therapy (trametinib + nintedanib, T/N) induces immunomodulatory chemokine secretion, promotes cytotoxic T-cell infiltration, and sensitizes mesenchymal PDAC to PD-L1 inhibition (Falcomatà et al., 2022). To map therapy-induced changes in the tumor microenvironment (TME) landscape, we conducted a time-course experiment examining T/N combination with immune checkpoint

blockade in vivo and in vitro. To assess dynamic transcriptional changes between the tumor cells and their TME in response to treatment, we employed lineage and RNA recovery (LARRY)-based barcoding.

Material and method

To investigate the characteristics of resistant and sensitive clones, we barcoded mesenchymal PDAC clones using the LARRY barcode and characterized their genomic features with low-coverage whole-genome sequencing (lcWGS). A pool of 40 distinct clones was orthotopically implanted into mice. We divided the cohorts into two experimental arms: control and treated (T/N + anti-PD-L1). Mice were sacrificed at specific time points, and in vitro samples were collected simultaneously. Samples were analyzed using Chromium 3'-scRNA-seq, FFPE-based Flex gene expression, and Xenium. To enable barcode detection with Flex, we designed specific probes for each clone.

Result and discussion

We successfully detected tumor barcodes using both 3'-scRNA-seq and Flex, thus allowing a clear distinction between tumor and non-tumor populations. Barcode analysis identified several treatment-resistant clones. Notably, two distinct populations emerged, including highly proliferative tumor cells with high Avil expression, predominantly composed of myc-amplified clones, and low-proliferative tumor cells with high Bgn expression, which showed more resistance to therapy. This analysis revealed gene signatures linked to resistance mechanisms against combinatorial therapy and immune checkpoint blockade.

Conclusion

Barcode-based tumor detection offers a powerful approach for tracking clonal dynamics, identifying resistant populations, and uncovering molecular mechanisms of therapy resistance in PDAC.

EACR25-0646

Unveiling the heterogeneity of pancreatic cancer initiation: a novel perspective on normal-mutant cell-cell Interactions

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Introduction

Pancreatic cancer (PC) is a devastating disease with abysmal survival rates, due to late diagnosis and early metastasis. Understanding PC development is crucial to identify biomarkers of early disease and prevent its progression. In most cases, PC initiates from KRAS mutant cells. These cells induce permanent acinar-to-ductal metaplasia (ADM). KRAS mutant cells drive the progression of ADM to pancreatic intraepithelial neoplasias (PanINs). Accumulation of other genomic alterations drive PanINs progression to invasive carcinoma. Our preliminary data, using a mouse model that more accurately mimics sporadic disease by

activating KRas mutations only in few cells of the pancreatic epithelium, unearthed the presence of non-mutant cells in PC initiation. This suggests a collaboration between non-mutant and KRas mutant cells in PanINs and tumours.

Material and method

FLASH tissue clearing was used to process banked mouse tissue samples, enabling 3D visualization of KRas mutant and non-mutant cell distribution in tumours and PanINs. BaseScope Duplex Assay *in situ* hybridization identified cells carrying the three most common point mutations in the KRas oncogene (G12D-40% of cases, G12V-35%, G12R-20%) and wildtype sequence in mouse and human samples.

Result and discussion

By analysing banked mouse tissue, we demonstrated how the 3D distribution of KRas mutant cells changes through neoplastic lesion development and tumour progression, unveiling mixed populations of mutant and non-mutant cells within PanINs and tumours. We observed an increase in the % of RFP+ cells overtime, with full RFP+, full RFP-, and mixed PanINs found. The transition from low-grade to high-grade PanIN is accompanied by an increase in non-mutant cells neighbouring mutant cells, suggesting a dynamic interplay between the two is required for tumour progression. We also unravel the heterogeneity of KRas wildtype and mutant cells in the same neoplastic lesion using BaseScope, confirming the presence of both KRas mutant and non-mutant cells. We observed that whilst some cells remain wildtype, others acquire KRasG12D, KRasG12V, and KRasG12C mutations.

Conclusion

To conclude, this work has highlighted the heterogeneity of PanINs and tumours in both a mouse model of sporadic disease and human tissue. The observed mixture of KRas mutant and non-mutant cells suggests a dynamic interplay between the two, and that KRas mutant cells may recruit non-mutant cells to PanINs to utilise them as support for lesion development. By beginning to understand this interplay further, we can begin to identify novel therapeutic targets and develop strategies for early detection and prevention of pancreatic cancer.

EACR25-0784

Development of an innovative microdissection platform to study tumor spatial heterogeneity

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Introduction

The intrinsic heterogeneity of tumors makes the study of the underlying mechanisms of tumor spread and molecular diagnosis a real challenge. Therefore, it is of great interest to identify and understand these mechanisms in order to determine the most appropriate treatment for each patient.

Material and method

Here, we describe an innovative microdissection platform called MiniPunch®. Based on the use of 200µm diameter and depth controlled single-use needles, this system is able to collect samples automatically in formalin-fixed paraffin-embedded (FFPE) blocks. Sampling of specific areas is enabled by the use of artificial intelligence that allows automatic alignment between the annotated virtual slide and the original FFPE block. A high sampling rate (~40 sec/sample) facilitates its routine use.

Result and discussion

Our work aims to demonstrate the relevance of this technology for the study of intrinsic tumor heterogeneity. To this end, after validating the applicability of the technology, we performed RNA-seq on MiniPunch® samples obtained from human tumors. We were able to show variations in transcriptome expression with respect to both location and stage of disease. We also demonstrated the potential of this technology for molecular diagnosis by comparing it with routine techniques. The results showed increased sensitivity with MiniPunch® samples and access to critical information on intra-tumor heterogeneity that can improve the accuracy of established diagnosis.

Conclusion

This platform, capable of investigating tumor heterogeneity, will contribute to a better understanding of the complex mechanisms involved in tumor evolution. In this way, by improving molecular diagnosis, this system will contribute to the global development of precision medicine and thereby improve patient care worldwide.

EACR25-0834

Barcode-based lineage tracing to track breast cancer clonal evolution

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Introduction

Despite significant progress in understanding tumor phylogeny through whole-genome sequencing, the cellular origins of breast cancer and their dynamic clonal evolution remain poorly understood. By integrating barcode-based *in vivo* lineage tracing with single-cell RNA sequencing, we aim to delineate the hierarchical organization of tumor cells and uncover the molecular dynamics driving tumor progression and metastatic dissemination.

Material and method

We utilize a novel mouse model that enables *in vivo* labeling of individual cells with a highly diverse set of genetic barcodes, allowing precise lineage tracing throughout tumor development and metastasis. Clonal tracking is performed in the MMTV-PyMT mammary tumor model at both pre-neoplastic and advanced tumor stages. This approach enables us to simultaneously assign each cell to a specific clone and determine its transcriptional signature.

Result and discussion

Our analysis reveals two distinct transcriptomic trajectories leading to EMT states. These trajectories are

associated with divergent molecular programs: one linked to an injury-associated state and the other to differentiation toward an alveolar lineage. Notably, these EMT trajectories correlate with distinct prognostic outcomes in human breast cancer, underscoring their potential clinical relevance.

Conclusion

By leveraging a novel lineage-tracing strategy, our findings provide fresh insights into the role of EMT in tumor heterogeneity and cancer progression. This study highlights the diverse transcriptional landscapes shaping breast cancer evolution and offers a framework for understanding how EMT contributes to metastatic potential.

EACR25-0879

Characterising the immunogenetic heterogeneity in liver cancer to understand the varied responses to immunotherapy, with a focus on multifocal disease

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Introduction

Liver cancer is the third-leading cause of cancer-related death worldwide. It arises in the context of a diseased liver, linked to alcohol, obesity or hepatitis viruses. Patients with advanced hepatocellular carcinoma are all offered the same standard-of-care immunotherapy, a combination of anti-VEGF and anti-PD-L1 monoclonal antibodies, but just a third of patients experience a slowing of tumour growth. Understanding what makes these patients different to each other is crucial in understanding how we can stratify them for treatment and identify opportunities for novel therapeutic strategies. These patients often present with multiple tumors at the same time, providing a unique opportunity to study this heterogeneity at both the inter- and intra-patient level.

Material and method

56 paired formalin-fixed, paraffin-embedded background liver and tumour samples were obtained from the National Health Service. DNA was extracted for Whole Exome Sequencing and a subtractive analysis was performed to identify tumour-specific variants. Sections were cut from the same samples and stained for proliferation (Ki-67) and immune cell (CD4, CD8, FoxP3) markers, using Immunohistochemistry. Stained images were quantified automatically using QuPath.

Result and discussion

Tumour mutation burden analysis for this cohort confirmed a low average value, compared to other tumour types, but a large range was seen. Genetic patterns were observed between different aetiological groups, with genes mutated at differing frequencies. Ki-67 staining revealed that the proliferation rate is significantly higher at the tumour periphery, compared to the core. This suggests that the tumours could align with

a 'boundary-driven' growth pattern that has been observed in other tumour types. When compared to background liver, the frequency of CD4+ and CD8+ immune cells was not significantly increased in the tumours and was highly variable. However, the frequency of regulatory FoxP3+ T cells was significantly increased, overall indicating a suppressive immune environment. In multifocal patients, the mutational data indicated a range of tumour relatedness and evolution contexts. Interestingly, many tumours with genetic similarity appear in separate lobes of the liver. The immune cell frequencies were often found to be highly variable, even in apparently metastatic tumours.

Conclusion

Immunogenetic profiles were found to be highly heterogeneous, both between separate patients and within the same patient. The finding that many tumours do not have an increase in CD4+ and CD8+ T cells, compared to the paired healthy liver samples, supports a mechanism whereby an initially 'immune cold' tumour immune microenvironment limits the benefit of immune checkpoint blockade. This suggests that a tumour-targeted treatment approach would be efficacious for many patients, with the genomic data providing strong rationale for personalisation within aetiological groups.

EACR25-1033

Patient-derived tumor xenografts as models of acquired chemoresistance – studying melanoma, renal cell carcinoma and non-small cell lung cancer

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Introduction

Cancer therapy was revolutionized in the last two decades by molecularly targeted therapies. However, in many cases, the efficient and manageable treatment declines within months or few years due to repopulation of the tumor with resistant cells, which leads to progression and metastasis – the two major factors of cancer mortality. Cell line model treatments can show individual cell changes following treatment, but found targets usually are failing to be translatable to clinics. Patient-derived tumor xenograft (PDTx) models are resembling heterogeneity, structure, plasticity of the tumors more than cell lines. Therefore, we wanted to see if resistance evolution in PDTx models assimilate to those in cell lines, or can reveal novel pathways of interest.

Material and method

We performed long-term treatment on melanoma (MM), renal cell carcinoma (RCC), and non-small cell lung cancer (NSCLC) with the respectively approved targeted drugs: vemurafenib, sunitinib and erlotinib. NOD-SCID mice used have limited timespan for the experiment, thus

tumors were serially transplanted and treated again. This fashion enabled us to collect flash-frozen samples from each generation of mice. The samples were either analyzed using mRNA-seq to show genetic background and changes in the landscape during treatment, or possible suspects were further confirmed by rt-pcr, western blot or immunohistochemistry.

Result and discussion

In melanoma, BRAF V600E mutation specific inhibitor usually is reported to enhance MAPK or AKT pathway activities, as well as loss of the mutation, or enhancement of CRAF. Intriguingly, none of these changes were recorded in PDTX resistance model, whilst upregulation of multidrug resistance determinant ABCB1, immune checkpoint stimulator CD27, or interferon inducible protein IFI27. In our RCC models, two different resistance strategies were identified, one described by the overexpression of MAPK pathway, the other by silencing cell cycle and apoptosis, generating the tumors from a slower, mesenchymal, motile cell phenotype. In NSCLC, the resistance mutation of EGFR was identified in one case, while altered MAPK and PI3K signaling in case of the other model.

Conclusion

Our PDTX resistance experiments show clear evidence that previously published resistance mechanism share role with unknown, patient-specific resistance strategies. With an increasing number of such model experiments, typical background landscapes might be identified to propose and intervene probable drug tolerance in solid tumors.

EACR25-1093

HPMA-based polymeric conjugate bearing derivative of HIV protease inhibitor lopinavir successfully reverses P-gp mediated multi-drug resistance in vitro and in vivo

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Introduction

Multi-drug resistance (MDR) is a common phenomenon in cancer. It may occur after several courses of chemotherapy (i.e. induced MDR) or could be an innate feature of some cancer types (i.e. natural MDR). MDR is often caused by overexpression of P-glycoprotein (P-gp), a member of ABC-transporter family, enabling cancer cells to efflux structurally unrelated anti-tumor drugs. Here we introduced a derivative (5-methyl-4-oxohexanoic acid ester) of HIV protease inhibitor lopinavir (LD) possessing high inhibitory potential for P-gp as well as STAT3 signaling covalently bound to N-(2-hydroxypropyl) methacrylamide (HPMA)-copolymer-based carrier (P-LD). We tested this conjugate in combination with similar conjugates bearing some conventional cyto-

static drugs in several cancer cell lines to evaluate its ability to overcome MDR in these lines and sensitize them to the drug action in vitro and in vivo.

Material and method

P388/MDR murine monocytic leukemia cell line with induced MDR and CT26 murine colon carcinoma as well as SCC7 murine head and neck squamous carcinoma with natural MDR were main model cancer cell lines used in this study. BALB/c, DBA/2 mice and NSG immunodeficient mice were used for in vivo testing. We used Real-Time PCR to assess relative P-gp expression in cancer cell lines. For evaluation of P-gp and STAT3 inhibitory activity of LD and P-LD calcein efflux assay and western blotting (as well as ELISA) were used, respectively. [3H]-thymidine assay and annexin V assay together with caspase-3 activity assays were used to determine cytostatic and cytotoxic activities of tested compounds. In vivo experiments involved treatment of tumor-bearing mice with polymeric conjugate combinations with subsequent monitoring of tumor growth, survival and body weight.

Result and discussion

Both LD and P-LD demonstrated potent P-gp inhibition. LD was confirmed to inhibit STAT3 signaling pathway. We also proved that tested compounds possess synergic cytostatic and cytotoxic effects in cancer cell lines in vitro when applied in combination with polymeric conjugates bearing several conventional cytostatic drugs (doxorubicin, mitoxantrone and docetaxel). In vivo experiments demonstrated an ability of combined treatment to slow down tumor growth and prolong survival in the models of cancer with both induced and natural MDR.

Conclusion

P-LD has a good potential as safe and effective P-gp inhibitor able to boost efficacy of anti-tumor drugs in P-gp expressing tumors. Further in vivo evaluation is required to ensure the best combination and application scheme for maximal efficacy of treatment using the compound.

The study was supported by the project National Institute for Cancer Research (Programme Exceles, ID Project No.LX22NPO5102) – Founded by the European Union – Next Generation EU and by the grant NU21-03-00273 from the Czech Health Research Council.

EACR25-1256

Unraveling Breast Cancer Heterogeneity: Microfluidic Sorting and Bioassay-Based Functional Analysis

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Introduction

The metastatic potential of cancer cells is closely linked to their mechanical properties, which evolve with cancer progression and heterogeneity. However, characterizing these properties requires robust tools for sorting and analysing distinct subpopulations. Small and large tumor cells play different roles, with smaller cells exhibiting higher proliferative potential and initiating new tumors, while larger cells may be more differentiated or adapted to specific microenvironments. This study presents a

deterministic lateral displacement (DLD)-based microfluidic device for the label-free sorting of MDA-MB-231 metastatic breast cancer cells into sub-populations based on morphological differences. We investigate the impact of sorting on cell behaviour and assess bio-functional heterogeneity using proliferation, migration and biomarker assays.

Material and method

A microfluidic device with three inlets and three outlets was utilized to fractionate breast cancer cells into sub-populations: small, medium and large. The sorting efficiency is validated using inverted microscopy and post-sorting behaviour is analysed through long-term culturing and functional assays. To assess the biological differences between subpopulations, we perform: Cell proliferation kit to monitor growth rates over one week. Cell migration assays to examine motility differences across subpopulations. Holographic microscopy for long-term tracking of cellular dynamics and their role in metastasis. Biomarker assays (Ki67 and YAP) to further explore differences in proliferation and mechanical signaling.

Result and discussion

Homogeneous subpopulations of small, medium and large cells were successfully isolated. All subpopulations demonstrated continued proliferation from day 0 to day 6. Large subpopulations exhibited significantly higher motility than small and medium subpopulations, suggesting that cell size influences metastatic potential. This is in line with previous reports highlighting the role of cytoskeletal adaptations in invasion dynamics. YAP labeling revealed that small cells experienced more mechanical stimulation than the larger groups, suggesting enhanced mechanotransduction pathways. This may indicate increased responsiveness to extracellular matrix stiffness, which could contribute to their invasive potential. The observed differences in migration, proliferation, and mechanotransduction highlight the functional heterogeneity of breast cancer subpopulations.

Conclusion

This study demonstrates the power of DLD-based microfluidics as a high-throughput, label-free approach for studying cancer heterogeneity. Future work will focus on generating 3D spheroid models from sorted subpopulations to further investigate their role in tumor progression, drug resistance and invasion mechanisms, ultimately aiding in the development of more targeted therapies for aggressive breast cancer.

EACR25-1301

The nucleolus connects inflammation and tumor cell plasticity in colorectal cancer

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Introduction

Colorectal cancer (CRC) is a major health problem and economic burden worldwide. The tumor micro-environment and cell heterogeneity are well-established determinants of cancer progression and an important cause of resistance to chemotherapies. Tumor hierarchies are fueled by a small population of self-renewing cancer stem cells (CSCs), reminiscent of the intestinal epithelium. Recent studies have uncovered the existence of zonations of biosynthetic active cells in defined niches of the tumors, with hyperactive ribosome biogenesis and protein synthesis defining cancer stem cells properties, whereas tumor cells undergo a progressive loss of their biosynthetic capacity during differentiation. We hypothesized that loss of ribosome biogenesis could act as a driver of differentiation in colorectal cancer.

Material and method

We used patient-derived organoids (PDOs) and preclinical models of CRCs including subcutaneous PDXs and liver metastasis derived from intrasplenic PDOs injection, in which we inhibited ribosome biogenesis by several approaches amongst which pharmacological inhibitors of RNA polymerase I.

Result and discussion

We showed in PDOs and PDXs that inhibition of ribosome biogenesis induces a reversible differentiation transcriptional program. We performed single cell RNA sequencing in PDOs which revealed that cell-autonomous inflammation programs are activated in response to ribosome biogenesis inhibition, suggesting a role in CRC differentiation plasticity. We then performed a 3D-HTS drug screening of 264 anti-inflammatory drugs in PDOs, and identified drugs, that in combination with ribosome biogenesis inhibition leads to terminal differentiation.

Conclusion

These findings show that:

- (i) ribosome biogenesis and translational control regulate CRC cell differentiation, and
- (ii) cell-autonomous inflammation contributes to CRC plasticity, opening an avenue to novel differentiation therapies in colorectal cancer.

EACR25-1414

Investigating Clonal Dynamics and Drivers of Oxaliplatin Resistance in Colorectal Cancer Using Cellular Barcoding Technology in SW480 Cell Line

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Introduction

Chemotherapy resistance in colorectal cancer (CRC) is a multifaceted process driven by the diverse contributions of individual cancer cell clones. The presence of cancer cell clone subpopulations with varying sensitivities to therapy allow resistant clones to survive and expand, ultimately reducing treatment efficacy and contributing to disease progression or recurrence. To investigate the mechanisms underlying this resistance, we established oxaliplatin-resistant SW480 cells and employed cellular DNA barcoding technology to track their clonal evolution. Our approach also aimed to identify drivers of

oxaliplatin resistance and to uncover secondary drug vulnerabilities to counteract resistance.

Material and method

We used cellular DNA barcoding technology to track SW480 individual clones during the resistance process. All analyses were performed in three drug resistant replicates and control groups. Using amplicon sequencing, we analyzed the barcode distributions across oxaliplatin-resistant and control groups. Whole-exome sequencing (WES) was employed to uncover genomic alterations that may have a pivotal role in resistance mechanisms. Additionally, a secondary drug screen of a 50-compound library was performed to explore therapeutic possibilities.

Result and discussion

We used amplicon sequencing to identify barcode distributions, and the pre-existing vs de novo nature of clones selected through the resistance generation process. Whole-Exome Sequencing (WES) was employed to uncover the genomic make-up of oxaliplatin resistance, highlighting the differences in mutational landscapes of the resistant groups. Finally, a secondary drug screening was performed using a 50-compound drug library, for an ultimate aim to exploit therapeutic vulnerabilities arising from oxaliplatin resistance.

Conclusion

To conclude, this study underscores the utility of cellular DNA barcoding as a powerful tool for dissecting the complex evolution of chemotherapy resistance in cancer. Our findings provide critical insights into the clonal dynamics and molecular drivers of CRC drug resistance, paving the way for targeted approaches to overcome resistance and improve treatment outcomes.

EACR25-1510

Homopolymer diversity at ultrafast evolving loci accurately reflects tumour mutation rate in mismatch repair-deficient (MMRd) CRC

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Introduction

Mismatch repair-deficiency (MMRd) occurs in approximately 15% of colorectal cancers (CRC). Extensive mutational damage fuels tumour growth but also increases neoantigen burden, rendering these cancers vulnerable to attack by the immune system. Recent work in our lab has uncovered a novel mechanism whereby coding homopolymers in the minor MMR genes, MSH3 and MSH6, are repurposed by MMRd tumours as cellular ‘gear switches’ to adapt mutation rates to immune selection (Kayhanian et al., 2024). Spontaneous mutation and reversion within these repetitive tracts modulates subclonal mutation rate, mutation bias and clonal HLA diversity during MMRd cancer evolution, accelerating immune escape. Building on this concept, our goal was to design a method to comprehensively map evolving genomic mutation rates in MMRd CRCs.

Material and method

Owing to their hypermutable nature, microsatellites are widely used to assess the tempo and mode of genomic

evolution. MMRd tumours exhibit elevated population heterogeneity, reflected by greater microsatellite length diversity, as well as an increased exonic mutation burden. Therefore, we reasoned that coding microsatellites in MSH3/MSH6-deficient clones undergo faster erosion than in their MSH3/MSH6-proficient ancestor lineages, allowing the derivation of a small targeted sequencing panel of homopolymers that together act as a proxy for tumour mutation rate. The final panel design consisted of coding microsatellites responsive to changes in mutation rate, alongside non-coding microsatellites included as an internal control. To quantify genomic diversity at these loci, we performed high-depth (1000x) sequencing of multi-region FFPE resection samples and derived ecological diversity indices, including the Shannon diversity index (SDI) and Jensen-Shannon distance (JSD).

Result and discussion

Preliminary results indicated that SDIs and JSJs across responsive loci strongly correlated with MSH3/MSH6 mutation status, with increased SDIs in MSH3/MSH6-deficient compared to MSH3/MSH6-proficient clones (i.e. regions of high and low mutation rates, respectively). Non-coding control loci showed no difference in SDIs or JSJs between tumour regions, and this was reproducible across all patient samples. Both intra-patient and inter-patient differences in SDIs and JSJs were observed across the cohort, with reconstructed phylogenies accurately reflecting the relationship between normal, MSH3/MSH6-proficient and MSH3/MSH6-deficient tumour samples.

Conclusion

Our findings suggest that genomic diversity at key ultra-fast evolving microsatellite loci can be used to infer genome-wide mutation rate. The small genomic footprint of the targeted panel enables affordable high-depth sequencing to reveal clonal diversity from small amounts of FFPE archival material. Further work is needed to validate our methodology in larger cohorts and explore its clinical application in MMRd CRC management.

EACR25-1606

Exploring the role of intratumor heterogeneity and tumor microenvironment crosstalk in the acquisition of cisplatin resistance in High-Grade Ovarian Carcinoma models

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Introduction

Ovarian cancer is the fifth leading cause of cancer-related deaths, affecting ~100,000 women annually. High-grade serous ovarian carcinoma, the most common histotype has a 5-year overall survival rate of around 35%.

Although most patients respond to first-line therapy, 70% relapse within 3 years with a resistant disease. Tumor heterogeneity has been described as a hallmark of cancer progression, impairing the efficacy of cancer therapy.

Material and method

Single-cell technologies were used on a patient-derived xenograft ovarian cancer model (MNHO124), well-representing the clinical setting of the evolution of platinum resistance. MNHO124 subcutaneously transplanted nude mice were randomized and treated or not with cisplatin (5 mg/kg q7dx3). Whole tumor masses were excised from euthanized mice at: 1) "Random Time" before treatment; 2) "Best Response" when tumor reaches about 90% of regression; 3) "Regrowing", when the tumor mass re-enters a proliferative state. Tumor samples ($n = 4$) were processed following 10x Genomics® protocols. RNA sequencing was performed by Illumina® technology. The bioinformatic analysis carried out with Seurat v5.

Result and discussion

Cells sequenced at the three time points were plotted in a Uniform Manifold Approximation and Projection (UMAP), and the clusters of the tumor and of the murine component were observed. The analysis on the human component has identified a total of 12 clusters. Some of these were predominantly composed of cells from a specific time point, leading us to classify them as time-point-specific. To identify their unique gene expression signatures, we performed differential gene expression and pathway enrichment analysis both for the top-expressed and downregulated genes. Signatures specific to the clusters associated with the "Regrowing" timepoint in the tumor component (e.g., DNA damage response, mitochondrial metabolism) are currently being validated using different molecular assays on different ex vivo and in vitro models, to understand if the mechanisms highlighted can represent a target of interest. The UMAP of the murine component revealed 13 distinct clusters, whose representation was uneven across the three timepoints. Both an automated and manual annotation of the murine component clusters are being performed to characterize the main populations composing the tumor microenvironment at the different timepoints and to investigate their evolution over time and their crosstalk with the human compartment.

Conclusion

These results provide new evidence on the role of tumor heterogeneity in the development of resistance to cisplatin in ovarian cancer. The scRNA sequencing analysis enabled the identification of distinct cell clusters, whose transcriptional signatures are still under investigation, and revealed potential markers and pathways involved in tumor progression and therapeutic response.

EACR25-1677

In Vivo, Time-Dependent Metabolic Flux Analysis of Normal and Tumor Tissue Using MasSpec Pen Technology

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Introduction

Pancreatic ductal adenocarcinoma (PDAC) is the most common and aggressive form of pancreatic cancer, accounting for ~90% of cases. It is a particularly lethal

disease, with a five-year survival rate of ~10%. Alterations in metabolic pathways are key to tumor growth, disease progression, and response to therapy. Metabolic flux analysis is a technique to quantify the rates of metabolic activity via isotopically labeled substrates. These assays are commonly performed on excised tissues with liquid chromatography mass spectrometry (MS). Here, we provide direct MS analysis of PDAC tissue using the handheld MasSpec Pen (MSPen), establishing the potential for *in vivo*, time-dependent metabolic flux analysis.

Material and method

KPC cell line-derived syngeneic orthotopic PDAC mouse model undergoes surgery for the implantation of tumor cells into the pancreas. Tumors grow for 4 weeks without treatment. Metabolic flux analysis is performed by injecting peritoneally 1.4 mg/g [$^{13}\text{C}_6$] glucose and sacrificing at 0.5h, 1h, 1.5h, and 3h for each respective mouse. Organs are harvested and flash-frozen in liquid N₂ for mass spectrometric analysis by the MSPen. Desorption electrospray ionization MS imaging was also obtained. This work led to *in vivo* tissue analysis under anesthesia (2% isoflurane 0.2 L/min in 100% oxygen), providing real-time temporal insight into the fate of 13C-glucose and its transformations in a live mouse.

Result and discussion

On *ex vivo* tissue, varying degrees of labeling for metabolites, such as glutamic acid and pyroglutamic acid were found by MSPen analysis. The brain was the most metabolically active organ, with enrichment of glutamic acid over 40% for the M+1 and M+2 isotopologues compared to the theoretical and a 12C-glucose control animal. Interestingly, while labeled alanine was detected, labeled pyruvate was not detected at the sampled timepoints. DESI-MSI of the brain shows the localization of glutamic acid in matching M+1 and M+2 isotopologues. Significant enrichment was also detected in other organs, such as the kidney and liver, between 6 to 10%. For example, pyroglutamic acid in the kidney peaked at 1 h and 1.5h, then dropped precipitously at the 3 h timepoint. For pancreatic cancer models, we analyzed n = 3 animals at 4 timepoints and 8 organs, including the tumor. An *in vivo* analysis was performed by anesthetizing a mouse isoflurane. Analysis during surgery with the MSPen revealed higher levels of enrichment *in vivo* at comparable timepoints, particularly for the higher isotopologues, M+3 and M+4.

Conclusion

Overall, the direct, longitudinal, and *in vivo* metabolic flux analysis in a single animal model we describe here may impact many areas of cancer research.

EACR25-1755

Characterizing polyp heterogeneity to identify those at higher risk of progressing to colorectal cancer

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Introduction

Colorectal cancer arises from precancerous lesions called polyps, most of which are benign, but some have the potential to progress to cancer. Early detection and polypectomy significantly reduce colorectal cancer incidence, but risk-based patient stratification could help avoid unnecessary and costly surveillance colonoscopies. Identifying high-risk polyps is therefore crucial, particularly through the identification of transcriptomic biomarkers, which may pave the way for predictive blood-based markers. Tumor microenvironment (TME) is a complex and dynamic ecosystem that is heterogeneous even at the earliest stages of tumorigenesis. In an Apc-mutated mouse model of intestinal tumorigenesis, we previously identified two distinct types of polyps, one of which exhibited a transcriptomic signature associated with immune infiltration. Given that inflammation is known to drive genomic instability, proliferation, and tumor progression, we aim to investigate whether this immune signature is also present in human polyps and whether it is linked to an increased risk of malignant transformation.

Material and method

Bulk RNA sequencing was performed on a distinct transgenic mouse model and on a human cohort, including 20 intestinal adenomas from Apc-mutated mice and 42 patient-derived samples comprising 21 advanced adenomas and 21 adenocarcinomas. To classify these lesions, unsupervised clustering and nearest-template prediction analyses were applied using our previously defined immune signature. Additionally, single-sample Gene Set Enrichment Analysis and multiple deconvolution approaches were used to characterize immune cell composition and functional cancer-related states.

Result and discussion

In both mice and humans, adenomas can be broadly classified into two main groups (~50%) based on the immune transcriptional profiles. The “immune-up” lesions showed heterogeneity in immune cell composition and activation, but B cells and myeloid cells appeared to be consistently present in contrast to the other immune cell types such as NK and CD8+ T cells. Importantly, “immune-up” polyps share transcriptomic profiles more closely resembling those of adenocarcinomas, with significant enrichment of signatures of hypoxia, invasion, differentiation, and EMT.

Conclusion

Our study reveals a transcriptional dichotomy among polyps in both a well-established mouse model and human patients, suggesting a subset of immune-enriched lesions that may share molecular features with adenocarcinomas. Given their similarity to malignant tumors, these lesions may represent high-risk polyps with an increased likelihood of progression to colorectal cancer.

EACR25-1808

Selective Inter-clonal Interaction is Crucial for the Metastatic Phenotype of BRAF-inhibitor Resistant Melanoma

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Introduction

Intra-tumoral heterogeneity is the major cause of therapy failure in malignant melanoma patients. Under chemotherapy pressure, the emergence of diverse drug-resistant clonal subpopulation and their intricate cooperation could contribute to a higher drug resistance, elevated tumorigenicity and metastasis. Multi-clonal interactions in tumors are very challenging to study, which demands relevant in vitro or in vivo models to investigate these events. Here we have shown how selective clonal interactions of BRAF-inhibitor resistant melanoma clones are crucial for melanoma progression and metastasis

Material and method

We have used a BRAF mutant malignant melanoma cell model, A375 and made them resistant towards the BRAF-inhibitor, Vemurafenib. The resistant cells were named A375VR. We have isolated three different clones (A375VR-C2, A375VR-C7, A375VR-C11), characterized them through whole exome sequencing, cell viability assays and ERK-activation analysis by western blotting. The clonal interaction was studied using 2D migration, spheroid expansion and in vivo CDX experiments (NIPER/BT/2022/2023). Signaling pathways involved with clonal interaction were evaluated by immunoblotting.

Result and discussion

Our results indicated that the A375VR cells comprised of multiple sub-populations of cells with different degrees of resistance towards Vemurafenib. Interestingly, our sequencing data revealed that the clones have different mutational spectra in the WNT and JAK/STAT3 genes and in addition, the metastatic signaling regulators of WNT and STAT3 cascades are variably expressed in these clones. As WNT and JAK/STAT signaling regulators significantly contribute to the metastatic phenotype of BRAF-inhibitor resistant melanomas, these molecular drivers could be involved with intra-clonal interaction driven tumorigenicity in BRAF-inhibitor resistant melanomas. Indeed, when the clones were grown in a co-cultured setup for cell migration and spheroid expansion studies, we observed that a few selective clones are positively interacting to increase coordinated migration or cell shedding from the spheroid. Our results showed that the clone A375VR-C11 interacts positively with clones A375VR-C2 and A375VR-C7 to increase migration. Based on these results, we conducted in vivo experiments and revealed that the combination of A375VR-C7 and A375VR-C11 induced higher tumorigenesis in animals compared to the individual clones. Altogether we have shown that selective clonal interactions in a heterogeneous tumor microenvironment are crucial for BRAF-inhibitor-resistant melanoma progression.

Conclusion

We have demonstrated a novel strategy to reveal the significance of clonal interaction and signaling involved during resistant melanoma progression. Similar approaches could be adopted to understand the clonal interaction driven metastasis in aggressive cancers and help formulating new therapeutic interventions.

EACR25-1820

Clonal evolution of metastasis in ovarian and colorectal cancers

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Introduction

Metastasis is the main cause of death from cancer. Patients with ovarian cancer (OVCA) and colorectal cancer (CRC) commonly develop metastases, resulting in a poor prognosis. Usually, OVCA disseminate early and directly into the peritoneal cavity, while CRCs most frequently disseminate to the liver through the portal vein. These different routes to metastasis are likely to impact the subclonal development of the cancers. Both cancer types have substantial tumor heterogeneity, and understanding the evolutionary processes is an important step in combating drug resistance and relapse. In light of this, we reconstructed the evolutionary trajectories to compare the modes and molecular determinants of dissemination in the two cancer types.

Material and method

Patients treated by debulking surgery for disseminated OVCA ($n = 23$) or resection of primary CRC and liver metastases ($n = 45$) at Oslo University Hospital were analyzed. Whole-exome sequencing was performed on a median of 5 samples from 2–3 tumor sites from each patient with OVCA (total $n = 104$), and a median of 4 samples per patient with CRC, including primary tumor and liver metastasis samples (total $n = 232$). The prevalence of different cancer cell populations was modeled based on the variant allele frequency of detected mutations, and adjusted for the DNA copy number at the mutated locus and tumor purity of the samples using PyClone. Phylogenetic relationships and subclonal ordering were inferred with ClonEvol. The data from OVCA have previously been published and analyses of CRCs are ongoing.

Result and discussion

Mutations in TP53 and BRCA1/2, as well as mutation signatures associated with homologous recombination deficiency, were predominantly clonal across OVCA. Similarly, mutations in CRC-critical genes such as APC, TP53 and KRAS were clonal and found across all cell subpopulations in the affected CRCs. The OVCA had particularly complex dissemination patterns with polyclonal dissemination in 73% of patients, including polyphyletic seeding of subclones with a branched evolutionary lineage in 23% and examples of bi-directional seeding. Preliminary analyses show that multiple modes of dissemination occur also in CRC,

including cases of monoclonal and polyclonal dissemination.

Conclusion

In conclusion, OVCA have complex evolutionary dissemination trajectories, consistent with frequent and a predominantly passive dissemination mechanism in the abdomen. Preliminary results from CRCs confirm that cases of polyclonal dissemination occur also in this cancer type, although the level of complexity has yet to be determined.

EACR25-1911

Metabolic and Tumor Heterogeneity-Driven Strategies to Overcome Platinum Resistance in Ovarian Cancer

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Introduction

Platinum resistance remains a major challenge in the treatment of this pathology, highlighting the urgent need for innovative therapeutic strategies. Preliminary results suggest the possibility to delay the development of resistance by adding metformin (MET) to cisplatin (DDP) in an upfront therapy setting in ovarian cancer PDXs. Our research focuses on the design of new strategies overcoming and preventing DDP resistance through metabolic interventions and the characterization of the evolution tumor heterogeneity during drug treatment.

Material and method

To reproduce our *in vivo* preliminary results in an *in vitro* setting, starting from sensitive (S) ID8 F3 murine ovarian cancer cells, we developed DDP-resistant (R) cells with multiple treatments. ID8 F3 cells were also treated with a combination of DDP and MET (SDM) to assess the possibility to delay the onset of resistance also *in vitro*. Metabolic profiling and molecular characterization were performed by Seahorse® flux analyzer on the S, R, and SDM cells, and samples derived from PDXs models. Single-cell RNA sequencing (scRNA-seq) was performed by 10X Genomics® technology on a S PDX (MNHOC124) at DDP different treatment stages to characterize tumor heterogeneity: Random time (untreated tumors); Best response (at the maximum of tumor regression after treatment); Regrowing (regrowing tumors after therapy challenge). The bioinformatic analysis was done with Seurat.

Result and discussion

Through multiple DDP treatments, we obtained a DDP-resistant cell line (R), showing an increase of 5 times of the IC50 respect to the parental one (S). As observed *in vivo*, the addition of MET to DDP was able to prevent the development of resistance, showing an intermediate IC50 (SDM cells). Through Seahorse assays we observed that the development of resistance in the ID8 R was associated with a metabolic rewiring, that included a

major use of OXPHOS as energy fuel. Similar results were observed in ex-vivo cultures derived from S, R and SDM PDX models. In the ID8 cells, the addition of MET was able to counteract the metabolic rewiring observed in the R cell line, and restore a more glycolytic phenotype in the SDM as the S cells. The scRNA-seq analysis identified 12 tumor clusters, with specific representation for each timepoint. Differential expression analysis revealed upregulation of genes involved in ribosomal function, mitochondrial metabolism, and DNA repair in clusters most represented at the regrowing timepoint.

Conclusion

Our findings underscore the critical role of tumor metabolism and heterogeneity in platinum resistance. The DDP+MET combination appears to delay resistance emergence, highlighting metabolism-targeting strategies as a potential preventive approach. Ongoing research on scRNaseq experiment and spatial omics aims to refine these strategies and analyse the murine tumor micro-environment contribution, to identify specific therapeutic targets within resistant subpopulations.

EACR25-1912 POSTER IN THE SPOTLIGHT

Longitudinal Dynamics of Transcriptome and Chromatin Accessibility in Ovarian High-Grade Serous Carcinoma

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Introduction

Ovarian high-grade serous carcinoma (HGSC) remains a leading cause of gynecological cancer mortality due to its marked heterogeneity and propensity for resistance to platinum-based therapies. Understanding the evolution of HGSC requires a comprehensive view of transcriptomic and epigenetic alterations that occur over time and across anatomical sites. Here, we present an integrated analysis that combines longitudinal bulk RNA-seq and single-cell multiome (snATAC-seq and snRNA-seq) data to delineate the dynamic landscape of HGSC.

Material and method

We analyzed 1,006 bulk RNA-seq samples from 308 HGSC patients enrolled in the prospective, multi-region DECIDER trial (NCT04846933). Samples spanned three treatment phases (treatment-naïve Tn, neoadjuvant-treated T, and relapse R) and represented multiple anatomical locations (adnexal, intra-abdominal tumors, and fluid metastases). Gene expression data was modeled with mixed scaled Poisson distributions and solved using expectation maximization, enabling to decompose expression profiles into patient-, tissue-, and treatment-specific components. In parallel, we generated single-cell multiome data from 16 samples from 6 HGSC patients, including Tn-T and Tn-R pairs, to capture epigenetic dynamics that underlie the transcriptomic changes under the treatment pressure.

Result and discussion

Adnexal sites, which included sites-of-origin, exhibited high proliferation potential, evidenced by a pre-dominance of the proliferative subtype, and marked patient specificity. We quantified intra-patient heterogeneity at diagnosis and found that 23% of patients exhibited homogeneous primary samples harboring a single transcriptomic subtype, and this was associated with poorer overall survival (log-rank p = 0.006) compared to patients with heterogeneous subtype profiles. Moreover, the mesenchymal subtype in adnexal tumors at diagnosis correlated with a worse prognosis (log-rank p = 0.026), an effect not observed in intra-abdominal tumors, where this subtype was more frequent (Fisher's exact test p = 2.6e-08). Using the Corneto pipeline for network inference, we identified key deregulated pathways that differentiate R from Tn samples and T from Tn samples based on transcription factor and pathway activities. Preliminary analysis of the 16 snATAC+snRNA multiome samples yielded distinct cell type-specific chromatin accessibility clusters on the UMAP, suggesting that integrated chromatin accessibility and transcriptomic profiling will further elucidate the regulatory mechanisms driving treatment response and disease progression in HGSC.

Conclusion

We evaluated intrinsic and acquired molecular heterogeneity in longitudinal HGSC samples. Our findings underscore the importance of dissecting spatial and temporal heterogeneity in HGSC to explain regulatory mechanisms driving resistance to chemotherapy.

EACR25-1970

Tumor subclonal heterogeneity as a cause of drug resistance in ovarian cancer

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Introduction

Ovarian high grade serous carcinoma (HGSC) is the most lethal gynecological malignancy. A key contributor to the high lethality is the resistance to treatment, which we aim to understand better by investigating the tumor subclonal composition. We previously showed that patients classify into 3 different evolutionary states based on the subclonal composition of their tumors at pretreatment and that these evolutionary states were significantly associated with treatment outcome. Integrating relapse samples to our previous results, and particularly focusing on the differences between primary and relapse, enhances our understanding of the mechanisms behind drug resistance. We hypothesize that greater tumor heterogeneity gives the cancer higher phenotypical plasticity, meaning the phenotypical changes caused by genomic aberrations are not the only cause of drug resistance. In this project we wish to quantify tumor heterogeneity, find clinically relevant heterogeneity trajectories between primary and relapse samples, and find explanation for drug resistance

other than the theory of a bottle neck effect, where resistance is solely based on acquisition of genomic aberrations.

Material and method

We are using primary and relapse tumor samples, ctDNA samples, and clinical data from 214 patients that are part of the DECIDER study [1]. The samples are collected from multiple anatomical sites, and the sampling is done longitudinally spanning years for most patients. Through whole genome sequencing we obtain copy number information and single nucleotide variants (SNVs) used to infer tumor subclones and phylogenetic trees.

Classifying the patients based on their heterogeneity trajectories over the course of their disease and combining with their clinical and transcriptomic information we aim to find patterns explaining treatment response.

Result and discussion

The set of phylogenetic trees based on primary and relapse samples will alone be among the largest and most comprehensive of its kind. In part of the patients we already see that a high heterogeneity in all treatment stages is associated with a poor treatment outcome. This supports the hypothesis of phenotypic plasticity being at least partly accountable for drug resistance, providing novel insight in our understanding of the resistance mechanisms in HGSC.

Conclusion

Our study provides compelling evidence of how tumor subclonal heterogeneity and evolutionary dynamics shape treatment resistance in ovarian HGSC. These findings support the existence of resistance mechanisms beyond the bottleneck theory.

[1] Multi-Layer Data to Improve Diagnosis, Predict Therapy Resistance and Suggest Targeted Therapies in HGSC; ClinicalTrials.gov identifier NCT04846933

EACR25-2014

Rhabdomyosarcoma tumours undergo endothelial trans-differentiation to evade treatment

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Introduction

Rhabdomyosarcoma (RMS) is the most common soft tissue sarcoma in children and adolescents. It arises from cells of mesenchymal origin across multiple organs where either a gene fusion of PAX3/7-FOXO1 (Fusion positive RMS [FP-RMS]) or RAS pathway mutations (Fusion negative RMS [FN-RMS]) drive aberrant myogenic developmental programs. In the relapse setting RMS is a tumour high unmet need with few treatment options. It is also noted for its plasticity with research focussed on modulation of the myogenic signature. We have identified an alternative avenue of plasticity, whereby RMS cells activate an endothelial developmental program, concurrent with the formation of vessel-like structures (vascular mimicry [VM]). We have shown this switch to be driven by anti-angiogenic receptor tyrosine kinase inhibitor (RTKI) treatment and strongly linked to therapy resistance in pre-clinical testing.

Material and method

Cells from 10 RMS tumour lines (RD, SMS-CTR, JR1, Rh36, T174, RMS01, Rh30, Rh4, Rh41, RMS01) were profiled for vascular mimetic capacity by in vitro Matrigel tube formation assays and in vivo imaging of tumour cell-derived conduits in transparent zebrafish xenografts. Response to regorafenib treatment was assessed by MTS and fluorescent tumour growth assessment assays. Matched transcriptomic (RNA-seq) and secretomic (proteome profiler) profiling was performed to identify markers of VM in RMS, which were validated by immunofluorescent imaging of cells and tissues. A VM gene expression signature based on co-expression of endothelial and tumour markers, was used to dissect VM niches from sc-RNA-seq data from 21 RMS clinical tumour samples.

Result and discussion

We have developed techniques to stratify RMS tumour samples by vessel forming / VM capacity both in vitro and in vivo and found the niche to be marked by the haematopoietic marker CD34 as well as the endothelial specific marker VE-cadherin at cell-to-cell junctions upon vessel maturation. Identification and profiling of the VM niche in sc-RNA data from 21 RMS clinical samples revealed it to be enriched in high-risk metastatic tumours and those that have relapsed from treatment. Profiling of In vitro and in vivo tumour response to the MRTKI regorafenib treatment, revealed VM capacity to be strongly and positively linked to resistance with treatment driving both haematopoietic and myogenic trans-differentiation in distinct tumour cell sub-populations.

Conclusion

Vascular mimicry in RMS provides a potential route towards uncoupling the tumour from dependence on traditional vascularisation, allowing for the greater level of aggression and treatment resistance that we see in our analyses. Some clinically used therapeutics, including regorafenib, can drive this behaviour. There is therefore an urgent need to better understand RMS endothelial plasticity and VM, to stratify patients and avoid detrimental therapeutic regimens, but also to investigate avenues for specific targeting.

EACR25-2144

Investigating the Role of Chromosomal Instability in Colorectal Cancer Drug Resistance Development

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Introduction

Chromosomal Instability (CIN) is the rate at which ongoing segregation errors occur over consecutive cell divisions, which can lead to significant genomic changes. While typically harmful to cells, CIN and its subsequent genomic reshuffling is thought to help cancer cells adapt to selection pressures, such as chemotherapy treatment.

Colorectal cancer (CRC), a leading cause of cancer-related deaths, frequently exhibits CIN – which is linked to poorer prognosis and drug resistance in this cancer. However, the exact mechanisms remain unclear. This study investigates the role of CIN in resistance to a key chemotherapy drug, fluorouracil (5'FU), in a CRC cell line.

Material and method

First, a chromosomally unstable CRC cell line was exposed to various 5'FU drug regimens, either as a population of cells or as isolated single cells (clonal outgrowth). Long-term IncuCyte proliferation imaging was used to track clonal outgrowth of single cells. A drug sensitivity screening was subsequently performed using cell proliferation assays, and rates of CIN were estimated by measuring segregation error rate in mitotic cells via immunofluorescence. Single cell DNA sequencing (scDNaseq) was used to compare genomic changes between drug-resistant vs non-resistant cells.

Result and discussion

A model of 5'FU-specific drug resistance has been developed in a chromosomally unstable CRC cell line. Interestingly, only 5'FU-resistant populations showed increased segregation error rate, when compared with control and non-resistant cells. Genomic changes in 5'FU-resistant vs non-resistant single cells have been characterised, showing some unique copy number alterations in drug-resistant cells. Clonal outgrowth experiments showed that single cells, grown into populations under 5'FU treatment, display various growth patterns and rates across treatment regimens; paired scDNaseq of these clones provides insight into clonal evolution, as well as genomic heterogeneity within and between 5'FU-resistant and non-resistant clonal populations.

Conclusion

This work highlights the propensity of a chromosomally unstable CRC cell line to adapt and develop resistance to the major CRC chemotherapy drug, fluorouracil. The rate of CIN shows a positive correlation with 5'FU resistance development in these cells, with future experiments underway to understand whether CIN is causative of this resistance. Cell-cell heterogeneity within and between 5'FU-resistant and non-resistant populations is highlighted by scDNaseq data, which indicates changes that may be key to 5'FU resistance. Given that CIN is present in 60-80% of cancers, understanding its contribution to cancer drug resistance is crucial to addressing the mortality caused by this drug resistance.

EACR25-2259

Elucidating the molecular mechanisms of whole genome doubling in PDAC

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Introduction

Genomic instability is a hallmark of pancreatic ductal adenocarcinoma (PDAC), driving tumor evolution, treatment resistance, and disease progression. One consequence is whole-genome doubling (WGD), leading to aneuploidy and chromosomal aberrations that fuel intratumoral heterogeneity. These genomic alterations play a critical role in shaping tumor evolution by generating genetic diversity, facilitating adaptation to selective pressures, and promoting metastatic potential. WGD and aneuploidy may also contribute to immune evasion and therapy resistance, making them key factors in PDAC progression. However, the mechanisms governing ploidy changes and their impact on tumor evolution remain poorly understood. Here, we investigated ploidy status and genomic alterations in genetically engineered mouse model (GEMM)-derived PDAC primary cell lines to elucidate their contribution to the evolution of aneuploidy in PDAC.

Material and method

We analyzed PDAC primary cell lines derived from GEMMs using flow cytometry and metaphase spreads to determine ploidy status. Whole-genome sequencing (WGS) was performed to characterize chromosomal alterations and structural variants associated with genomic instability. Additionally, phosphoproteomic analysis was conducted using mass spectrometry to investigate alterations in signaling pathways associated with ploidy changes.

Result and discussion

Whole-genome doubling was identified in all PDAC cell lines, though to varying degrees. As expected, cell lines with genomic loss of p53 correlated with higher rates of WGD and increased chromosome numbers. However, in contrast to established models, we also observed cell lines with p53-independent increases in chromosome numbers, driven by genomic activation of Kras and PI3K signaling pathways. Phosphoproteomic analysis revealed distinct differences in kinase activity profiles between PDAC cell lines with varying ploidy levels. Notably, key kinases regulated by PI3K signaling were differentially phosphorylated in high-ploidy cell lines, suggesting a potential role in maintaining genomic instability and tumor evolution.

Conclusion

Our study provides novel insights into aneuploidy and genomic instability in PDAC. The identification of both p53-dependent and p53-independent occurrences of WGD challenges current models and highlights the role of Kras and PI3K signaling in driving chromosomal instability. Further studies into early evolutionary mechanisms during tumor development could provide a more mechanistic understanding of whole genome doubling in PDAC.

EACR25-2327

Characterization of breast cancer tumoroid subclones by proteomics for personalized medicine

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Introduction

Introduction: Breast cancer (BC) is the most common cancer among women causing the highest number of cancer-related deaths. Currently, BC research is mainly conducted using 2D cultures or mouse models. Both have proven to have limitations: 2D culture lacks cell-cell or cell-matrix interactions and mice are associated with ethical problems and poorly mimic the human diseases. There is an unmet need to develop novel more accurate, realistic, standardisable and robust experimentation models.

Material and method

Methodology: We have developed BC tumoroids made from a mixture of tumor cells isolated from human and canine BC tissues. In the tumoroid model, we conducted studies to isolate tumoroid-derived subclones with heterogeneous profiles. We analyzed these subclones using proteomics and mass spectrometry imaging to compare them with their parent tumoroids. Our goal was to determine whether the study of these subclones could lead to a more comprehensive treatment strategy that targeting all subclones, potentially reducing post-treatment resistance compared to standard hospital treatment.

Result and discussion

Results: Our results show that intratumoral heterogeneity is maintained in the tumoroid model. While many tumoroids exhibited a homogeneous profile, others showed heterogeneity within tumoroids derived from the same patient, which is critical for recapitulating in vivo drug responses. This led us to investigate different subclones within the tumoroids that represent intra-tumoral heterogeneity. We isolated different sub-populations and used mass spectrometry imaging to demonstrate their similarities to the parent tumoroids. Additionally, proteomic profiling revealed distinct differences between the subclones. Finally, we showed that not all subclones grow and respond uniformly to treatments. The next step is to propose new treatments based on our proteomics analysis that can affect all subclones, thereby preventing relapse in patients.

Conclusion

Conclusion: Our knowledge of biology, combined with our skills in proteomics and mass spectrometry imaging helped us to better understand the BC tumoroids and to study the different intratumoral populations that are so problematic today.

EACR25-2436

Tackling gastric cancer heterogeneity using patient-derived organoids

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Introduction

Gastric cancer remains a major clinical challenge, with its incidence and mortality predicted to increase significantly by 2040. Currently ranked fifth globally in both incidence and mortality, the disease is characterised by rising case numbers and inherent heterogeneity.

Gastric cancer exhibits molecular heterogeneity driven by diverse oncogenic drivers and clinical heterogeneity influenced by anatomical location, highlighting the critical need for personalised treatment strategies.

Material and method

To study tumour heterogeneity in gastric cancers, patient-derived organoids (PDOs) were established from multiple locations of treatment-naïve tumours. Morphological assessments were performed to evaluate intra-patient heterogeneity. Drug response studies were conducted on at least three PDOs per patient to assess their reactions to the standard-of-care chemotherapy FLOT (5-Fluorouracil, Leucovorin, Oxaliplatin, and Docetaxel) as well as to alternative therapies, including targeted drugs.

Additionally, sequencing analyses were performed to examine mutational differences between PDOs from the same patient.

Result and discussion

PDOs demonstrated significant intra-patient heterogeneity in their morphology. While most PDOs from the same patient exhibited similar responses to FLOT chemotherapy, distinct responses were observed when exposed to alternative therapies, including targeted drugs. Sequencing data further revealed mutational differences among PDOs derived from the same patient, providing insights into tumour heterogeneity and its impact on therapy resistance.

Conclusion

The use of multiple PDOs per patient highlights the intra-patient heterogeneity of gastric cancer and its influence on therapy responses. This approach enhances our understanding of primary and secondary resistance mechanisms, driven by tumour heterogeneity, and may facilitate the identification of alternative treatment strategies targeting specific oncogenic pathways. These findings underscore the potential of PDO models to inform personalised treatment decisions for gastric cancer patients.

EACR25-2441

SP2G: A Tool to Unveil Glioblastoma Migratory Diversity

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Introduction

Glioblastoma (GBM) is the most aggressive primary brain tumor, with a median survival of 8–14 months after diagnosis. Its treatment failure is mainly due to two factors: high heterogeneity, which hinders the identification of effective molecular targets, and strong invasiveness, leading to frequent recurrences observed in over 90% of patients. To migrate efficiently, GBM cells mostly exploit the abluminal surface of the blood vessels, which is particularly enriched in laminin, and the white matter tracts. In this study, we present a biomimetic approach based on gridded micropatterns to controllably recapitulate the surface and the topology of blood vessel walls, aiming to differentiate distinct inter- and intra-patient motility behaviours and link them to specific molecular signatures.

Material and method

We developed an analytical platform called SP2G (SPheroid SPreading on Grids) to investigate the motility of patient-derived GBM cells. Tumor spheroids were seeded onto laminin-coated micropatterns mimicking the structure of the brain vasculature, and analyzed using a semi-automated Fiji-based toolkit. This approach allows precise measurements of migratory parameters and enables to distinguish the most invasive clones from the less motile ones, highlighting differences which can be potentially linked to molecular signatures.

Result and discussion

GBM cell analysis revealed significant variability in migration patterns among different patients. Three different patient-derived cell lines already known for their diverse motility modes were tested and confirmed in term of distinct migratory behaviours, with the most aggressive one spreading further away compared to the others. SP2G also showed to be sensitive to drug assays and variations in laminin concentration, confirming the bona-fide of the assay. Intra-patient heterogeneity was also tested with five clones coming from the same tumor. Once again, SP2G revealed differences in migratory behaviours, identifying three motile and two non-motile clones. These differences were further confirmed by molecular signatures showing the motile clones to be enriched in specific integrins compared to non-motile ones. Most interestingly, SP2G also revealed spreading diversity of motile clones between each other, grouping them in two categories which were further confirmed by Principal Component (PC) and Gene Set Enrichment (GSE) analysis.

Conclusion

SP2G has proven to be an effective tool for characterizing the migratory diversity of GBM cells, providing a fast, simple and reproducible approach to identify invasive subpopulations. It may be used to for motility screenings, in order to correlate cell migration with

cancer dissemination, being potentially extendable also to pan-spheroids analysis.

EACR25-2530

Comprehensive molecular landscape of acquired resistance to sotorasib in pancreatic cancer

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Introduction

Sotorasib, a KRASG12C inhibitor, represents a breakthrough in treating pancreatic ductal adeno-carcinoma (PDAC); however, acquired resistance limits its clinical efficacy.

Material and method

To investigate resistance mechanisms, we generated a sotorasib-resistant MIA PaCa-2 (KRASG12C) cell line through progressive sotorasib exposure (2–512 nM) and isolated resistant clones via single-cell sorting. Phenotypic changes were assessed using proliferation, migration, and invasion assays, while molecular alterations were analyzed via the PanCancer NanoString panel, protein arrays, western blotting, and fluorescence microscopy.

Result and discussion

Following the resistance induction protocol, 33 of 400 clones remained viable after re-exposure to sotorasib (512 nM). Clone E9 was selected for further analysis due to its higher IC₅₀, elevated levels of active endogenous Ras post-sotorasib treatment, and a protein expression profile indicative of KRAS signaling bypass. In the absence of treatment, the resistant cell line exhibited downregulation of 15 genes, including growth factors (e.g., FGF2), cytokines (e.g., CSF3, IL1A), and genes associated with proliferation (e.g., RAC2, PAK7, MET), cell signaling (e.g., LRP2, PRDM1, GAS1, SOCS3, NFKBIA, KLF4), and gene regulation (e.g., DTX4, JAG2, RPS6KAS). Conversely, the resistant line showed upregulated expression of 5 genes: JAG1 (Notch signaling), IL1R1 (inflammatory response), RUNX1 (hematopoiesis and gene regulation), DDIT4 (stress response and mTOR modulation), and XRCC4 (DNA repair). Protein analysis identified elevated phosphorylation of insulin receptor, IGF-1R, Axl, and mTOR, confirming KRAS signaling bypass. Computational modeling demonstrated that combining sotorasib with mTOR inhibitors restored sensitivity in resistant cells.

Conclusion

These findings elucidate the molecular mechanisms underlying sotorasib resistance in PDAC and provide a rationale for developing combinatorial therapies to overcome resistance, offering new hope for patients with KRASG12C-mutated pancreatic cancer.

EACR25-2594

Spontaneous telomere dysfunction promotes acrocentric chromosome arm

fusions and aneuploidy in cell cycle checkpoint impaired human astrocytes

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Introduction

Telomere dysfunction (TD) is considered to be a potent mutator mechanism in cancer and a driver of aneuploidy and structural variation (SV). However, it is unclear which SVs are a consequence of TD and which are simply bystanders to other mutational processes. We sought to investigate how TD reshapes cell cycle checkpoint impaired human astrocytes over time using an *in vitro* model system.

Material and method

Normal human astrocyte (NHA) cells were transduced with retroviral vectors containing HPV-18 E6/E7 to impair cell cycle checkpoints. Limited dilution cloning was used to isolate single cells that were independently cultured alongside bulk cultures and grown over six months to induce telomere attrition. Representative clones were selected for short-read genome sequencing and copy number profiling. M-FISH karyotyping was performed to assess genome structure. Immunofluorescence (IF) microscopy was used to quantify metaphase and anaphase defects. Primary NHA cells and a selected bulk and single-cell clone of E6E7-transduced samples with unique copy number profiles were further analyzed using Hi-C, Oxford Nanopore ultra-long and Pacific Biosciences HiFi long-read sequencing. Diploid phased genome assembly was performed on all long-read sequenced samples to determine the full-length chromosome sequences. Pangenome graphs were constructed from assembled genomes to evaluate large-scale structural variation.

Result and discussion

NHA cells began showing aneuploidy after 42 doublings following transduction with E6/E7 (82 days in culture). In particular, we observed partial deletions of all acrocentric chromosome arms (13, 14, 15, 21 and 22) independently in several single cell and bulk clones, most prominently affecting the small arm of chromosome 13, progressing to a complete del(13) after 60–70 doublings. Long read data showed telomere attrition, initially 12 Kb in NHA primary cells and dropping to 6 and 4 Kb at 42 and 70 doublings after E6/E7 transduction, respectively. M-FISH karyotyping showed significant chromosomal abnormalities in every cell examined, including t(2,13) in 40%, del(13) in 30%, t(1,21) in 20% of cells analyzed. Conversely, no chromosomal abnormalities were observed in primary NHA cells. IF showed anaphase bridges in 30% of cells, further implicating TD as the root cause of the observed phenotypes. Long-read sequencing and genome assembly of primary NHA cells produced a diploid genome assembly comparable in quality with the HG002 diploid genome assembly from

the Human Pangenome Reference Consortium. Genome graph-derived and haplotype-specific variant calling confirmed an elevated number of SVs in E6/E7 transduced cells compared to primary NHA cells. Intriguingly, we observed an enrichment of SVs with telomeric sequence at the breakpoint junction, reaffirming TD as the causal mechanism of SV.

Conclusion

Our results suggest that telomere dysfunction promotes fusions involving acrocentric chromosome arms.

Tumour Immunology

EACR25-0008

TRIB1 leads to M2 skewing of macrophages in LUSC

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Introduction

Building upon the bioinformatics insights, this study aims to delve into the role of tribbles pseudokinase 1 (TRIB1) in M2 macrophages in the context of lung squamous cell carcinoma.

Material and method

M0 macrophages were prepared and co-cultured with LUSC cells, after which the M2-polarized macrophages and the associated markers were analyzed. The proliferation and mobility of the co-cultured LUSCs were analyzed via functional experiments. Loss- or gain-of-function assays of TRIB1 and its putative regulator KLF transcription factor 6 (KLF6) were conducted to analyze their impacts on M2 phenotype shift of macrophages and the malignancy of tumor cells. A mixture of macrophages and LUSC cells was injected into immunocompromised mice for *in vivo* assays.

Result and discussion

Co-culturing with LUSC cells led to an M2 shift of macrophages, accompanied by an upregulation of TRIB1. Knocking down of TRIB1 suppressed this M2 polarization, and it inhibited proliferation and mobility of the co-cultured LUSC cells. The aberrant upregulation of TRIB1 in M2 macrophages was induced by the loss of KLF6, which binds to the TRIB1 promoter for transcriptional repression. Artificial upregulation of KLF6 similarly reduced M2 macrophages and the LUSC cell malignancy, and it weakened tumor growth and dissemination in mice. However, these effects were counteracted by TRIB1 upregulation.

Conclusion

This research suggests that the TRIB1 upregulation upon KLF6 leads to M2 shift of macrophages and LUSC progression.

EACR25-0039

TRIB1 leads to M2 skewing of macrophages in LUSC

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Introduction

RNA editing is one of the most prevalent post-transcriptional modifications observed in normal physiological processes. Many studies have suggested that widespread RNA editing is an important factor in the occurrence and development of cancer. However, the value of RNA editing in the development and prognosis of bladder cancer has not been clarified.

Material and method

BLCA RNA-seq data and RNA editing data were downloaded from the TCGA database and synapse database to find prognostic signature RNA edits by single/multi-factor Cox analysis and to perform prognostic risk model construction. Differentially expressed genes (DEGs) were screened from high and low risk groups based on the risk scores of the prognostic risk model and subjected to functional and pathway enrichment analyses. In addition, survival analysis, ROC analysis, independent prognostic analysis, ADAR gene correlation analysis, and nomogram curves were used to test the prognostic model effects. RNA editing gene differences, immune infiltration analysis, and immuno-therapy response analysis were used to investigate in depth the mechanisms by which RNA editing leads to poor prognosis.

Result and discussion

Univariate/multivariate Cox analysis was performed to construct prognostic risk models for nine RNA edits. Survival analysis and independent prognostic analysis showed that risk model scoring was an independent prognostic factor in predicting the survival of BLCA patients. In addition, the calibration curves showed high agreement between actual survival and predicted rates, and the ROC curves further demonstrated the model's good predictive ability. Meanwhile, ADAR gene expression and risk score showed a positive correlation, and the 2 RNA edits in the model also differed significantly between the tumor and normal groups. In particular, there were differences in immune cell infiltration in patients with high and low expression of GM2A (dist = 1596), SLC36A3 (dist = 4377) |chr5: 150651549, and different immunotherapy responses.

Conclusion

In short, we have developed a new prognostic model of bladder cancer, which may be helpful for personalized counseling and treatment of BLCA, and is expected to bring a new direction to bladder cancer research.

EACR25-0071

Intra-tumoral bacteria in high grade serous ovarian carcinoma

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Introduction

While tumors were once thought to be sterile, it has now been shown that many cancers, including high-grade serous ovarian cancer (HGSOC), contain microbiota. Importantly, tumor-resident bacteria could be a potential source for DNA damage that may contribute to more aggressive tumor behavior, such as resistance to chemotherapy and immunochemotherapy, and metastatic potential. Little is known about the interplay of these factors, especially for ovarian cancer. To provide better understanding of this aspect of disease biology, and ultimately to develop better targeted treatments for better outcomes, we investigate in this project the HGSOC microbiome and its link with treatment response.

Material and method

Clinical HGSOC samples (n = 80) were collected as part of the ONCOSYS-OVA prospective study. To investigate the microbiome of each HGSOC tumor, we use three independent approaches: immunofluorescence (IF), DNA amplification-based methods, and amplicon sequencing. Gram-positive and -negative bacteria are detected in formalin-fixed, paraffin-embedded (FFPE) tumor samples by IF. Total bacteria present in our fresh frozen tumor samples are quantified using nested qPCR and digital PCR (dPCR). From the same samples we also have estimates of DNA damage as well as DNA repair capacity (homologous recombination scores). Bacterial identification and diversity assessment is done by high-throughput sequencing. Filters are put in place to eliminate common environmental contaminants, with the help of including positive (fecal sample), negative controls (blood samples), and non-template controls (NTC), with the addition of normal healthy tissue samples in order to differentiate between the healthy tissue microbiome and the tumor specific resident bacteria.

Result and discussion

Preliminary findings indicate that individual HGSOC samples contain different levels and composition of bacteria compared to positive and negative controls and NTCs. These findings were concordant with IF staining results, showing presence of Gram positive and negative bacteria, from the same samples. Next, we performed high-throughput sequencing to identify which bacterial species are present in these samples. The results showed that most samples harbor various bacterial species at different levels of positivity, confirming the previous findings we obtained using IF, nested PCR and dPCR.

Conclusion

Our results so far suggest that most HGSOCs contain bacteria. Our future work is to expand the analysis to include more samples to assess the diversity of tumor-resident bacteria, and its putative direct impact on DNA damage, DNA repair and treatment resistance.

EACR25-0118

New mouse models to define the function of CCR7+ dendritic cells in anti-tumour immunity

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Introduction

Conventional dendritic cells (cDCs) constitute crucial orchestrators of adaptive immune response against tumours. Recent single-cell transcriptional analyses have unraveled an activation state of tumour-infiltrating cDCs (referred to herein as actDCs a.k.a. CCR7+ DCs, mregDCs, or cDC3s) derived from both cDC1 or cDC2 subsets. Their transcriptional profile suggests putative opposing T cell-stimulating or inhibitory roles, and due to their intratumoural scarcity and lack of experimental tools, the actual contribution of actDCs to tumour immunity remains unknown.

Material and method

Exploiting in-house scRNAseq data of tumour-infiltrating immune cells and intersectional genetics, we here developed new genetically engineered mouse models to conditionally and selectively track, isolate or deplete actDCs. We also devised a primary bone marrow culture system to develop actDCs *in vitro*. We employed our novel actDC reporter to separate actDCs from their resting counterparts and performed tumour antigen presentation assays. We further used our new mouse model for targeted actDC depletion to evaluate their contribution to T cell-mediated tumour control and the response to dual αPD-1/αCTLA-4 blockade or CD8+ T cell adoptive transfer.

Result and discussion

We show that the ability of cDCs to promote CD8+ T cell activation in response to tumour antigens is confined to the actDC state. Mechanistically, actDC1s cross-present, and actDC2s cross-dress tumour-derived material to CD8+ T cells in a tumour cell-type dependent manner. Crucially, we demonstrate that specific and acute depletion of actDCs impairs T cell priming and tumour eradication *in vivo*. Moreover, we show that actDCs are necessary for a successful response to both antibody-mediated and cell-based immunotherapy.

Conclusion

Overall, our work formally establishes an essential, non-redundant contribution of actDCs to T cell-mediated, anti-cancer immunity.

EACR25-0119

Prognostic role of Immunoscore in colon cancer

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Introduction

CRC is world-leading health problem. Treatment of CRC is based on TNM classification. For patients with stage 1 disease treatment is well established, but for patients with stage 2 and 3 there are controversies about need of post-operative chemotherapy. We need more prognostic factors which patients are in high risk and which are in low risk. Immunoscore is method based on density of CD3+ and CD8+ TILs in tumor and its invasive margin. It makes quantitative evaluation and results are converted in five predefined threshold from Immunoscore 0 to Immunoscore 4. Several trials are establishing its value as prognostic factor in CRC. This is first retrospective trial studying role of Immunoscore in Bulgarian population of CRC patients.

Material and method

This is single center retrospective analysis of 35 patients with stage II and stage III CRC. All of them have undergone radical surgery in period 2016-2021. Immuno-score analysis is done to tumor samples from each patient. Data from each patient demographics, tumor characteristics and DFS were collected.

Result and discussion

There were patients in all categories of Immunoscore from 0 to 4. The most prevalent were patients with Immunoscore 2 and the patients in highest and lowest values were minority. There were 14 women and 21 men. Distribution of Immunoscore is similar in both sexes. Median result in women is 2.07 and in men 2.10. The study finds no significant differences in Immunoscore distribution between genders. The number of patients in stage II was 20 and in stage III was 15. Results of Immunoscore were similar between both groups- 2.0 in stage II and 2.1 in stage III. There is no significant correlation between age and Immunoscore, and the distribution of Immunoscore is similar in both stage II and III patients. The patients with left side tumors have median three years DFS 33,43 months and these with right sided tumors have DFS 33,36m. It is observed significant difference in results of Immunoscore in patients with left and right sided tumors. The patients with left location have median IS 1,90, but these with right location have median IS 2,36. This difference remains valid when compared according to stage and gender. The results show that patients with a high Immunoscore have best disease-free survival rates. After four years surveillance there are no progression in this group(median DFS-48months). While those with low or intermediate Immunoscore have a higher risk of disease progression. The worst result are for men with right sided tumors and IS-2, which have median DFS of 20 months.

Conclusion

The study concludes that Immunoscore is a valuable prognostic tool independent of tumor stage and could potentially guide decisions on adjuvant chemotherapy. High Immunoscore correlates with excellent survival rates, independent of the tumor stage and location. There is significant difference in Immunoscore for left and right sided tumors. This could be one of explanations for different prognosis according to sidedness.

EACR25-0131**Glucocorticoids trigger CD8+ T cell-dependent tumour control**

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Introduction

Melanoma is the deadliest form of skin cancer. Treatment with immune checkpoint blockade (ICB) has transformed outcomes. However, half of melanoma patients do not derive long-term benefit, and improved understanding of this is required to identify novel mechanisms of resistance and treatment. We screened a range of topical treatments, used in non-melanoma skin diseases, for anti-cancer activity in an ICB-refractory murine melanoma model.

Material and method

We used an intradermal melanoma model that we found to be fully unresponsive to the dual combination of anti-PD1 and anti-CTLA4 to assess the effects of topical imiquimod, 5-fluorouracil, diclofenac and glucocorticoid (GC) treatment. Multiparametric flow-cytometry, immunofluorescence and whole transcriptome and single-cell RNA sequencing of mouse tumours were used to assess the effects of GCs in the tumour micro-environment (TME). Bioinformatic analysis of publicly available datasets and IHC of in-house patient samples was employed for human analysis.

Result and discussion

Of all topical treatments tested, only topical GCs resulted in acute tumour growth inhibition. After just two doses, GC-treated tumours shrank, whereas control-treated tumours doubled in volume. Intriguingly, this effect was lost in Rag1^{-/-} mice (deficient in B and T cells) or in mice depleted of CD8+ T cells, uncovering a key role for T cells in GC-induced tumour growth control. Of 8 cancer models tested for GC-responsiveness, half experienced immune-dependent control, and half did not. Genetic ablation of the glucocorticoid receptor in tumour cells, but not in immune cells, abrogated this effect, suggesting GCs act directly on tumour cells to stimulate anti-tumour immunity. Analysis of cancer patient transcriptomic datasets showed melanoma patients with high GC receptor expression, signalling and activation have better overall survival than those with low expression. GC treatment spared CD8+ T cells in the TME, but reduced the presence of conventional and regulatory CD4+ T cells. Sequencing, genetic knockout and targeted mutagenesis experiments revealed GCs downregulated the expression of GARP on the surface of melanoma cells. This reduced TGFβ signalling, allowing CD8+ T cell tumour killing. Supporting this, in single-cell RNA sequencing analysis of human patients, tumours with high glucocorticoid receptor expression had lower TGFβ signalling in tumour infiltrating CD8+ T

cells. Of the 8 tumour models tested, only GC-responsive tumours also responded to TGFβ inhibition, suggesting that GCs triggered immune-dependent tumour control when tumours are reliant on TGFβ signalling for immune evasion.

Conclusion

We discover a paradoxical immune-dependent shrinkage induced by GCs in certain tumour models. Given the widespread use of GCs in patients receiving ICB, these unexpected findings may have significant clinical impact.

EACR25-0154**Five dominant amino acid substitution signatures shape tumour immunity**

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Introduction

Numerous mutational mechanisms are identified in cancer, but their functional impacts are unclear. We hypothesize that certain mutation sources preferentially generate amino acid substitutions that are unlikely to produce immunogenic neopeptides, leading to immune-cold tumours regardless of origin or mutation burden.

Material and method

To test the hypothesis, we studied characteristic patterns of amino acid substitution in 9300 cancer exomes and derived a map of functional associations between environmental mutagens, therapeutic agents, and deficiencies in DNA repair mechanisms.

Result and discussion

Despite the immense diversity of mutational sources, only five primary amino acid substitution signatures emerged, with major differences in their functional properties. One notable signature, AAS4, primarily linked to alkylating agents and mismatch repair deficiency, is especially prevalent in kidney and liver cancers. AAS4-type tumours are less likely to accumulate hydrophobic amino acids, leading to lowered neoantigen immunogenicity. These tumours typically exhibit immune-desert microenvironments and respond poorly to immune checkpoint therapies. However, we identified certain combinations of HLA class I variants and mutational processes which are prime spots for immune response. For instance, HLA-B*07:02, a common variant in European populations with atypical peptide binding can uniquely induce T-cell proliferation in AAS4-type cancer cells.

Conclusion

In summary, our findings indicate that the quality of neo-antigens, rather than their quantity, is a key determinant of cancer immunity. Our study helps explain why mismatch repair-deficient tumours fail to respond to immunotherapy despite having a high mutational burden, and highlights the need to use amino acid substitution patterns to improve predictive biomarkers and therapeutic strategies.

EACR25-0183**Interleukin-15 signaling in
Immunosurveillance**

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Introduction

IL-15 is one of the most promising candidate in cancer immunotherapy due to its ability to promote the proliferation and cytotoxicity of Natural killer (NK) and CD8+ T cells. The mode of action of IL-15 is very special: IL-15 signals through a heterotrimeric receptor complex composed of three subunits, the α chain (IL-15R α), the β chain and the common γ chain. During biosynthesis, IL-15 associates with IL-15R α and is transported to the cell surface where IL-15R α ‘presents’ IL-15 to target neighboring cells expressing the β chains, referred as ‘trans-presentation’ (mostly known to occur in lymphoid cells). However, in myeloid cells, IL-15 is presented to its receptor on the same producer cell, known as ‘cis-presentation’. Our group previously showed that ‘trans-presentation’ by IL-15R α is dispensable in early innate immune responses to infections. We hypothesize that IL-15 signaling occurs in ‘cis’ via the IL-15R $\beta\gamma$ c complex independently of IL-15R α and is critical for tumor immunosurveillance.

Material and method

Spontaneous fibrosarcomas were induced with Methylcholanthrene in WT, II15-/- and II15ra-/- mice. Tumor development was monitored, and tumor samples were assessed for histopathological, immunological and proteomic features. Cell lines derived from MCA-induced tumors in WT, II15-/- and II15ra-/- mice were established.

Result and discussion

Tumor incidence is reduced in II15ra-/- ($n = 10/50$) compared to II15-/- ($n = 14/34$) and WT ($n = 16/47$) mice. Histologically, tumors from the three mice groups look comparable but we observed reduced NK and CD8+ T cells in II15-/- and II15ra-/- tumors. The proteomic data analyses show that antigen processing and presentation and cellular responses to Interferons are differentially regulated in II15ra-/- compared to II15-/- tumors.

Established cell lines from MCA-induced fibrosarcomas of WT, II15-/- and II15ra-/- mice express MHC-I, PD-L1 and have comparable proliferation rates in vitro.

Following implantation in WT mice, WT, II15-/- and II15ra-/- tumor cell lines do not show any significant differences in the growth. These observations suggest that IL-15 signaling does not play a significant role in tumor immune surveillance. Nonetheless absence of IL-15 ‘trans-presentation’ reduces the incidence of spontaneous tumors.

Conclusion

Our work highlights the subtleties of IL-15R α -independent IL-15 signaling in early innate immune response in immunosurveillance.

EACR25-0261**Immunosurveillance shapes the genetic landscape of intestinal tumors in replication error-deficient mice**

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Introduction

Carcinogenesis is normally suppressed by cancer immunosurveillance, a crucial defense mechanism in which the immune system identifies and eliminates neoplastic cells, by negatively selecting the ones presenting immunogenic peptides. However, neoplastic cells eventually devise immune evasion strategies, enabling their progression into detectable tumors. This emphasizes the importance of identifying and understanding the precise mechanisms underlying cancer cell recognition and evasion.

Material and method

Our study employed a mouse model of intestinal carcinogenesis with replication error deficiency (RER+), in immunocompetent (IC) and immunodeficient (ID) backgrounds. Whole-exome sequencing (WES) was performed to compare the tumor genetic landscape and determine distinctive attributes, either positively or negatively selected by the adaptive immune system. Preclinical findings were validated through a mutational screening performed in a cohort of human RER+ intestinal tumor samples. Immunogenicity of mutation-derived peptides was measured by IFN γ production upon co-culture of splenocytes from peptide-vaccinated mice with peptide-loaded murine dendritic cells.

Result and discussion

ID mice exhibited a higher incidence of cancer, an increased number of intestinal lesions, and a shorter lifespan compared to IC mice. Additionally, tumors from ID mice displayed a greater somatic mutation burden, unique mutation profiles, and an enrichment of specific mutations. These mutations occurred in genes commonly altered in human RER+ intestinal tumors. In vivo experiments confirmed the immunogenic potential of specific mutations identified in ID mice tumors.

Conclusion

Our findings suggest that immunosurveillance actively influences intestinal carcinogenesis, possibly through selective immune-mediated editing of the tumor genetic landscape, with elimination of cells presenting specific immunogenic mutations. It might be thus important to consider such mutations for the design of novel therapeutic approaches.

EACR25-0277**Small cell lung cancer evades immune surveillance through an ERBB2-dependent mechanism**

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Introduction

Small-cell lung cancer (SCLC) is characterized by systemic metastases and its high aggressiveness, therefore SCLC is frequently identified at an advanced stage, making treatment and prognosis extremely difficult. Chemotherapy, radiotherapy, and immunotherapy are the current treatment options for SCLC but research on targeted treatment options is still proceeding. Immune checkpoint blockage targeting programmed cell death-1 (PD-1) or programmed death-ligand 1 (PD-L1) plus chemotherapy has shown promise in extending overall survival of SCLC patients. However, SCLC cells develop resistance and the capacity to evade immune surveillance.

Material and method

We examined molecular profiles of matched primary and metastatic SCLC samples obtained from patients and an autochthonous SCLC mouse model using phosphokinase arrays, proteomics and immunohistochemistry. We used CRISPR-Cas9 knock-outs to functionally confirm MHC-I and ERBB2-dependent effects in vivo and in co-culture assays. Lastly, we examined the therapeutic potential in mice by combining PD-1 and ERBB2-blockade and analyzed tumor cells and T cells extensively using flow cytometry, scRNA and TCR sequencing.

Result and discussion

MHC-I expression was decreased in matched SCLC metastases. We found that SCLC metastases enhanced ERBB2 signaling, which suppressed MHC-I expression by a STING-dependent mechanism in SCLC cells and stimulated immune modulating gene expression. Combining ERBB2 inhibition with anti-PD-1 immune checkpoint blockade in the autochthonous SCLC mouse model resulted in an improved survival rate by stimulating an anti-tumor T-cell response and clonal expansion.

Conclusion

Our results address the unmet need to identify targetable mechanisms that govern tumor immune evasion in SCLC and clearly show that combined inhibition of ERBB2 and PD-1 represents a valid therapeutic approach to enhance outcomes for SCLC patients.

EACR25-0299

The profile of gut microbiota in carcinogenesis driven by mutant EGFR in non-small cell lung cancer

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Introduction

Accumulating evidence has clarified that gut dysbiosis is involved in lung cancer development and progression. Although the relationship between tumors and gut microbiota has been extensively studied using clinical samples, no studies have examined the association between mutant EGFR-induced lung carcinogenesis and dysbiosis in gut microbiota. Therefore, we investigated the gut microbiota profiles in stool samples from human lung-specific conditional EGFR-mutant transgenic mice during lung tumor carcinogenesis.

Material and method

Stool samples were collected before tamoxifen treatment (V1) and at each time point following mutant EGFR expression in lung tissue (V2) and lung tumor appearance (V3). Fecal 16S rRNA taxonomy was analyzed to assess microbial diversity, composition, and dynamic changes at each time point.

Result and discussion

We found that microbiota richness and diversity were significantly elevated when tumors developed and grew in the lung. Phylogenetic analysis of the microbial community revealed that Lachnospiraceae, Ruminococcaceae, Porphyromonadaceae, Rhodospirillaceae, Odoribacteraceae, and Desulfovibrionaceae showed a significant increase at the V3 stage compared to the V1 stage at the family level. In contrast, Lactobacillaceae, Bacteroidaceae, Muribaculaceae, Coriobacteriaceae, and Rikenellaceae significantly decreased at the V3 stage compared to the V1 stage. Furthermore, Lactobacillus species, also known as SCFA-producing bacteria, were relatively abundant at the V1 stage but were depleted with the occurrence of lung tumors at the V3 stage.

Conclusion

Changes in gut microbiota, such as Lactobacillus species, may be a predictive factor for the emergence and progression of tumors in an animal model of lung adenocarcinoma induced by mutant EGFR.

EACR25-0344**Tumor-associated *Staphylococcus* spp. and its potential role in breast cancer progression**

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Introduction

Recent research has unveiled a compositional change of bacteria residing in the mammary tissue in the presence of a growing tumor, suggesting their potential role in tumor progression. We recently demonstrated that oral antibiotic administration in 4T1 mammary tumor-bearing mice significantly reduced tumor growth and enhanced anti-tumor immunity by locally depleting *Staphylococcus epidermidis*. Herein, we investigated the presence of DNA from *Staphylococcus* genus (spp.) and its association with outcome in breast cancer (BC) patients, and analyzed the impact of *Staphylococcus* spp. on tumor-infiltrating immune populations.

Material and method

The presence of *Staphylococcus* spp. was investigated by RT-PCR on already available DNA from tumor tissues of a BC patient cohort (n=45), consecutively collected in 2010 at our Institute. The survival analysis was performed by stratifying patients into positive and negative groups according to bacterial presence, using Kaplan-Meier curves. Tumor-infiltrating immune cells were investigated via CIBERSORTx deconvolution analysis of tumor gene expression data. A standard curve in a 2-fold series was set up by qPCR on DNA extracted from 4T1 mammary tumor-isolated *S. epidermidis*. Conditioned medium from *S. epidermidis* isolated from murine mammary tumors was used to evaluate the induction of regulatory T cells (Tregs) from spleen-derived naive CD4+CD25- T cells and polarization of macrophage derived from bone marrow cells of healthy mice by flow cytometry and RT-PCR.

Result and discussion

The presence of *Staphylococcus* spp. in BC specimens was associated with significantly worse relapse-free survival ($p=0.04$, log-rank test). *Staphylococcus*-positive tumors exhibited higher T reg infiltration and increased M1/M2 macrophage ratio. To correlate *Staphylococcus* DNA quantity to patients' outcomes in a second independent BC patient cohort, we set up a q-PCR and analyses are in progress. Conditioned medium of *S. epidermidis* culture induced FoxP3+ T cells differentiation and promoted a mixed macrophage phenotype, characterized by upregulated IL12b, IL1b, IL10 and IL6 expression. These results are indicative of an association between the bacterium and the enhanced immunosuppressive tumor microenvironment. To validate these observations, a study on mice injected into the mammary fat pad with 4T1 tumor cells is ongoing to

determine whether there is a causal link between the progressive accumulation of *Staphylococcus* spp. during mammary tumor growth, the increased Treg infiltration and the acquisition of a pro-tumor phenotype by tumor-associated macrophages.

Conclusion

Our findings suggest that breast cancer-associated *Staphylococcus* may contribute to an immunosuppressive tumor microenvironment by promoting Treg expansion and macrophage polarization. Further investigation is needed to fully evaluate the potential of *Staphylococcus* as a biomarker for predicting patient outcomes.

EACR25-0399**PTP1B inhibition in tumour cell-conditioned macrophages promotes breast cancer by modulating tumour-promoting genes**

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Introduction

In breast cancer, tumour associated macrophages (TAMs) are the most abundant immune cells, accounting up to 50% of the tumour mass. Due to their highly immunosuppressive phenotype, TAMs are the major promoters of tumour growth and high infiltration is linked to resistance to immune checkpoint inhibitors and chemotherapy. This study aims to determine whether TAMs are re-programmed to adopt an anti-tumour phenotype using a PTP1B inhibitor, which has already been shown to reduce breast cancer progression through its effects on cancer cells.

Material and method

Human monocyte-derived macrophages (hMDM) were conditioned to TAM-like cells by co-culture with T47D (ER+, PR+) and triple negative breast (TNBC) cancer cell line MDA-MB-231. Effects of PTP1B inhibition of T47D and MDA-MB-231 conditioned TAMs on mammosphere growth were examined by area and viability measurements. Mechanisms by which cancer cell conditioning and PTP1B inhibitor treatment affect macrophage phenotype and mammosphere growth were assessed by gene expression arrays.

Result and discussion

Tumour cell-conditioned macrophages significantly decreased T47D mammosphere area by $19 \pm 6.4\%$ but did not affect MDA-MB-231 mammosphere growth. In contrast, PTP1B inhibitor treatment of tumour cell polarised macrophages enhanced the growth of both T47D and MDA-MB-231 mammospheres by $80 \pm 13.5\%$ and $8 \pm 4.1\%$ respectively. Gene expression arrays revealed T47D mammospheres, generated following co-culture with TAMs downregulated the expression of distinct cancer promoting genes including TGF β , HIF1 α and vimentin. MDA-MB-231 mammosphere generated following co-culture with TAMs downregulated expression of the same cancer promoting genes in addition to Snail2 and STAT5A. PTP1B inhibitor treatment of TAMs upregulated expression of these

cancer promoting genes in both T47D and MDA-MB-231 mammospheres formed post co-culture. PTP1B inhibitor treatment of tumour polarised hMDM enhanced expression of genes including PPAR- γ and IL-10 that result in tumour promoting macrophages which consequently contributed to the increased mammosphere growth.

Conclusion

Therapeutics based on PTP1B inhibition may lose efficacy for treatment of those breast cancers with high infiltration of macrophages (e.g. TNBC) by polarizing macrophages to a more tumorigenic phenotype which drives breast cancer progression.

EACR25-0418

Distinct bladder tumor microbiome profiles between responders and non-responders to intravesical Bacillus Calmette-Guérin therapy in high-risk non-muscle invasive bladder cancer

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Introduction

Intravesical Bacillus Calmette-Guérin (BCG) therapy is a cornerstone treatment for high-risk non-muscle invasive bladder cancer (HR-NMIBC). BCG functions by stimulating the host's immune system. Increasing evidence suggests that bladder microbiome interacts with the host immune system and may impact the efficacy of BCG therapy. We compared the bladder tumor microbiome profiles between responders and non-responders to BCG therapy.

Material and method

We used taxonomic classification tools, Kraken2 and Bracken, to generate a microbiome counts matrix from RNA-sequence data of HR-NMIBC tissue samples naïve to BCG therapy, obtained from the Gene Expression Omnibus series accession number GSE176178. We conducted alpha and beta diversity analyses on responders and non-responders, followed by differential analysis using ANOVA-Like Differential Expression tool (ALDEx2).

Result and discussion

In the study of bladder tumor microbiome profiles in HR-NMIBC naïve to BCG therapy, significant differences were found between responders and non-responders in microbial diversity and composition. In responders, alpha diversity indices, including the Chao1 and Shannon scores, which measure microbiota richness and evenness, were significantly lower than in non-responders ($p = 0.035$ and 0.038 , respectively), indicating reduced microbiota richness and evenness. For beta diversity, multi-dimensional scaling revealed distinct microbiome compositions between the two groups ($p = 0.001$). Stacked bar charts highlighted the differences in the top

12 genera and 19 species for each group, showing the variations in relative abundance of the microbiome at the genera and species levels between responders and non-responders. Differential analysis using ALDEx2 identified 10 species predominant in either group: *Curtobacterium flaccumfaciens*, *Bacillus velezensis*, *Bacillus cereus*, and *Plasmodium yoelii* in responders, versus *Actinoplanes* sp. OR16, *Neurospora crassa*, *Ascochyta rabiei*, *Malassezia restricta*, *Purpureocillium takamizusanense*, and *Streptomyces tanashiensis* in non-responders.

Conclusion

Our findings revealed significant differences in the bladder tumor microbiome composition between BCG responders and non-responders in the HR-NMIBC. While microbiota richness and evenness were significantly lower in BCG responders compared to non-responders, certain species were still more predominant in responders. These results suggest that the bladder tumor microbiome may influence treatment outcomes, underscoring its potential as a predictive biomarker for BCG therapy.

EACR25-0441

Starch degrading bacteria and prebiotic diet synergistically promote anti-tumor immune microenvironment and response to Immune Checkpoint Blockade

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Introduction

Emerging evidence indicates that the gut microbiota plays a critical role in shaping antitumor immune responses, offering an innovative avenue for enhancing Immune Checkpoint Blockade (ICB) efficacy. Recent multi-omics profiling of a large cohort of patients with primary colon cancer revealed that a specific consortium of bacteria known to degrade resistant starch was associated with improved survival and a tumor-suppressive microenvironment (Roelands et al. Nat. Medicine 2023). Here, we validated the immunomodulatory effects of these bacteria, specifically *Ruminococcus bromii*, and dietary interventions with castalagin, a microbiota-modulating compound, in shaping tumor microenvironment and response to ICB.

Material and method

To assess the impact of gut microbiota and microbial interventions on tumor immune response, C57BL/6 mice

received a five-day antibiotic treatment (Day -19 to -14) via oral gavage to deplete microbiota, followed by a washout period. They underwent fecal microbiota transplantation (FMT) or received a vehicle control, either with or without *R. bromii* colonization. On Day 0, MC38 tumor cells were injected, and received anti-PD1/CTLA4 therapy. A subset received daily castalagin gavage. Fecal samples were collected at multiple time points. Mice were sacrificed on Day 17. Gene expression and 16S profiling were performed for four tumors per treatment group ($n = 20$).

Result and discussion

Four treatment regimens were considered: aPD1/aCTLA4 (ICB), ICB+castalagin, ICB+*R. bromii*, ICB+*R. bromii* and castalagin-enriched diet. Gene expression profiling and immunohistochemistry showed that microbiota remodeling enhances the immune response to ICB by increasing CD8+ T-cell infiltration and up-regulation of immune-related genes. We observed a higher estimated proportion of CD103+ dendritic cells and a reduction of the immuno-suppressive myeloid compartment. Supplementation with *R. bromii* alongside ICB significantly reduced tumor growth compared to checkpoint blockade alone. The combination of ICB, *R. bromii*, and castalagin diet leads to the most substantial suppression of tumor growth.

Conclusion

Our findings suggest a synergistic effect between microbiota modulation and immune checkpoint blockade, and that gut bacteria's degradation of resistant starch induces beneficial metabolic alterations that activate anti-tumor immune responses.

EACR25-0462

Prognostic Significance of NK-T Cell Differentiation in Oropharyngeal Squamous Cell Carcinoma: A Transcriptomic Analysis

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Introduction

Checkpoint inhibitors have recently been introduced for the treatment of advanced oropharyngeal squamous cell carcinoma (OPSCC), with treatment decisions guided by the combined score of PD-L1 expression in cancer cells and the tumour immune microenvironment (TIM). However, a transcriptome compared with other parameters of OPSCC could provide additional criteria and indicate novel therapeutic targets. This study aimed to compare the transcriptome of OPSCC with clinicopathological findings and signalling pathways of TIM cells.

Material and method

Clinicopathological data and OPSCC transcriptome of 81 patients were collected from cBioPortal and compared with single-sample Gene Set Enrichment Analysis (ssGSEA), using the GenePattern online platform.

Survival analysis was performed for each selected signalling pathway connected to B cells, T cells, neutrophils, NK cells and NK-T cell activation, differentiation, and proliferation. Statistically significant pathways were associated with clinicopathological data. Statistical significance was set at $p < 0.05$.

Result and discussion

Patients with a higher enrichment score for NK-T cell differentiation had better survival outcomes than those with a lower enrichment score, with a median of survival 5.62 years compared to 4.68 years ($p = 0.015$). NK-T cell differentiation enrichment was negatively correlated with alcohol consumption ($p = 0.0202$), and positively correlated with clinical stage ($p = 0.011$) and tumour grade ($p = 0.0079$). Further investigation of this pathway highlighted three key genes: ATF2, ITK, and ZNF683. Higher Z scores for these genes were associated with improved survival, with ITK showing the strongest association.

Conclusion

Our findings suggest that NK-T cell differentiation might have a significant role in OPSCC prognosis, with higher enrichment scores and gene expression being associated with improved overall survival. Further studies are needed to explore the role of NK-T cell function in OPSCC progression and to evaluate its potential as a potential therapeutic target.

EACR25-0542

Molecular and immune landscape of recurrent and/or distant metastatic squamous cell carcinoma of the head and neck: an IMMUCAN project

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Introduction

Squamous cell carcinoma of the head and neck (SCCHN) arises from the epithelium of the oral cavity, oropharynx, hypopharynx and larynx. Most patients present with locally advanced disease, but less than 60% of the patients remain free of cancer at 3 years. Recurrent and/or metastatic (R/M) SCCHN has poor prognosis, with a median overall survival of 10–17 months. While large-scale multimodal studies have characterized primary treatment-naïve SCCHN, the molecular and immune landscape of R/M SCCHN remains under-explored.

Material and method

We analyzed 253 R/M SCCHN patients (57% with at least 2 lines of treatment in the R/M setting prior to sampling) using the IMMUCAN multimodal-omics workflow, which integrates molecular and immune profiling. More specifically, we used Whole Exome Sequencing (WES), bulk RNA sequencing (RNAseq), multiplexed immunofluorescence (IF) and expression of tumor markers by Imaging Mass Cytometry. We extracted features from these modalities, encompassing mutations and copy number alterations (WES); deconvolution results, pathway activation scores and unsupervised gene modules (RNAseq), and cell proportions in the tumor and stroma compartments (IF).

Result and discussion

Our findings indicate that HPV status, primary tumor site, substance abuse, relapse pattern, and systemic treatments significantly influence tumor biology. Specific genomic alterations were observed in laryngeal cancer and non-smokers/non-drinkers. HPV-positive tumors retained a strong genomic and transcriptomic identity, but showed no difference in immune infiltrate, immune activity or prognostic benefit compared to HPV-negative tumors. 11q13.3 amplification was more frequent in HPV-negative metastatic tumors and hypopharyngeal cancer, and the genes in this region exhibited two different patterns of expression. HPV-negative SCCHN with locoregional recurrence showed elevated EGFR and CXCL12 pathway activity, and EGFR activity correlated to EGFR ligand expression but not EGFR copy number. Systemic therapy led to a decrease in immune features, such as lymphocyte infiltration (IF) and interferon gamma activity (RNAseq). TGF β pathway activation was specifically linked to patients prior to any R/M treatment line. High expression of genes involved in cell migration and adhesion correlated with poor survival, while B and CD8+ T cell infiltration predicted improved survival. Furthermore, prognostic factors were dependent on the specific R/M SCCHN pattern, with important dichotomies observed between distant metastatic and locoregional prognostic features.

Conclusion

This study highlights the heterogeneity of R/M SCCHN, provides deeper characterization of tumor biology at this stage and finds potential biomarkers for novel therapies.

EACR25-0561

Genome-wide CRISPR screens identify N-glycosylation as regulator of T cell:tumor cell interactions

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Introduction

The efficacy of immunotherapy fundamentally depends on effective T cell:tumor cell interactions. However, the molecular determinants orchestrating this engagement remain incompletely understood. Understanding the regulatory mechanisms of these interactions is crucial for optimising T cell-mediated cytotoxicity in cancer treatment.

Material and method

To systematically map and perturb key regulators of the functional T cell:tumor cell interactome, we employed

two innovative cell:cell interaction assays. Using these assays, we conducted genome-wide CRISPR screens to identify genes critically required for both physical and functional interactions between T cells and tumor cells. Additionally, we utilised TurboID proximity labeling and T cell-tumor cell conjugates to investigate the molecular landscape influencing these interactions.

Result and discussion

Our study identified the complex N-glycan biosynthesis pathway as a crucial regulator of T cell:tumor cell interactions. Given the known role of N-glycans in protein trafficking and surface retention, we further explored their impact using cell surface proteomics and TurboID-based interactome profiling. These analyses revealed increased expression of cell adhesion and co-stimulatory molecules in complex N-glycan-deficient T cells. Functionally, T cells with perturbed N-glycans exhibited a shift towards an effector memory phenotype, characterized by elevated granzyme and integrin expression. Moreover, both genetic and pharmacological interventions reducing complex N-glycans enhanced intra-tumoral infiltration of T cells *in vivo*, supporting their role in modulating T cell effectiveness in tumor environments.

Conclusion

This study provides a comprehensive functional atlas of molecules modulating T cell:tumor cell interactions. Our findings highlight the complex N-glycan biosynthesis pathway as a key regulatory mechanism and a potential therapeutic target to enhance T cell-mediated cytotoxicity in cancer immunotherapy.

EACR25-0571

Bacteroides Ovatus Is Associated with Clinical Response to Cancer

Immunotherapy in Renal Cell Carcinoma

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Introduction

Renal cell carcinoma (RCC) is an immunogenic malignancy responsive to immunotherapy, but durable responses and predictive biomarkers remain insufficient. The gut microbiota influences cancer progression and immunotherapy efficacy, yet its role in RCC is unclear. This study explores microbiome-based insights to enhance treatment prediction and overcome resistance in advanced RCC (aRCC) by profiling gut microbiota after immunotherapy, analyzing its correlation with outcomes, and identifying key microbes influencing treatment response.

Material and method

Study Cohort We conducted a baseline follow-up of 28 aRCC patients receiving immunotherapy combined with targeted therapy and collected their fecal samples. Treatment response was assessed at 12 months using iRECIST. Tumor Model SPF mice pretreated with antibiotics received a cocktail (metronidazole, vancomycin, ampicillin, neomycin) in drinking water for one

week. Renca cells were subcutaneously injected into BALB/c mice, followed by intraperitoneal PD-1 blockade or isotype control twice weekly. For targeted therapy experiments, axitinib or sunitinib was administered daily. *Bacteroides ovatus* (B.o, ATCC-8384) was orally administered (1×10^8 CFU) twice weekly.

Result and discussion

We categorized 28 patients receiving immunotherapy combined with targeted therapy into responders (n=20) and non-responders (n=8) based on iRECIST criteria, and analyzed their post-treatment fecal metagenomes. LEfSe identified B.o as enriched in responders. Cox regression revealed a significant negative correlation between high B.o abundance and post-treatment progression; patients with higher B.o abundance had improved PFS. Oral B.o administration enhanced anti-PD-1 mAb efficacy in tumor-bearing mice, an effect lost upon B.o clearance with metronidazole. However, B.o failed to improve targeted therapy efficacy. Only live B.o and conditioned media treatments showed comparable immunotherapeutic efficacy. Untargeted LC-MS/MS identified arginine as a key B.o-derived metabolite influencing immunotherapy response, further validated by colorimetric assay and HPLC. Previous studies suggest that B.o enhances immunotherapy by increasing arginine levels and CD8+ T cell activity. Flow cytometry of tumor tissues from PD-1 mAb-treated mice confirmed this, showing higher CD8+ T cell numbers and increased GZMB and TNF α expression in the B.o-treated group.

Conclusion

Our study reveals a strong correlation between increased gut B.o and enhanced immunotherapy response in aRCC. B.o promotes progression-free survival by modulating immune responses through arginine secretion. Mouse models confirm its role in augmenting PD-1 blockade efficacy, with elevated arginine levels enhancing CD8+ T cell function. These findings highlight B.o and its metabolites as potential adjuncts to improve immunotherapy outcomes in aRCC, supporting their clinical application.

EACR25-0603

Expression analysis and functional characterization of CD25 on conventional dendritic cells in liver cancer

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Introduction

Hepatocellular carcinoma (HCC) is the fourth leading cause of cancer-related mortality worldwide. Despite recent advancements, treatment approaches including those based on immune checkpoint blockade (ICB) therapy remains largely ineffective in HCC patients.

Given the need for alternative or complementary strategies, we propose that enhancing conventional dendritic cell (cDC) function could potentiate anti-tumor immunity. However, cDCs are often dysfunctional in cancer, leading to impaired immune recognition and response. Therefore, restoring cDC function is a promising strategy to improve anti-tumor immunity. Evidence from our lab reveals that activated/mature cDCs (mDCs) within tumor tissue exhibit elevated expression of IL2ra (CD25), the α -subunit of the IL-2 receptor, but lack the signaling component IL2rb. The role of CD25 in mDC-mediated anti-tumor responses, however, remains unexplored. Therefore, our study aims to define the contribution of CD25 to cDC effector functions in HCC.

Material and method

Hypotheses regarding CD25 contribution in cDC effector functions have been tested using either subcutaneous or orthotopic injection of syngeneic the liver cancer cell line HepG2. To analyze CD25 function in primary liver cancer, mice have been challenged with chemical-induced carcinogenesis through diethyl-N-nitrosamine (DEN) treatment. We have recently generated a Clec9acexII2rafl/fl mouse model to specifically target CD25 in cDCs and assess its role in liver cancer progression and response to ICB. A bone marrow-derived cDC (BMDC) in vitro differentiation protocol enabled exploration of mDC crosstalk with T cells and NK cells, as well as IL-2 trans-presentation.

Result and discussion

In our chemically induced HCC model, we observed a significant reduction in CD25 expression on cDCs during tumor progression, accompanied by impaired NK cell infiltration. Similarly, in our Clec9acexII2rafl/fl mouse model, we found decreased infiltration of cytotoxic CD11b $^{+}$ NK cells and activated T cells, along with a delayed rejection of highly immunogenic tumors. Furthermore, bone marrow-derived cDCs from Clec9acexII2rafl/fl mice exhibited defective maturation/activation, characterized by reduced expression of key cDC markers, including CD40, CD86, MHC-II, and PD-L1. CD25-deficient cDCs also displayed impaired antigen presentation to both CD8 $^{+}$ and CD4 $^{+}$ T cells, leading to diminished activation and proliferation. In contrast with previous evidence that describes CD25 as an immune regulatory molecule, our results support CD25 anti-tumor function in cDCs, suggesting novel therapeutic opportunities for HCC patients.

Conclusion

Collectively, our findings suggest that CD25 is crucial for cDC effector functions and the coordination of anti-tumor immunity, particularly by influencing NK and T cell responses in liver cancer.

EACR25-0636

Immune landscape of a SHH medulloblastoma mouse model: a preclinical platform for Immuno-Oncology studies

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Introduction

Medulloblastoma (MB) is the most common malignant brain tumor in children, with approximately 650 new cases being diagnosed annually in the European Union. It is classified by the World Health Organization as a grade 4 malignancy and divided into four molecular subgroups: WNT, SHH, Group 3, and Group 4. Current standard treatment causes severe and long-lasting side effects, which makes urgent the need for novel therapeutic strategies, such as immunotherapy. Our group previously characterized the immune profile of MB, identifying low expression of CD274 and CTLA4, and identified elevated expression new putative immune targets CD24 and CD276, in a large cohort of patients. Nonetheless, there is a lack of standardized preclinical models for immuno-oncology research, and the tumor microenvironment (TME) of existing models remains poorly understood. To address this gap, we characterized the immune profile of the Math1-cre:Ptch1lox/lox mouse model of SHH-MB.

Material and method

RNA was isolated from FFPE samples of normal cerebellum ($n=9$) and tumors ($n=14$) from mice of different ages (P21, P42, and P56). The expression of 561 immune-related genes was analyzed using the NanoString® Immunology Panel, and candidate genes were further validated by RT-qPCR. Additionally, a multiplex cytokine/chemokine array, assessing 111 analytes, was performed using protein extracted from fresh tissue of normal cerebellum ($n=4$) and MB tumors ($n=4$) from mice aged P42.

Result and discussion

Transcriptomic analysis identified 329 differentially expressed genes between normal cerebellum and MB. Consistent with human MB data, Cd24a, Cd276, Cxcl1, Cxcr4, and Tgfb2 were significantly upregulated in tumor samples. Notably, Cd24a and Cxcr4 were the most differentially upregulated genes in the tumor samples. Immune checkpoint analysis revealed low expression of Pdcd1, Cd274, and Ctla4, suggesting an immuno-suppressive TME. Immune cell infiltration estimates showed reduced T cells, CD8+ T cells, and B cells in MB, with myeloid cells being the predominant immune population, mirroring the immune landscape of human MB. Furthermore, publicly available single-cell RNA-seq data analysis showed that Cd24 expression was restricted to tumor cells in both human MB and a comparable SHH-MB mouse model. Proteomic analysis via multiplex array revealed decreased levels of TF, FGF1, CX3CL1, and AHSG, while CHI3L1, ICAM-1, IGFBP-2, LYX, and MPO were upregulated in MB. Functional enrichment analysis linked these proteins to pathways involved in the acute inflammatory response, regulation of AKT signaling, and ERK1/2 cascade modulation.

Conclusion

In conclusion, the immune profile of our SHH-MB mouse model mirrors the immune landscape of human MB, further supporting CD24 as a potential immunotherapeutic target. This preclinical model provides a valuable platform for advancing immuno-oncology research and the development of novel immunotherapies for MB.

EACR25-0712

Oncogenic MYC depletes amino acids from the tumor microenvironment to foster immune evasion in PDAC

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Introduction

Treatment of pancreatic ductal adenocarcinoma (PDAC) has seen only marginal improvements over recent decades, resulting in a dismal 5-year survival rate of merely 11%. A significant obstacle in tumor therapy is the insufficient infiltration with cytotoxic lymphocytes. PDAC stands as a paradigm example of a human tumor characterized by dysregulated expression of the MYC oncogene. Genetic depletion of MYC in an orthotopic transplant model of PDAC induces T cell-mediated tumor regression (Krenz et al., 2021, Gaballa et al., 2024). While the intracellular functions of MYC proteins are well-characterized, the molecular intricacies of MYC's non-cell-autonomous role in promoting immune evasion remain to be elucidated.

Material and method

To unravel the underlying interaction networks within the tumor ecosystem, we employed a multi-faceted approach to investigate orthotopically transplanted murine PDAC tumors after depletion of MYC. Our methodology encompassed bulk RNA sequencing, multi-color flow cytometry and immunofluorescence, single-cell sequencing, mass spectrometry, and spatial transcriptomics.

Result and discussion

Single-cell sequencing of murine PDAC tumors unveiled that infiltrating T cells exhibit attenuated activation, particularly in translation and rRNA biogenesis pathways, which is reversed following MYC depletion. These tumor ecosystems are characterized by severe amino acid scarcity. This is alleviated upon MYC depletion, since MYC depletion curtails the expression of metabolic enzymes and solute carriers in PDAC tumor cells both in vivo and in vitro. In culture experiments mimicking these metabolic conditions demonstrate that increasing amino

acid availability promotes activation of the PI3K/AKT/mTOR pathway and upregulates granzyme expression in effector T cells. Notably, selective inhibition of amino acid uptake by tumor cells *in vivo* elevates their levels in the interstitial fluid, reinvigorating immune surveillance. This triggers rapid and sustained regression of PDAC tumors in our aggressive orthotopic transplant model. The MYC oncogene drives cell-autonomous growth of tumor cells with heightened nutrient demands, particularly amino acids. This creates a nutrient-depleted environment that renders T cells in PDAC ecosystems dysfunctional. Corroborating these findings, retrospective analysis of human single-cell sequencing data reveals that the T cell signature from our nutrient-replete PDAC ecosystems bears closer resemblance to T cells in healthy tissue than those within the tumor ecosystem.

Conclusion

Our findings highlight the potential of selectively targeting tumor cell metabolism as a promising therapeutic strategy for pancreatic ductal adenocarcinoma (PDAC). Moreover, they also emphasize the critical role of amino acid uptake by lymphocytes, underscoring the need for drugs that can more selectively deplete nutrients in tumor cells while sparing immune cells.

EACR25-0722

Impact of smoking status on microbiome diversity and taxonomic shifts in bladder cancer

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Introduction

Smoking, as a key risk factor for bladder cancer, causes tobacco-derived carcinogens to accumulate in the bladder via urine. Emerging research suggests a significant role of the microbiome in bladder cancer, with distinct microbial profiles identified in cancer tissues. While smoking is known to alter microbiomes in the gut and other tissues, its effects on the microbiome of bladder cancer remain underexplored. This study investigates the influence of smoking status on the composition, diversity, and distribution of specific bacterial taxa within bladder cancer tissues.

Material and method

RNA expression profiles and associated clinicopathological data of bladder cancer samples were obtained from the TCGA database, a publicly available cancer genomics resource [1]. Samples from 399 bladder cancer patients were included in this study. We calculated Chao1 and Shannon indices to assess richness and evenness, respectively. Beta diversity was analyzed using multidimensional scaling (MDS), with p-values determined via the Adonis test. Differential abundance (DA) analysis was conducted using ANOVA-Like Differential Expression tool (ALDEEx2).

Result and discussion

Analysis of alpha diversity metrics, including the Chao1 and Shannon indices, revealed no statistically significant

differences in species richness or evenness among current smokers, former smokers, and non-smokers (all $p > 0.05$). Similarly, beta diversity analysis using MDS showed no significant clustering or compositional differences between these groups (all $p > 0.05$), suggesting that smoking history does not markedly influence the overall microbiome diversity in bladder cancer tissues. However, taxonomic-level analyses revealed notable patterns. At the phylum level, variations in the relative abundance of Firmicutes, Bacteroidetes, Actinobacteria, and Proteobacteria were observed, with a significantly higher abundance of Proteobacteria in current smokers compared to non-smokers and former smokers, particularly those with a cessation period of over 15 years. At the class, order, family, and genus levels, distinct shifts were identified, including a reduced abundance of Bacilli, Lactobacillales, Lactobacillaceae, and Lactobacillus in current smokers. In contrast, Proteobacteria-related taxa such as Enterobacteriaceae and Pseudomonas were more prevalent in current smokers, indicating smoking-induced alterations in specific microbial taxa within bladder cancer tissues.

Conclusion

These findings suggest that while smoking status does not significantly impact overall microbiome diversity in bladder cancer tissues, it induces specific taxonomic shifts, particularly affecting Lactobacillales and Proteobacteria. These microbiome alterations may contribute to the understanding of microbiome-mediated mechanisms underlying smoking-related bladder cancer development.

[1] URL: portal.gdc.cancer.gov

EACR25-0728

Cancer vaccines target to a disintegrin and metalloprotease domain (ADAM) could induce anti-tumor immunity

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Introduction

Immune modulation in cancer is a critical strategy aimed at enhancing the immune system to combat tumors and reduce metastasis. One promising approach is immune checkpoint blockade, particularly focusing on the activation of CD8+ T cells. Our research investigates the molecular mechanisms of the metastatic-associated gene, a disintegrin and metalloprotease domain 9 (ADAM9), in regulating the immune response within the tumor microenvironment and its role in cancer progression. ADAM9 overexpression is linked to poor outcomes in cancer patients, specifically in lung and breast cancers.

Material and method

Through RNA-seq analysis comparing control and ADAM9 knockout (KO) lung tumors, we identified genes influenced by ADAM9 that contribute to immune suppression. ADAM9 appears to impact immune cells by modulating cytokine production, interferon response, and lymphocyte activation, thereby influencing the tumor microenvironment and facilitating tumor growth and

metastasis. Additionally, we generated recombinant ADAM9 to assess its therapeutic potential.

Result and discussion

Our findings showed that knocking out ADAM9 in lung cancer cells alters cytokine profiles, reduces neutrophil infiltration, increases CD8+ T cell presence, and enhances the interferon-gamma (IFN- γ) pathway activity. These changes may explain the smaller tumor sizes and decreased metastasis observed in syngeneic mouse tumor models. Remarkably, the recombinant protein ADAM9 with adjuvants completely inhibited tumor growth in our models, highlighting ADAM9's significance in tumor progression and its potential as a candidate for cancer vaccine development.

Conclusion

In summary, our research underscores the critical role of ADAM9 in shaping the immune landscape of tumors. Targeting ADAM9 may provide new therapeutic approaches, enhancing the effectiveness of existing cancer treatments.

EACR25-0743

POSTER IN THE SPOTLIGHT

COX-2/PGE2 Activity in Cancer Cells: A Key Driver of Metastasis via Immune Escape

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Introduction

Metastasis, the leading cause of cancer-related deaths, remains a significant clinical challenge due to the lack of effective therapies. A critical question that remains unanswered is why some tumours evade immune control and metastasise, while others do not. Our previous work demonstrated that COX-2/PGE2 activity in cancer cells promotes progressive tumour growth through immune escape in preclinical models of primary tumours.

Building on this, we explored the role of this inflammatory pathway in regulating the immune response to metastasis.

Material and method

We evaluated the metastatic potential of breast, pancreatic, and renal cancer cells following orthotopic transplantation or intravenous injection into immune-competent wild-type mice or genetically engineered to lack specific immune cells or inflammatory signalling pathways. We used bioluminescent cancer cells to explore the steps of the metastatic cascade influenced by cancer cell-COX2/PGE2 activity. Furthermore, we conducted antibody-mediated depletion of specific immune cells to assess their functional contribution to metastasis.

Result and discussion

Using loss- and gain-of-function approaches, we show that cancer cell COX-2 expression is both necessary and sufficient to drive metastatic dissemination across

multiple cancer models. Mechanistically, we found that PGE2 supports metastatic colonisation by enabling cancer cells to escape NK and T cell recognition.

Interestingly, in contrast to primary tumours, we provide evidence that neutrophils play a central role in facilitating metastasis by COX-2-expressing cells. This highlights the distinct immune microenvironmental interactions between primary and metastatic tumours.

Conclusion

Our findings establish PGE2 production by cancer cells as a critical mediator of neutrophil-driven pro-tumorigenic activity and immune escape. In addition, they suggest that targeting the COX-2/PGE2 axis could offer a therapeutic strategy to enhance the efficacy of immunotherapies for patients with metastatic cancer.

EACR25-0782

Observational study comparing sequential boost versus SIB

chemoradiotherapy induced toxicity and its association with oral candidiasis in Head and Neck Cancer Patient

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Introduction

Radiotherapy for LAHNC has changed considerably over the past two decades with the advent of conformal radiotherapy techniques. Whereas these techniques differ in treatment related outcomes including survival, however the toxicity remains unanswered. Our objective of this study was to compare the sequential versus SIB chemoradiotherapy induced toxicities and their association with the oral candidiasis in HNC patients.

Material and method

A single institutional prospective observational data on histologically proven squamous cell LAHNC treatment with definitive chemoradiation 66-70 Gy was done. Treatment was delivered via sequential boost using IMRT/VMAT/IGRT. Sequential Arm was delivered in 3 Phases, Phase I- 46-50 Gy, Phase II- 10-14 Gy, and Phase III- 10 Gy, while SIB Arm with 66 Gy (High risk), 60 Gy (Intermediate risk), and 54 Gy (Low risk) was given. Both arms received concurrent chemotherapy with weekly injection of Cisplatin 40 mg/m². Toxicity was graded weekly during treatment and a three monthly follow-ups.

Result and discussion

With both the Arms of our study, the oropharynx was the most common subtype followed by the larynx and hypopharynx. Most of the patients had good performance score with ECOG 0-1 and 70-75% of patient were locally advanced with the stage IVA and stage IVB. More than 70-75% of the patient consumed tobacco/ tobacco related products and 70-75% of patients received chemotherapy in both sequential and SIB Arms. In terms of toxicity,

there were no treatment-related deaths in either cohort. Grade III dermatitis occurred in 40% vs 50% of patients receiving sequential vs SIB chemoradiotherapy respectively while Grade IV dermatitis was observed in 2% vs 20% of the cases receiving sequential boost vs SIB treatments. Likewise, Grade III mucositis was observed in 60% vs 55% with sequential vs SIB treatments while Grade IV mucositis was found in 5% vs 25% of cases with sequential vs SIB treatments. Grade IV dysphagia occurred in 0% of cases with sequential treatment compared to 15% with SIB. These toxicity profiles suggest that the Grade III/IV dermatitis and dysphagia were higher in SIB Arm in comparison with the sequential boost arm. Grade III/IV mucositis associated with the oral candidiasis was more common in the SIB arm compared to the sequential arm. Among the Candida species, *Candida albicans* outnumbered the non-albicans *Candida*. Overall, we find that these techniques appear equivalent with respect to treatment outcomes though sequential boost using Rapid Arc/IMRT technique is associated with some improvement in rates of acute toxicity.

Conclusion

There was no difference in the disease related outcomes between the two treatment delivery approaches. A higher rates of acute toxicity with Grade III/IV RT dermatitis, mucositis, dysphagia and associated oral candidiasis were observed to be higher in SIB arm compared to sequential arm.

EACR25-0801

Reprogramming and therapeutic relevance of immunosuppressive cells in the process of prostate cancer metastasis

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Introduction

Prostate Cancer is the most cancer diagnosed worldwide being the second cancer-cause of death. Although 90% of prostate cancer have good prognosis, 20-10% of the prostate cancer cases are early diagnosed metastatic prostate cancer(mTNPC) which only 50% of these patients survive. Thus, it's important the understanding of the mTNPC immune landscape to develop new immunotherapies to improve the patient's survival rate.

Material and method

The Lab developed an animal model which mimics the aggressiveness and metastatic capacity of mTNPC. This animal model consists of the complete knock out of PTEN and LKB1 tumor suppressors generating tumors within 3-5 months.

Result and discussion

This genetic model showed high infiltration of MDSC cells and low lymphocytes infiltration, compared to different genetic mouse models with different degree of aggressiveness (PTENpc-/- LKB1pc+/-, PTENpc-/- LKB1pc+/-). The dissociation of the PTENpc-/- LKB1pc-/- tumors and reinjecting them in the ventral prostate of the C57bl/6 mice produced a fast-growing tumor, within 15-20 days, maintaining the same

metastatic capacity to lungs and lymph nodes. Furthermore, the reinjected tumors present the same immune cells infiltration of the genetic model. This reinjected model when treated with androgen deprivation therapy responded but expressed resistance at the end of the experiment. Also, immunotherapy against MDSCs, anti-GR1, seemed to reduce the metastasis and the infiltration of pro-tumoral immune cells. We generated a cell line from one of these reinjected tumors, the T051 cell line. This new line, having the genetic model genotype and AR expression, was injected in mice obtaining more aggressive tumors with a higher dissociation capacity to several organs than reinjected tumors. When treating mice-bearing T051 subcutaneous tumors with anti-PD1 as a single agent and in combination with Androgen Deprivation Therapy, tumor growth is partially controlled with an improvement in T-Cell response. Furthermore, the T051 tumors presents high infiltration of MDSCs as well as the genetic model. Thus, this model allows to test different therapeutic strategies to reduce MDSC functions and improve treatment response.

Conclusion

In conclusion, these new animal models and the cell line having an aggressive immune landscape, capacity to metastasize and responding to different therapies will help us to get a deeper knowledge of mTNPC.

EACR25-0829

The role of immune checkpoints interplay in modulating NK cell activity in ovarian cancer environments

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Introduction

Ovarian cancer (OC) is the most lethal gynaecological malignancy. To date, there are no screening methods or biomarkers to diagnose the disease at an early stage. NK cells play a pivotal role in antitumor immunity in OC. Their activity is modulated by activating and inhibitory signals, including the programmed death pathway (PD-1/PD-L1/PD-L2) and TIGIT/CD155/DNAM-1 axis. DNAM-1 acts as a co-stimulatory receptor. After binding to ligands such as CD155, it enhances NK cytotoxicity, while TIGIT has an inhibitory effect. Moreover, these

two pathways work synergistically, leading to immune evasion in the ovarian tumor microenvironment (TME). In this study, we compared the distribution of NK cells with DNAM-1, TIGIT, and PD-1 expression in different OC environments.

Material and method

The study group consisted of 34 patients with histopathologically confirmed OC. Mononuclear cells (MNCs) were isolated from three OC environments, i.e. peripheral blood (PB), peritoneal fluid (PF), and tumor tissue (TT), using density gradient centrifugation. The isolated MNCs were stained with specific monoclonal antibodies (anti-CD3, anti-CD16+56, anti-DNAM-1, anti-PD-1, anti-TIGIT) and analyzed by flow cytometry. The percentages of the NK cells in different environments were compared using the Wilcoxon paired test.

Result and discussion

The highest %NK cells was detected in PB in comparison to PF (median 14.88% vs. 4.90%; $p < 0.001$), and TT (median 14.88% vs. 3.06%). We observed the highest %NK cells with TIGIT expression in PF in comparison to PB (median 90.15% vs. 75.86%; $p < 0.01$) and TT (median 90.15% vs. 64.19%). Similarly, we found the highest percentage of NK cells with DNAM-1 expression in PF in comparison to PB (median 69.76% vs. 60.86%) and TT (median 69.76% vs. 48.13%); however, the differences did not reach the statistical significance ($p > 0.05$). We also found higher percentage of PD-1 positive NK cells in TT than PB (median 41.01% vs. 21.43%; $p < 0.05$) and PF (median 41.01% vs. 26.44%). The accumulation of NK cells with TIGIT expression in PF may create an immunosuppressive microenvironment, facilitating immune evasion and metastatic spread. The accumulation of PD-1 positive NK cells among OC infiltrating cells suggests that NK cells may be chronically exhausted within TME. The highest percentage of TIGIT or DNAM-1 positive NK cells in PF may indicate that NK cells' activity is dependent on the availability of the ligands in the environments. Moreover, TIGIT has a higher affinity to CD155 than DNAM-1, which promotes immunosuppression within TME.

Conclusion

The high accumulation of NK cells with TIGIT expression in PF indicates that PF is a strongly immunosuppressive environment. The high %NK cells with PD-1 expression among OC infiltrating cells may be related to NK cell exhaustion within the ovarian TME.

Financed by the National Science Center, Poland. Grant no. 2021/41/N/NZ6/01727.

EACR25-0866

Advancing treatment in high-grade serous ovarian cancer through targeting of RNA splicing

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Introduction

The RNA binding protein RBM39 regulates alternative splicing and is targeted by the small aryl sulphonamides E7070 (indisulam) and E7820. These molecules display strong anticancer activity by acting as a molecular glue between RBM39 and the E3 ubiquitin ligase DCAF15, leading to the polyubiquitination of RBM39 and

ultimately resulting in defects in RNA splicing. Ovarian high grade serous carcinoma (HGSC) is the most prevalent, as well as the most aggressive and lethal subtype of ovarian cancer. Current standard therapeutic options include cytoreductive surgery and chemotherapy, however, even patients who initially respond well to treatment are likely to develop recurrence. Ovarian HGSC tumours are highly heterogeneous lesions traditionally categorised as 'immune-cold' due to the low immune infiltrate observed. Given the challenges in treatment arising from the high heterogeneity and low immune infiltrate observed, as well as the frequent late-stage diagnosis of ovarian HGSC, the development of new treatment regimens is essential. Previous studies have shown that depletion of RBM39 via indisulam treatment gives rise to highly immunogenic neoepitopes capable of increasing the response to immunotherapy in murine models of neuroblastoma. Additionally, in house data has shown that RNA-splicing derived neopeptides are expressed in ovarian cancer models in vitro. Based on this data, I hypothesise that RBM39 depletion via molecular glue indisulam modulates the tumour immune microenvironment by generating neoepitopes capable of eliciting an immune response in ovarian HGSC models.

Material and method

The tumour immune microenvironment was profiled in vivo following indisulam treatment via flow cytometry and immunohistochemistry.

Result and discussion

These results show an increase in the level of specific immune cell populations including conventional dendritic cells or tissue resident macrophages. An increase in the levels of conventional dendritic cells (cDCs), a type of antigen-presenting cell, as well as macrophages, could suggest an increase in immune activation as well as tumour immunogenicity potentially due to the presence of novel immunogenic neoepitopes.

Conclusion

An increase in antigen-presenting cells (APCs) such as cDCs may reflect a rise in neoantigens available for identification, aligning with our hypothesis. Overall, these results could suggest a complex immuno-modulatory effect following RBM39 depletion, which could potentially benefit patients receiving immunotherapies.

EACR25-0888

An integrated organoid and stool-derived culture platform for investigating microbiota-driven metabolite effects in colorectal cancer

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Introduction

Colorectal cancer (CRC) progression is influenced by the gut microbiome via direct bacteria–host interactions, secretion of metabolites/toxins, and immune cell recruitment that drives pro-inflammatory signalling. Yet, the impact of microbiome-derived metabolites on tumour progression remains poorly understood. Previous studies examined single bacterial or metabolite species, overlooking the complexity of community dynamics. To address this, we developed and validated a novel platform to assess community-culture derived bacterial metabolite supernatants on CRC adenoma and carcinoma organoid models.

Material and method

Matched caecal content from tumour-bearing mice with mutations in *Apcfl/fl*, *BrafV637E*, *KrasG12D* and *Pik3caH1047R* was collected at humane endpoint for whole metagenome sequencing and targeted and untargeted metabolomics. Linear discriminant analysis of the metagenomic dataset identified genotype-specific bacterial species signatures with differential abundance relative to controls. Stool-derived *in vitro* communities (SICs) were also established from the caecal content of tumour-bearing and wild-type mice, with their species composition validated by 16S-rRNA analysis. Finally, the bacterial metabolite supernatants were integrated with the colorectal cancer organoid models *in vitro*, with viability being assessed by CellTiter Glo® assays.

Result and discussion

Metabolomic and untargeted metagenomic data of matched samples reveal that driver mutations shape gut microbiota composition and tumour progression. We identified species signatures uniquely linked to each genotype, showing either enrichment or reduction relative to wild-type. Remarkably, these signatures persisted in SICs as confirmed by 16S-rRNA analysis. Moreover, *BrafV637E*, *KrasG12D*, and *Pik3caH1047R* organoids exhibited enhanced, disease-state-specific survival when treated with matched-genotype SIC metabolites versus wild-type. This confirms that microbiome changes generate genotype-specific pro-oncogenic niches that support tumour progression.

Conclusion

Key mutations in colorectal cancer – *Apcfl/fl*, *BrafV637E*, *KrasG12D* and *Pik3caH1047R* – drive changes in gut microbiome composition that promote tumour cell survival and progression. These alterations also drive notable modifications in the secretory metabolite profile, affecting the biochemical environment and metabolic interactions within the ecosystem. Our platform reintegrates these metabolites with colorectal cancer organoids *in vitro*, enabling study of their functional implications. Additionally, our findings suggest that bacterial metabolites from healthy donors may complement current therapies, improving patient outcomes.

EACR25-0903

Unveiling the Landscape of Tumor-Associated Myeloid Lectins in Immunosuppressive Breast Cancer

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Introduction

Breast Cancer (BC) subtypes with poor prognosis are often associated with tumor microenvironments (TMEs) rich in myeloid immune cells. Cumulating evidence points to their role in promoting pro-tumorigenic immunosuppressive (IS) TMEs. Immunotherapies targeting immune suppression, typically cytokine signaling, have been proposed. Yet they faced challenges due to signaling redundancy and compensatory mechanisms, hampering clinical success. Recent findings emphasize the role of myeloid lectins, immune-modulating carbohydrate receptors that recognize aberrant glycans on tumor cells, in sustaining IS TMEs. As such, lectins are proposed as potential targets for immunotherapies, but the systematic characterization of their expression pattern remains underexplored. In this work, we profiled the lectin landscape of the BC myeloid cell population to identify potential targets to block myeloid-mediated immunosuppression.

Material and method

We analyzed an RNA-Seq dataset and classified samples as IS and Non-IS, by applying a published classifier gene panel [1]. We then combined differential gene expression analysis (DGEA) of the lectins in the IS vs Non-IS group with analysis of the top 25% over-expressed lectins in the myeloid cells of a single-cell RNA-Seq cohort [2]. For biological validation of the identified lectins, we employed a BC human 3D cell model (3D-3) using alginate microencapsulation and agitation-based cultures [3]. Both stromal (fibroblast) and immune components (blood-derived monocytes) were co-cultured with spheroids of BC cell lines MDA-MB-231, HCC1806 & BT474. The lectin subset was probed in the 3D-3 model of BC by flow cytometry. Top hits were validated for clinical outcome in patient tumor samples.

Result and discussion

Bioinformatic analysis of 3207 patients [4] revealed HER2 overexpressing and Triple Negative tumors overrepresented in the IS group (44%), associated with decreased overall survival and relapse-free survival. DGEA identified 13 lectins of interest enriched in the IS myeloid subset. Along 1 week of culture, an IS TME was acquired depicting cancer-associated fibroblasts and tumor-associated macrophages (TAM) phenotype. Probed lectins showed differential protein expression upon TAM polarization (CD163+CD206+) and the top hits correlated with clinical outcome. Functional validation by CRISPR gene knockout and evaluation of the impact on TAM polarization is ongoing.

Conclusion

In sum, we deliver a comprehensive characterization of the lectin landscape of myeloid cells within IS TME in BC, with putative targets to tackle IS, rendering tumors sensitive to novel immunotherapies.

We acknowledge funding from FCT/MECI (PT): iNOVA4Health (UID/04462); LS4Future (LAP/0087/2020); PTDC/BTM-TEC/0432/2021; 2022/11642/BD to G.T. I Tekpli, X. et al., *Nat Commun* 10, 5499 (2019) 2 Xu, L. et al., *Cell Rep Med* 5, 5 (2024) 3 Domenici, G. et al., *Adv Biol* 8, 12 (2024) 4 Dalal, H. et al., *Sci Rep* 12, 4696 (2022)

EACR25-0906

Osteopontin reprogrammes macrophages to a dysfunctional state in intrahepatic cholangiocarcinoma for patients with longer survival outcomes on chemotherapy

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Introduction

Heterogeneous benefit from chemotherapy in intrahepatic cholangiocarcinoma (iCCA) stems from differences in tumour and systemic biology. Previously, using pre-treatment biopsies, we identified the RPLS transcriptomic signature which distinguishes patients who would become rapid progressors (RP) from long survivors (LS) on chemotherapy. These differences predominantly originate from altered tumour-macrophage dynamics which remain to be mechanistically tested.

Material and method

We transcriptionally annotated iCCA cell models as RP-like (HUCCT1, KKU213, HUH28) or LS-like (KMCH1, CCLP1, SNU1079, YSCCC). To generate tumour-educated macrophages, conditioned media transfer was performed from these iCCA cell lines to THP1-derived and primary macrophage models. THP1 cells and primary monocytes were differentiated into macrophages by phorbol 12-myristate 13-acetate and colony stimulating factor 1, respectively. THP1 macrophages were further polarized into M1 (using interferon gamma and lipopolysaccharides) or M2 (using interleukin-4 and interleukin-13) states. Secreted proteins in conditioned media were profiled by NULISA-seq (200-plex inflammation panel). Macrophage phenotypes were assessed by viability (WST-1), morphology (IncuCyte® live cell imaging), phagocytosis (Zymosan substrate colorimetric assay), and transcriptional reprogramming (RNA-seq). The impact of recombinant osteopontin (SPP1) treatment on macrophage phenotypes was further evaluated.

Result and discussion

Conditioned media from RP-like iCCA cell lines contained a higher abundance of 52 extracellular proteins linked with pro-inflammatory signalling. Exposure of THP1-macrophages to RP-like media led to increased viability and altered morphology (more elongated and amoeboid-like shape) without any effect on their phagocytic capacity. Contrasting with these observations, conditioned media transfer from LS-like iCCA more extensively reprogrammed the transcriptomes of THP1- and primary macrophages. This suggested either a greater immunostimulatory effects of secreted proteins in LS-like conditioned media or greater immunosuppressive effects of secreted proteins in RP-like conditioned media.

Importantly, SPP1 was the only higher abundant protein in LS-like conditioned media. Recombinant SPP1 treat-

ment decreased viability and the phagocytic capacity of M0 THP1-macrophages (but not M1 or M2).

Conclusion

LS-like iCCA secrete higher amounts of SPP1, directly compromising the immune functions of macrophages in the M0-state. These data support a unique immune evasion strategy employed by a subset of tumours associated with better outcomes on chemotherapy. Understanding these cellular dynamics may offer insights into potential immunodulatory effects of SPP1 and its role in the tumor-macrophage-mediated iCCA progression.

EACR25-0941

Sympathetic-β2 adrenergic receptor drives the formation of pro-metastatic niche in liver metastasis

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Introduction

Liver is the most common site for cancer metastases, which may be attributed to the hepatic immuno-suppressive microenvironment. Recent studies have highlighted the immunomodulatory of innervation in various cancers. Given that the liver is richly innervated, we aimed to investigate their possible involvement in the metastatic-prone liver microenvironment.

Material and method

Integrative single-cell RNA sequencing (scRNASeq) and multiplex immunofluorescence (mIF) analysis in patients with colorectal cancer (CRC) liver metastasis (CRLM) was utilized to profile the expression patterns of neurotransmitter receptors. Orthotopic CRLM mouse models, high-dimensional flow cytometry, spatial transcriptomics, β2-adrenergic receptor (β2-AR) blocker and chemical sympathectomy were applied to investigate the functional roles and possible molecular mechanisms of β2-AR in CRLM.

Result and discussion

Our integrative analysis uncovered that β2-AR, the receptor of norepinephrine, was significantly upregulated in myeloid cells from patients with CRLM. Using our established spontaneous CRLM mouse models, we found that the upregulation of β2-AR was restricted to hepatic neutrophils, induced by the existence of primary CRC tumors and correlated with tyrosine hydroxylase (TH)+ sympathetic nerve fibers in the liver. Most importantly, blockade of sympathetic-β2-AR via β2-AR blockers or chemical sympathectomy in CRC-tumor bearing mice showed a potential to reduce liver metastatic incidence without suppressing primary CRC. Mechanistically, β2-AR activation may upregulate the expression of S100a8/9 to enhance the immunosuppressive activity of hepatic neutrophils via regulating neutrophil degranulation, thereby promoting CRC liver metastasis.

Conclusion

Our findings reveal the potential of targeting β2-AR signaling for the prevention and treatment of liver metastasis.

EACR25-0944**Translation Dysregulation In Cancer as a Source for Targetable Antigens**

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Introduction

Recent clinical successes of cancer immunotherapies have highlighted the importance of identifying cancer neoantigens – novel epitopes of self-antigens that derive from mutant proteins – for anti-tumor immunity. Indeed, identifying neoantigens derived from tumor-specific somatic mutations has enabled the advent of personalized therapeutic cancer vaccines. However, these immunotherapies are still limited to certain cancer types and are seldom curative. A major contributing factor to this limitation is the scarcity of identified targetable antigens. Thus, expanding the neoantigen search to non-mutational sources could greatly increase immunotherapy applications. This can be achieved by exploiting antigens derived from dysregulated protein synthesis in malignant cells.

Material and method

To investigate the role of translational dysregulation in immunological tumor control, we disrupted translation fidelity by deleting tRNA- γ W synthesizing Protein 2 (TYW2) in human and murine tumor cells and characterized the downstream impact on translation fidelity and immunogenicity using immunopeptidomics, genomics, and functional assays.

Result and discussion

Our analyses revealed that TYW2-KO cells generate immunogenic out-of-frame peptides. Furthermore, Tyw2 loss increased tumor immunogenicity and Tyw2 KO tumor-bearing mice exhibited a superior response to immune checkpoint blockade (ICB) therapy compared to Tyw2 WT tumor-bearing mice, indicating involvement of antigen-specific anti-tumor responses. Importantly, stimulation of T cells from ICB-treated Tyw2 KO tumor-bearing mice with out-of-frame peptides induced antigen-specific proliferation and activation in different ex vivo assays. Finally, our observations were corroborated by primary melanoma patient data analyses showing that reduced TYW2 expression was associated with increased response to ICB in patients.

Conclusion

Together, we demonstrate that defects in translation fidelity in cancer cells drive tumor immunogenicity. This study highlights the potential of tumor translational regulators as an attractive target for immunotherapy development, for enhancing the repertoire of tumor neoantigens.

EACR25-0960**16S rRNA sequencing based characterization of human gastric microbiome in patients with autoimmune atrophic gastritis and gastric neuroendocrine tumors**

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Introduction

Autoimmune atrophic gastritis (AAG) is characterized by the development of anti-parietal cell antibodies. The continuous loss of parietal cells leads to oxytic atrophy, eventually resulting in the development of sites with metaplasia, an event highly associated with the development of gastric tumors. The exact mechanisms leading to the development of autoimmunity and AAG's transformation into gastric neuroendocrine tumors (G-NETs) remain to be elucidated. Furthermore, the chronic increase in gastric pH caused by hypochlorhydria is expected to result in noticeable changes within the composition of the gastric microbiota. There is a scarce number of studies that have explored the changes in the composition of the gastric microbiota in relation to AAG and G-NETs, an interesting aspect that may further contribute to the development of gastric tissue atrophy and the acquisition of malignant potential. In this study, we aimed to investigate the composition of gastric microbiota to elucidate potential microbial determinants that are involved in the development of AAG and G-NET.

Material and method

Gastric juice aspirates (GA) were collected during endoscopic visits. All participants provided signed informed consent before participating in the study. The study was approved by the Central Medical Ethics Committee of Latvia [1]. DNA from GA samples was extracted using the QIAamp PowerFecal Pro DNA kit, followed by 16S rRNA V3 and V4 region sequencing on the Illumina MiSeq system with 100k reads per sample. In bioinformatics analysis, the Host DNA contamination was detected and removed using Bowtie2 (v2.5.1) and GRCh38 (v44) GENCODE human genome reference. This was followed by read merging and concatenation using PANDAseq (v2.11). De-noising, chimera removal, and amplicon sequence variant (ASV) detection were performed using DADA2 as part of the QIIME2 wrapper (v2023.09). Only ASVs with a sequence frequency of 10 or more were retained. Remaining ASVs were classified using the naïve Bayes classifier available in QIIME2, with a V3-V4 region-filtered SILVA (v138.1) database as a reference.

Result and discussion

In our data, we observed that the number of identified species was uniform across the sample groups; however, significant changes were observed in the evenness score of the identified species: Benjamini-Hochberg (BH) adjusted P-values of 0.02 (controls vs. AAG) and 0.006 (controls vs. G-NET). Differential abundance analysis

revealed that both AAG and G-NET groups showed an increase in the abundance of the *Rothia* genus compared to healthy controls. Furthermore, a reduction in the abundance of the *Haemophilus* genus was observed in the AAG group, and a reduction in *Gemella* was observed in the G-NET group.

Conclusion

The changes in gastric microbiota in patients with AAG and G-NET can be observed in species evenness.

Moreover, patients with AAG and G-NET have an increased abundance of *Rothia* genera.

[1] Approval no.: Nr. 01-29.1.2/2265

EACR25-0977

Infiltration of PD1+T cells after neoadjuvant FOLFIRINOX treatment associates with improved clinical outcomes in pancreatic cancer

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Introduction

Pancreatic ductal adenocarcinoma (PDAC) has limited therapeutic options and a dismal prognosis. In recent years, immunotherapies have revolutionized the treatment of various cancers, but, to this date, PDAC patients have not benefited from these breakthroughs. Modulation of the PDAC microenvironment with conventional therapies may break immunosuppressive barriers that thwart the success of immunotherapeutic approaches. Accordingly, we investigated the impact of neoadjuvant therapy regimens on the immune micro-environment of PDAC.

Material and method

We applied imaging mass cytometry (IMC) with 41 cellular markers to assess the immunological landscape of tissues derived from 32 PDAC patients, including patients that underwent neoadjuvant treatment with FOLFIRINOX or Gemcitabine combined with radiotherapy. We further examined the T cell compartment by analyzing an independent cohort of 133 PDAC patients by multiplex immunofluorescence (IF).

Result and discussion

We identified a clear impact of neoadjuvant therapy on several immune subsets, including a remarkable effect on the T cell compartment. CD4+ and CD8+ T cell infiltration within the tumor microenvironment (TME) was particularly pronounced in a subset of patients treated with FOLFIRINOX. In contrast, Gemcitabine-based chemoradiotherapy associated with reduced T cell infiltration, highlighting the distinct immunological consequences of these treatment regimens. Strikingly, tissues from FOLFIRINOX-treated patients also exhibited a higher frequency of PD-1-expressing T cells. Importantly, T cell infiltration was associated with an improved clinical outcome specifically in the FOLFIRINOX-treated group.

Conclusion

Our findings underscore the immunomodulatory capacity of standard-of-care therapies in PDAC. Importantly, our findings could inform the rational design of combinatorial strategies integrating chemotherapy (e.g., FOLFIRINOX) and immunotherapy. Furthermore, we demonstrated the need for patient stratification as the immunologic effects of neoadjuvant therapy appear to be restricted to a subset of patients.

EACR25-0994

Unraveling Tissue-Resident Memory to Combat Metastatic Breast Cancer

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Introduction

Primary breast cancer is the most frequently diagnosed cancer in women, with prognosis worsening once metastases form. Despite advances in immunotherapy that enhance CD8 T cell responses, these treatments often fail against metastatic disease. CD8 T cells can suppress metastases, but the tumor microenvironment drives exhaustion, limiting their effectiveness. Exhausted T cells produce fewer pro-inflammatory cytokines and express inhibitory receptors that restrain anti-tumor activity. A key challenge in cancer therapy is reinvigorating tumor-specific T cells that sustain function after metastases progress. However, it remains unclear which T cell subsets are essential for maintaining immune responses against metastatic tumors. We investigated the recruitment, origin, and effectiveness of CD8 T cells in combating disseminated tumor cells.

Material and method

We utilized the EO771 breast carcinoma model, an aggressive tumor line that metastasizes in mice. EO771 cells were genetically modified to express ovalbumin, allowing tracking of OVA-specific CD8+ T cells from OT-I mice. Tumor cells were orthotopically engrafted, leading to primary tumors that spontaneously metastasized to the lungs. This model enabled us to study tumor-specific CD8+ T cell recruitment, phenotype, and function at primary and metastatic sites. We used spectral flow cytometry to analyze CD8+ T cells in tumors and lymph nodes. Exhaustion markers PD-1 and Tim-3 identified terminally exhausted T cells, while precursor exhausted T cells were defined by CD62L expression in tumor-draining lymph nodes. Migration and localization of CD8+ T cells in lung metastases and lung-draining lymph nodes were examined to assess their role in anti-tumor immunity.

Result and discussion

At the primary tumor, tumor-specific CD8 T cells exhibited robust responses but were mostly terminally exhausted, co-expressing PD-1 and Tim-3. These cells were maintained by precursor exhausted T cells in tumor-draining lymph nodes expressing CD62L. After metastases developed, tumor-specific CD8 T cells were recruited to the lungs and lung-draining lymph nodes, displaying a similar exhausted phenotype. These results suggest that CD8 T cells respond to metastases by colonizing local lymph nodes, from which they establish immune responses against disseminated tumor cells.

Conclusion

Our findings highlight tumor-specific T cell precursors in metastatic site lymph nodes as critical players in immune responses against metastatic breast cancer. Targeting these precursors may enhance immunotherapy efficacy in advanced disease.

EACR25-0995

Reprogramming of Pro-tumoral Monocyte-derived Macrophages: A Novel Strategy Targeting Cathepsin B and Non-Apoptotic Caspases

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Introduction

Monocyte-derived macrophages (MDMs) are key innate immune cells involved in essential biological processes such as tissue repair, host defense and anti-tumor immunity. However, tumor or leukemia-associated anti-inflammatory macrophages (TAMs, LAMs) promote cancer growth by stimulating angiogenesis, inhibiting anti-tumor immunity and contributing to treatment resistance. Recently, several strategies have been developed to target these macrophages, by blocking their recruitment, reducing their population, activating them or reprogramming them. Therefore, a better understanding of the underlying mechanisms involved in the generation and polarization of anti-inflammatory MDMs is crucial for the development of new therapeutic avenues.

Material and method

Our team used an ex vivo model to generate and polarize human MDMs from the peripheral blood of healthy donors. After purification, primary human monocytes are differentiated into immature macrophages (M0) in response to CSF-1. These M0 macrophages are then polarized into a pro-inflammatory phenotype (M1) with LPS + IFN- γ , or an anti-inflammatory phenotype (M2) with IL-4. Flow cytometry, RT-qPCR, RNA sequencing, ELISA and various functional assays were used to characterize these different types of macrophages.

Result and discussion

Using this model, we demonstrated the crucial role of non-apoptotic caspase-8 in both monocyte-to-macrophage differentiation and their M2 polarization and identified a novel non-canonical activation of caspase-8 (CASP8) by cathepsin B (CTSB). Specific activation of caspase-8 by cathepsin B leads to the non-canonical cleavage and activation of caspases 3 and 7 and the cleavage of several substrate proteins at sites distinct from those observed during apoptosis. Finally, we evidenced that targeting the CTSB / CASP8 axis with pharmacological agents (CA-074, Emricasan or our own original and specific non-apoptotic caspase inhibitors) or with genetic approaches (siRNA) not only inhibits the generation of anti-inflammatory macrophages but also reprograms them towards a pro-inflammatory profile. We are currently working on characterizing and targeting of LAM populations via the CTSB / CASP8 axis in bone

marrow samples from patients with acute myeloid leukaemia.

Conclusion

Our work identifies a novel CTSB / CASP8 axis as a key regulator of monocyte-to-macrophages differentiation and M2 polarization. By targeting this pathway, we have successfully reprogrammed anti-inflammatory macrophages to a pro-inflammatory state, providing novel therapeutic strategies to counteract immuno-suppressive macrophages, reshape the tumor micro-environment and enhance anti-tumor immunity.

EACR25-1017

Deciphering the cellular and molecular mechanisms leading to immune exclusion and Tertiary Lymphoid Structure organization in Non-Small Cell Lung Cancer

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Introduction

The Tumor MicroEnvironment (TME) of Non-Small-Cell Lung Cancer (NSCLC) is a highly complex and heterogeneous ecosystem, consisting of tumor cells, immune cells, cancer-associated fibroblasts (CAF), and endothelial cells. Among immune cells, B and T lymphocytes can be organized into Tertiary Lymphoid Structures (TLS), associated with improved prognosis and response to immunotherapy. Conversely, low immune infiltration and absence of TLS have been linked to poor clinical outcomes. However, the mechanisms underlying immune cell infiltration and TLS formation within the TME are not fully elucidated. We hypothesize that tumor cell phenotype, blood/lymphatic vessels, CAF subsets, and extracellular matrix composition collectively influence immune cell infiltration and TLS organization. This study therefore aims to identify, using a multiomics approach, the molecular mechanisms involved in immune cell infiltration and TLS formation.

Material and method

We are analyzing a retrospective cohort of 211 NSCLC patients who underwent surgery. Immune cell infiltration in the TME is characterized using multiplex immunofluorescence, to quantify B and T cell densities, TLS presence and maturity, high endothelial venules and tumor cell densities. Additionally, we are quantifying stromal components, including CAF subsets and blood/lymphatic vessels. These features are analyzed using HALO software, followed by bioinformatics analyses to classify the cohort based on immune cell density and TLS characteristics. We have initiated spatial transcriptomics analysis using Visium HD on 18 patients and are conducting bulk transcriptomics on the entire cohort.

Result and discussion

Preliminary results indicate that 204 out of 211 patients exhibit at least one mature TLS, with 81% of these showing secondary follicle-like TLS (SFL-TLS), the highest stage of maturity. As expected, high density of SFL-TLS correlates with better overall survival. Based

on this classification, we have selected two patient groups for Visium HD analysis: one with low and one with high TLS density (9 patients in each group).

Conclusion

This study will provide valuable insights into the relationships between various TME components, and immune cell infiltration and TLS formation.

Additionally, it will provide transcriptomic profiles of distinct TME subtypes, contributing to a deeper understanding of the NSCLC immune landscape and supporting the identification of potential new therapeutic strategies.

EACR25-1021

HDAC6 inhibition alters T cell function and metabolism in non-small cell lung cancer

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Introduction

Non-small cell lung cancer (NSCLC) accounts for nearly 85% of lung cancer cases and is the leading cause of cancer-related deaths worldwide. Histone deacetylase 6 (HDAC6) has been described to drive cancer development through multiple pathways, such as oncogenic tumorigenesis, cell motility and proliferation. Recently, HDAC6 has also been shown to play a role in cancer cell metabolism. While HDAC6 appears to be a promising cancer drug target, the regulatory and metabolic role of HDAC6 on T cell activity remains largely unknown. This study aims to investigate the metabolic influence of HDAC6 inhibition in mediating T cell function in NSCLC.

Material and method

Whole blood samples were obtained from patients recruited for the PLAN clinical trial (NCT05542485) and GAMBIT clinical trial. Peripheral blood mononuclear cells were isolated from the blood samples to study CD8+ T cell activation/function following HDAC6 inhibitor (HDAC6i) treatment ex vivo. In addition, we used Single Cell ENergetIc metabolism by profiling Translation inHibition (SCENITH), a flow cytometry-based method that functionally profiles single cell metabolism ex vivo to examine metabolic alterations caused by HDAC6i in NSCLC. Metabolic changes within CD8+ T cells following HDAC6i treatment in NSCLC were also assessed using the Seahorse analyser.

Result and discussion

Interestingly, CD8+ T cell activation was augmented following treatment with HDAC6i. This was accompanied by changes in metabolism of CD8+ T cells. Furthermore, CD8+ T cells treated with HDAC6i demonstrated enhanced activity, consolidating the regulatory role of HDAC6 on CD8+ T cell function.

Conclusion

In short, the findings from this research elucidated the potential of HDAC6i as an anticancer agent in NSCLC by strengthening tumour killing ability of CD8+ T cells via metabolic modulation.

EACR25-1053

Beyond multidrug resistance: the role of ABCC1 in metabolic detoxification and immune modulation

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Introduction

Head and neck squamous cell carcinoma (HNSCC) is the seventh most common cancer worldwide characterized by a high mortality rate and resistance to chemotherapy. In recent years immune-checkpoint inhibitors (ICIs), have been approved for recurrent or metastatic HNSCC. However, the highly immunosuppressive micro-environment of HNSCC limits the efficacy of immunotherapy. The ABC transporter, ABCC1, has been widely studied for its role in promoting the extracellular efflux of chemotherapeutic agents contributing to the multidrug resistance phenotype observed in many tumors, including HNSCC. ABCC1 also regulates the efflux of various endogenous metabolites, such as leukotriene C4, GSH and sphingosine, which may affect key cancer-related processes, like inflammation

Material and method

The role of ABCC1 was analyzed by siRNA and CRISPR/Cas9-mediated KO in SCC cells. Metabolomic profiling and RNA-seq were performed on ABCC1 KO cells to investigate metabolic and transcriptional changes. To assess the in vivo impact of ABCC1, ABCC1 KO murine cells were inoculated into immunocompetent mice. After three weeks, tumors were excised and analyzed for immunophenotype

Result and discussion

We found that ABCC1 is highly expressed in the classical subtype of HNSCC, which is typically characterized by a gene expression profile related to detoxification. By integrating mass spectrometry (MS)-based metabolomic analysis and transcriptomic profiling, we found that HNSCC ABCC1KO cells exhibits dysregulation of methylglyoxal detoxification pathway and decrease of a gene signature associated with TNF- α signaling and purine metabolism genes. In detail, ABCC1 deletion leads to a significant reduction of the expression a class of cytokines (CXCLs), which are key mediators of the recruitment of tumor-associated neutrophils. Consistent with this, syngeneic transplantation studies revealed that SCC tumors lacking ABCC1 exhibit a significant reduction in neutrophil populations. Along the same line, we found that ABCC1 expression is positively

correlated with neutrophil levels in human HNSCC patients. Remarkably, high neutrophil infiltration and elevated neutrophil-to-lymphocyte ratio (NLR) have been linked to poor prognosis and reduced response to immunotherapy in HNSCC patients. In addition to regulating inflammatory chemokines, ABCC1 genetic deletion also affects the expression of a group of genes (CD39, CD73 and ENPP1) playing a crucial role in the production of extracellular adenosine (eADO), a key mediator of immune escape. Taken together, our findings suggest that ABCC1 is embedded in signaling cascades linking the detoxification pathway to immune regulation in HNSCC.

Conclusion

Our findings uncover the interconnected mechanisms through which ABCC1 shapes the immunophenotype and the inflammatory status of HNSCC, providing the proof of principle to exploit an ABCC1-based targeting approach to improve the immunotherapy efficacy in HNSCC.

EACR25-1061

Trem2+ Macrophages as a Novel Therapeutic Target for Metastatic Prostate Cancer

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Introduction

Prostate cancer (PCa) is the most prevalent cancer in men, where metastasis is the leading cause death. Metastatic hormone naïve PCa (mHNPC) is determined at the time of first diagnosis, with limited therapeutic options available. Clinical trials testing immunotherapy, even in combination with standard of care, has showed limited clinical benefits. Among this resistant micro-environment, tumor-associated macrophages (TAM) have emerged as major mediators of immunosuppression that promote progression of various tumor types.

Material and method

In our group we generated novel conditional mouse models with different degree of aggressiveness of PCa. Pten and Lkb1 loss (Ptenpc-/-Lkb1pc-/-) in the prostate epithelium works as a model of mHNPC, as it results in very aggressive and fast-growing prostate cancer, developing metastasis in lumbar lymph nodes and lung. As a control model, the heterozygous loss of Lkb1 generated PCa, but with no signs of metastasis. Single RNAseq was performed in these two models, and also in localized and metastatic PCa human patients from Basurto Hospital. For preclinical studies, T051 cells derived from Ptenpc-/-Lkb1pc-/- model were injected subcutaneously and treatments were administered. Immunophenotyping studies were performed in BD FACSymphony and Attune NxT cytometers. Human monocytes from healthy donors were purified by Robosep from peripheral blood mononuclear cells and differentiated into macrophages for 7 days with M-CSF.

Result and discussion

In this work, we firstly demonstrated that macrophage function is crucial for tumour growth in Ptenpc-/-

Lkb1pc-/- subcutaneous model, as CSF1R blockage reduces ~ 50% tumour growth as a single agent, and ~ 85% when combining with PD1 blocking agent. Cytometry analysis confirmed macrophage depletion, accompanied by an increase in Ly6C+CD11b+ monocytic myeloid-derived suppressor cells (MDSC), suggesting a compensatory mechanism. Notably, CSF1R blockage increased CD8+CD3+ lymphocytes, which was exacerbated even more when combining with PD1 blockage. For the discovery of TAM-specific targets in this model, single cell RNAseq highlighted Trem2 as one of the key players in the metastatic mouse model, further validated in human metastatic patients. Cytometry analysis confirmed the increased expression of TREM2 in F4/80+CD11b+ TAMs, compared to CD11C+ dendritic cells, or Ly6G+CD11b+ granulocytic and Ly6C+CD11b+ monocytic MDSCs. In fact, PDL1+ TAMs presented enriched TREM2 levels, which may support its role in aggressiveness. In human healthy macrophages, the association between TREM2 and pro- or anti-inflammatory stimuli presents a different behaviour, suggesting the specificity of TREM2 towards TAMs.

Conclusion

In conclusion, we identified TREM2 as TAM marker in a novel mouse model of mTNPC, suggesting that its blockage might be a promising therapeutic strategy in combination with conventional immunotherapy.

EACR25-1070

Analysis of intratumor immune heterogeneity reveals spatial organization of immunosuppression in breast cancer

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Introduction

Recent advances in single-cell transcriptomics have shed light on breast cancer intratumoral heterogeneity (ITH). However, the spatial distribution of immune cell populations and the associate T-cell repertoire (TCR) in the breast tumor-microenvironment (TME) remain still poorly understood.

Material and method

Here, we performed paired single-cell RNA/TCR sequencing on 60,000 cells isolated from three distinct tumor regions (central- proximal to the tumor core, peripheral- proximal to the invasive margins, and intermediate- between the first two regions), in five

patients with early stage, untreated, luminal breast cancer.

Result and discussion

Our analysis revealed a distinct spatial organization of immune cells within the TME. The tumor periphery was enriched in monocytes and dendritic cells, as well as naïve CD4+ and memory-like CD8+ T cells, including both bystander and pre-dysfunctional populations. In contrast, the tumor core contained higher numbers of M2-like macrophages, clonally expanded Tregs, and late-dysfunctional CD8+ T cells with tumor-reactive properties. Peripheral regions exhibited increased expression of tertiary lymphoid structure (TLS) signatures, while the tumor core showed higher glycolysis activity. Multiplex IHC further confirmed key findings, including the enrichment of TCF1+ pre-dysfunctional CD8+ T cells in the periphery, highlighting a progressive accumulation of immunosuppressive cell populations toward the tumor core.

Conclusion

Our study reveals a region-specific immune organization in luminal breast cancer, with a progressive accumulation of macrophages, Tregs, and tumor-reactive late-dysfunctional CD8 T cells toward the core. These findings offer new insights into TME heterogeneity, with potential implications for personalized immunotherapy strategies.

EACR25-1217

Glutamine scarcity modulates innate immunity in lung cancer

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Introduction

KRAS mutations are common in non-small cell lung cancer (NSCLC), often diagnosed late, making it hard to treat and deadly. Chemotherapy and targeted therapies frequently fail. Immunotherapy, like Immune Checkpoint Blockade (ICB), is a breakthrough, yet 70% of advanced NSCLC patients don't respond due to poor T-cell activation. The cGAS/TBK1 pathway, key in innate immunity, senses threats and aids anti-tumor immunity via T-cells. In the tumor microenvironment (TME), glucose and glutamine are vital for tumor growth, but scarcity, especially of glutamine, alters cancer and immune behavior. We studied how glutamine lack affects immunity in NSCLC.

Material and method

We tested glutamine restriction's impact on the cGAS/TBK1 pathway in KRAS-mutant NSCLC cell lines. Cells were grown in low-glutamine media mimicking TME stress. Phospho-TBK1 levels were measured to assess pathway activity, and interferon-gamma (IFN- γ) expression was quantified for T-cell effects. We compared glutamine-rich and -poor conditions to simulate TME nutrient shifts.

Result and discussion

In this study, we found that glutamine restriction in the TME blocks the cGAS/phospho-TBK1 axis in lung cancer cells. Low glutamine reduced phospho-TBK1 activation, cutting IFN- γ levels and weakening immune surveillance. It also shifted cancer cell metabolism and

innate signaling, likely boosting immune evasion. This shows glutamine controls the cGAS/TBK1 pathway, tying nutrient stress to poor T-cell activation in NSCLC. This metabolic-immune link may explain ICB resistance.

Conclusion

Glutamine scarcity in the TME inhibits cGAS/TBK1 signaling, impairing T-cell activation and aiding immune evasion in NSCLC. This metabolic-immune interplay reveals why immunotherapy fails in many cases. Targeting glutamine metabolism could improve ICB success, offering new hope for NSCLC treatment.

EACR25-1218

Taming the metabolism of tumor-associated macrophages to fight NF1-related tumors

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Introduction

Malignant peripheral nerve sheath tumors (MPNSTs) are aggressive sarcomas developing either sporadically or in the context of Neurofibromatosis type 1 (NF1) against which there are no current treatment options. Despite being highly infiltrated by macrophages endowed with an anti-inflammatory M2-like phenotype, the potential role of these immune cells in MPNST growth has been elusive. Accumulating evidence shows that factors released in the tumor microenvironment (TME) skew tumor associated macrophages (TAMs) toward a protumoral phenotype sustained by specific metabolic adaptations. Here, we evaluated the phenotypic, transcriptional and functional properties of macrophages associated to MPNSTs identifying the role of the mitochondrial chaperone TRAP1 in sustaining several protumoral TAM actions.

Material and method

We exposed bone marrow derived macrophages isolated from WT and TRAP1 KO mice to MPNST cell medium and studied the phenotypic and transcriptional traits of resulting TAM-like cells. In addition, we performed functional characterizations of TAMs employing Boyden chamber and Matrigel assays of tumor/endothelial cells co-cultured with macrophages to investigate the ability of MPNST-conditioned macrophages to tune MPNST cell invasion/migration and endothelial cell angiogenesis.

Result and discussion

We have found that the conditioned media from MPNST cells drives the up-regulation of a set of conventional M2-like TAM markers, and of the metabolic enzymes glutamine synthetase (GLUL) and Arginase 1 (ARG1) in naïve and M2 anti-inflammatory macrophages. Such TAM-like cells sustain MPNST migration and exert proangiogenic properties both in vitro and in vivo through succinate accumulation and HIF-1 α stabilization. By ablating the mitochondrial chaperone TRAP1, these protumoral TAM functions are impaired, along with a reduced succinate accumulation and HIF-1 α expression.

Conclusion

Our data provide proofs of macrophage pro-tumoral polarization following exposure to MPNST cell released factors that remain to be identified. TRAP1 expression sustains the transition of macrophages toward the TAM-like state whereby TRAP1-dependent succinate accumulation leads to HIF-1 α stabilization and a pseudo-hypoxic response following MPNST conditioning. This work provides the first insight into TRAP1 role in pro-neoplastic TAMs, thus identifying this mitochondrial chaperone as novel therapeutic target in the macrophage cell compartment.

EACR25-1245

Tumor miRNAs in small-extracellular vesicles dampen the CD8+T cell response against tumor and predict response to immunotherapy in NSCLC

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Introduction

Despite the development of immune checkpoint inhibitors therapy (ICI) in non-small cell lung cancer (NSCLC) patients, most of them eventually experience relapse. Resistance mechanisms to ICI are associated with low immune response. Antitumoral immune response is mainly promoted by CD8+ T lymphocyte (CD8 T cells). However malignant cells can escape this immune surveillance due to microRNA (miRNA) loaded into small extracellular vesicles (sEV) secreted by malignant cells. sEV are currently emerging as promising biomarkers. They are released by all cell types, participate at the cell-cell communication and can be found in all body fluids, reflecting at distance the state of the tumor. Here, we investigated i) the immunosuppressive role of sEV derived from patient's NSCLC (NSCLC-sEV) on antitumoral CD8+ T cells and ii) their use as biomarkers of response to ICI in the BREATHE (Biocollect for REseArch in THoracic cancer) biocollection.

Material and method

NSCLC-sEV were purified by ultracentrifugation from NSCLC patient-derived cell lines, from tumor resection or from plasma (ethics agreement DC-2011-1399, DC-2017-2987). Their immunosuppressive effects were studied by exposing CD8+ T cells to NSCLC-sEV, where their activation, proliferation, and viability were assessed. Their use as biomarker was investigating by NGS sequencing of their miRNA cargo. Using bioinformatic analysis, we identified potential immunosuppressive microRNAs loaded into sEV that were tested by direct transfection in CD8+ T cells.

Result and discussion

CD8+ T cells exposed to NSCLC-sEV showed reduced expression of their activation markers CD25, CD45 and CD226, and decreased secretion of antitumoral TNF α , granzyme B, and perforin-1, suggesting that they are unable to conduct an efficient anti-tumoral response.

NSCLC-sEV also upregulated the CD8+ immune checkpoint markers, such as PD-1, TIGIT, and TIM-3, and decreased the viability and proliferation of exposed CD8+ T cells. By bioinformatic prediction, we selected miRNAs loaded into NSCLC-sEV that could promote an immunosuppressive effect. Transfection of our candidate miR-29c-3p and miR-181a/b-5p, into CD8+ T cells induced an inhibition similar to that induced by native NSCLC-sEV. NSCLC-sEV and their miRNA cargo, at diagnosis, are also promising biomarkers of response to ICI since we identified 17 miRNAs which discriminate efficiently non-responders (patient having a disease progression by RECIST criteria on first CT evaluation) and long-term responders (patients free-from-progression at 18 months after the beginning of treatment).

Conclusion

To conclude, NSCLC-sEV can impair antitumoral CD8+ T cell response. We demonstrated that miRNAs included into sEV derived from tumor cells can impair CD8+ T cell response against cancer and are promising biomarkers of response to immunotherapy in NSCLC.

EACR25-1270

Distinct gut microbiota in breast cancer patients and its dynamics during endocrine treatment

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Introduction

The effects of gut microbiota on cancer progression and treatment are increasingly recognized, with associations reported across various diseases and cancer types. However, its modulation in breast cancer remains under-explored. In this study we examined the gut microbiome of breast cancer patients compared to healthy controls and analyzed longitudinal changes in microbiome composition and clinical parameters during endocrine treatment.

Material and method

Newly diagnosed hormone receptor-positive (HR+) breast cancer patients (n=92) treated at the Sheba Breast Oncology Institute were recruited. Fecal and blood samples, along with extensive clinical data and detailed questionnaire, were collected at diagnosis and at multiple time points during endocrine treatment. Fecal samples underwent full shotgun metagenomic sequencing. The microbiomes of breast cancer patients were compared to those of a large control group (n = 920) of healthy age- and BMI-matched women collected at the Weizmann Institute. A machine learning algorithm was employed to differentiate between breast cancer and healthy microbiomes. Longitudinal changes in clinical parameters were correlated with gut microbiome alterations during treatment.

Result and discussion

Breast cancer patients exhibited a distinct microbiome composition with specific microbial signatures. Alpha diversity, a measure of microbial richness, was significantly lower in breast cancer patients compared to the healthy control group. The relative abundance of several microbial species differed significantly between breast cancer patients and controls. A predictive model successfully distinguished microbiome profiles of breast cancer patients from those of healthy women.

Longitudinal analysis revealed that certain species enriched in breast cancer samples, particularly from the *Bifidobacterium* family, decreased in abundance during endocrine therapy, shifting toward levels observed in healthy women. Additionally, changes in clinical parameters, such as decreases in LDL cholesterol and increases in HbA1c levels, were correlated with microbiome alterations. Functional analysis of these microbial species suggests a potential link between gut microbiota and the host's response to endocrine treatment.

Conclusion

The gut microbiome of women with breast cancer exhibits distinct characteristics with potential predictive value, warranting further investigation for future use in early detection and prevention strategies. The microbiome also appears to play a role in the physiological response to endocrine treatment, opening new avenues for personalized treatment approaches.

EACR25-1337

Indisulam evokes T cell dependent cytotoxicity through mis-splicing in neuroblastoma

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Introduction

Neuroblastoma is a highly lethal pediatric tumour of the sympathetic nervous system. Multimodal therapy has had limited success, while development of immunotherapies has proven challenging, owing to the low surface human leucocyte antigen type I expression and negative thymic selection of T cells. However, high-risk MYCN amplified neuroblastoma is sensitive to splicing inhibitors. Indisulam, a molecular glue, degrades RNA binding motif 39 (RBM39), an RNA splicing co-factor, leading to aberrant splicing. This mis-splicing leads to the generation of neopeptides, some of which have been shown to be immunogenic in mouse melanoma. Here we assessed the presence of mis-spliced transcripts, coding for immunogenic neopeptides, generated from RBM39 degradation across cancer cell lines, and validated their presence in neuroblastoma *in vitro*. Additionally, we confirmed the ability of indisulam-treated neuroblastoma cells to trigger proliferation and activation of CD8+ T cells.

Material and method

We performed an in-silico analysis, matching events producing immunogenic neopeptides, to corresponding events identified in a literature search of studies with RNA splicing data on cell lines treated with RBM39 degraders. The presence of events identified in multiple

cell lines was confirmed through PCR using custom primers. The immunogenicity of indisulam treated cells was established through a Dendritic cell (DC) assay. DCs were isolated from human Peripheral Blood Mononuclear Cells (PBMCs) and cocultured with indisulam treated KELLY cells. The DCs were isolated and cocultured with syngeneic naïve T cells. The primed T cells were then cocultured with indisulam-treated KELLY cells. Flow cytometry was used to assess their proliferation and activation.

Result and discussion

Our in-silico analysis revealed that RBM39 degradation generates a robust profile of transcripts derived from mis-splicing, common across several cancer cell lines. The presence of two of these events was identified in two neuroblastoma cell lines, IMR-32 and KELLY. Interestingly, KELLY was not one of the cell lines included in our in-silico analysis, indicating that some of these events may be present across many neuroblastoma cell lines. Lastly, our DC assay revealed a significant increase in proliferation and activation of naïve human T cells exposed to KELLY cells treated with indisulam compared to the control.

Conclusion

Our findings indicate that splicing inhibitors can induce production of transcripts common across neuroblastoma and other cancer cell lines. We aim to identify the presence of the immunogenic neopeptides, these mis-spliced transcripts code for, through immunopeptidomics. Our findings show that combination of splicing inhibitors with immunotherapies, such as vaccines, could leverage these immunogenic neoantigens, paving the way towards the development of highly efficacious and specific immunotherapies against solid pediatric tumours.

EACR25-1385

Analysis of the spatial characteristics of the DLBCL tumor microenvironment utilizing the SignalStar® multiplex immunohistochemistry assay

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Introduction

Diffuse Large B-Cell Lymphoma (DLBCL) is the most prevalent form of non-Hodgkin lymphoma, with over 18,000 cases diagnosed annually in the United States. While approximately 60% of DLBCL patients achieve long-term remission through standard chemotherapy, those with refractory DLBCL face a poor prognosis. The immune landscape of DLBCL is highly complex, requiring a deeper understanding of the dynamic interplay between T cell activation and immuno-suppression within the tumor microenvironment (TME) to improve patient stratification and predict therapeutic outcomes. Given this complexity and the importance of spatial relationships among various cell types in the TME, it is essential to employ multiplex immunohistochemical

(mIHC) assays for simultaneous visualization of biomarkers and phenotypic markers.

Material and method

In this study, we developed a 12-plex SignalStar mIHC panel incorporating T cell phenotypic, activation, and suppression markers to characterize the TME in FFPE DLBCL cores. Immuno-oncology markers were visualized within the tumor and in the context of myeloid cells, distinguished by CD68 expression. The assay involved a single primary incubation of all 12 antibodies, followed by the application of fluorescently labeled oligonucleotide networks to amplify the signals of four antibody-oligo conjugates across three imaging rounds. After imaging the first four conjugates, the fluorescent signal was enzymatically removed, and the process was repeated for two additional rounds. The three resulting images were aligned using Visiopharm's Tissuealign™ platform. Quantitative analysis, including positive cell counts and marker co-localization, was performed using Visiopharm's Phenoplex™. Additionally, biomarker expression patterns were assessed with Phenoplex™, and the data were correlated with patient demographics, disease progression, and treatment responses.

Result and discussion

Our findings highlight the SignalStar mIHC assay as a valuable tool for unraveling the complexities of the immunosuppressive TME.

Conclusion

This assay is able to provide researchers with deeper insights into responses to immunotherapy and chemotherapy combination strategies.

EACR25-1396

IL1RAP targeting is a new therapeutic strategy for advanced triple negative breast cancer

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Introduction

The interleukin-1 (IL-1) family, with IL-1 α and IL-1 β as key members, plays a critical role in shaping the inflammatory tumor microenvironment in cancers, including breast cancer (BC). IL-1 signaling supports tumor growth and therapy resistance, requiring IL-1 receptor 1 (IL1R1) to dimerize with the IL-1 receptor accessory protein (IL1RAP). IL1RAP is overexpressed in several cancers, including triple-negative breast cancer (TNBC), but its role in TNBC progression remains unclear.

Material and method

Here, we investigate IL1RAP activation in TNBC using patient data, cell lines, and in vivo models. Analysis of TCGA and METABRIC databases with CANCERTOOL and TIMER 2.0 revealed elevated IL1RAP mRNA expression in estrogen receptor-negative (ER-) BC and TNBC, correlating with reduced disease-free survival. IL1RAP expression also associates with cancer-associated fibroblasts (CAFs), inflammatory mediators

(IL-1 α , IL-1 β , IL-6, IL-8), and the Oncostatin M receptor (OSMR), a key BC progression factor.

Result and discussion

In TNBC cell lines, RT-qPCR and western blot confirmed high IL1RAP expression, while Olink Target 48 Cytokine panel analysis showed increased pro-inflammatory cytokine production. IL-1 α /IL-1 β stimulation further upregulated IL-1 α , IL-1 β , IL-6, and IL-8 expression. Functionally, IL-1 β treatment of the MDA-MB-231 cell line enhanced cell migration in Transwell assays, whereas IL1RAP inhibition, using siRNA knockdown, significantly impaired this effect. Furthermore, in an *in ovo* tumor growth model, where TNBC cells were seeded onto the chorioallantoic membrane (CAM) of fertilized chicken embryos, IL1RAP inhibition reduced tumor growth *in vivo*. These results further validate the importance of IL1RAP in TNBC and highlight the relevance of the *in ovo* model in rapidly assessing tumor progression. This model allows us to explore IL1RAP-driven mechanisms in a complex microenvironment and provides a solid foundation for refining and guiding future studies in murine models with greater precision. Additionally, we identified a compelling interaction between IL-1 and Oncostatin M (OSM), a cytokine implicated in BC progression and metastasis. OSM promoted the expression of IL1RAP, IL1R1, IL-1 α , and IL-1 β in cultured TNBC cells and tumor xenografts, as shown by RT-qPCR and western blot analysis, further reinforcing the role of IL-1 signaling in BC-associated inflammation.

Conclusion

This study demonstrates that IL1RAP is overexpressed in TNBC and highlights the central role of IL-1 signaling in tumor-promoting inflammation within the tumor microenvironment. Overall, our findings suggest that IL1RAP represents a promising therapeutic target in TNBC and that its inhibition could serve as a potential treatment strategy for TNBC patients.

EACR25-1408

LIF promotes immunosuppression through C1q+TAMs in GBM

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Introduction

Glioblastoma (GBM), the most common brain cancer in adults, has a mean survival of 14.6 months, which reflects its unmet therapeutic needs. Leukemia-inhibitory factor (LIF) is a pleiotropic cytokine part of the IL-6 family of cytokine that is expressed in GBM. Previously, it has been shown that LIF can act as an oncogenic factor and promote immunosuppression in the GBM tumor microenvironment. In recent years, a population of tumor-associated macrophages (TAMs) characterized by the expression of C1q has gained attention due to their immunosuppressive properties and correlation with poor prognosis. Moreover, Dexamethasone (DEX), which is widely used as a symptomatic treatment for GBM, has also been linked to immunosuppression.

Material and method

We studied the effect of LIF alone or in combination with DEX on bone-marrow derived macrophages (BMDMs) and GBM animal models. We then analyzed the immunosuppressive effect of C1q on T-cells using both isolated T-cells and mouse-derived tumor tissue cultures (MDTTCs) from tumors derived from wild-type and C1qa-/ mice co-cultured with T-cells. Finally, we studied the effect of LIF blockade in vivo alone or in combination with αPD1, comparing wild-type and C1qa-/ mice and assessing the anti-tumoral effect by measuring tumor growth and analyzing T-cell activation.

Result and discussion

LIF induced the expression of C1q both by RNA and protein in BMDMs. Neutralization of LIF in GBM animal models led to a decrease in the expression of C1q in CD11b+ cells and tumor growth. DEX synergized with LIF and increased the expression of C1q and M2-markers by BMDMs in vitro, as well as the C1q+TAM population in vivo. Moreover, C1q repressed the activation of T-cells in vitro, and blockade of LIF in MDTTCs showed an increased activation of co-cultured T-cells which was higher in the wild-type tumors compared to the C1qa-/ ones. In vivo, tumors were significantly smaller in C1qa-/ mice compared to wild type, and blockade of LIF led to an increased T-cell activation in wild-type, but not in C1qa-. Combination with αPD1 led to an increased anti-tumoral response both by tumor growth and T-cell activation.

Conclusion

Our results indicate that LIF promotes an immunosuppressive C1q+ TAMs population, that is involved in T-cell dysfunction. Treatment with DEX increases this population and the sensitivity of macrophages to LIF. Blocking LIF through neutralizing antibodies results in a decrease of the C1q+ TAM population, as well as in an anti-tumoral response and increased activation of T-cells. In the absence of C1q, tumor growth is delayed and the effect of αLIF is decreased, supporting both the role of C1q in tumor progression and that the antitumoral effects of αLIF can be partially attributed to the decrease in C1q+ TAMs. Therefore, targeting this population via LIF antagonists may contribute to improve immunotherapies in GBM.

EACR25-1442

Decoding Immune Evasion in Mismatch-repair Deficient Colorectal Cancer Organoid-Immune Co-culture Models

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Introduction

Mismatch repair deficient (MMRd) colorectal cancer (CRC) accounts for 15% of all CRC cases. MMR status carries both prognostic and predictive value as patients with MMRd tumours, compared to MMR proficient CRC, display better outcomes and are sensitive to immune checkpoint inhibitors (ICIs). However, questions regarding optimal ICI duration and regimen, in particular

single or doublet ICI, remain unresolved. There is now an urgent need for predictive biomarkers that allow rational stratification.

Material and method

To understand ICI immune biology and immune escape in MMRd tumours in granular molecular detail, we set out to functionally study cancer and immune system interactions using mass cytometry profiling. We leverage a unique patient cohort with locally advanced MMRd CRC from the NEOPRISM-CRC trial (NCT05197322). Eligible patients are offered three cycles of neoadjuvant pembrolizumab before surgical tumour resection. Peripheral blood mononuclear cells (PBMCs) are collected and patient-derived organoids (PDOs) are isolated from pre- and post-treatment tumour samples. Patient-matched PDOs and tumour-stimulated PBMCs are co-cultured with and without pembrolizumab to recapitulate the immune micro-environmental niche in vitro. We carry out TOBis (Thiol-reactive Organoid Barcoding *in situ*) Mass Cytometry to characterise immune cell phenotypes and functional states of immune evasion and resistance to ICIs. These experiments are replicated in bulk and single cell cloned organoids to investigate the impact of (sub)clonal neoantigen complexity on immune response and pembrolizumab efficacy. We employ high-depth sequencing to map the neoantigen landscape of patient tumours and organoids.

Result and discussion

We have successfully established a collection pipeline and created a cohort of pre- and post-treatment matched PDOs alongside patient-matched PBMCs. We have generated a library of single cell cloned daughter organoids, >20 monoclonal organoid lines per parent bulk PDO. This approach allows in-depth dissection of the (sub)clonal neoantigen complexity and its impact on the anti-tumour immune response. Pilot co-culture experiments of PDOs and healthy donor PBMCs confirm the suitability of this platform to study tumour-immune cell interactions. TOBis Mass Cytometry will be used to capture shifts in cell cycle phases, immune cell phenotypes and cytotoxicity in the presence or absence of pembrolizumab. Corresponding fresh tumour samples were sequenced to interrogate the predictive value of neoantigen profiles of MMRd colorectal tumours. Data analysis is currently underway and results will be correlated with clinical outcomes and ICI response.

Conclusion

Our integrative approach provides a powerful framework to dissect immune evasion and ICI resistance in MMRd CRC, with potential implications for biomarker discovery and treatment optimisation.

EACR25-1473

TREM1 neutrophils drive multi-organ metastasis

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Introduction

Metastasis remains incurable, and there is an urgent need to identify biomarkers that can predict metastasis across different organs. Among metastatic lesions, neutrophils are one of the major immune infiltrates, and their presence is associated with poor prognosis and therapy resistance. Recently, there has been increasing recognition of neutrophil heterogeneity in solid tumors. However, the specific organs in which neutrophils are reprogrammed and the factors responsible for this reprogramming to promote metastasis remain poorly understood. In our lab, we identified that breast cancer (BC) patients with lung metastasis exhibited increased expression of Triggering Receptor Expressed on Myeloid Cells 1 (TREM1). TREM1 is primarily expressed in neutrophils in both humans and mice, and its bi-modal expression in tumors led us to investigate the functional heterogeneity of TREM1+ neutrophils.

Material and method

To explore this, we used well-established pre-clinical models of metastatic BC (E0771 orthotopic tumors and MMTV-PyMT GEMM, which closely mimic human breast cancer progression).

Result and discussion

We found that both experimental and spontaneous models of lung and liver metastasis triggered TREM1 expression in neutrophils. In contrast, full-body or neutrophil-specific deficiency of TREM1 inhibited metastasis growth in these organs. Next, to determine if tumor-derived factors are responsible for TREM1 upregulation, we co-cultured bone marrow neutrophils with conditioned media from BC metastatic cell lines. Tumor-derived factors induced TREM1 expression in vitro. We are currently screening for potential TREM1 ligands in vivo by analyzing the secretome of TREM1-deficient tumors and comparing it to wild-type (WT) tumors. TREM1 signaling is activated when its ligand binds to the receptor, amplifying inflammation. Thus, we hypothesize that TREM1-driven inflammation could be pro-metastatic. Indeed, TREM1 depletion impaired pro-interleukin-1 β (pro-IL-1 β) production in neutrophils. Interestingly, when using β -glucan to reprogram neutrophil progenitors, we observed that "trained" neutrophils exhibited reduced expression of TREM1+IL-1 β + pro-tumoral neutrophils. Since trained immunity reprograms the metabolism of hematopoietic progeny, we investigated the metabolic state of TREM1-deficient neutrophils. We found that TREM1-deficient neutrophils were fully mature, displayed increased OXPHOS metabolism and ROS production, but showed no differences in mitochondrial membrane potential compared to TREM1-sufficient neutrophils.

Conclusion

Taken together, our results suggest that TREM1 could serve as a potential biomarker for metastasis. We propose that TREM1 functions as a metabolic "rheostat" for neutrophils at metastatic sites, and that its activity may explain the heightened metastatic sensitivity of the lung through a TREM1-controlled regulatory axis involving glycolysis and IL-1 signaling.

EACR25-1511

The single cell landscape of intrahepatic NK cells in colorectal cancer metastasis: diversity and candidate therapeutic targets

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Introduction

Colorectal cancer is the third most frequent human malignancy characterized by high rates of hepatic metastasis and still lacks a curative approach. Patients with colorectal cancer liver metastases (CRLMs) have a 5-year overall survival between 20 and 45%, a recurrence of 60% and poor clinical benefits from immunotherapies. The liver is a preferential tissue for Natural Killer (NK) cells that are classified into two main subsets, namely CD56Bright/CD16- (CD56Bright) and CD56Dim/CD16+ (CD56Dim). Exploring the heterogeneity of intrahepatic NK cells beyond this dichotomy is key to identify specific immune checkpoints (ICs) and to unveil novel therapeutic targets.

Material and method

We conducted in depth single cell RNA-sequencing combined with multiparametric flow cytometry analysis of NK cells and total CD45+ immune cells. In addition, functional studies were performed to assess the relevance of immune-surveillance by NK cells and to leverage their anti-tumor potential against CRLMs.

Result and discussion

We demonstrated that tissue-resident CD56Bright NK cells in CRLMs are mature and potent anti-tumor effectors, showing minimal developmental relationship with intrahepatic CD56Dim and circulating NK cell subsets. In particular, unique transcriptional profile and peculiar expression of ICs were observed in CD56Bright and CD56Dim liver NK cell clusters, in line with their specific end-terminal differentiation programs in the metastatic niche. Among ICs, CXCR4 emerged as targetable inhibitory receptor involved in CXCL12-dependent mitigation of intrahepatic NK cells. CXCR4 negatively correlated with Interferon- γ (IFN γ) and increased production of this anti-tumor molecule was reported after CXCR4 blockade with the antagonist plerixafor. In addition, we identified IL-1R8 as an IC ubiquitously expressed by NK cells in CRLMs, demonstrating that its pharmacological inhibition with an original monoclonal antibody significantly improved NK cell anti-tumor effector functions.

Conclusion

In summary, the present study discloses the origin, heterogeneity and effector potential of matched circulating and intrahepatic NK cells from CRLM patients by using unbiased analytic approaches. It sheds light on an abundant tissue-resident IFN γ + CD56Bright NK cell population with an anti-tumor signature, and provides two possible therapeutic targets (i.e., IL-1R8 and CXCR4) to complement current CRLM immunotherapy.

EACR25-1513**POSTER IN THE SPOTLIGHT****Depleting tumour-infiltrating Tregs to enhance the efficacy of KRAS-mutant targeted therapies in lung cancer**

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Introduction

Non-small cell lung cancer (NSCLC) is the leading cause of cancer-related deaths, and around 15% of NSCLC patients present with a KRAS-G12C mutation. Recently, drugs selective against the KRAS-G12C mutant protein have been approved for clinical use. However, resistance development to these KRAS-G12C inhibitors (G12Ci) remains a prevalent and unresolved issue. Interestingly, although G12Ci only target mutant cancer cells, pre-clinical data show that they profoundly remodel the tumour microenvironment, diminishing the immunosuppressive myeloid compartment and increasing effector cytotoxic cells. However, this G12Ci-driven remodelling seems insufficient to drive durable responses in non-immune hot models, even when combined with anti-PD1.

Material and method

In this study, we make use of an immune-excluded orthotopic murine model of KRAS-G12C NSCLC and immunophenotyping approaches to explore potential mechanisms restraining the immunostimulatory potential of G12Ci.

Result and discussion

Here, we identify regulatory T cells (Tregs) as restrictors of an effective G12Ci-driven anti-tumour immune response. We show that treatment with G12Ci results in the rapid increase of tumour-infiltrating Tregs. Furthermore, we show that, in the proximity of Tregs, CD8 T cells lose their interactions with both dendritic cells and tumour cells, suggesting a disruption of the local cancer-immunity subcycle. Mechanistically, the elevation in Tregs was driven by an increase in CXCR3/CXCL9-11 signalling upon G12Ci treatment and was at least partially independent of external recruitment. Finally, we show that depleting tumour-infiltrating Tregs in combination with G12Ci was enough to re-invigorate the CD8 T cell response and drive long-term responses.

Conclusion

Overall, these results provide a strong rationale and showcase the potential for using Treg depleting strategies to improve the efficacy of G12Ci for the treatment of NSCLC.

EACR25-1534**Immune surveillance is actively impaired by the secretome of mismatch repair proficient colorectal cancers**

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Introduction

Patients affected by colorectal cancer (CRC) with DNA mismatch repair deficiency (MMRd), often respond to immunotherapies based on immune checkpoint inhibitors (ICI), while mismatch repair proficient (MMRp) tumors do not. It is widely accepted that the high mutational burden and the elevated neoantigen levels associated with MMR deficiencies are the main determinants of sensitivity to immune-based therapies. Here, we asked whether factors beyond the neoantigenic landscape could account for the lack of response of MMRp CRC to immune checkpoint blockade.

Material and method

We developed a model in which MMRd and MMRp CRC cells express equal levels of a given antigen recognized by engineered T cells. This system allowed us to functionally dissect whether the lack of T cell-mediated recognition in MMRp tumors stems from an intrinsic resistance to T-cell attack rather than solely from a low mutational burden. We evaluated immune activation, cytotoxic capacity, and the impact of tumor-derived soluble factors on antigen-specific immune responses.

Result and discussion

MMRp tumors consistently displayed reduced immune recognition and resistance to T-cell-mediated killing. Impaired immune activation correlated with a distinct biological profile, characterized by reduced expression of key effector molecules. Functional assessments showed that while MMRd tumors were efficiently eliminated by engineered T cells, MMRp tumors remained unaffected, highlighting an intrinsic resistance mechanism. Further analysis revealed that soluble factors secreted by MMRp cells actively impaired neoantigen-mediated recognition in MMRd CRC cells. Our data suggest that the MMRp-derived secretome interferes with T-cell binding to tumor cells, disrupting the formation of productive immunological synapses and thereby preventing effective tumor recognition and killing.

Conclusion

The presence of an immunogenic antigen is per se not sufficient to overcome the intrinsic resistance of MMRp tumors to immune checkpoint blockade. Our study demonstrates that MMRp tumors exhibit both intrinsic resistance to T-cell attack and active suppression of immune activation through secretome-mediated mechanisms. These findings reveal a previously unrecognized role of the tumor secretome in shaping immune evasion, highlighting potential therapeutic targets to restore T-cell function in MMRp CRC.

EACR25-1540**Integrative molecular and spatial analysis of intratumoral tertiary lymphoid structures: insights into immunotherapy response**

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Introduction

Intratumoral tertiary lymphoid structures (TLS) have been associated with improved outcome and response to immunotherapy in various cohorts of patients with cancer. Predominantly, mature TLSs (mTLSs) developing germinal centers represent privileged sites for local antigen presentation by dendritic cells, contributing to the generation of tumor-targeting CD8+ effector memory T cells. In line with this notion, further understanding of key quantitative and qualitative differences between mTLSs formation and progression in immune checkpoint inhibitor (ICI)-responsive vs ICI-resistant neoplasms may guide the development of alternative therapies in poorly responsive carcinomas, such as metastatic high-grade serous ovarian cancer (mHGSOC).

Material and method

We harnessed spatial transcriptomics Xenium *in situ* platform and 35-plex immunofluorescence PhenoCycler Fusion technology alongside with advanced digital pathology to characterize spatial interplay between immune components within early and mTLS structures within mHGSOC and NSCLC tumor samples.

Result and discussion

Specifically, mTLSs of mHGSOC are associated with an enriched population of naïve (IgD+MUM-CD38-CD20+) and PDL1+CD20+ B cells, suggesting impaired level of antigen presentation. Regarding this notion, the majority of CD8+ T cells within mTLSs of mHGSOC are associated with TIM3+PD1+, terminally exhausted and ICI-resistant T cell phenotype. The active immuno-suppression might be further amplified by the presence of tumor associated macrophages within invasive zone of mTLS of mHGSOC, which might negatively contribute to TLS propagation leading to cancer evasion.

Conversely, spatial B-cell profiling identifies patterns of *in situ* maturation and differentiation, characterized by abundance of mature CD79a+CD20+ B cells and CD138+CD19+ plasma cells signatures in mTLS of NSCLC. As such, mTLSs of NSCLC preserve and ICI-responsive TCF1+PD1+CD8+ T cells phenotype, suggesting a robust anti-tumor immune response within these aggregates with subsequent propagation within the tumor.

Conclusion

Integration of high-dimensional multiplex immuno-fluorescence technology with spatial transcriptomics provides a unique tool for characterization of TLS cellular composition and functional relevance in shaping anti-tumor immunity. Further understanding of cancer

driven differences in mTLS composition can lead to the development of immunotherapeutic interventions tailored to unique immune landscapes of particular malignancies.

EACR25-1578

Develop feeder-free differentiation of human hypoimmunogenic iPSCs into natural killer (NK) cells with cytotoxic potential for advanced hepatocellular carcinoma (HCC) treatment

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Introduction

Immunotherapy, particularly T cell-based therapies, has revolutionized cancer treatment, but faces challenges like severe adverse effects and high costs. NK cell-based therapy offers advantages such as safety and off-the-shelf feasibility. NK cells can identify and eliminate tumor cells without HLAs matching, reducing GvHD risk. Clinical-grade methods are producing large numbers of NK cells from various sources, with iPSC-derived NK cells showing promise due to their self-renewal ability and potential for gene editing. Hepatocellular carcinoma (HCC) is a common and deadly cancer with poor prognosis despite advanced treatments. HCC is immunologically cold, with lower NK cell numbers and function. Enhancing NK cell activity improves survival rates in HCC patients, and tumor-infiltrating NK cells predict better responses to sorafenib therapy.

Material and method

We developed a scalable NK cell production protocol from hypoimmunogenic iPSCs without feeder cells stimulation for clinical use. Hypoimmunogenic iPSCs formed embryoid bodies (EBs) and then differentiated into CD34+CD43+ hematopoietic progenitor cells (HPCs), followed by differentiated into CD56+CD3- NK cells (named iNK), using a combination of cytokines. Furthermore, we screened out specific nanobodies (Nbs) against B7-H3 (also known as CD276) from camelid gene library to equip iNK cells for targeting HCC cells.

Result and discussion

During the iNK differentiation period, we observed 2 to 10-fold cell increase in the number of iNK cells compared with iPSCs. After expansion, iNK cells became more homogenous and the purity of iNK cells can be up to 80%. More than 95% of cells expressed both CD45 and CD43. Expression of NKG2D and NKp44 was also high in iNK cells. Although the expression of CD16 was about 20%, iNK cells exhibited good cytotoxicity effect against HCC cell lines, HepG2. *In vitro* cytolytic ability of iNK cells was comparable to that of NK-92, an immortalized cell line exhibiting high activity of blood NK cells. On the other hand, we observed CD276 expressed higher lever in HCC compared to normal liver cells via western blot. And anti-CD276 Nbs can recognized the native CD276 epitope through flow cytometry method.

Conclusion

We established a feeder-free and scalable iNK differentiation platform from human hypoimmunogenic iPSCs for clinical use. In addition, we identified the anti-CD276 Nbs for enhancing the targeting of iNK cells. Using CD276-equipped iPSC-derived NK cells may hold significant potential for developing effective and accessible immunotherapies for HCC.

EACR25-1628

Mapping T Cell Diversity in Sarcoma

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Introduction

Sarcomas are rare and heterogeneous neoplasms originating from diverse mesenchymal tissues. Standard therapies often fail to cure advanced diseases, underscoring the need for novel, biologically informed treatment options. Sarcoma tumor cells form a complex ecosystem with non-cancerous cells in their micro-environment, which may create unique dependencies and potential therapeutic vulnerabilities. In particular, tumor-infiltrating T cells have emerged as potent immunotherapeutic targets through immune checkpoint inhibition. Additionally, chimeric antigen receptor T cell therapy is effective in certain sarcomas. However, response rates to these treatments vary greatly within and across sarcoma subtypes and the roles of T cells in sarcoma ecosystems remain poorly understood. An informed selection of sarcoma patients for immunotherapies remains challenging due to a lack of predictive biomarkers.

Material and method

Viable sarcoma tissue, non-cancerous tumor-adjacent tissue, and peripheral mononuclear cells from patients' blood were collected under written consent and ethics approval and dissociated into single-cell suspensions. Sarcoma-infiltrating T cell states were analyzed using high parametric spectral flow cytometry and a panel of 36 antibodies. Transcriptional profiling of T cells and T

cell receptor sequencing were performed using single-cell transcriptomics with simultaneous surface protein detection (CITE-Seq).

Result and discussion

We used a combination of spectral flow cytometry and CITE-Seq to generate a comprehensive single cell map of sarcoma infiltrating T cells. T cell profiling using spectral flow cytometry revealed phenotypically diverse T cell states in 118 sarcomas spanning 28 sarcoma entities. We identified activated and potentially tumor-reactive T cells, as well as PD-1+ exhaustion-like T cells. Single cell transcriptomics of selected sarcomas uncovered additional heterogeneity within T cell populations, suggesting differences in their functionality that may influence immune surveillance and therapeutic outcomes. T cell receptor sequencing demonstrated clonal expansion of distinct T cell clones, underpinning an active role in sarcoma ecosystems.

Conclusion

Our findings shed new light on the complexity of T cell states in sarcoma ecosystems and will be used to advance T cell-directed precision-medicine approaches for sarcoma therapy.

EACR25-1637

Exploiting Tumour Ablation to Augment Anti-cancer Immunotherapy in Hepatocellular Carcinoma

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Introduction

Hepatocellular carcinoma is responsible for 75–85% of primary liver cancers and arises from chronic liver disease. Many patients with chronic liver disease often do not qualify for surgery due to liver fragility and instead undergo ablative therapy. Examples of ablative therapies include stereotactic ablative radiotherapy, cryoablation, oncolytic virus treatment and histotripsy. These therapies can enhance the anti-tumour immune response, via the release of damage-associated molecular patterns (DAMPs) from ablated tumour cells, however the underlying mechanisms remain unclear. Despite the growing use of these techniques, there is limited understanding of the immune-stimulatory effect and limited guidance for clinicians. The aim of this project is to characterise tumour immunology post-ablation directly comparing each therapy.

Material and method

To assess the immune-stimulatory effects of each ablative therapy we used flow cytometry, ELISAs, and immune assays to quantify key DAMPs from ablated tumour cells and assess immune activation. Mature dendritic cells were incubated with tumour supernatants to evaluate activation and immune checkpoint markers. Next, we will experiment on primary tissue and conduct immunophenotyping of PBMCs from patients undergoing ablative therapies in clinical trials.

Result and discussion

Our findings reveal significant increases in key DAMPs, particularly DNA and heat shock protein 70, in the supernatant of ablated tumour cells. This increase correlates

with enhanced expression of the early activation marker CD69 on PBMCs. Furthermore, dendritic cells exposed to supernatants from cryoablated tumour cells displayed elevated expression of multiple activation and immune checkpoint markers, including CD80, CD86 and PD-L1, suggesting a role in modulating the anti-tumour immune response.

Conclusion

This project has shown a significant increase in DAMP release from cell lines, triggering effective immune cell activation. Next, primary patient tissue will be treated to assess DAMP release and immune cell stimulation. Alongside this, PBMC immunophenotyping from treated patients will highlight a potential immune response at the systemic level. Future comparisons of in vitro and patient-derived data will help define the molecular mechanisms of ablative therapies at the cellular, tissue, and systemic levels. This will help aid the design of early-phase clinical trials combining immunotherapy with ablative treatment for HCC treatment.

EACR25-1674

NKG2A inhibition promotes NK cell-CD8+ T cell interactions in support of improved anticancer immunity in ovarian carcinoma

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Introduction

Immune checkpoint inhibitors (ICIs) have demonstrated remarkable activity in a subset of patients with solid malignancies, especially in the context of abundant tumor infiltration by CD8+ cytotoxic T lymphocytes (CTLs) – and consequent expression of PD-L1 by neoplastic and myeloid cells – at baseline. While natural killer (NK) cells have also been shown to control oncogenesis and tumor progression, at least in some oncological settings, their phenotypic and functional heterogeneity and its impact on ICI sensitivity remains incompletely understood.

Material and method

Here, we harnessed a variety of transcriptomic, spatial and functional assays to characterize the phenotypic and functional properties of NK cells in ICI-sensitive and ICI-resistant solid carcinomas.

Result and discussion

We demonstrate that advanced non-small cell lung carcinoma (NSCLC), which is generally considered an immunologically “hot” tumor – contains elevated levels of intratumoral cytotoxic CD56dim NK cells that (1)

express high levels of NKp46 and CD57, (2) exhibit patterns of in situ activation associated with mature tertiary lymphoid structures (mTLSs), and (3) correlate with favorable prognosis. Conversely, the NK cell compartment of immunological “cold” tumors such as high-grade serous ovarian carcinoma (HGSOC) – which generally contains immature TLSs and ICI-resistant TCF1-TIM3+CD8+ CTLs – is enriched in functionally exhausted CD56bright NK cells expressing high levels of TIM3 and NKG2A. In this latter context, while PD1 blockers fail to restore anticancer immunity, targeting NKG2A results in improved NK cells cytotoxicity coupled with the (at least partial) recovery of CD8+ CTL functions. Consistent with this notion, an NKG2A blocker positively interacted with a PD1 inhibitor in a syngeneic model of ovarian cancer.

Conclusion

Collectively, our findings delineate key phenotypic and functional differences in the NK cell compartment of ICI-responsive vs ICI-resistant tumors that may be harnessed therapeutically to restore anticancer immunity in the context of a functional interaction between NK cells and CD8+ CTLs.

EACR25-1727

PARP inhibitors as immune modulators in metastatic ovarian cancer therapy

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Introduction

Epithelial ovarian carcinoma (EOC) is one of the top five causes of cancer-related death in women. Most patients with EOC achieve initial remission after primary or interval cytoreductive surgery combined with platinum-taxane chemotherapy. However, mutations in BRCA1 or BRCA2 genes, which lead to homologous recombination (HR) defects, play a crucial role in platinum sensitivity and justify the use of poly (ADP-ribose) polymerase (PARP) inhibitors as maintenance therapy, commonly linked to extended progression-free survival in EOC. Besides their direct cytotoxic and cytostatic effects, PARP inhibitors (PARPi) have shown significant immunostimulatory properties by disrupting DNA repair in cancer cells, and opening possibilities for synergy with immune checkpoint inhibitors (ICIs).

Material and method

We harnessed a variety of transcriptomic, spatial and functional assays to characterize the immunomodulatory effects of PARPi on immunological configuration of experimental BR5BRCA1/- syngeneic mouse model of ovarian cancer and human EOC tumor samples.

Result and discussion

Our finding demonstrates, that PARPi increase the mutational burden in EOCs due to unresolved DNA damage and releases of damage-associated molecular patterns (DAMPs), thereby increasing T cell infiltration within tumor microenvironment. In addition, PARPi

appear to promote potent type I interferon (IFN) secretion through the activation of cGAMP synthase and the stimulator of the interferon genes (STING) pathway. In combination with ICIs, PARPi showed a beneficial effect on the balance between adaptive anti-tumor immunity and innate myeloid components, leading to an improved cytotoxic T-cell response, as observed in mouse models and HGSOC tumor samples.

Conclusion

These observations emphasize the role of strategically designed combinations of PARPi and immunotherapeutic agents, which could be the key to overcoming immunosuppression in the EOC microenvironment, thereby improving clinical outcomes.

EACR25-1742

Integration of Bacterial and Fungal Microbiota and Lipidomic Data in Consecutive Saliva and Stool Samples of Lung Cancer Patients

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Introduction

Microbiota-targeted biomarkers are emerging as promising non-invasive tools for the early detection of cancers. Increasing evidence suggests that alterations in microbiota can lead to significant disruptions in lipid metabolism. This study aims to investigate the oral and intestinal microbiome, lipidome, and their interactions, which hold substantial potential for providing clinical guidance.

Material and method

To achieve this, lipid profiles, as well as prokaryotic and eukaryotic microorganisms, were determined by collecting consecutive stool and saliva samples from lung cancer (LC) patients and healthy controls (HC) at three time points: pre-treatment, and at 6- and 12-months post-treatment. In all these samples, the 16S rRNA and 18S rRNA genes for bacteria and eukaryotes, respectively, were sequenced with an oxford nanopore platform. Lipid profile was investigated using nanoflow ultra-high performance liquid chromatography-electrospray ionization-tandem mass spectrometry. All data were evaluated and integrated by bioinformatics analysis.

Result and discussion

18S rRNA analyses indicated that stool samples from LC patients had significantly higher evenness and diversity, with comparable OTU counts to HC. Saliva samples from LC patients showed a higher OTU count but similar evenness and diversity. In contrast, 16S rRNA analysis

revealed lower OTU counts in both stool and saliva samples from LC patients compared to HC; however, OTU counts increased over time in consecutive samples from LC patients. Beta-diversity analysis showed no significant differences in microbiota composition between HC and LC patients, although initial and second samples from LC patients exhibited similar clustering patterns. 18S rRNA analyses revealed that Ascomycota was less abundant, while Mucoromycota was more abundant in the saliva of LC patients compared to HC; the opposite was true for stool samples. Pichia kudriavzevii was less abundant, while Carcinomyces was more abundant in saliva samples from LC patients. 16S rRNA analyses showed that Firmicutes were significantly more abundant in both samples from LC patients, while Euryarchaeota were less abundant in saliva. Streptococcus and Blautia were more abundant in both samples of LC patients, respectively, while Levilactobacillus was more abundant in stool from HC. Lipidomic analysis revealed increased diacylglycerol levels in saliva, potentially due to bacterial stimulation. Ceramide in LC patients correlated positively with Firmicutes, a correlation not seen in HC. Phosphatidylserine, found in saliva, may serve as a biomarker for LC, with significantly reduced levels in LC patients and a correlation with gram-negative bacteria.

Conclusion

This study demonstrates that microbiota may play critical roles in cancer development through their impact on lipid metabolism; however, further research is needed to explore this relationship in greater depth.

Supported by TUBITAK-120N924.

EACR25-1758

Making 'cold' tumors 'hot': ERAP1 modulation as an innovative strategy to enhance tumor immunogenicity

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Introduction

Although immune checkpoint blockade (ICB)-based immunotherapy has led to remarkable clinical results across various cancer types, its efficacy remains limited to a subset of patients. This underscores the urgent need to explore novel strategies to enhance therapeutic responses and expand the pool of responders. In this context, we investigated the modulation of ERAP1, a key enzyme in the antigen processing and presentation pathway. While loss of this enzyme activity is frequently correlated with reduced in MHC-I surface expression, which could contribute to tumor immune evasion,

impairment of ERAP1 expression in tumor cells has recently been shown to improve ICB efficacy in a murine neuroblastoma model. Therefore, ERAP1 represents an intriguing target for enhancing antitumor immune responses. Here, we examined how ERAP1 influences the tumor microenvironment (TME) in both ‘cold’ or immune excluded and ‘hot’ or immune inflamed murine melanoma models.

Material and method

Two murine melanoma cell lines expressing ERAP1 were genetically engineered using the CRISPR/Cas9 machinery targeting the ERAP1 gene to knock-out the enzyme. The efficacy of the gene editing was confirmed by Sanger sequencing and the loss of ERAP1 at the protein level by western blot. To understand to which extent ERAP1 inhibition impacts antigen presentation and immune responses, we performed peptide-dependent stability assays, immunopeptidomics, bulk RNA-seq analyses, ex vivo co-culture assays and in vivo studies. The tumors derived from the in vivo studies were further analyzed by flow cytometry and spatial proteomics to characterize the immune compartment of the tumor microenvironment.

Result and discussion

Our findings reveal that ERAP1 inhibition increases the susceptibility of melanoma cells to immune-mediated killing by altering the stability of peptide-MHC-I complexes and reshaping the tumor immunopeptidome. This, in turn, enhances the activation of T cells and NK cells. In ‘hot’ melanoma tumors, ERAP1 inhibition leads to tumor growth reduction by recruiting activated NK cells. In contrast, while ERAP1 inhibition alone is not sufficient to control tumor growth in ‘cold’ melanoma, it significantly reshapes the immune landscape by increasing immune cell infiltration and enhancing the recruitment of cytotoxic T cells into the tumor core.

Conclusion

These results support ERAP1 inhibition as a promising strategy for converting ‘cold’ tumors into ‘hot’ tumors, reinforcing its potential as a safe and effective approach for combination with immunotherapies. Additionally, they highlight the importance of identifying predictive biomarkers of response to ERAP1 inhibition.

EACR25-1759

A Nanopore Based Approach for Detection of *Fusobacterium nucleatum* Subspecies in Colorectal Cancer Tissue

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Introduction

The common oral bacteria *Fusobacterium nucleatum*, has long been associated with colorectal cancer – a leading cause of cancer death worldwide. Traditionally, *F. nucleatum* has been divided into four subspecies, namely *F. nucleatum* subspecies *animalis*, *F. nucleatum* subspecies *polymorphum*, *F. nucleatum* subspecies *vincentii* and *F. nucleatum* subspecies *nucleatum*. Recently, suggestions have been made to reclassify the four into separate species. However, accurately distinguishing between them remains challenging, especially utilizing

partial 16S regions. To address this problem, we have developed a Nanopore full length 16S sequencing workflow, which enables reliable classification of the four subspecies.

Material and method

Our method allows multiplexing of 192 samples on one flow-cell. Further, we have refined the initial PCR step using a high-fidelity polymerase. We also developed a novel demultiplexing tool which allows reuse of forward and reverse barcodes. For species classification, we provide a custom bioinformatic pipeline, using a comprehensive and curated 16S database.

Result and discussion

Our method successfully differentiates the four subspecies of *F. nucleatum* with a high sensitivity. To confirm the reproducibility of our method, we used a positive control consisting of a known microbial community mixed with human cells, which shows consistent results across sequencing runs. Given the strong association between *F. nucleatum* subspecies *animalis* and colorectal cancer, our method is well suited for detecting bacteria in clinical samples with low bacterial content and high presence of host DNA, e.g. patient fresh frozen colorectal tissue.

Conclusion

We present a full length Nanopore 16S sequencing workflow, from wet to dry lab, offering a cost effective and sensitive approach for detecting *Fusobacterium* subspecies, as well as other species. Given *F. nucleatum*'s link to colorectal cancer, our method enables studying its role and clinical implications. Given that the method is eligible for samples with low microbial content, it is well suited for examining bacteria in tissue samples from patients.

EACR25-1852

Teneurin-4 as a key player in cancer progression and target for gene-based vaccines to halt tumor growth and progression in preclinical models

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Introduction

Cancer Stem Cells (CSC) play a crucial role in sustaining tumor growth and progression, highlighting the urgent need to identify novel functional targets expressed on CSC. By comparing the transcriptomes of differentiated Triple Negative Breast Cancer (TNBC) cells and CSC, we identified upregulated transmembrane proteins as promising candidates for cell- and antibody-based therapies. Among these, Teneurin-4 (TENM4), emerged as a compelling target due to its elevated expression in TNBC patients with poorer prognosis. This study aims to

unveil new molecular mechanisms in cancer biology and validate TENM4 as a therapeutic target across different tumor types.

Material and method

TNBC, Colorectal Cancer (CRC) and Neuroblastoma (NB) cells were silenced using RNAi and CRISPR/Cas9 technology. The role of TENM4 in self-renewal and migration was assessed in vitro through tumorsphere-forming ability assay and transwell invasion assay. Tumor growth and lung metastasis were evaluated following the subcutaneous injection of TENM4-proficient and deficient cells. Moreover, a retrospective analysis (n=59) evaluated the correlation between TENM4 expression and patient prognosis. Finally, the immunogenicity of a DNA-based vaccine against TENM4 was tested by Flow cytometry and ELISPOT assay.

Result and discussion

Our results demonstrate that TENM4 plays a crucial role in CSC self-renewal, migration, and invasion in TNBC, CRC, and NB. Moreover, we demonstrated TENM4 role in vivo in CRC growth and in TNBC metastasis, associated with fewer circulating tumor cells in mice injected with TENM4-deficient cells compared to controls. Additionally, we also demonstrated a decreased phosphorylation of FAK, AKT, ERK and a reduced expression of MMP9, suggesting an involvement of TENM4 in Extracellular Matrix (ECM) remodeling, potentially explaining the involvement of TENM4 in metastasis. Further data also suggest a role of TENM4 in TNBC chemoresistance, potentially involving endoplasmic reticulum stress and autophagy pathways. The clinical relevance of TENM4 in TNBC is underscored by the increase recurrence rates in TNBC patients with tumors with a higher TENM4 expression. Furthermore, TENM4 targeting via gene- and virus-based vaccination resulted safe and effective in eliciting anti-TENM4 response. Different experiments are ongoing to evaluate the efficacy of TENM4 targeting.

Conclusion

Overall, these data identify TENM4 as pro-tumorigenic in TNBC, CRC and NB, playing a role in stemness, migration, chemotherapy and metastasis. Moreover, the immunogenicity of the vaccination against TENM4 point out the possibility to use TENM4 as a valuable immune-target in different tumor subtypes.

EACR25-1862

Eosinophil deactivation by the breast tumor microenvironment can be reversed by interferons

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Introduction

Eosinophils, granulocytes mainly studied in the context of allergy and helminth infection, were recently

recognized as important players in the response to immune checkpoint blockade (ICB) in breast cancer. However, in contrast to colorectal or lung tumors, depletion of eosinophils or induction of eosinophilia in murine models of breast cancer does not impact tumor progression. These data indicate that the breast tumor microenvironment (TME) can evade eosinophil-mediated cytotoxicity, although the underlying mechanisms remain poorly understood.

Material and method

Using NT193, E0771 and 4T1 murine syngeneic models of breast cancer, we explored phenotypical and functional heterogeneity of eosinophils in TME by spectral flow cytometry and bulkRNA sequencing of sorted subpopulations. Subset-specific signatures from transcriptomic analysis were a) validated on protein level and b) recapitulated in vitro, through development tracking of bone-marrow derived eosinophils and cytokine stimulation. Furthermore, predicted cell killing abilities were tested ex vivo and in vitro. Finally, we examined the impact of ICB on eosinophil subset regulation in NT193 treatment-responsive tumors.

Result and discussion

We identified two novel subsets of tumor-associated eosinophils. In the bone marrow and blood eosinophils express relatively high levels of Ly6C receptor, however they gradually lose the expression in TME during tumor progression, resulting in Ly6C+ and Ly6C- eosinophil populations. This phenotypic change correlates with higher tumor volumes and reduced tumor cell killing ability. Moreover, Ly6C+ eosinophils are more responsive to interferons and upregulate pathways involved in activation of adaptive immunity. We show that Ly6C+ eosinophils represent a long-lived population that naturally differentiates into Ly6C- deactivated state. This transition can be prevented and partially reversed in presence of interferons. Mice bearing NT193 tumours were treated with anti-PD-L1 to induce an interferon-rich TME, and whilst the proportion of Ly6C+ eosinophils was unchanged, these cells overexpressed Ly6C and shifted towards higher expression of MHC-I.

Conclusion

Our data provides new evidence that eosinophils are a highly plastic population that are shaped into a less active state by the progressing breast TME. We present an interferon-guided mechanism by which eosinophils escape differentiation and restore a more active Ly6C+ phenotype. This model could explain why depleting eosinophils in treatment-naïve breast cancer does not result in tumor regression, while re-activated eosinophils play an important role in responses to ICB.

EACR25-1872

Non-haematopoietic cell drivers of immune-dysfunction in Hepatocellular carcinoma

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Introduction

In 2020, Hepatocellular carcinoma (HCC) was the sixth most frequent cancer and third leading cause of cancer-related deaths worldwide. Many factors contribute to the development of HCC, including viral infection, alcohol intake, metabolic-associated disease, autoimmunity and genetic liver disorders. Despite the differences in these underlying causes, HCC develops in the context of chronic inflammation resulting in non-haematopoietic cell and tissue alterations. This opposing role of the immune system in both the development and control of chronic liver disease and HCC is not fully understood. A better understanding of this intricate network of cell interactions and tissue regions is required to enhance the efficacy of current therapeutic options.

Material and method

We evaluated the tumour microenvironment (TME) using Imaging Mass Cytometry (IMC) on tumour and non-tumour tissue specimens from 16 advanced HCC patients and single-cell RNA sequencing on cells from tumour and non-tumour tissue as well as paired peripheral blood mononuclear cells from 10 HCC patients

Result and discussion

Single-cell RNA sequencing identified a CD34+ cancer-associated fibroblast (CAF) cell population that was present in higher proportions in HCC tumour tissue when compared to non-tumour tissue. Using IMC, we quantified and spatially mapped CD34+ inflammatory CAF cell-interactions in both non-tumour and tumour tissue from HCC patients to determine its potential role in HCC development.

Conclusion

The development and progression of liver disease is underpinned by an inflammatory cascade that results in aggressive CAFs. Specific cell signatures in the liver tissue (non-tumour and tumour), unique to this cascade can be used to identify patients at risk of disease progression to HCC.

EACR25-1891

Transcripts containing Retrotransposable Elements play a regulatory role in modulating the functions of Tumor Infiltrating Lymphocytes

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Introduction

Unravelling Tumor Infiltrating Lymphocytes (TIL) heterogeneity is essential to develop more effective immunotherapies. We have recently highlighted the role of LINE1, the predominant class of Retrotransposable Elements (RE), in regulating TIL exhaustion by integrating transcripts related to T cell activation and downregulating their canonical isoforms. Nevertheless, the role of RE expression as a determinant of TIL diversity and function remains poorly explored.

Material and method

We have isolated CD3+ and CD45+ T cells from tumor resections and normal adjacent tissues of Non-Small Cell Lung Cancer (NSCLC), Colorectal Cancer (CRC) and Esophageal Adenocarcinoma (EAC) patients. Due to the challenging task of studying transcripts containing RE with current single-cell technologies, we performed both long- and short-read scRNA-seq. We have developed a custom pipeline to analyze long-read data to generate a pan-cancer Atlas of RE-transcripts that we quantified at isoform level using full-length short-read data.

Recognizing that TIL phenotypes are plastic and dynamic, we have introduced a novel strategy of integrating dimensionality reduction performed on novel RE-transcripts with trajectory inference tools.

Result and discussion

We described approximately 50,000 novel transcripts that are specific to NSCLC, CRC and EAC, with some shared instances. Almost 46% of them included REs, resembling their genomic distribution. Trajectory inference analysis confirmed the positive correlation between LINE1 and exhausted TILs, but it also revealed an enrichment of Alu-containing transcripts in active T cells, while regulatory T cells (Treg) and follicular T helper cells (Tfh) mostly expressed transcripts containing the LTR class. Our results point into the direction of a strong connection between immune phenotypes and RE-transcripts expression. To elucidate the relevance and function of RE-transcripts in TIL identity, we will leverage CRISPR-Cas13 system to specifically modulate specific RE-transcripts and identify potential non-coding regulators.

Conclusion

Long-read sequencing technologies are revolutionary for the evaluation of RE-containing transcripts, that are often understudied because of the repetitive and interspersed nature of retrotransposons. By the integration of cutting-edge sequencing technologies and computational workflows, this project unveil the biological relevance of RE-transcripts in TILs identity. In doing so, it also introduces the innovative concept that RE-transcripts may serve as novel immunological checkpoints and a promising avenue for cancer therapy.

EACR25-1895

b2-microglobulin interaction with HLA class I influences the antigenic landscape and the presentation of cancer associated peptides

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Introduction

B2-microglobulin (B2m) is the light chain of the human leukocyte antigen I, and despite being a well-known molecule of the peptide loading complex (PLC), the granularity of its impact on the antigenic landscape of human cells is not well known. Different attempts have been carried out to understand the role of B2m, mainly regarding its modulatory affinity to the heavy chain (HC) and its importance in stabilising the peptide HLA-I complex, promoting HLA-I recycling and preventing degradation of the heavy chain. Here, we aim to investigate how the interaction of B2m with the HLA-HC changes the quality of the peptide repertoire and the HLA-I ability to present endogenous and exogenous cancer-related epitopes.

Material and method

We transduced K562 and C1R cell lines with two different constructs of the HLA-A*02:01 allele, either coding for the HLA-HC only, in which case the HLA-I assembly relies on the endogenous expression of B2m, or HLA-HC fused to B2m by a linker. We then analysed the characteristics and features of the entire peptide repertoire presented by the HLA-I molecules. We also investigated the efficiency of these cell lines to present exogenous synthetic peptides pulsed, peptides derived from the proteasomal degradation of endogenous antigens, and peptides derived from the cross-presentation of exogenous antigens. For this, we used an indirect system of detection consisting in GFP-reporter Jurkat cells transduced with TCRs that recognise 4 epitopes of interest derived from cancer-related antigens, NRAS, KRAS and PMEL.

Result and discussion

Results and discussion: When we analysed the entire peptide repertoire by eluting the HLA-I immunopeptidomes, we found that the antigenic landscape of cell lines with B2m-HC fusion had been greatly impacted. We then computed the distribution of the predicted binding affinity of the identified peptides and found that these cell lines presented an overall weaker peptide repertoire, as the median of the predicted binding affinity of all the immunopeptidome was significantly higher compared to cell lines without the B2m-HC fusion. We observed how this system hindered the presentation of strong peptide binders derived from the proteasomal degradation of endogenous antigens by co-transducing monoallelic cell lines (4 different alleles) with minigene sequences that expressed known epitopes and observing higher presentation of these epitopes in cell lines without B2m-HC fusion. Finally, we confirmed this effect in a cross-presentation experimental setting where cell lines with and without the B2m fusion differentially presented known epitopes.

Conclusion

Overall, results suggested that fusing B2m to the HLA-HC appears to be enough to escape the ER quality control mechanisms and present poorly loaded HLA-I molecules, which impinges upon the presentation of strong peptide

binders that could potentially be therapeutic immunogenic targets.

EACR25-1898

Exploring the dual role of Toll-like receptor 2 in tumor cell autonomous and microbiota-driven chemoresistance in breast cancer

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Introduction

Besides immune cells, Toll-Like Receptor (TLR)2 is also expressed on breast cancer (BC) cells, correlating with poor prognosis and chemoresistance in patients. Indeed, chemotherapy (CT) induces DAMPs release by damaged cells, including TLR2 ligands. In addition, CT induce dysbiosis, increasing PAMPs release that may activate immune receptors like TLR2. We investigated the TLR2-mediated mechanisms in BC progression and chemoresistance and whether the breast microbiota composition influences CT response via TLR2 activation.

Material and method

Combination of TLR2 inhibitor CU-CPT22 and CT were tested both in vitro and in vivo in a 4T1 BC model.

Mammary dysbiosis was induced in BALB/c mice via antibiotics local administration. WT or TLR2-silenced 4T1 cells were injected into the dysbiotic mammary gland, and mice were treated with doxorubicin. Tumor growth was monitored, and tumor samples were analyzed for microbial composition by 16S rRNA sequencing. BC patients undergoing or not neoadjuvant CT were recruited, to collect both pre-operative biopsies and surgical resection samples for intra-tumoral microbiome analysis by 16S rRNA sequencing.

Result and discussion

TLR2 blockade increased tumor cell sensitivity to doxorubicin in vitro and in vivo, indicating that TLR2 targeting could enhance CT efficacy. Mammary dysbiosis exacerbated tumor progression and chemoresistance in 4T1 tumor-bearing mice, suggesting a potential role for dysbiosis in these mechanisms. Silencing TLR2 within the tumor abolished this effect, indicating that TLR2 expression by tumor cells may drive dysbiosis-dependent chemoresistance. Preliminary analysis of patient tumor samples revealed that CT can drastically alter intra-tumoral microbial composition. Notably, Sphingobacteriales and Lactobacillales were enriched in CT-treated patients compared to patients that did not receive CT.

Conclusion

TLR2 promotes BC progression and chemoresistance, and its inhibition restores BC sensitivity to chemotherapy. Furthermore, antibiotic-induced mammary dysbiosis promotes tumor progression and enhances chemoresistance in 4T1 tumor-bearing mice. However,

silencing TLR2 in tumor cells reverses these effects, suggesting that altered PAMPs release within the dysbiotic mammary gland binds to TLR2, exacerbating tumor growth and chemoresistance. Moreover, CT can reshape the intra-tumoral microbiome toward a more tumor-promoting composition, as suggested by the enrichment of Lactobacillales, linked to tumor growth in gastric cancer, and Sphingobacteriales, which secrete atypical lipopolysaccharides capable of activating TLR2. Further RNA sequencing will provide insights into chemoresistance mechanisms and TLR2-related pathway activation in relation to altered microbiome composition. These findings pave the way for therapeutic strategies combining microbiome modulators with TLR2-targeting approaches to enhance BC treatment efficacy.

EACR25-1915

Deciphering the complex relationship between diet, the gut microbiota and anti-tumour immunity

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Introduction

Diet and the gut microbiota are key factors that regulate systemic immune homeostasis. Diet is therefore closely linked to the capacity of the microbiota to regulate anti-tumour immunity during immune checkpoint inhibitor (ICI) immunotherapy. We therefore sought to use differing dietary contexts to assess the impact of long-term pre-established immune homeostasis on anti-tumour immunity.

Material and method

To model a healthy balanced diet versus a poor unbalanced diet, C57BL/6 mice were fed either a standard chow (CD) or high fat (HF)/high sugar (HS) diet for 6–8 weeks prior to inoculation with a syngeneic cancer cell line and the impact on tumour growth and responsiveness to ICI immunotherapy assessed. Microbiome samples were analysed using 16S rRNA gene sequencing. Immune populations across the mesenteric lymph nodes (MLN), blood (PBMC), spleen, tumour draining LN and tumour were profiled using mass/spectral flow cytometry. Oral glucose tolerance tests were also performed.

Result and discussion

Prior to tumour inoculation, mice on CD versus HF/HS long term have distinct gut microbial communities and immune repertoires across all sites. CD mice were enriched in fibre-utilisers/SCFA producers and associated with greater frequencies of ROR γ T+ Tregs in the MLN. In contrast, HF/HS mice were enriched with intestinal mucin degraders and bile tolerant microbes and had higher overall B cell frequencies and increased PD-1 and Ki67 expression across the B and T cell compartments. Surprisingly despite these differences, no differences in tumour growth or responsiveness to ICIs were observed

across 5 different melanoma cell lines of varying genetics and immunogenicity (YUMM1.7, YUMMER1.7, YUMM3.3, YUMM3.3-UVR, B16-F10). We postulated that the metabolic impacts of diet components, such as fat source and fatty acid composition, may be one aspect that plays an important role. We thus sought to compare the impact of differing high fat sources (coppa vs lard) on systemic metabolism and tumour growth. After only 2 weeks on diet, HF-Lard mice exhibited impaired glucose tolerance and reduced insulin sensitivity compared to CD and HF-Coppa mice. Notably, HF-Lard significantly accelerated the growth of YUMM3.3-UVR tumours. In contrast, both HF diets accelerated the growth of MC38 colorectal adenocarcinomas, and no differences were observed between diets for B16-F10 tumours in this short-term dietary context. This suggests that dietary fat source and its impact on metabolism, as well as tumour type/genetics are important factors in how the diet-gut axis influences tumour growth.

Conclusion

Together this highlights the complex relationship between diet, the gut microbiota and anti-tumour immunity. Ongoing work will provide valuable insight into the impact of different fat sources and diet durations on metabolism, the gut microbiota and anti-tumour immunity during ICI treatment and ultimately inform the design of more specific dietary interventions.

EACR25-1924

In-silico functional profiles of anaerobic bacteria in prostate cancer

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Introduction

Prostate cancer is among the most common cancers globally (1 in 8 men in the UK will be diagnosed in their lifetime). Major risk factors include age, ethnicity, and family history. Microbial infections contribute to many cancers, and the prostate microbiome influences prostate cancer. Identifying the Anaerobic Bacterial Biomarker Set (ABBS) bacteria – *Fenollarria*, *Ezakiella*, *Peptophilus*, *Anaerococcus*, *Porphyromonas*, and *Fusobacterium* – may help predict aggressive prostate cancer at diagnosis. ABBS-positive samples are linked to more aggressive disease, and the mechanisms of actions of these bacteria are important to investigate.

Material and method

Whole genome sequencing data from 650 tumour samples in the Pan-Prostate Cancer Group (PPCG) were analysed for microbial content. Unmapped reads were quality-trimmed, human-depleted, and classified using Kraken. The metabolic profiles of ABBS-positive and negative samples were assessed using HUMAnN (v3.7). Gene families and pathways were analysed with Maaslin2 (v1.12.0) in R (v4.2.0).

Result and discussion

ABBS-positive tumours had 888 significantly enriched gene families and 336 metabolic pathways, including genes for tRNA synthetases for all 20 amino acids and methionine synthesis/salvage pathways. This was

significantly higher than the 75 gene families and 17 pathways found in ABBS-negative tumours. Key ABBS-associated pathways include methionine salvage/synthesis and tRNA charging. Cancer cells rely heavily on external methionine (Hoffman effect) and arrest in the S/G2 cell cycle phase under methionine restriction. Methionine metabolism may be a core feature of cancer development. tRNA charging, facilitated by aminoacyl-tRNA synthetases (ARSs), enables peptide bond formation. Aberrant ARS expression is linked to the transformation from benign to malignant cells, with ARS-encoding genes exhibiting expression profiles similar to Cancer-Associated Genes (CAGs) in 10 cancers.

Conclusion

These findings suggest microbial metabolism may contribute to prostate cancer progression when ABBS bacteria are present. Future work will look at mutational profiles in the PPCG that are associated with ABBS positive tumours, identifying additional bacteria with a similar metabolic profile and investigating the relationship of enzymes and pathways affected in ABBS positive tumours with long term survival in ABBS positive patients.

EACR25-1926

Unlocking Immune Resistance: The Role of PIRB Deletion in Pancreatic Cancer Therapy

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Introduction

Paired Immunoglobulin-like Receptor B (PIRB) is the murine ortholog of the human Leukocyte Immunoglobulin-like Receptor B (LILRB) 2 and 3, immunological receptors with diverse tissue-specific functions. These receptors are physiologically expressed by hematopoietic cells, such as macrophages, granulocytes, dendritic cells, and B lymphocytes. Thanks to their intracellular Immunoreceptor Tyrosine-based Inhibitory Motives (ITIM), these molecules act as negative receptors, physiologically preventing premature activation and exaggerated responses. In addition, they have been associated with an immunosuppressive behavior of various cell types. Recently, these receptors have also been reported to be highly expressed by cancer cells, where they directly support their aggressive behavior, aerobic glycolytic metabolism, and metastatic spread.

Material and method

To better characterize its role in modulating the anti-tumoral immune response in models of pancreatic ductal adenocarcinoma (PDA) and to assess the capacity to elicit immunological memory, non-proliferating ovalbumin (OVA)-expressing PDA cells were inoculated subcutaneously into C57/BL6 WT and Pirb^{-/-} mice. After three weeks, mice were challenged with live cells, and tumor growth was measured biweekly. At time of sacrifice, ELISPOT, cytotoxicity, and ELISA assays

were performed to investigate both cellular and humoral responses. The intrinsic pro-tumoral role of PIRB when expressed by PDA cancer cells was investigated through the generation of a Pirb^{-/-} murine PDA cell line (K-PIRB) and the assessment of its proliferative and invasive behavior with a MTT, wound healing, and soft agar clonogenic assay. Pirb^{-/-} cells' sensitivity to both pro-tumoral Transforming Growth Factor (TGF) β and anti-tumoral Interferon (IFN) γ was assessed with RT-PCR and FACS analyses.

Result and discussion

The absence of PIRB significantly improved immunization against the well-known experimental antigen OVA. Indeed, immunized Pirb^{-/-} mice showed a reduced growth of subcutaneous living tumor cells, together with an enhanced number of IFN γ -secreting T cells and higher levels of OVA-specific IgG. In addition, K-PIRB cells displayed a significantly slower growth, proliferation, and migration properties in comparison to wild-type counterpart. In line with this, K-PIRB cells grew significantly less when injected orthotopically into both wild-type and Pirb^{-/-} mice. Furthermore, K-PIRB cells were less responsive to the pro-metastatic TGF β , while increasing their FAS and MHC-I levels upon stimulation with IFN γ .

Conclusion

Overall, PIRB represents a promising target not only to enhance the anti-tumor immune response, but also to directly impact on PDA growth and aggressive behavior. Further analyses will assess its clinical transferability and its potential synergistic effect when combined with approved therapies.

EACR25-1953

Harnessing Mitophagy in Tumor-Associated Macrophages to Enhance Anti-Tumor Immunity

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Introduction

Tumor-associated macrophages (TAMs) and tumor cells are crucial in creating an immunosuppressive tumor microenvironment (TME) through metabolic programming. Understanding the metabolic changes within the TME and the impact of tumor-derived metabolites on the functional phenotypes of TAMs is essential for the development of effective cancer therapies. Recent studies highlight enhanced lipid metabolism in TAMs, which is linked to their protumorigenic functions. However, the mechanisms by which lipid metabolism shapes TAM phenotypes and functions during tumor progression remain inadequately understood.

Material and method

In our study, we found that tumor-derived lipids lead to increased lipid content and enhanced oxidative phosphorylation in TAMs. Simultaneously, we observed elevated levels of mitochondrial reactive oxygen species (ROS) and signs of mitochondrial dysfunction in these cells. Notably, TAMs displayed increased mitophagy activity, a selective autophagic process that removes damaged mitochondria.

Result and discussion

We hypothesize that excessive lipid accumulation or impaired fatty acid oxidation results in mitochondrial stress in TAMs. The upregulation of mitophagy appears to be a mechanism through which TAMs ensure their survival and maintain pro-tumorigenic functions despite mitochondrial dysfunction. Moreover, inhibiting X-mediated mitophagy may disrupt mitochondrial fitness, subsequently affecting the pro-tumorigenic phenotype of TAMs and potentially leading to reduced tumor progression.

Conclusion

These findings suggest that targeting mitophagy in TAMs could be a promising strategy to overcome immune suppression and metabolic barriers in the TME, ultimately enhancing anti-tumor immunity.

EACR25-1977

Bacterial Colonization in Breast Cancer: Exploring *Fusobacterium nucleatum* and Hypoxic Niches

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Introduction

Standard clinical parameters such as tumour size, age, lymph node status, and molecular markers are used to predict progression risk and treatment response. However, exploring additional markers could offer a more comprehensive understanding of the tumour microenvironment (TME). The TME influences tumour development, progression, disease severity, and survival, with tumour-associated bacteria playing significant roles. Studies on tumour-associated microbiota have focused on high bacterial-load sites such as the gut, oral cavity, and stomach, but interest is growing in non-gastrointestinal (GI) solid tumours, such as breast. Species present have been shown to contribute to DNA damage, immuno-suppression, and treatment resistance. *Fusobacterium nucleatum* is an opportunistic anaerobe, enriched in periodontal lesions, oral cancer, colorectal cancer, and has been shown to promote treatment resistance and metastatic progression. Also found in breast cancer, *F. nucleatum* potentially plays a role in immune evasion by inhibiting immune cell populations. Hypoxia, a common hallmark of solid tumours, poses a hospitable habitat for such anaerobic bacteria. Furthermore, tumour necrosis releases nutrients that further support bacterial colonisation. While *F. nucleatum* has been associated with immune suppression and treatment resistance in

colorectal cancer, its role in breast cancer remains unclear. This study aims to establish robust detection methods to enable further exploration of its impact in the breast TME.

Material and method

Due to the discrete nature of bacterial presence in breast cancer, we established robust cell line controls for *F. nucleatum* (ATCC 25586) and *F. animalis*, formerly *F. nucleatum* subsp. *animalis* (ATCC 51191) positivity, confirmed via qPCR. Cell lines were then used to optimise staining and digital image analysis techniques.

Result and discussion

We optimised immunofluorescence (IF) staining protocols and investigated the utility of both proprietary (provided by Professor Daniel Slade, Virginia Tech, USA) and commercially available (Diatheva, Italy) antibodies for *Fusobacterium* staining, with the former being deemed suitable to progress to tissue staining due to its capacity to detect both *F. nucleatum* and *F. animalis*. Multiplex-IF (mIF) and multiplex fluorescent in situ hybridization (mFISH) protocols were also developed for the co-detection of *F. nucleatum* and Carbonic Anhydrase-IX (CA-IX), a marker for hypoxia. We implemented digital pathology workflows to quantify bacteria-positive cells, CA-IX, and double-positive populations.

Conclusion

By establishing robust methods to target and quantify bacterial presence and hypoxia in the tumour micro-environment, we can begin to investigate the impact bacterial species have on the immune landscape in the TME. This work provides a foundation for future research into microbial influence on breast cancer its impact on treatment efficacy.

EACR25-1984

Low-density neutrophils: a valuable clinical tool for monitoring breast cancer progression and treatment decisions

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Introduction

Metastatic breast cancer (mBC) remains a leading cause of cancer-related deaths among women globally. Despite therapeutic advances, many treatments fail to improve

patient outcomes, emphasizing the need for innovative approaches and reliable biomarkers to better predict treatment response. Recent studies highlight the critical role of neutrophils in cancer, with low-density neutrophils (LDN) acting as key modulators in the tumor microenvironment. Indeed, LDN are associated with tumor growth and metastasis, contributing to an immunosuppressive environment that supports cancer progression. Here, we further investigated the clinical significance of LDN in mBC by assessing their association with disease progression and patient outcomes.

Material and method

Blood samples from 72 metastatic BC patients were analysed, following LDN isolation via density gradient centrifugation. Flow cytometry was used to quantify and characterize these populations. The obtained results were associated with patients' clinical data.

Result and discussion

Our results demonstrated a significant accumulation of LDN in the blood of metastatic BC patients, particularly those with faster disease progression. Notably, elevated LDN levels were linked to reduced life expectancy independent of metastatic sites (bone, lung, or liver). Longitudinal analysis of LDN fluctuations in patients' blood regarding their clinical data further revealed that increased LDN frequency is often associated with adverse clinical events, disease progression, and non-response to treatment.

Conclusion

This study underscores the prognostic value of LDN in BC, with elevated levels of these cells indicating advanced disease and poorer outcomes. These findings suggest that LDN could serve as valuable biomarkers for monitoring disease progression and treatment response. Ultimately, integrating LDN assessment into the clinical practice could help better identify patients at higher risk of faster progression and may help guide therapeutic strategies toward a more personalized approach in BC management, potentially improving patient therapeutic outcomes.

EACR25-2026

Investigate the effect of alcohol-altered proteins from extracellular vesicles on the immune response and initiation of oral cancer

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Introduction

The processes involved in carcinogenesis depend on changes in tumor cells and the dynamic communication in the microenvironment. Studies report smoking and alcohol consumption as risk factors for oral squamous cell carcinoma (OSCC). Still, the mechanism of alcohol on the OSCC initiation needs to be elucidated.

Material and method

Peripheral blood mononuclear cells (PBMC) from healthy donor buffy coats were treated with immunopeptides selected from a protein identified to be more present in the extracellular vesicles isolated from biopsy tissue from oral cancer patients and primary fibroblasts

treated with alcohol. TCD4 and TCD8 modulation of activation (CD25 and CD69), exhaustion (PD-1, LAG-3 and Tim-3), senescence (CD57) and differentiation (FOXP3) were analyzed by flow cytometry. Cytokines (IL-2, IL-4, IL-6, IL-10, IL-17, TNFα and IFNγ) present in the supernatant of PBMC treated with immunopeptides were measured by Cytometric Bead Array (CBA).

Result and discussion

PBMC treated for 72 hours with 10 μmol/L of immunopeptides showed a reduction in the percentage of TCD4+ cells expressing Granzyme B and decrease in the mean fluorescence intensity of Tim-3 in TCD4+ cells (treated vs. control; p≤0.05; paired Student's t-test). Moreover, a reduction in the percentage of TCD8+ cells expressing Granzyme B and increase in the percentage of TCD8+ CD25+FOXP3+ cells were noted (treated vs. control; p≤0.05; paired Student's t-test). Treatment of PBMC with immunopeptides also resulted in significant lower detection of IL-10, TNFα and IFNγ (treated vs. control; p≤0.05; paired Student's t-test) in the supernatant. These results are in line with other findings from our group that showed PBMC co-cultured for 72 hours with EtOH-treated fibroblasts or EVs showed lower mRNA expression of CD25 and CD69 (treated vs. control; p≤0.05; paired Student's t-test) and a decrease in the percentage of TCD3+ and TCD4+ cells expressing CD25 (treated vs. control; p≤0.05; Wilcoxon test) and TCD4+ cells expressing CD69 (treated vs. control; p≤0.05; paired Student's t-test).

Conclusion

The results indicate that immunopeptides can modulate the activation of T cells and might be related to initiation of tumorigenesis in oral cancer, and in future may be used as a therapeutic target for prevention and treatment of OSCC.

EACR25-2054

Tissue Microenvironment Shapes PMN-MDSC Function, Maturation, and NETosis Capacity

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Introduction

PMN-MDSCs are a heterogeneous population of immature myeloid cells that suppress immune responses in various pathological conditions, including cancer. These cells exhibit significant heterogeneity and plasticity, with functional diversity dependent on their tissue origin and the signals they receive from the surrounding microenvironment. The study examines the heterogeneity and dynamic differentiation patterns of PMN-MDSCs in different tissue microenvironments, including bone marrow (BM), apheresis (AF), and peripheral blood (PB).

Material and method

PMN-MDSCs were identified by flow cytometry and isolated using magnetic beads separation or cell-sorting. Cells were obtained from BM, AF, and PB of tumor patients. Functional tests were performed using NK cells

as effector cells and tumor cell lines as target cells. scRNA-seq analysis was employed to identify distinct cell clusters within each tissue source, taking into consideration differentially expressed genes (DEGs). Pseudotime analysis was performed to predict the developmental trajectory of cells from the least differentiated to the most differentiated state. To simulate the tumor microenvironment, PMN-MDSCs derived from BM and AF were conditioned with pleural fluid (PF)

Result and discussion

ScRNA-seq analysis revealed that each PMN-MDSC subpopulation was segregated separately based on tissue origin, highlighting significant heterogeneity and functional diversity. Notably, conditioning with PF induced maturation of PMN-MDSCs and significantly increased their immunosuppressive activity. The identified factors were shown to promote PMN-MDSC maturation. A key result is that cytokines involved in the maturation of PMN-MDSC induce the expression of CD88a, a receptor involved in the formation of NETs (Neutrophil Extracellular Traps), and increases their NETosis capacity. PMN-MDSCs conditioned with PF showed gene expression patterns similar to those of PMN-MDSCs derived from peripheral blood (PB), suggesting the activation of immunosuppressive pathways.

Conclusion

PMN-MDSCs do not represent a monolithic population but rather a diverse collection of cells with distinct functional capabilities, which are influenced by their location and the surrounding tissue microenvironment. The results obtained highlight that modulating cytokines present in TME or CD88a signaling could represent a promising therapeutic approach to potentiate anti-tumor immunity. The study provides a detailed overview of the heterogeneity and dynamism of PMN-MDSCs, with a focus on the impact of the tissue microenvironment on their function, maturation, and ability to form NETs, suggesting new therapeutic strategies targeting these cells in pathological settings.

EACR25-2058

Chemotherapy Modulates the Immune Microenvironment and Epigenetic Landscape in MLL-AF9-Driven Acute Myeloid Leukemia

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Introduction

Acute Myeloid Leukemia (AML) is an aggressive blood cancer characterized by the expansion of immature myeloid cells in the bone marrow (BM), leading to hematopoietic failure. AML remains associated with poor prognosis, due to early treatment failure and high relapse rates following intensive chemotherapy. Here, we investigated how chemotherapy reshapes the immune microenvironment in a murine model of AML to identify tumor vulnerabilities and enhance immune responses and treatment outcomes.

Material and method

MLL-AF9-transformed murine AML cells were transplanted into immunocompetent mice without myeloablation, followed by a standard chemotherapy regimen (5+3 days of Daunorubicin and Cytarabine). BM and spleen (SPL) were analyzed two days post-treatment for immune cell composition using flow cytometry. Single-cell RNA sequencing (scRNA-seq) examined transcriptional changes, and histone modifications were assessed via western blot.

Result and discussion

scRNA-seq identified five leukemic clusters: one monocytic non-cycling and four granulocyte-monocyte progenitor (GMP)-like clusters with distinct cell cycle and inflammatory states. While chemotherapy reduced overall leukemic burden, all clusters were maintained but cycling and inflammatory GMP-like clusters were slightly increased. However, none of the tumor clusters upregulated senescence signatures, and instead down-regulated inflammatory and interferon (IFN) pathways, indicating weak immune activation. In the non-leukemic compartment, T and NK cells showed no significant IFN activation post-chemotherapy, while inflammatory pathways were upregulated in monocyte/macrophage subpopulations. Quantitative Flow cytometry revealed a decrease in macrophages and an increase in monocytes in the BM. To investigate potential immune suppression mechanisms, we analyzed the epigenetic remodeling in leukemic cells. Expression of EZH2, the functional enzymatic component of the Polycomb Repressive Complex 2 (PRC2), was heterogeneous but upregulated in most GMP-like tumor clusters post-chemotherapy. Additionally, genes associated with H3K27me3 histone modification were downregulated, suggesting increased H3K27me3 levels, further confirmed via western blot analyses, highlighting EZH2 as a potential immunotherapeutic target.

Conclusion

Our findings indicate that chemotherapy reshapes the immune microenvironment in MLL-AF9 AML by modulating myeloid populations but fails to induce a strong IFN response or robust immune activation. The weak senescence signature in BM-derived leukemic cells suggests that chemotherapy alone is insufficient to trigger immunogenic senescence. Additionally, the upregulation of EZH2 and increased H3K27me3 levels suggest an epigenetic mechanism contributing to immune evasion and leukemia persistence. Targeting EZH2 may enhance immune activation, offering a potential therapeutic strategy to improve AML clearance.

EACR25-2060

HLA-DR-expressing Cytotoxic T Lymphocytes as drivers of the effectiveness of T cell-based therapies in Breast Cancer

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Introduction

Neoadjuvant chemotherapy remains the standard of care for high-risk or inoperable breast cancer (BC) tumors, yet its success rate falls below 50%, highlighting the need for better strategies. T cell-based therapies, involving ex vivo expansion of patients' T lymphocytes, have emerged as a promising alternative, particularly for chemotherapy-resistant tumors, but their efficacy is often limited. Building on our previous findings that the antigen-presenting molecule, HLA-DR, is crucial in tumor infiltrating cytotoxic T lymphocytes (CTLs) for a favorable response to neoadjuvant chemotherapy, we further investigated the role of HLA-DR-expressing CTLs in anti-tumor responses and evaluated strategies to amplify these cells.

Material and method

To elucidate the role of HLA-DR in improving CTL-mediated tumor elimination, we conducted in vitro and in vivo experiments using zebrafish xenograft models. Then, aiming at increasing the population of HLA-DR-expressing CTLs, we optimized an ex vivo stimulation protocol using cells isolated from peripheral blood. Additionally, we tested the potential of different immune agents to boost their cytotoxicity against BC cells. This study included 209 BC patients, from whom both blood and tumor biopsy samples were collected, and 32 healthy donors who provided blood samples. These samples were used across all in vitro assays, including CTLs expansion, functional evaluation, and screening experiments.

Result and discussion

Our findings revealed that only CTLs expressing HLA-DR effectively eliminate tumor cells. Moreover, CD4⁺ T cells were identified as essential drivers of CTLs activation and cytotoxicity. Indeed, blocking HLA-DR or depleting CD4⁺ T cells impaired CTLs function, underscoring the importance of this interaction for therapeutic success. Notably, our study revealed that short-term stimulation increased HLA-DR expression while preserving CTLs functionality, whereas prolonged expansion diminished their effectiveness, underscoring the importance of prioritizing cell quality over quantity. Furthermore, we demonstrated that PD-1 blockade with

nivolumab further upregulated HLA-DR levels, amplifying CTL-mediated tumor cell killing.

Conclusion

Overall, our results emphasize the critical role of HLA-DR in T cell-based therapies and highlight the synergistic benefits of ex-vivo PD-1 treatment along with short-term stimulation in enhancing CTLs efficacy. These insights pave the way for improved immunotherapeutic strategies for chemotherapy-resistant BC.

EACR25-2069

Profiling the tumor-resident intracellular microbiota in small cell lung cancer and its influence on chemotherapy sensitivity

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Introduction

Small cell lung cancer (SCLC) is an aggressive subtype of lung cancer with a poor prognosis, often characterized by rapid relapse and resistance to chemotherapy. At present, the mechanism of chemoresistance in small cell lung cancer has not been fully elucidated. Notably, increasing evidence suggests that tumors harbor diverse microbiomes. Similar to the gut microbiome, the intratumoral microbiota has recently garnered attention for its capacity to modulate metabolism and the tumor microenvironment, thereby influencing the biological characteristics of tumor development. However, the role of the intratumoral microbiota in SCLC remains far less understood.

Material and method

Tumor samples were collected from 71 patients with SCLC at Shanghai Pulmonary Hospital between 2020 and 2022. A comprehensive analysis of the tumor-resident intracellular microbiota in SCLC was conducted using 16S rRNA gene sequencing. The intracellular bacterial communities were profiled, and their correlation with clinical outcomes was assessed.

Result and discussion

Our analysis revealed a diverse intracellular microbiota within SCLC tumors, with significant variations in bacterial composition among patients. Smoking significantly alters the intratumoral microbiota in SCLC, with LDA Effect Size analysis indicating higher abundance of Lactobacillales and Rothia in smoking patients. The presence of certain bacterial taxa, particularly Clostridium species, was associated with increased chemotherapy sensitivity. Additionally, survival analysis showed that patients with a higher abundance of Clostridium had longer progression-free survival. As Clostridium is primarily involved in short-chain fatty acid metabolism, we speculate that it may influence chemotherapy sensitivity by modulating tumor cell metabolism through the production of metabolites such as butyrate and propionate.

Conclusion

In conclusion, the tumor-resident intracellular microbiota in small cell lung cancer SCLC is associated with chemotherapy sensitivity and clinical outcomes, with certain Clostridium species linked to enhanced chemotherapy response. Our findings suggest that tumor-resident microbiota, albeit at low biomass, play an important role

in chemotherapy response, intervention of which might therefore be worth exploring for advancing oncology care.

EACR25-2090

Targeting CAIX in tumor microenvironment stimulates anti-tumor immune response

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Introduction

In solid tumors, the tumor microenvironment (TME) is often characterized by inflammation, hypoxia, and acidosis, which significantly impact cancer progression. These attributes of the TME substantially contribute to forming an immunosuppressive environment and influence cytokine production, immune cell recruitment, and their function. Carbonic anhydrase IX (CAIX), a hypoxia-induced enzyme, plays a crucial role in tumor cell adaptation to low oxygen conditions and generating an acidic tumor microenvironment. However, the role of CAIX in regulating anti-tumor immune responses has not been extensively investigated, and thus, it was a goal of this study.

Material and method

Spheroids of pancreatic ductal adenocarcinoma cells (PDAC) were established and treated with anti-CAIX combinatorial therapy using an inhibitor of CAIX enzymatic activity, SLC0111, and CAIX-specific antibody, M75. The infiltration of spheroids with immune cells and spheroid cell viability were evaluated using a Cytation instrument. RNA samples of PDAC cells were analyzed for cytokine expression by RT2 Profiler Array. RNA samples of CAIX+ and CAIX-silenced fibrosarcoma cells were analyzed by Clariom followed by unsupervised clustering. Gene Set Enrichment Analysis (GSEA) was used to analyze gene expression. The relationship between CAIX levels and gene sets identified by GSEA was also analyzed in TCGA sarcoma samples and pancreatic cancer. Human T cell responses were evaluated in the presence of culture media collected from CAIX+ and CAIX- cells by flow cytometry.

Result and discussion

We found that combination therapy against CAIX, or silencing CAIX expression, led to enhanced infiltration of immune cells into spheroids. CAIX-suppressed spheroids exhibited a higher degree of cancer cell death. Differential gene expression analysis of PDAC cells treated with combinatorial therapy and CAIX-silenced fibrosarcoma cells revealed the upregulation of genes involved in anti-tumor immune responses. T cell activation assays showed that conditioned media from

CAIX+ tumor cells dramatically inhibited T cell responses, while conditioned media from CAIX-silenced cells increased activation of CD8 T cells. Furthermore, GSEA analysis identified that attenuation of CAIX expression is associated with the upregulation of genes involved in IFN-γ, IFN-α, and inflammatory responses. A negative correlation between CAIX levels and these immune pathways, which predicted better overall survival, was confirmed by *in silico* analysis of cancer patient samples.

Conclusion

We found that besides the known effects of CAIX on the survival of tumor cells in hypoxia, the activity of this enzyme plays an important role in the suppression of anti-tumor immune responses. Thus, targeting CAIX may represent a new opportunity to create a favorable milieu for anti-tumor immune responses in the TME.

Funding: The Slovak Research and Development Agency 20-0480.

EACR25-2114

Rapid and Ficoll-free Magnetic Isolation of Human Monocytes from Whole Blood in High Purity and Yield

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Introduction

Monocytes and their derived differentiated cell types, such as monocyte-derived dendritic cells (moDCs), myeloid-derived suppressor cells (MDSCs), macrophages, and tumor-associated macrophages (TAMs) play a crucial role in cancer research and cell-derived therapies. Thus, ensuring robust, reliable and efficient procedures for their process generation is fundamental. Herein, we present a magnetic separation procedure based on new technology for a rapid, scalable and efficient isolation of human monocytes from whole blood in a ficoll-free process. This method enables the isolation of pan-monocytes (CD14+/CD16+) from human whole blood within 35 minutes. It offers a significantly shorter processing time compared to other magnetic cell separation techniques, which involve labor- and time-consuming steps to obtain peripheral blood mononuclear cells (PBMCs).

Material and method

The isolated monocytes were analyzed via flow cytometry for cell count and purity and tested for unspecific cell activation. Moreover, the differentiation potential of the isolated monocytes was evaluated by *in vitro* cell differentiation assays to yield moDCs, classically activated macrophages (M1) and alternatively activated (M2) type macrophages, as well as MDSCs. Additionally, co-culture experiments of moDCs with autologous T-cells were performed to investigate the stimulation performance of moDCs.

Result and discussion

Our isolation method resulted in a highly pure (>90%) untouched CD14+ monocyte population, with no unspecific cell activation, demonstrating their suitability for downstream applications. Moreover, isolated monocytes could be successfully differentiated into moDCs, M1 and M2 type macrophages, as well as MDSCs. Co-culture experiments of moDCs with autologous T cells demonstrated their successful ability to stimulate antigen specific T cells proliferation.

Conclusion

Based on these results, we introduce a new and highly efficient magnetic cell separation protocol that enables the rapid, scalable and ficoll-free isolation of human monocytes from whole blood. This will facilitate downstream applications including immunotherapy and cancer research.

EACR25-2120

Two-in-One: New Opportunities for Autologous Co-Isolation of T and B Cells from Small Blood Samples

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Introduction

Immunomagnetic cell isolation plays a key role in studying specific cell populations, enabling their respective contributions driving immunological reactions. Current separation methods are restricted to isolate one target population at a time, necessitating sample splitting or sample relabeling. Thus, these conventional approaches compromise on cell yield and limiting studies on autologous derived cells. With the MACSprep™ CD3 and CD19 Selection Kit, Miltenyi Biotec offers a new cell separation workflow to generate T and B cells from limited human blood samples in manual and automated processes. This newly developed workflow was established on the autoMACS® NEO as a fully automated cell separation device therefore also enabling simultaneous multi-cell separations as shown here.

Material and method

Expanding the previously developed StraightFrom® REAlease® approaches, the described innovative cell separation technology of the selection kit combines orthogonal release mechanisms that allow simultaneous labeling and sequential elution of target cells from a single MACS column. In this study, we demonstrate the efficacy of the cell separation process by co-isolating CD3+ T cells and CD19+ B cells from human whole blood with high purities and the comparability between manual and automated processes via the autoMACS NEO device. Furthermore, isolated cells were dissociated from separation labels using the benefits of the REAlease technology and taken into cell culture, to verify their naïve expression of activation markers and maintenance of full functionality upon stimulation.

Result and discussion

The results showcase high purities and recoveries achieved through the co-isolation of CD3+ T cells and CD19+ B cells from human whole blood by the MACSprep™ CD3 and CD19 Selection Kit even down

to small starting volumes of 0.5 mL. The fully automated separation via the autoMACS NEO device allows comparable results to manual separation with minimal hands-on-time. Upon cell culture, no expression of specific surface activation markers in absence of stimuli for either CD3+ T cells or CD19+ B cells was observed.

Conclusion

This novel cell separation technology represents a significant advancement in immunomagnetic cell isolation, offering a smart workflow solution for classical multi-cell isolation from single complex and quantity-limited blood samples accompanied with walk-away, standardized automation processes. Therefore, it holds promise for accelerating research in cancer immunology, investigation of autologous derived cell populations and vaccines dealing with limited starting materials requiring precise cell isolation techniques.

EACR25-2125

In vitro investigation of *Parvimonas micra*'s role in colorectal cancer progression

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Introduction

Colorectal cancer (CRC) is the second-leading cause of cancer related deaths. Several risk factors contribute to CRC development, some of which can be non-modifiable (e.g. age, genetic predisposition, inflammatory bowel), while others can be modifiable (e.g. smoking, alcohol consumption, western-style diet, low fiber intake, physical inactivity). Recent studies have shown an association between gut microbiota and CRC progression. Those risk factors can disrupt the bacterial symbiotic relationship in the human gut, leading to imbalances in microbial composition called dysbiosis. Dysregulation in the gut microbiota has been linked to CRC development. Recent studies have highlighted some onco-pathogens, such as *Fusobacterium nucleatum*, *Parvimonas micra*, and *Peptostreptococcus stomatis*, as being associated with CRC, but their exact role in tumor progression remains unclear.

Material and method

Full-length 16S sequencing alongside qPCR analysis has been used to identify and quantify the relative abundance of *P. micra* in clinical samples. The presence of *P. micra* was also correlated with other CRC-associated bacteria. To further investigate these microbial interactions and their potential role in CRC progression, multiple clinical strains of *P. micra* were evaluated for their ability to form biofilms, as well as their interactions with other CRC-related bacteria in biofilm formation. To assess the impact of *P. micra* on CRC progression, cell proliferation and migration assays were conducted using different CRC cell lines. Western blot analysis was performed on protein extracts from CRC cell lines infected with *P. micra* to evaluate the activated pathway.

Result and discussion

Results from qPCR analyses and 16S rDNA sequencing confirmed the association of *P. micra* with CRC, in line with our previous findings. Biofilm analysis showed an increase in biomass when *P. micra* was co-cultured with *F. nucleatum* and *P. stomatis*, suggesting a possible synergistic microbial interaction. Factors secreted by *P. micra* were found to enhance CRC cell proliferation and migration, indicating that the bacterium may promote tumorigenic process. Preliminary protein expression analysis showed upregulation of autophagy-related markers (P21, LC3B), suggesting activation of autophagy pathways. Additionally, a high concentration of *P. micra* heat shock protein HSP60 (also known as GroEL) was detected in the bacterial supernatant, suggesting a potential role in host-pathogen interactions.

Conclusion

Our findings support a role for *P. micra* in CRC progression and suggest a possible link to other onco-pathogen through the biofilm synergy. Moreover, *P. micra* might induce autophagy as a survival mechanism via the release of bacterial HSP60. These findings highlight a potential crosstalk between microbial biofilm formation, autophagy induction, and CRC progression positioning *P. micra* as an important factor within the tumor microenvironment.

EACR25-2127

Targeting TLR2 improves the efficacy of breast cancer therapies

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Introduction

Despite significant progress in its treatment, breast cancer (BC) remains the leading cause of cancer death in women. Therefore, the development of combination therapies able to target key cancer-inducing or cell-sustaining pathways is needed. We have previously demonstrated that Toll-like receptor 2 (TLR2) promotes CSC self-renewal, breast cancer progression and resistance to chemotherapy due to DAMPs binding. TLR2 inhibition restores and potentiates doxorubicin anti-tumor effect in vitro and in vivo. In addition, TLR2 seems to regulate the expression of the cystine/glutamate antiporter xCT, which controls redox balance and mediates several pro-tumoral mechanisms. The role of xCT in breast cancer is largely studied by our group and different vaccines have been developed to target this antigen with promising results in pre-clinical models. In this study, TLR2 targeting is exploited to improve the efficacy of both chemo- and immunotherapy as a new strategy for breast cancer treatment.

Material and method

We analyzed the effects of TLR2 inhibitors in combination with chemotherapies or inhibitors of the cystine-glutamate antiporter xCT, a promising BC target. 4T1 tumor-bearing mice were treated with nanosystems

delivering TLR2 inhibitors in combination with chemotherapy and xCT immunotargeting.

Result and discussion

TLR2 conferred resistance to immunogenic cell death inducers such as some chemotherapies or xCT inhibitors in BC cells. Treatment with TLR2 inhibitors induced apoptosis of BC cells, and a synergistic effect was observed with chemotherapy or xCT inhibitors. Nanoparticle-mediated delivery of TLR2 inhibitors significantly impaired tumor progression in vivo, and their combination with chemotherapy or xCT immunotargeting further improved these effects.

Conclusion

In conclusion, TLR2 mediates several pro-tumoral mechanisms including chemoresistance. TLR2 inhibition improves the efficacy of chemo- and immunotherapies opening new perspectives for the treatment of breast cancer.

EACR25-2129

Impact of the intratumoral bacteria on immunotherapy outcomes in hepatocellular carcinoma

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Introduction

Intratumoral bacteria are increasingly recognized as influential within the tumor microenvironment (TME). However, their role remains poorly unexplored in hepatocellular carcinoma (HCC), the third most common cause of cancer-related death worldwide. We recently reported two distinct HCC TME subtypes in patients

treated with atezolizumab+bevacizumab (atezo+bev): ImmunePos, enriched with CD8+ T effector cells and CXCL10+ macrophages and associated with atezo+bev responses, and ImmuneNeg, lacking immune cell infiltration. Here, we aimed to explore the clinical relevance of tumor resident bacteria in response to atezo+bev in HCC.

Material and method

We characterized the intratumoral microbiome in 290 HCC samples from patients treated with atezo+bev (n=247) and atezo alone (n=43) from the IMbrave150 and GO30140 clinical trials. RNAseq was used to profile the HCC resident microbiome at the genus level.

Microbial content was identified by screening transcriptomic reads using the PathSeq pipeline within the Genome Analysis Toolkit. Reads aligning to the human GRCh38 genome were removed, and the remaining reads were mapped to microbial reference genomes. To filter out contaminants and adjust for batch effects, we retained only genera with a relative abundance of $\geq 10\%$. The association between bacterial prevalence (relative abundance present in > 10% of samples) and abundance with progression-free survival (PFS), overall survival (OS), and our TME classification was evaluated.

Result and discussion

Here we show that the prevalence of *Bacillus* in HCCs of atezo+bev treated patients, was associated with a significantly longer PFS ($p = 0.006$) and a trend toward prolonged OS ($p = 0.075$) compared to those without *Bacillus*. In addition, *Bacillus* prevalence and abundance were significantly higher in atezo+bev responders (prevalence: $p = 0.0029$, abundance: $p = 0.0032$ vs non-responders) but not in responders to atezo alone. Especially, ImmunePos responders to atezo+bev, were significantly enriched with *Bacillus* (prevalence: $p = 0.0009$ vs non-responders; abundance: $p = 0.01$ vs ImmuneNeg responders; $p = 0.0002$ vs non-responders).

Conclusion

We identified *Bacillus* specifically associated with a significantly longer PFS and a trend towards longer OS in atezo+bev-treated HCC patients. Moreover, responders to atezo+bev, particularly those with ImmunoPos characteristics, showed significantly higher abundance and prevalence of *Bacillus*, which suggests that TME has a role in determining the bacterial landscape.

EACR25-2137

Tumour microenvironment and neoantigens to develop targeted immunotherapy for paediatric neuroblastoma patients

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Introduction

Immunotherapy has revolutionized treatment of adult cancers in the last decades. Nevertheless, it is not as effective for paediatric cancers, probably due to differences in their immune system and intrinsic characteristics of tumours. Indeed, tumour microenvironment of paediatric tumours has not been fully studied, and little is known about neoantigenicity of mutations. The main aim of this work was to characterize the tumour microenvironment of paediatric neuroblastoma using spatial transcriptomics. The final goal was to identify putative tumour neoantigens in paediatric neuroblastoma patients to study their role as biomarkers or therapeutic strategy.

Material and method

We analysed four FFPE samples of paediatric neuroblastoma patients from Hospital Miguel Servet (Zaragoza, Spain). Spatial transcriptomics was conducted using Visium Spatial Gene Expression kit (10x Genomics) and sequenced using NovaSeq (Illumina). Resulting data were analysed using Space Ranger (10X genomics), Loupe Browser (10x Genomics), and the R package Seurat. To identify potential neoantigens, we used a training cohort ($n = 1089$) and a validation cohort ($n = 225$) from cBioPortal and bioinformatic tools such as NetMHCpan.

Result and discussion

Preliminary results from spatial transcriptomics allowed identification of different transcriptomic clusters, confirming the heterogeneity and complexity of the tumour microenvironment. In some samples, the antigen presentation pathway mediated crosstalk between two adjacent tumour regions, one of them showing a greater infiltration of immune cells. We are currently evaluating differential gene expression among the different types of cells of the tumour microenvironment. We found that patients with a higher number of neoantigens had worse overall survival. To evaluate the feasibility of neoantigens as potential therapeutic strategies, we studied the recurrence of neoantigens among patients. Despite most neoantigens were private, two immunogenic mutations were recurrent. Interestingly, these mutations associated with worse patient prognosis. We then confirmed neoantigenicity of these mutations using other prediction tools focused on different steps of antigen presentation.

Conclusion

Altogether, studying the tumour immune microenvironment could be useful to identify patients that will benefit from immunotherapy. Furthermore, targeting neoantigens could be a therapeutic strategy for paediatric solid tumours.

This study was supported by Asociación de Padres de Niños con Cáncer de Aragón (ASPAHOA).

EACR25-2151

Paracrine and Neoplastic Effects of D-2HG

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Introduction

In intrahepatic cholangiocarcinoma (iCCA), 13% of patients exhibit Isocitrate Dehydrogenase 1 (IDH1) mutations, resulting in the production of the onco-metabolite R-2-hydroxyglutarate (2-HG). Ivosidenib (Tibsovo®) targets this mutation and lowers 2-HG levels, yet often only achieves partial clinical responses. Here we hypothesize that despite potent reduction of 2-HG by ivosidenib, remaining residual levels of 2-HG inhibiting sensitive enzymes result in suboptimal efficacy. This research examines 2-HG's targets and its effects on neoplastic and immune cells.

Material and method

Neoplastic Effects: We will compare the effect of genetic versus pharmacological (ivosidenib) depletion of 2-HG in genetically modified iCCA mouse cell lines expressing mutant IDH1 (mIDH1). The role of 2-HG gene targets will be investigated using sub-targeted screens, transcriptomics, epigenetic profiling, and in-vivo studies.

Paracrine Effects: Clinical findings suggest that the immunosuppressive tumor microenvironment of mIDH1 tumors is due in part by the inhibitory effect of 2-HG on T-cells. We will explore T-cell immunity in mIDH1 tumor-harboring mice and engineer T-cells to prevent intrinsic 2-HG accumulation. Using genetic screens in T-cells, we aim to identify mechanisms to overcome 2-HG-induced immunosuppression that could enhance T-cell infiltration and function.

Result and discussion

Anticipated findings include identifying genes inhibited by 2-HG despite ivosidenib treatment, clarifying their roles in mIDH1 biology and treatment outcomes. We also expect to reveal the effects of 2-HG's influence on immune evasion and immunological mechanisms impacting treatment efficacy.

Conclusion

This study aims to elucidate 2-HG's targets, proposing strategies to enhance clinical outcomes by minimizing 2-HG levels and uncover new pathways that, when inhibited, could increase ivosidenib efficacy.

EACR25-2158

Differential Impact of *Staphylococcus aureus* Infection on Lung Cancer and Normal Bronchial Cells

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Introduction

Staphylococcus aureus (*S. aureus*) infection is a frequent complication in lung cancer patients, accounting for up to 70% of infectious issues. Traditionally seen as an

extracellular pathogen, *S. aureus* is now recognized for its ability to survive and replicate intracellularly within tumor tissues. This intratumor invasion can trigger inflammatory pneumonia, obstructive pulmonary diseases, and secondary infections, potentially leading to organ dysfunction. Despite advances in cancer treatments, these infections remain a major cause of mortality, emphasizing the urgent need for improved strategies to manage bacterial complications in lung cancer patients. This study aims to elucidate the molecular and cellular effects of *S. aureus* infection on lung cancer and normal bronchial epithelial cells.

Material and method

The effects of *S. aureus* infection at 1:25, 1:50, and 1:100 ratios on A549 lung cancer and BEAS-2B bronchial epithelial cell lines were established. Intracellular infection was visualized using Giemsa staining. The intracellular *S. aureus* burden was quantified through CFU analysis, and the infection index was subsequently calculated. Cell cycle and apoptosis assay using flow cytometry. Immunofluorescence staining was conducted to detect DNA damage induced by the infection. To assess metastatic potential, colony formation assays, invasion and migration assays were performed. CD44/133 staining was utilized to explore stem cell-like properties. The expression levels of key genes involved in cancer progression, apoptosis, and migration were examined using RT-PCR.

Result and discussion

The findings of this study highlight the significant effects of *S. aureus* infection at varying rates on both the A549 and the BEAS-2B cell lines. The results demonstrated that *S. aureus* infection significantly impacted various cellular processes, including apoptosis, cell cycle, DNA damage, and metastatic / stemness potential in A549 cells than BEAS-2B cells. Additionally, alterations in gene expression related to cancer progression, apoptosis, and migration were detected, providing valuable insights into the molecular mechanisms underlying the interaction between *S. aureus* and both lung cancer and normal bronchial epithelial cells. These findings contribute to a deeper understanding of the role of bacterial infection in cancer biology and its potential implications for cancer progression and treatment.

Conclusion

This study revealed that *S. aureus* infects both lung cancer and normal bronchial epithelial cells, but lung cancer cells are more vulnerable to intracellular invasion and dysfunction. These differential effects underscore the need for further research into the underlying molecular mechanisms to develop targeted therapies that address both cancer progression and associated bacterial infections, ultimately improving outcomes for lung cancer patients.

EACR25-2187

Glucocorticoid Receptor: An Oncosuppressor 'Immune-dependent' in EGFR-driven Lung Adenocarcinoma

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Introduction

Lung cancer is the leading cause of cancer-related deaths worldwide due to its aggressive nature, with oncogenic mutations in EGFR being one of the most common genetic drivers of this disease. In particular, lung adenocarcinoma (LUAD), the most common subtype of lung cancer, often forces patients to endure significant physiological stress, leading to the production of cortisol. However, the consequences of physiological glucocorticoid receptor (GR) mediated signaling in LUAD is not well described. Interestingly, GR expression is downregulated in tumors in comparison to healthy parenchyma with lower GR expression correlating with poorer patient survival outcome.

Material and method

We use autochthonous mouse models of lung tumorigenesis to study the impact of tumor cell intrinsic GR signaling in EGFR driven lung tumors, supplemented by *in vitro* experiments.

Result and discussion

GR deletion in EGFR driven lung tumor cells promotes tumor initiation and progression, resulting in drastically reduced survival, in immunocompetent mice. *In vitro*, however, GR deficient tumor cells proliferate slower, and also following transplantation into immunodeficient mice. This discrepancy suggests a pivotal role of the tumor microenvironment in GR's tumor-suppressive effects. Indeed, loss of GR results in an immuno-suppressive microenvironment characterized by lower cytotoxic and helper T cells and increased myeloid derived cells and tumor associated macrophages. To stronger emphasize the impact of the immune microenvironment, we then implemented more immunogenic mouse models where cancer cells express the neoantigen ovalbumin. Intriguingly, mice harboring GR deficient, OVA expressing tumors exhibited increased tumor burden not only compared to GR and OVA expressing controls, but also compared to the non-OVA, GR deficient group. This suggests that GR deficient tumor cells hijack infiltrating T cells to boost tumorigenesis. In addition, by multiplex cytokine assay, we discovered that bronchoalveolar lavage derived from tumor intrinsic deficient GR mice present an higher level in TGF β and CXCL12 which might promote the tumor immunosuppressive microenvironment and the tumor growth.

Conclusion

We identified tumor intrinsic GR signaling as a powerful tumor suppressor in EGFR driven LUAD, with its loss resulting in a tumor promoting tumor microenvironment. These findings may have major impact on current treatment strategies, including the outcome of osimertinib therapy.

EACR25-2189

BAP1 deletion disrupts IFN- γ signaling and sensitizes ASXL1 mutated myeloid leukemia cells to NK cell cytotoxicity

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Introduction

Natural Killer (NK) cell recognition and killing of malignant cells involve multiple structures expressed by NK cells and target cells. Here, we analyzed multiple genome wide CRISPR screens to identify genes of importance for K562 cell susceptibility and resistance to NK cell cytotoxicity. A recurrent hit in multiple screens was the BRCA1-associated protein 1 (BAP1) gene that was found to protect K562 cells from NK cell cytotoxicity. BAP1 is a tumor suppressor gene that encodes a deubiquitinating enzyme that modulates multiple cellular processes. Germline BAP1 mutations are associated with tumor predisposition syndrome and somatic loss of function mutations have been frequently associated with various tumors. BAP1 is rarely mutated in leukemia, but it is reportedly implicated in the presence of Additional sex combs-like 1 (ASXL1) mutations.

Material and method

To determine how BAP1 affected cell susceptibility to NK cells, we generated BAP1 knock-out (KO) in K562 cells and exposed them to NK cells in functional assays. We also characterized BAP1 deficient cells with immunostaining and proteomic analysis. Moreover, we performed BAP1 knock-down (KD) in a series of ASXL1 wild-type and mutated myeloid leukemia cell lines.

Result and discussion

In the absence of interferon gamma (IFN- γ), BAP1 KO cells did not present with enhanced sensitivity to NK cells as suggested by the screen. However, IFN- γ -treated BAP1 KO cells triggered increased NK cell degranulation and were more sensitive towards NK cell killing than corresponding wild-type cells. Interestingly, in the presence of IFN- γ , BAP1-deficient cells failed to upregulate HLA class I expression that otherwise inhibited NK cell cytotoxicity. Further experiments revealed that these cells displayed reduced expression of the IFN- γ receptor 1. Interestingly, BAP1 knockdown in a series of myeloid leukemia cell lines selectively decreased HLA-E and IFN- γ receptor 1 expression in cell lines harboring ASXL1 mutations.

Conclusion

This study proposes a new role of BAP1 and provides further insight into biological consequences of targeting BAP1 in ASXL1-mutated myeloid leukemia suggesting a rationale for combining BAP1 inhibition and NK cell-based immunotherapy.

EACR25-2190

Notch3-Regulated miRNAs Target CXCR4 to Enhance Leukemic Cell Dissemination in T-ALL

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Introduction

T-cell Acute Lymphoblastic Leukemia (T-ALL) is an aggressive malignancy driven by Notch-activating mutation, leading to thymocyte maturation arrest and uncontrolled proliferation. A key feature of T-ALL progression is the trafficking of leukemic cells between the thymus and bone marrow, where a protective microenvironment may support their expansion. We hypothesize that miRNA-mediated regulation of CXCR4 plays a crucial role in thymocyte escape and engraftment in the BM. In a Notch3-transgenic (N3-ICtg) mouse model, we observed an aberrant expansion of DNCD3 ϵ^+ CXCR4 $^-$ cells, suggesting a potential link between CXCR4 downregulation and Notch3 hyperactivation, along with a panel of upregulated miRNAs.

Material and method

To validate our mouse model findings in human T-ALL, we transfected TALL1 cells with miR150-5p mimics (100nM-200nM) and analyzed CXCR4 expression using qRT-PCR and FACS analysis. In Molt3 cells we silenced Notch3 and quantified miR139-5p and miR150-5p levels by qRT-PCR. ChIP assay was performed to assess the binding of RBPJK to the miR139-5p promoter in both TALL1 and Molt3 cells. To further investigate the Notch/CXCR4/miRNAs crosstalk, experiments were conducted using SupT1 cells stably expressing specific miRNAs via lentiviral transduction, along with T-ALL patient-derived xenograft and human T-ALL primary samples.

Result and discussion

We assessed the combined effect of the two miRNAs in transfected N3-dependent TALL1 cells, characterized by high miR139-5p and low miR150-5p, showing down-regulation of CXCR4. N3-knockdown in Molt3 cells resulted in decreased expression of both miRNAs, indicating a functional connection between Notch3 signaling and miRNA expression. ChIP assay revealed that RBPJK (Notch transcriptional effector) is efficiently recruited in both TALL1 and Molt3 cells, confirming its direct regulation by Notch signaling. To extend our results to human T-ALL patients, we analyzed and detected the inverse correlation between CXCR4 expression and miR150-5p and miR139-5p expression in a panel of PDX and patients' samples.

Conclusion

Our results confirm that miR139-5p and miR150-5p regulate CXCR4 in human T-ALL. Future studies will be conducted on human SupT1 cell line, possibly to explore miRNA release via extracellular vesicles (EVs) in the thymic microenvironment. In conclusion, these results suggest that Notch-induced miRNA deregulation may play a crucial role in the advanced stages of T-ALL by promoting leukemic cell dissemination and thymic dysfunction, ultimately identifying potential biomarkers for targeted new therapeutic strategies.

EACR25-2195

Macrophage-Mediated Fibrosis Drives Immunotherapy Resistance in Melanoma

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Introduction

Macrophages represent the primary infiltrating immune subset in most cancer types and tumor-associated macrophages (TAMs) sustain tumor growth, promote invasion and mediate immune suppression. Cellular metabolism is implicated in the education of macrophages and dictates their phenotype. Interestingly, macrophages engulfed with lipids have been recently described in various cancers, including prostate, gastric cancer and melanoma. However, the mechanisms that drive lipid accumulation in macrophages and the consequences of such metabolic phenotype on cancer therapy remain uncovered.

Material and method

In this study, we applied high-throughput techniques, including single cell RNA sequencing and proteomic analysis to unravel the mechanisms responsible for lipid loading in macrophages and to explore the implications of lipid accumulation on functional activity of TAMs in melanoma.

Result and discussion

Our investigation confirmed that lipid accumulation is a distinct feature of tumor-infiltrating macrophages in melanoma. Notably, we observed that lipid-accumulation in TAMs correlates with tumor size and with the expression, by macrophages, of markers associated with immunosuppression, including Arginase-1 and PD-L1. Bulk RNA sequencing revealed that the CLEAR signaling pathway is deregulated in lipid-loaded macrophages compared to the lipid-deprived counterpart. Accordingly, we observed that the transcription factor EB (TFEB), master regulator of the CLEAR network, is dysfunctional in tumor-conditioned macrophages. In addition, autophagic flux is impaired in macrophages exposed to tumor cells, thus indicating a potential role of TFEB in promoting autophagy deficiency and lipid accumulation in TAMs. In the second part of the project, we explored the mechanisms triggered by lipid accumulation in TAMs. Notably, we discovered that lipid loaded macrophages can sustain a pro-fibrotic program and directly contribute to fibrosis, producing collagen and extracellular matrix (ECM)-related proteins. Importantly, inhibition of this pro-fibrotic program effectively prevented expression of genes related to ECM in macrophages and improved response to immunotherapy in melanoma models.

Conclusion

In conclusion, this project sheds light on mechanisms that contribute to lipid accumulation in TAMs and introduces innovative approaches to target lipid-loaded macrophages in melanoma. Based on our findings, we propose to target lipid accumulation in TAMs as a strategy to improve efficacy of current available immunotherapies, thus

representing an opportunity for advancing cancer therapy.

EACR25-2199

The ILC compartment in mismatch repair-deficient colorectal cancers is dominated by CD127-negative ILC1-like cells with cytotoxic activity

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Introduction

The immune microenvironment of colorectal cancer (CRC) plays a critical role in patient prognosis. Our previous study identified an unconventional innate lymphoid cell (ILC) population ($\text{Lin}^- \text{CD7}^+ \text{CD127}^- \text{CD103}^+ \text{CD45RO}^+$) enriched in mismatch repair (MMR)-deficient CRCs, exhibiting hallmarks of cytotoxic activity. However, the specific role of this ILC subset in anti-tumor immunity remains poorly defined.

Material and method

To further characterize this subset, we employed single-cell RNA sequencing (scRNA-seq), single-cell mass cytometry, and imaging mass cytometry to define the phenotypic hallmarks of CD127⁻ ILCs in CRC. Additionally, we flow-sorted ILCs from colon tumors to assess their tumor-killing capacity in relation to Human Leukocyte Antigen (HLA) Class I expression on target cells.

Result and discussion

The predominant ILC population in MMR-deficient CRCs consists of CD127⁻CD103⁺ ILC1-like cells, followed by CD127⁻CD103⁻ NK-like cells and a minor subset of CD127⁺CD103⁻ conventional ILCs. These cells express the transcription factor TBX21 (T-bet) but lack Eomes expression. Furthermore, CD127⁻CD103⁺ ILCs exhibit a distinct molecular profile, expressing Killer-Immunoglobulin-like Receptors (KIRs), various co-inhibitory and co-stimulatory molecules, NKG2A, and HLA class II molecules. Functionally, CD127⁻CD103⁺ ILCs demonstrate cytotoxic activity against colorectal cancer cells.

Conclusion

Our findings reveal that CD127⁻CD103⁺ ILC1-like cells, distinct from NK cells and conventional ILCs, are enriched in MMR-deficient CRC. Their high NKG2A/CD94 expression suggests a potential mechanism for recognizing HLA Class I-deficient tumor cells. These results highlight the active role of ILCs in anti-tumor immunity and suggest their potential as targets for cancer immunotherapy.

EACR25-2215

Comprehensive Profiling of Transgenic Mouse Antibody Repertoires with High-Throughput Single Cell BCR and Whole Transcriptome Sequencing

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Introduction

Transgenic mouse models are excellent tools to model human antibody response against antigens or disease of choice, given their ability to undergo diversification, in vivo affinity maturation, clonal selection and clonal expansion in their natural immune environment. Current antibody discovery methods lack the scale required to fully screen the antibody repertoire of transgenic mouse models to uncover maximum desirable antibody hits per discovery campaign.

Material and method

Here, we demonstrate our novel combinatorial barcoding approach to enable high-throughput single cell BCR and whole transcriptome profiling of transgenic mouse samples with unprecedented sensitivity, scalability, and flexibility. As a proof of concept, we profiled over 1 million cells from multiple transgenic mice that were either naive or immunized with antigens of interest to obtain hundreds of thousands of paired BCR clonotypes.

Result and discussion

Using the whole transcriptome data, we detected all B cell subpopulations including plasma cells at single cell resolution. We then used the paired heavy and light chain BCR data to detect productive chains in the majority of B cells revealing both unique and expanded clonotypes. In the expanded clonotype pool we found specific patterns of VDJ utilization and amino acid motifs compared to the naive pool.

Conclusion

This method paves the way for high throughput screening of antibody repertoires from transgenic mice to allow for deep mining of repertoires for antibody discovery and therapeutic outcomes.

EACR25-2230

The lipid droplet metabolic hub regulates human NK cell function

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Introduction

Metabolic perturbations, including aberrant lipid metabolism, are characteristics of the tumor microenvironment and promote immune cell dysfunction. Natural killer (NK) cells can infiltrate tumors and play an integral role in tumor immunosurveillance. Metabolic dysregulation has been associated with impaired NK cell activity, resulting in enhanced tumor growth and an increased rate of metastasis. The effects of metabolically challenging environments on NK cell metabolism and pro-inflammatory and cytotoxic functions remain poorly understood.

Material and method

Blood samples from HDs were obtained from the Establisement Francais du Sang (Paris, France). Peripheral blood mononuclear cells (PBMCs) were isolated with Ficoll-Paque (GE Healthcare). Metabolomics: FACS-sorted fresh or cytokine activated NK cells (250,000 cells) were washed in ammonium carbonate (75 mM). Preheated (70 °C) 70% ethanol extraction solvent was added to extract metabolites. The

analysis was performed by General Metabolics as previously reported (Surace, *Nature Imm*, 2021). FACS: Cells were stained with surface antibodies and Viability Dye eFluor 506 (eBioscience) in phosphate-buffered saline/2% fetal calf serum for 30 minutes on ice. For intracellular staining, cells were stimulated for 18 hours, and Golgi Plug/Golgi Stop (BD) were added for the last 3 hours. Cells were fixed/permeabilized using Cytofix/Cytoperm (BD) and then stained. Samples were acquired using the LSRFortessa (BD) and analyzed by FlowJ10 software (Tree Star). Bodipy dyes (493 and FLC16 or 12) were used to assess fatty acid uptake and lipid droplet content.

Result and discussion

We profiled the metabolome of the two main circulating human NK cell subsets, the highly cytotoxic CD56Dim CD16+ and the cytokine-producers CD56Bright CD16-NK cells, by mass spectrometry. We found that both NK cell subsets are equipped with distinct diet- and host-derived metabolites at steady-state. Neutral lipids appeared to be enriched in CD56Dim NK cells compared with the CD56Bright NK cells and are stored in lipid droplets. Upon activation, both subsets showed a loss of metabolites and increased lactate production, consistent with previously reported high glycolytic rates in proliferating cells. However, only activated CD56Bright NK cells intensified lipid uptake and lipid-droplet biogenesis. Inhibition of fatty acid oxidation affected only cytokine production in CD56Bright NK cells, while Diacyl-glycerol O-acyltransferase 1 (DGAT1)-mediated lipid storage was crucial in orchestrating cytotoxicity and cytokine production in both subsets. Altogether, our data suggest lipid droplets are an essential organelle regulating NK cell biology.

Conclusion

Understanding how NK cell function is regulated and reprogrammed by lipid metabolism is beneficial for developing strategies to reverse NK cell dysfunction in tumors and inflammatory settings characterized by aberrant metabolism.

EACR25-2269

Immunomodulatory effects of HuR inhibition in EGFR-TKI resistant NSCLC cells

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Introduction

Immunotherapy has produced positive results in patients with advanced non-small cell lung cancer (NSCLC),

except for those bearing a mutant epidermal growth factor receptor (EGFR). In particular, once resistance to EGFR-tyrosine kinase inhibitors (TKIs) is established, EGFR-mutant NSCLC patients do not benefit from therapy with immune checkpoint inhibitors (ICIs) despite PD-L1 expression. Acquired resistance to EGFR-TKI is associated with an immune suppressive phenotype, involving downregulation of class I HLA antigens expression and increased secretion of interleukin(IL)-6, IL-8 and transforming growth factor-(TGF) β . These factors are all targets of the RNA-binding protein (RBP) HuR, which regulates mRNA stability/translation of bound transcripts. Given the mounting role of HuR in cancer immune evasion, we investigated its function in modulating two mechanisms contributing to the poor response to ICIs in EGFR-TKI resistant cells: cytokine secretion and class I HLA expression.

Material and method

EGFR-TKI-resistant cell lines (HCC827GR/PC9GR/H1975OR) were generated by treating HCC827/PC9/H1975 cells with Gefitinib or Osimertinib. PC9- and H1975-HuR-KO were generated by CRISPR/Cas9 technology. KH-3 and SRI-42127 commercially available HuR inhibitors were used. Protein expression was analyzed by immunoblotting, confocal microscopy, flow cytometry. Cytokine secretion on cell supernatants was analyzed by ELISA assay and flow cytometry-based multiplex immunoassays.

Result and discussion

Confocal microscopy analysis identified increased cytoplasmic HuR, a feature of functional activation, in EGFR-TKI-resistant cells compared to parental cells. Levels of HuR targets IL-6 and IL-8 were significantly increased in EGFR-TKI-resistant cells respect to parental cells ($p < 0.01$), while their levels were reduced in PC9- and H1975-HuR-KO cells ($p < 0.05$). Furthermore, the loss of HuR impaired in vitro acquisition of resistance to both gefitinib and osimertinib in PC9 and H1975 cells, together with a selective decrease (>80%; $p < 0.01$) of IL-8 secretion. HuR inhibitor SRI-42127 (2.5 μ M, 24 hours) reduced only IL-8 release from both PC9GR and H1975OR, whereas the inhibitor KH-3 significantly reduced, in a dose-dependent manner, the levels of both IL-6 and IL-8 in H1975OR cells. In addition, flow cytometric analysis showed PD-L1 upregulation and class I HLA antigens downregulation in H1975OR compared to H1975 cells. Intriguingly, pharmacological HuR inhibition induced a 2 fold increase of class I HLA antigens in both cell lines suggesting an improvement in antigen presentation.

Conclusion

Our results indicate that HuR contributes to EGFR-TKIs resistance acquisition and modulates IL-8 and class I HLA expression, which are determinants of poor response to ICIs. These studies support the evaluation of HuR inhibitors to restore the EGFR-TKI responsiveness to immunotherapy.

Study supported by AIRC

EACR25-2286**Divergent Metabolic States of Circulating vs. Tumor-Infiltrating Lymphocytes in Cancer***P. Ritter^{1,2,3}, S. Oliveto^{1,3}, S. Biffo^{1,3}*¹*National Institute of Molecular Genetics, fondazione "Romeo ed Enrica Invernizzi", Milano, Italy*²*Politecnico di Milano, Department of Chemistry, Materials and Chemical Engineering "Giulio Natta", Milano, Italy*³*University of Milan, Department of Biosciences, Milano, Italy***Introduction**

Understanding the metabolic differences between tumor-infiltrating lymphocytes (TILs) and peripheral blood lymphocytes (PBLs) is crucial for characterizing immune cell functionality in the tumor microenvironment. TILs often exhibit distinct metabolic profiles compared to their circulating counterparts due to the hypoxic and nutrient-deprived conditions within the tumor. To investigate these metabolic adaptations, we employed a microfluidic droplet-based single-cell analysis platform, enabling real-time assessment of metabolic activity at a single-cell resolution. This approach allows for direct comparison of lymphocyte populations in physiologically relevant conditions, providing insights into how the tumor microenvironment influences immune cell metabolism.

Material and method

Single-cell metabolic activity was analyzed using a microfluidic droplet-based system (Lighthouse Biotech, Italy). Lymphocytes were resuspended in a working mix containing a pH sensitive fluorescent dye and encapsulated into 20–30 pL droplets within a microfluidic chip. After incubation, the droplets passed through a fluorescence-based detection zone, where metabolic activity was assessed in real time based on extracellular acidification dynamics. Fluorescence signals were recorded at the single-cell level, allowing for quantitative comparisons across lymphocyte populations. Statistical analysis confirmed a significant difference in extracellular acidification between TILs and PBLs, supporting the metabolic divergence induced by the tumor microenvironment. Based on these measurements, metabolically active cells were identified and sorted into distinct subpopulations, enabling further downstream analyses of their functional and phenotypic characteristics.

Result and discussion

Single-cell metabolic profiling revealed higher extracellular acidification in TILs compared to circulating PBLs, suggesting a metabolic shift associated with the tumor microenvironment. Statistical analysis confirmed that TILs exhibited a significantly greater acidification rate, likely reflecting adaptation to hypoxic and nutrient-deprived conditions. Additionally, the assay detected metabolic heterogeneity within both populations, indicating the presence of distinct subpopulations with varying acidification rates. Sorting these cells based on metabolic activity enables further functional studies, providing valuable insights into immune adaptation in cancer and the potential for therapeutic targeting of metabolically reprogrammed lymphocytes.

Conclusion

Our findings highlight a metabolic shift in TILs, emphasizing the role of metabolic adaptation in tumor immunity. By enabling quantitative assessment of metabolic activity at a single cell level, this approach provides a valuable tool to identify immune subpopulations with therapeutic potential, offering insights for refining immune-based cancer therapies.

EACR25-2294**Enhancing immunotherapy in KRAS G12C-driven lung cancer through B cell responses and Tertiary Lymphoid Structure formation.***A. Alonso de la Vega¹, J. Boumelha¹, K. Ng¹, P. Anastasiou¹, C. Moore², M. Molina Arcas¹, G. Kassiotis¹, J. Downward¹*¹*Francis Crick Institute, London, United Kingdom*²*Francis Crick Institute, United Kingdom***Introduction**

Lung cancer remains the leading cause of cancer-related mortality worldwide, with mutations in the KRAS oncogene accounting for 20–30% of lung adenocarcinoma (LUAD) cases. The development of targeted therapies and the use of immune checkpoint blockade (ICB) inhibitors has dramatically improved the clinical benefit and outcome of KRAS mutant lung cancer patients. Unfortunately, most patients do not respond to immunotherapy highlighting the importance of identifying predictive markers for patient response. Recent studies have identified tertiary lymphoid structures (TLS) to be good prognostic markers for ICB response. However, cause-and-effect relationships of the associations between TLS, KRAS inhibitors and immunotherapy have not yet been established.

Material and method

To investigate the mechanistic basis behind these associations, we have established a novel immunogenic mouse model for KRAS-driven lung adenocarcinoma that expresses antigens derived from endogenous retroviruses (ERV) and stimulates TLS formation *in vivo*. Using multiplexed imaging and genetically engineered models, we evaluate the contribution of TLS, B cells and anti-tumour antibodies to immune protection and how this is enhanced by KRAS-G12C inhibitor (G12Ci) treatment in KRAS-mutant lung cancer.

Result and discussion

We found that these tumours elicit a potent humoral response through the formation of germinal center structures and the generation of specific anti-ERV envelope protein antibodies. This antibody-mediated response is pivotal in establishing immunological memory, ensuring sustained protection against tumour relapse. Notably, Cas9-mediated deletion of specific proviruses confirmed that TLS formation and B cell responses are triggered by ERV expression. Furthermore, forced expression of ERV in an immune-cold model was sufficient to generate specific antibodies against ERV tumour antigens. Additionally, we explored the therapeutic potential of KRAS inhibition, which has been shown to elicit potent antitumor immune responses and synergize with anti-PD-1 therapy. We demonstrated that G12Ci treatment promotes anti-tumour B cell responses.

and the formation of mature and functional TLS structures.

Conclusion

Overall, our findings shed light on the potential of ERVs and G12Ci in enhancing the efficacy of immunotherapeutic approaches for KRAS mutant lung cancer. Exploring effective methods to induce ERV-derived antigens and understanding the mechanisms by which G12Ci influences B cell responses and TLS formation holds promise for predicting and improving patient response.

EACR25-2304

Study on sex-based myeloid cell differences in a clinically relevant glioblastoma mouse model

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Introduction

Glioblastoma (GBM) is the most common malignant primary tumor of the central nervous system with an extremely poor prognosis. Sex differences in GBM incidence, outcome and clinical data are well recognized, however the biological mechanisms which drive these discrepancies are not fully understood. The impact of gender on GBM immunosuppressive tumor microenvironment, is still among the unmet clinical needs.

Material and method

To investigate sex differences in GBM progression and its implication on the innate and adaptive immune landscape we exploited the preclinical GBM model obtained injecting orthotopically the syngeneic SB28 glioma cells in the striatum of C57BL/6J mice. Male/Female differences in terms of engraftment rate and tumor growth were analyzed in-vivo by bioluminescence intensity analysis at different time-point. By immunofluorescence analysis we were able to define this model in terms of tumor infiltrative capacity and progressive myeloid cell recruitment. High-dimensional flow cytometry detection of 18 parameters allow to better investigate innate/adaptive immune compartments.

Result and discussion

We observed that in a timeframe of 14-days SB28 murine model recapitulates the infiltrative features, the modest immune visibility, the proportion of immune cell populations and the spatially progressive myeloid recruitment of human GBM. We then stratified mice according to sex, and we were able to identify differences in tumor growth in the early phase of tumor progression which were maintained throughout the duration of tumor growth assessment (Two-way ANOVA Bonferroni's multiple comparison test, *p < 0.05). Preliminary data at early time-point showed higher infiltration of myeloid population in the tumor core and border of male mice, while lymphoid populations showed higher frequencies in females.

Conclusion

Investigating the sex-specific phenotype of myeloid and lymphoid cells longitudinally across the tumor growth'

timeline and their interaction with glioma cells in this clinically relevant GBM model will permit to better understand the sex-based differences in human GBM, paving the way for sex-personalized approaches that may lead to improve therapeutic efficacy.

This work is supported by Fondazione AIRC per la Ricerca sul Cancro (IG 25979 2021)

EACR25-2309

Primary human M1 and M2 macrophage to evaluate inflammatory and pro-resolving mediators and their role in tumor growth

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Introduction

Introduction: Tumor-associated macrophages (TAMs) play a crucial role in the tumor microenvironment (TME) and are predominantly associated with an M2-like phenotype, which supports tumor growth, immune suppression, and metastasis. Macrophages exhibit remarkable plasticity and can polarize into M1 (pro-inflammatory, anti-tumor) or M2 (immunosuppressive, pro-tumor) subtypes. M1 macrophages secrete pro-inflammatory cytokines, engage in tumor cell destruction, and sustain inflammation, whereas M2 macrophages contribute to tissue repair, immune suppression, and tumor progression. Given the resemblance of TAMs to M2 macrophages, understanding their functional properties is critical for developing therapeutic strategies targeting the TME. The aim of this study was to develop a primary human macrophage model mimicking M1 and M2 characteristics and to evaluate their functional and secretory profiles, with a focus on their role in tumor progression.

Material and method

Monocytes from human donors were differentiated into M1 macrophages using GM-CSF and IFN- γ , while M2 macrophages were obtained through M-CSF, IL-10, and dexamethasone treatment. The expression of M1- and M2-specific markers was analyzed to confirm polarization. Morphological and functional characterizations were performed, including NADPH oxidase activity (superoxide anion production), phago-cytosis assays, and tumor cell growth modulation. Additionally, bioactive lipid secretion from arachidonic acid metabolism was assessed using liquid chromatography-tandem mass spectrometry (LC-MS/MS) following cell stimulation.

Result and discussion

M1 macrophages exhibited higher secretion of IL-12p70 and superoxide anion compared to M2 macrophages. M1 cells also expressed elevated levels of SLAM and HLA-DR, whereas M2 macrophages showed increased CD14 expression. Functionally, M2 macrophages demonstrated enhanced phagocytic activity against *Candida albicans* yeast, while co-culture experiments with the HCT116 tumor cell line revealed that tumor cell proliferation was greater in the presence of M2 macrophages than M1. Furthermore, M1 macrophages were found to produce higher levels of TXB2, PGE2, PGD2, and 15-HETE.

Conclusion

These findings confirm that primary human macrophages differentiated in vitro exhibit functional M1 and M2 characteristics consistent with their physiological roles. Notably, M1 macrophages display tumoricidal activity, whereas M2 macrophages support tumor growth, reinforcing their resemblance to TAMs. This model provides a valuable tool for investigating macrophage plasticity in the TME and for screening strategies aimed at reprogramming TAMs toward a pro-inflammatory, anti-tumor phenotype.

EACR25-2318

Exploiting 'Myeloid-Derived Suppressor Cells'-targeted therapies in pre-clinical models of Notch-dependent T-Cell Acute Lymphoblastic Leukemia

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Introduction

Notch receptor family members regulate essential steps of T cell development and their aberrant activation inside this subset represents the main causative event for 'T-Cell Acute Lymphoblastic Leukemia' (T-ALL). The features of juvenile T-ALL are recapitulated in the Notch3 transgenic (N3tg) mice, in which tumoral T-cells drive the accumulation of Myeloid-Derived Suppressor Cells (MDSCs), that in a cancer context can inhibit anti-tumor immune response, thus promoting disease progression. Programmed cell death 1 (PD-1) is an inhibitory receptor that suppress T-cell activation, upon binding to its ligand, PD-L1. MDSCs contribute to tumor progression through different mechanisms, including expression of PD-L1 which suppresses PD-1+ immune cells, such as T- and NK-cells (NKs). Targeting the PD1/PD-L1 axis with immune checkpoint inhibitors (ICIs), such as anti-PD-L1 antibodies, represents a promising approach to enhance immune response against tumor, that however, is poorly studied in T-cell leukemia. Thus, our aim is to explore the role of MDSCs as possible target of immunotherapy in T-ALL.

Material and method

We administrated both a single anti-PD-L1 antibody and a combined anti-Gr-1/anti-PD-L1 antibody therapy to N3tg mice by intraperitoneal injections. At the end of the treatment, N3tg mice and relative controls, were sacrificed and appropriate spleen subsets were isolated to evaluate suppressive function of MDSCs and NK cytotoxic activity. To extend results to humans we also performed co-culture experiments between human NK-92 and Notch3-dependent T-ALL cell line, TALL-1, to assess the effects of tumor cells on PD-1 expression on NKs.

Result and discussion

We demonstrate an inverse correlation between MDSC suppressive function and NK activity during T-ALL progression, that correlates with significant accumulation of PD-L1+ MDSCs and PD-1+ NK cells. Treatment with anti-PD-L1 antibodies leads to a reduction in the absolute

number of both tumor T-cells and MDSCs, while increasing NK cytotoxic function. Further, preliminary data indicate that combined therapy with anti-Gr-1 and anti-PD-L1 antibodies, targeting MDSC differentiation and suppressive function respectively, may further enhance disease control. Additionally, in humans we observed that T-ALL cells induce PD-1 expression on NK-92 cell line, suggesting that, as in murine model, MDSCs can inhibit NK cell function through the PD-1/PD-L1 interaction.

Conclusion

Our findings suggest that targeting the PD-1/PD-L1 axis on MDSCs may modulate NK antitumor immune response, resulting in the inhibition of T-ALL progression. Further studies are required to better define therapeutic efficacy of MDSC-targeted combined therapy and to assess their influence on ICI-side effects.

EACR25-2376

Carcinogenic Effects of UV Radiation on *S. epidermidis* Infected Keratinocytes and Melanoma Cell Line

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Introduction

Skin cancer is a major public health concern, with melanoma being one of the most aggressive and deadly forms. Ultraviolet (UV) radiation is recognized as a primary environmental risk factor for melanoma, inducing DNA damage, oxidative stress, and immune suppression. In addition to UV exposure, recent studies suggest that the skin microbiota, particularly *Staphylococcus epidermidis*, may play a role in cancer progression and modulation. While some strains of *S. epidermidis* exhibit protective effects against skin neoplasia, others have been associated with cancer-related mechanisms. This study aims to investigate the effects of *S. epidermidis* infection and UV radiation on keratinocytes and melanoma cells, focusing on cellular responses and potential carcinogenic interactions.

Material and method

In this research, human keratinocyte (HaCaT) and melanoma (SK-MEL-30) cell lines were cultured under standard conditions and infected with *S. epidermidis*. Following bacterial infection, the cells were exposed to controlled doses of UV radiation to assess their response to photodamage. Then, total RNA isolation, cDNA synthesis, and qPCR were done. Depending on these methods, the determination of gene expression level of major genes which play roles in the cancer mechanism was provided. Various cellular analyses, including profiling, migration assays, and cell cycle analysis, were conducted to evaluate the impact of *S. epidermidis* and UV exposure on cancer-related pathways.

Result and discussion

The results of this study provide insights into the interplay between skin microbiota and environmental carcinogens, contributing to a better understanding of microbial involvement in melanoma pathophysiology.

Conclusion

Findings from this research may have implications for the development of novel therapeutic strategies targeting microbial interactions in skin cancer prevention and treatment.

EACR25-2394

Exploring the potential of common spliceosome mutations as biomarkers for directing immune checkpoint blockade therapies in myeloid malignancies

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Introduction

DNA damage repair deficiencies (DDRD) can elicit an innate immune response via activation of the cytosolic dsDNA-sensing cGAS-STING pathway. However, activation of this pathway can induce the up regulation of immunosuppressive checkpoint proteins, promoting an inflamed but immunosuppressed state. This provides a rationale for the use of immune checkpoint inhibitors (ICIs) for DDRD-positive tumours. Interestingly, mutations in the spliceosome, a premature-mRNA splicing complex, are commonly found in myeloid malignancies. Previously, we described how mutations in a key spliceosome component, SF3B1, conferred a DDRD. Therefore, this study aims to explore how common spliceosome mutations in SRSF2 and SF3B1 can be exploited to guide the use of ICIs in myeloid malignancies.

Material and method

Using CRISPR/Cas9-generated isogenic models harbouring the SRSF2P95H and SF3B1K700E mutations, DNA damage repair kinetics, micronuclei induction and cytosolic dsDNA/dsRNA accumulation were assessed using immunofluorescent labelling. qRT-PCR and ELISA analysis were used to assess expression and secretion of inflammatory cytokines, respectively, following treatment with daunorubicin, a topoisomerase II inhibitor. The immune competent embryonic chick chorioallantoic membrane (CAM) model was used for in vivo validation of the benefit of ICIs in spliceosome-mutant models.

Result and discussion

Similar to previous findings in our SF3B1K700E model, the SRSF2P95H mutation conferred a reduced ability to repair DNA damage, indicating a potential DDRD. DNA damaging agents have been shown to boost cGAS-STING activation, particularly in DDRD tumours. DNA damage induction by daunorubicin induced significant increases in micronuclei and cytosolic dsDNA in SRSF2P95H and SF3B1K700E models compared to WT controls. Significant increases in expression and secretion of inflammatory cytokines, CCL5 and CXCL10, were observed in both our SRSF2 and SF3B1-mutant models, compared to WT counterparts post-daunorubicin. Interestingly, no significant reductions in CCL5 transcription or secretion were observed following

STING-depletion in the SRSF2P95H model but were significantly reduced following depletion of MAVS, an adaptor protein involved in cytosolic dsRNA sensing. Indeed, cytosolic dsRNA accumulation was significantly increased in SRSF2P95H cells post-daunorubicin.

Furthermore, ICI with nivolumab resulted in SF3B1K700E tumour control, but interestingly induced more aggressive SRSF2P95H tumours in the immune competent CAM model.

Conclusion

To date, our data suggests that the SRSF2P95H and SF3B1K700E mutations confer an increased capacity to induce an innate immune response compared to their WT counterparts. However, this response may be reliant on the dsRNA-sensing MAVS pathway rather than the STING-dependent pathway. Together, this data is paving the way for use of SRSF2 and SF3B1 mutational status as a biomarker for the use of ICIs.

EACR25-2414

The immunomodulatory role of tumor-derived extracellular vesicles in breast cancer: insights into dendritic cell dysfunction and immune evasion

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Introduction

Dendritic cells (DCs) play a pivotal role in antitumor immune responses by acting as key antigen-presenting cells that initiate T-cell activation and immune surveillance. DC vaccines are being investigated in cancer immunotherapy due to their ability to stimulate antigen-specific T-cell responses. However, tumor cells can evade immune surveillance by modulating tumor microenvironment, which impairs effectiveness of DC-based therapies. In breast cancer, DC function is compromised, and monocyte-derived DCs (MoDCs) from patients also exhibit tolerogenic phenotype. Tumor extracellular vesicles (TEVs) have emerged as key mediators of intercellular communication, but their role in modulating MoDC function remains incompletely understood. This study aimed to evaluate the impact of TEVs isolated from MCF-7 (luminal A) and MDA-MB-231 (MDA) (triple-negative) breast cancer cell lines on MoDC differentiation in vitro. MDA cells are highly invasive and serve as a model for metastasis and therapy resistance.

Material and method

TEVs were isolated using the ExoQuick-TC kit and characterized by Western blotting, Nanosight, and Transmission Electron Microscopy. MoDCs derived from

healthy donors were cultured with IL-4, GM-CSF, and LPS, with or without TEVs (30 µg/mL). MoDCs cultured without or with HEK-293 EVs were used as controls. Phenotypic markers (CD11c, HLA-DR, CD80, CD86, and PD-L1) were analyzed by flow cytometry. miRNAs expressed in MDA EVs were quantified by qPCR.

Result and discussion

The results showed that TEVs from both cell lines differentially modulated the phenotype of mature dendritic cells. MDA EVs significantly reduced HLA-DR, CD11c, CD80, and CD86 expression, while increasing PD-L1 levels, suggesting a shift toward an immunosuppressive phenotype. MCF-7 EVs only decreased CD80 expression. Both types of TEVs upregulated PD-L1, indicating potential immune evasion. MoDCs treated with MDA EVs exhibited impaired ability to induce CD4+ and CD8+ T cell proliferation but induced the differentiation of Treg lymphocytes in mixed lymphocyte reaction. qPCR analysis revealed an up-regulation of let-7i, miR-10a, miR-10b, miR-126, miR-155, miR-21, miR-29b, and miR-34a. Bioinformatics analysis, using miRTarBase, the GSE22886 dataset in GEO2R, Python and GSEAPY package, identified pathways associated with interferon signaling, interleukin-6 and co-stimulatory molecule expression as potential targets of upregulated miRNAs.

Conclusion

This study underscores the role of TEVs in modulating MoDC phenotype and function, suggesting that these nanovesicles contribute to establishment of an immunosuppressive tumor microenvironment and evasion. The miRNAs identified in these vesicles may represent a potential mechanism through which they induce a tolerogenic DC profile. The ability of TEVs to modulate DC function highlights their potential as critical regulators of immune responses in cancer and opens new therapeutic avenues

EACR25-2547

The role of TRAIL/TRAIL-receptor bidirectional signaling for NK-cell cytotoxicity against neuroblastoma cells

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Introduction

Neuroblastoma (NB) is the most common pediatric extracranial solid malignancy, described as a cold tumor due to low MHC class I expression and therefore an ideal target for natural killer (NK) cell-based therapies. NK cells kill cancer cells using two different mechanisms, by release of cytotoxic granules and by ligating death receptors using ligands, such as Fas and TRAIL. Recent reports suggest that TRAIL ligation may result in bi-directional signaling. Here we investigated the role of TRAIL/TRAIL-receptor interplay and reverse signaling for NK cell responses towards neuroblastoma cells with the long-term goal of developing novel NK-based immunotherapeutic strategies in neuroblastoma.

Material and method

We performed degranulation and cytotoxicity assays using neuroblastoma cells (SK-N-AS or KELLY)

incubated with NK cells isolated from the buffy coats of de-identified healthy donors and pre-activated IL-2 and IL-15 to maximize TRAIL expression. To pinpoint the involvement of TRAIL-Rs in the NK degranulation process, we either disabled the binding to TRAIL with TRAIL-R1/R2-blocking antibodies or knocked down the receptors. To estimate the significance of death receptors for the degranulation process, we independently blocked signaling from NK cell receptors, such as NKG2D, NKp46, or DNAM-1, and compared the results.

Result and discussion

We found that neuroblastoma cell lines SK-N-AS and KELLY express TRAIL-R2 and that they trigger substantial NK cell degranulation and cytotoxicity. TRAIL-R1/R2 blockade reduced NK cell degranulation more than blockade of the major activating receptors NKG2D and DNAM-1, and to a similar extent as NKp46 blockade. Additionally, combined blockade of TRAIL-Rs and activating receptors, almost completely rescued neuroblastoma cells from NK cell killing. Furthermore, the knock-down of TRAIL-R1/R2 molecules reduced neuroblastoma cell death killing and significantly reduced the NK cell degranulation towards neuroblastoma cells. All data point towards a notable role of the TRAIL-TRAIL-R1/R2 complex in NK-cell activation.

Conclusion

Our findings suggest bidirectional TRAIL signaling upon NK cell interaction with neuroblastoma cells. Blockade/knock-down of TRAIL-R1/R2 did not only reduce NK cell killing of SK-N-AS and KELLY but also strongly reduced granule-mediated cytotoxicity of NK cells, highlighting the importance of TRAIL in NK cell signaling.

EACR25-2584

Combination therapy with EPHA3 monoclonal antibody and anti-CD47 antibody reprograms tumor-associated macrophages to inhibit recurrent head and neck cancer

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Introduction

This study investigates the combination efficacy of EPHA3 monoclonal antibody and anti-CD47 antibody treatments in recurrent head and neck cancer through the reprogramming of TAMs (tumor-associated macrophages).

Material and method

We utilized various cancer cell lines to assess the expression of EPHA3, CD47, and markers for M1 and M2 macrophage subtypes through western blotting, FACS, and FISH in both parent and radioresistant cell lines. The effects of EPHA3 and CD47 treatments were evaluated both in vitro and in vivo on human and mouse cell lines, with a focus on tumor growth, phagocytosis, and changes in immune cell infiltration

Result and discussion

Our results indicated that overexpression of EPHA3 and CD47 is associated with decreased phagocytosis in radioresistant cancer cells, particularly within the M2

macrophage subset. However, combined treatment with EPHA3 and CD47 antibodies significantly reduced tumor growth and enhanced phagocytosis both in vitro and in vivo. This was accompanied by an increase in M1 macrophage markers (iNOS, TNF- α , IL-6) and a decrease in M2 markers (arginase 1, CD206, IL-10, P-STAT1), as demonstrated by flow cytometry. Additionally, EPHA3 antibody treatment was found to potentiate CD47-mediated repolarization towards the M1 phenotype. In conclusion, our findings suggest that treatment with EPHA3 and anti-CD47 antibodies promotes tumor cell phagocytosis and inhibits tumor growth by shifting TAMs from a tumor-promoting (M2) state to a tumor-inhibiting (M1) state in vivo.

Conclusion

This highlights the potential therapeutic value of targeting TAM polarization in the treatment of recurrent head and neck cancer.

EACR25-2595

CD4 T cells in mediating direct anti-tumor activity and MHC class II loss in melanoma patients

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Introduction

CD4 T cells, recognizing antigens presented by MHC-II molecules, are well-defined for their role as helpers or regulators in cancer immunity. However, single cell sequencing studies found intratumoral CD4 T cells with cytolytic markers present in various cancers, suggesting also their direct anti-tumor activity, similar to cytotoxic CD8 T cells. Strikingly, a subset of melanomas shows strong constitutive expression of MHC-II molecules, despite of the general restriction to professional antigen presenting cells.

Material and method

Using autologous human tumor-T cell systems, we expanded tumor-reactive CD4 T cells from patients with advanced metastatic melanoma and analyzed their cytokine production via flow cytometry. Additionally, we performed FACS staining and targeted next-generation DNA sequencing to examine the expression of MHC-II genes in short-term cultured melanoma cell lines derived from consecutive patient samples.

Result and discussion

We demonstrate that CD4 T cells with tumor antigen specificity are present in patient blood and among tumor-infiltrating lymphocytes (TILs). These T cells directly kill autologous melanoma cells that constitutively express MHC-II molecules. Longitudinal sample analyses reveal the genetic loss of tumor antigen-presenting MHC-II molecules over the course of the disease, suggesting that melanoma immune evasion occurs due to strong selective pressure from CD4 T cells. Strikingly, MHC-II molecule

expression exhibits both intra- and inter-metastasis heterogeneity in the absence of genetic alterations, suggesting that non-genetic mechanisms may regulate MHC-II loss in tumor cells to evade CD4 T cell surveillance.

Conclusion

Overall, our study highlights the importance of tumor-reactive CD4 T cells as direct anti-tumor effectors and emphasizes the need for deeper analyses of CD4 T cells and MHC-II regulation in melanoma.