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Contents

Talk Session I

Hois Victoria

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Functional and metabolic characterization of beta cells during type 1 diabetes progression

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Type 1 diabetes (T1D) is an autoimmune disorder that is characterized by a progressive loss of insulin-producing beta cells. The decline in beta cells is accompanied by the infiltration of immune cells within pancreatic islets and an increase in ER stress. To date the mechanism behind the decline in beta cell function is poorly characterized. As part of the BioTechMed consortium <U+0093>MIDAS - Inflammatory Mechanism involved in Diabetes Uncovered by Tissue Imaging and Machine Learning<U+0094>, we want to link systemic immune cell response during T1D progression to functional and metabolic alterations within beta cells. To resemble the pathogenesis of human T1D we use non-obese diabetic (NOD) mice. Recent data from our consortium indicate that the decline in beta cell function occurs before any symptomatic manifestation of diabetes. Hence, we characterized the systemic and beta cell-specific immune- and molecular phenotype of NOD mice at defined non-diabetic blood glucose levels. Multiparameter flow cytometry analyses revealed only minor changes in immune cell populations in the blood of NOD mice before the onset of T1D. In contrast, pancreatic islets isolated from non-diabetic NOD mice exhibit already a decline in insulin secretion and storage ability associated with an upregulation in ER stress. Notably, plasma from these NOD mice induced ER stress and impaired cell viability of murine beta cells and pancreatic islets obtained from healthy mice. Thus, plasma from NOD mice comprises metabolic factors that modulate functional and metabolic pathways in pancreatic islet before the onset of T1D. However, whether these factors are coming from immune cells needs further investigations.

Klaczynski Michaela

Abstract ID: 92782

Fetoplacental-endothelial derived small extracellular vesicles (fp-sEVs) as modulators in the development of the fetal immune system

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Introduction: During pregnancy the placenta releases small extracellular vesicles (sEVs) into the maternal circuit. The main function of those placental sEVs is to maintain the immune tolerant environment towards the fetal allograft. The cell-to-cell communication between the placenta and the fetus is weakly understood, therefore, we aim to investigate the contribution of fetoplacental-endothelial derived sEVs (fp-sEVs) in the development of the fetal immune system. Methods: Primary fetoplacental-endothelial cells (fpECs) were isolated from placentae at term, preeclamptic (PE) and preterm (PT) pregnancies. fp-sEVs were enriched from media supernatants of fpECs by differential ultracentrifugation (100,000?g, 22 hours, 4°C pellet) and characterized by size (nanoparticle tracking analysis) and sEVs markers like ALIX, Syntenin, CD9, CD63 and CD81. In addition, a lectin microarray was used to profile glycosylation pattern of fp-sEVs and their parent fpECs cell membrane. Results: fpECs secrete fp-sEVs with a mean size of 125.3±3.9 nm (term, n=4), 162.4 nm (PT, n=1) and 153.4±20.44 nm (PE, n=3). Specific sEVs markers verified the presence of fp-sEVs. Moreover, on protein level we identified the placental specific marker Siglec-6, a sialic acid binding protein, and the endothelial marker CD31. The lectin microarray revealed that fpECs and fp-sEVs differ in their glycosylation profile in both term and PE. Compared to their parent fpECs, fp-sEVs are enriched with sialic acids, indicating a selective cargo loading in the sEVs biogenesis. Conclusion: These preliminary results suggest that the signature of fp-sEVs resembles their origin and the state of the parent fpECs. Based on the notion that glycans are important for intercellular communication; obtained sialic acid signatures on fp-sEVs may indicate an interplay of these vesicles and respective receptors on immune cells. Together, our findings could indicate an effective priming of the fetal innate immune system in utero.

Michenthaler Helene

Abstract ID: 93516

Multi-omics in one assay: qPRO-seq to decipher chromatin and transcriptional landscapes of fasting in adipose tissue

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Adipose tissue (AT) plays a central role in the regulation of systemic energy homeostasis. Transcriptome studies have shown that AT globally rewires its gene expression (GE) program upon fasting, but the regulatory determinants are not well understood. Key players of the fasting response are specific transcription factors (TFs) that bind to promoter and enhancer regions. Enhancer RNAs (eRNAs) are short and unstable RNAs, that are generated from TF-bound enhancers. For genome-wide determination of nascent RNAs in genes, promoters, and enhancers, quick precision nuclear run-on sequencing (qPRO-seq) of visceral AT of mice fed and fasted at different timepoints was performed. Data were analyzed using a customized bioinformatics pipeline. Validating our method, we found genes involved in ß-oxidation (e.g. Acads and Slc25a20) upregulated after three hours of fasting, while genes involved in lipid storage, including GK, were downregulated after one hour of fasting onset. Interestingly, GE analysis indicates signatures of immune cell infiltration (e.g. Il1r1, Reg4) as early as three hours after nutrient withdrawal. Moreover, we detected about 250 fasting-activated eRNAs cumulative over 6 hours of fasting. Motif enrichment analysis of fasting-selective enhancers identified several known AT-specific TFs, such as GRE, CEBP, RXR, as well as previously undescribed players, like the golgi-associated olfactory signaling receptor (GFY). Hence, we are first to apply qPRO-seq to complex tissues and show that, in combination with bioinformatics analyses, GE as well as the underlying regulatory events can be revealed in one genome-wide assay. In future, we will focus on analysis of fasting-evoked super enhancer clusters, TF networks, and their downstream pathways as well as on wet lab experiments to validate novel findings revealed by qPRO-seq. Since AT is very heterogenous and undergoes extensive remodeling upon fasting, cell-type specific approaches and models are currently tested.

Talk Session II

Eberhard Anna

Abstract ID: 92721

Paired analysis of blood plasma and urine reveals complementary information in prostate cancer

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Recently, liquid biopsy using blood, plasma, urine, saliva, and various other bodily fluids has shown utility to detect, diagnose, and monitor many types of cancers. So far, blood has been studied most, but recently cfDNA in urine has been extensively investigated. In particular, in urological cancers, ucfDNA might improve the sensitivity of liquid biopsy by proximal sampling, i.e., analysis of biofluids collected proximal to the tumor site. While evidence suggests that ctDNA in urine can be informative in renal and bladder cancer, less is known about ucfDNA in prostate cancer patients. Here, we aimed to determine the presence, levels, and potential clinical applications of ctDNA in plasma and urine of metastasized prostate cancer patients. To this end, we analyzed matching urine and plasma samples from 82 metastatic prostate cancer patients using a shallow whole genome sequencing approach. The tumor fraction and somatic copy number alterations (SCNA) were assessed using the ichorCNA algorithm. Our data shows that even though the tumor fractions do not significantly differ in plasma and urine, there is a great variability at patient level. SCNAs were detected with a high concordance in plasma and urine in 12% of patients, while in 26% and 17% of patients SCNAs were detected in plasma or urine only, respectively. In addition, in a subset of patients (n=26) longitudinal sampling was performed for monitoring purposes. ctDNA detection was slightly more likely in samples who coincided with clinical progression. Our data indicate that the presence of tumor-derived DNA in urine provides complementary information about the tumor that the sole analysis of plasma may miss. Since urine represents a desirable proposition given the quantities that can be collected at great ease, ucfDNA holds promise in the clinical management of prostate cancer.

Haitzmann Theresa

Abstract ID: 91958

Combined inhibition of gluconeogenesis and glycolysis suppresses lung cancer spheroid growth.

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Background/Aims Due to the heterogeneous blood supply in solid tumors, cancer cells often face a shortage of important nutrients such as glucose. It is known that when the access of lung cancer cells to glucose is cut off, they can still bypass glucose metabolism and synthesize vital metabolites using some steps of gluconeogenesis by expression of phosphoenolpyruvate carboxykinase (PEPCK, PCK2). However, the effect of simultaneously suppressing gluconeogenesis and glycolysis in cancer cells has not been studied yet. We hypothesize, that combined inhibition of the former two pathways may lead to a synergistic effect in suppression of lung cancer spheroid growth.

Method/Results Glycolysis was inhibited by 2-Deoxyglucose, a widely studied glycolytic inhibitor, and gluconeogenesis was either inhibited using shRNA mediated silencing of PCK2 or pharmacologically with the PEPCK-inhibitor Axon1165. The effect of dual inhibition was assessed as growth of 3D lung cancer spheroids and proliferation rate of 2D cultured lung cancer cells. Combined inhibition of glycolysis and gluconeogenesis led to a synergistic effect in suppression of lung cancer spheroid growth and reduction of the proliferation rate in both inhibitor combinations.

Conclusion Inhibiting glycolysis and gluconeogenesis simultaneously seems to represent a promising approach to circumvent the metabolic flexibility of cancer cells. However, the underlying mechanisms behind the observed interaction need to be studied more extensively to understand the process leading to the synergy.

Lind Karin

Abstract ID: 93406

Impact of TP53 aberrations on the transformation of human hematopoietic stem and progenitor cells (HSPCs)

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Background/Aims: Acute myeloid leukemia (AML) is an aggressive hematopoietic malignancy derived from transformed HSPCs. AML patients with TP53 aberrations face an exceedingly poor prognosis, even when treated with intensive regimens or novel strategies. To improve their outcome, deeper insight into underlying pathogenetic mechanisms is needed. Therefore, we aim to investigate if particular TP53 aberrations - mutations, deletions and a combination thereof - representing mono- and bi-allelic states, have a different impact on HSPC function and influence malignant transformation.

Results: Using an innovative CRISPR-Cas9 genome-editing approach combined with flow cytometry, allowed us to successfully enrich for human HSPCs comprising hot spot mutations (TP53 p.R175H and p.R273H) and gene knock-outs in a mono- and bi-allelic manner. Successful editing was confirmed on DNA and RNA levels via PCR and Sanger sequencing and p53 protein expression was demonstrated by Western blot analysis. In cell cycle analysis, bi-allelic TP53 aberrant HSPCs showed increased proliferation. These aberrations also form significantly more total colonies in methylcellulose assays with a propensity for erythroid differentiation. Furthermore, they revealed serial replating capacity indicating enhanced self-renewal.

Conclusion: We could successfully generate and characterize mono- and bi-allelic TP53 alterations in healthy, primary human HSPC using a CRISPR-Cas9-mediated knock-in strategy. We showed that bi-allelic mutant TP53 in HSPCs resulted in increased proliferation and enhanced self-renewal potential. These data clearly demonstrate the impact of TP53 aberrations on HSPCs and confirm the loss of function (LOF) phenotype. Current work focuses on the impact of these aberrations on genomic instability.

Poster Session I

Lembeck Anna

Abstract ID: 93283

The prevalence of pincer and fish-shaped red blood cells in various hematologic disorders.

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Background: In peripheral blood smears the morphology of red blood cells (RBC) provides key information for differential diagnosis of anemia but also other diseases. Distinct poikilocytes such as fish- and mushroom-shaped RBC, also known as pincer cells, are rarely observed. Methods: The smears of 255 blood specimens, grouped into various types of anemia (iron deficiency anemia (IDA), ?-thalassemia minor (BTM), sickle cell disease (SCD), microangiopathic hemolytic anemia (MAHA), autoimmune hemolytic anemia (AIHA), hereditary spherocytosis (HS), hereditary elliptocytosis (HE), vitamin B12/folate deficiency (VBFD)and other diseases myelodysplastic syndromes (MDS), primary myelofibrosis (PMF), malaria, liver disease (LD)) as well as a control group, were systematically reviewed. Abnormal RBCs were counted as cells per 20 high-power fields at 1000-fold magnification. The prevalence in different diseases as compared to controls was assessed by the Mann-Whitney-U Test and potential correlations were determined by using the Spearman correlation. Results: While fish cells were found with statistically increased numbers in blood smears of patients with IDA, BTM, MDS, HE, PMF and VBFD, numbers of pincer cells were significantly elevated in IDA, BTM, MDS, HS, HE, PMF and VBFD. Accordingly, numbers of fish and pincer cells displayed a strong correlation in IDA, BTM, MDS, PMF and VBFD ($p<0.01$). A negative correlation of hemoglobin levels independent from the underlying disorders was shown for numbers of fish ($p < 0.0001$) as well as pincer cells ($p < 0.0001$). Conclusion: These results demonstrate that fish- and pincer cells represent an abnormal RBC morphology indicative of pathologic conditions in distinct hematological disorders. Their numbers are associated with the severity of anemia. Additionally, numbers of pincer- and fish cells strongly correlate, assuming they are either the same kind of RBC abnormality or at least share the same etiopathogenesis.

Phan Minh Phuong

Abstract ID: 93422

Impact of inflammation on disease progression and extracellular matrix remodeling in failing hearts

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Background: Cardiovascular disease including heart failure (HF) are the most frequent cause of death in Europe. There are several pathophysiologic conditions which contribute to the pathogenesis of myocardial dysfunction such as ischemia, volume and/or pressure overload, aging<U+0085> Recently, immune cells and immune mediators have been reported to play important roles in these. Here, we aim to identify the role of cardiac antigen-specific T-cell activation and recruitment in the development of left ventricle (LV) hypertrophy, cardiac remodeling in non-ischemic HF.

We hypothesize that boosting protective immune responses by transferring antigen-specific T-cells into recipient mice subjected to pressure-overload induced heart failure enhances extracellular matrix remodeling.

Aim: Evaluate the effects of adoptive transfer of myosin heart-specific T-cells in an experimental murine model, to check whether these specific T-cells infiltrate the heart, modulate the inflammatory cardiac micromilieu and thereby healing outcomes.

Methods: Evaluate the effects of adoptive transfer of heart-specific T-cells in an experimental model of pressure-overload induced heart failure by transferring T-cells expressing transgenic TCRs specific for a cardiac myosin heavy chain alpha antigen (TCR-M cells). TCR-M cells will be purified from spleens and LN of donor mice using magnetic cell-sorting under sterile conditions and then transferred into syngeneic infarcted mice via the tail vein. As control group, we will adoptively transfer CD4+ T-cells specific for ovalbumin as this is an irrelevant antigen. Survival rate will be recorded. Cardiovascular outcome of recipient mice will be monitored by serial echocardiography (primary readout) on D7 and D28. Cardiac mass (hypertrophy) will be determined by LV weights, and lung congestion by wet lung weight. Interstitial fibrosis in the remote area will be quantified by histology (picrosirius red) and confirmed by collagen mRNA expression.

Phan Thi Thanh Huyen

Abstract ID: 92919

PPAR-? SIGNALING TRIGGERS A METABOLIC SWITCH ESSENTIAL FOR BRONCHIOLAR REGENERATION

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NA

Dysregulation of energy homeostasis often leads to development of metabolic syndrome, associated with cardiovascular disease, diabetes, liver steatosis, and cancer. The influence on the lung, however, is poorly characterized. Nevertheless, central obesity, type-2 diabetes, hypertension, and enhanced blood triglyceride levels are linked to reduced lung function in several epidemiological studies. Our data show that Peroxisomal Proliferator Activated Receptor-? (PPAR-?) signaling is essential for lung regeneration (Kanti et al., 2022). Upon binding of fatty acids the nuclear receptor PPAR-? drives the transcriptional program for fatty acid ?-oxidation and mitochondrial biosynthesis. To induce airway epithelial injury we challenged mice with naphthalene (NA). This led to bronchiolar epithelial denudation after few days. In the following week, the bronchiolar epithelium fully recovered. Using RNA in situ hybridization we found that PPAR-? target genes were selectively upregulated in the bronchiolar epithelium during repair. Pyruvate Dehydrogenase Kinase 4 (Pdk4) mRNA showed the strongest induction post NA exposure. Intriguingly, PDK-4 inhibits Pyruvate Dehydrogenase activity, and thereby, induces a metabolic shift from sugar to fatty acid catabolism. Pharmacological activation of PPAR-? signaling enhanced the bronchiolar regeneration potential in mice (Kanti et al., 2022). Therefore, we hypothesize that PPAR-? signaling triggers a metabolic switch, essential for bronchiolar regeneration. Adopting a powerful and novel combination of RNAscope in situ hybridization and immunofluorescence, we aim to understand which cell type(s) of the airway epithelium undergo metabolic shift for injury-repair. Our ongoing mechanistical analyses in vivo and in bronchiolar organoids should clarify the metabolic requirements for bronchiolar regeneration. In future this may allow us to develop targeted treatment strategies for diseases linked to failure of lung epithelial regeneration.

Pilic Johannes

Abstract ID: 92624

Hexokinase 1 rings prevent mitochondrial fission during energy stress

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Background: To meet cellular needs, metabolic enzymes must constantly adapt to changes in substrate availability. It is unknown how changes in substrate availability impact the subcellular location of the mitochondrial-bound enzyme hexokinase 1 (HK1). To answer this question, we use live-cell imaging and a substrate-free perfusion buffer.

Results: HK1 surprisingly clustered into ring-like structures that constricted mitochondria during glucose depletion. HK1-rings rapidly disassembled upon glucose readdition. The formation of HK1-rings was observed in multiple cell types and was confirmed with immunofluorescence. Using genetically encoded biosensors, we observed that the appearance of HK1-rings correlated more tightly with mitochondrial ATP than with cytosolic glucose levels, indicating that a lack of energy and not glucose drives the formation of HK1-rings. A single point mutation in the ATP-binding site increased that probability of HK1-ring formation. In contrast, mutating bulky residues in the mitochondrial binding domain abolished the formation of HK1-rings. HK1-rings were localized at mitochondrial fission sites, characterized by the contact with endoplasmic reticulum. Finally, we could demonstrate that HK1-rings prevented mitochondrial fission during energy stress.

Conclusion: HK1 forms rings in response to changes in substrate availability and may help cells in resisting energy stress by preventing mitochondrial fission.

Reynders Michelle

Abstract ID: 92899

Liver-adipose tissue communication through mTORC1

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Background/Aims: The mammalian target of rapamycin complex 1 (mTORC1) serves as a nutrient-sensing hub responsible for the switch between anabolism and catabolism. Dysregulation of mTORC1 results in metabolic disorders such as fatty liver, obesity and diabetes. Much is known about the regulation of mTORC1 within the liver, but little is known about how hepatic mTORC1 affects other tissues. Our research focuses on adipose tissue, due to its role in energy storage and its <U+0093>feedback<U+0094> relationship with the liver. We studied the role of mTORC1 within the liver-adipose tissue axis using different dietary regimes and knockout mice. Results: Feeding a ketogenic diet strongly activates mTORC1 in adipose tissue and, to a much smaller extend, in the liver. In liver-specific mTORC1 knockout animals, the adipose mTORC1 activation is preserved. These results coincide with our previous research, which shows that even if tissues are subjected to similar dietary conditions, metabolic regulation can differ between those tissues. Some diets show changes in gluconeogenic and lipogenic gene expression in liver but do not show these changes (or show opposite changes) in white adipose tissue, including expression of Pepck, Fasn and Scd1. One possible explanation for the effect of ketogenic diet on adipose tissue might be an increased secretion of the hepatokine fibroblast growth factor 21 (FGF21) that occurs under this diet. We therefore injected mice with recombinant FGF21, resulting in similarly strong activation of mTORC1 in adipose tissue. However, we rejected the hypothesis that hepatic FGF21 is solely responsible for mTORC1 activation, as ketogenic diet in FGF21 knockout mice showed increased adipose mTORC1 activation. Conclusion: The activation of adipose mTORC1 due to a ketogenic diet is neither dependent on hepatic mTORC1 nor solely dependent on increased FGF21 expression. Nutritional regulation of molecular metabolism is not similarly affected amongst investigated tissues.

Stockner Alina

Abstract ID: 92934

Regulation of lipid alterations in heart failure with preserved ejection fraction

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Obesity and aging are major risk factors contributing to the pathogenesis of heart failure with preserved ejection fraction (HFpEF), a predominant form of cardiac insufficiency with only a limited number of therapies. High levels of fatty acids and toxic lipid species which are present in obesity have been associated with HFpEF. Recently, the interorgan crosstalk between the white adipose tissue (WAT) and the heart has emerged as a novel therapeutic target to reduce the growing burden of this disease. In this regard, we hypothesize that pharmacological inhibition of adipose triglyceride lipase (ATGL) by Atglistatin (ATGLi) and thus reducing rates of lipolysis in WAT decreases lipotoxic effects and ameliorates the HFpEF phenotype. For this purpose, to generate experimental HFpEF driven by obesity and hypertension, respectively, 6-weeks-old C57BL6/J mice were administered a high-fat diet (HFD) and the nitric oxide synthase inhibitor L-NAME in the drinking water for 8 weeks. Upon the development of HFpEF phenotype, mice fed HFD and L-NAME were treated or not with ATGLi for 4 weeks. In vivo examinations, including weekly monitoring of body weight, transthoracic echocardiography and non-invasive blood pressure measurements, were used to analyze the effect of ATGLi on cardiometabolic health. Compared to control mice fed standard diet, HFpEF mice had significantly increased body weight gain, which was markedly reduced by ATGLi. Preliminary data from echocardiography showed improved diastolic dysfunction in response to ATGLi treatment, suggesting that inhibition of lipolysis in WAT has the potential to ameliorate the pathogenesis of HFpEF in mice. Future studies will focus on the mechanisms by which ATGL improves cardiometabolic health in experimental HFpEF.

Voglhuber Julia

Abstract ID: 92147

Hypertension and heart failure are accompanied by alterations of the mitochondrial membrane potential in cardiomyocytes

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Background Hypertension is the number one risk factor for the development of heart failure and one of the largest unmet medical needs in cardiovascular medicine. One of the hallmarks of hypertension and heart failure is mitochondrial dysfunction in cardiomyocytes. Although initial studies recognized the importance of different mitochondrial subpopulations, a direct comparison of the role of intrafibrillar (IF) vs perinuclear (PN) mitochondria in cardiac disease is lacking.

Methods/Results We used live cell imaging to investigate changes in mitochondrial membrane potential (??m) of IF vs PN mitochondria. We isolated cardiomyocytes from hypertensive rats, failing mice, and non-failing and failing human hearts. Using a ??m-sensitive dye, we stained the cells and acquired confocal images while subjecting the cells to physiological stress in the form of high-frequency electrical stimulation. Thereby, we demonstrated that ??m was generally more susceptible to alterations in PN mitochondria of diseased hearts than the respective healthy controls. In cells from hypertensive rats, we found depolarization of PN mitochondria upon stress, but not at baseline. However, in cardiomyocytes from both mouse and human failing cardiomyocytes, we found alterations of ??m already at baseline. Application of stress further exacerbated the depolarization of PN mitochondria in failing mice. IF mitochondria showed signs of deterioration only upon high-frequency pacing in failing cardiomyocytes of mice.

Conclusion Taken together, we propose that changes in ??m of PN mitochondria are highly prominent in cardiac disease and may be implicated in the progression of cardiac remodeling. IF mitochondria appear to be affected only under stress conditions in heart failure, indicating incapability to cope with increased workload. Normalization of PN mitochondrial function by ameliorating ??m depolarization emerges as an interesting new strategy to prevent or stall cardiac remodeling.

Wawrzen Alicja Katarzyna

Abstract ID: 93420

Unraveling the Role of Epigenetic Mechanisms as Potential Driver of Female Predominance in Pulmonary Arterial Hypertension

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Gender bias is observed in numerous conditions and diseases, however exact mechanisms underlying this predisposition are often unclear. Pulmonary arterial hypertension (PAH) is a rare lung vascular disease with female predominance. It is characterized by vessel well thickening and lumen narrowing, resulting mainly from pulmonary artery smooth muscle cells (PASMCs) expansion and matrix deposition. Despite improved therapy, PAH still represents a severe and progressive disorder in which the causative mechanism is not entirely understood. We investigated whether normal epigenetic inactivation of the second chromosome X in females might be disturbed in PAH patients. We hypothesized that the resulting aberrant biallelic expression of X-linked genes could thus drive PAH pathogenesis and female predominance.

Genes escaping X chromosome inactivation (escapees) were identified using a combination of single cell RNA sequencing and whole exome sequencing in PASMCs isolated from donors and PAH patients, eight subject in each group. Escapees were identified based on a cutoff of 5% or higher for biallelic expression that was found in at least 4% of the sequenced cells. Our preliminary results show that on average 11% of genes expressed in PASMCs under normal condition escape chromosome X inactivation, while in diseased samples this averaged to 9%. Most of the escapees are common and shared between conditions (53%). PAH-unique escapees (21% of all detected escapees) were defined by enrichment mainly in protein metabolism and biosynthesis processes. Within them only MAOA and SMS genes were already connected with PAH pathogenesis. Rest of identified escapees in PAH group could represent novel research target.

Obtained data revealed that disturbances in chromosome X inactivation pattern are unlikely to be the cause of female predominance in PAH, however it provides a valuable contribution to understanding PAH pathogenesis.

Zoidl Philipp

Abstract ID: 92966

The influence of Tramadol on platelets´ function

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Background Tramadol is one of the major opioids and a ligand with weak μ-opioid receptor affinity. In addition there is also an analgesic mechanism via serotonin reuptake inhibition. Serotonin affects blood clotting via several mechanisms: first, platelets carry serotonin receptors, activation of which leads to enhancement of platelet aggregation triggered by other neurotransmitters such as adenosine diphosphate (ADP) and thrombin; and second, serotonin-mediated vasoconstriction reduces blood flow in smaller blood vessels and facilitates wound healing.

In recent years, various analgesics have been investigated for their potential to affect the coagulation cascade or platelet function. Alterations have been demonstrated for NSAIDs such as metamizole as well as acetaminophen and co-analgesics such as SSRIs. On the question of the influence of tramadol on platelet function, interestingly, only a few papers with opposite results can be found.

Methods The aim of our study is to quantify the effect of tramadol on platelet aggregation. To this end, the effect is demonstrated in an ex vivo study using optical aggregometry (LTA) on whole blood.

In addition, two further questions will be investigated: -A titration series will be used to attempt to establish a dose-response curve for the effect of tramadol on platelet function. - Tramadol is often co-administered with other drugs such as ibuprofen, novalgin, or SSRIs. A number of studies are being conducted to determine if these combinations alter the effect of tramadol on platelet function.

Due to the lack of studies to date, there is no indication of the magnitude of the effect, therefore, based on studies with comparable methodology, as well as the statistical limitations of the study methodology (standard deviation LTA, interindividual differences in platelet function), we plan to study 15 subjects.

Conclusion Our study is currently ongoing, we expect first results by approximately Q1 2023.

Gaksch Lukas

Abstract ID: 93293

Immune cell composition in patients with and without steroid-refractory acute graft-versus-host disease after allogeneic hematopoietic cell transplantation

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Background One of the most life-threatening complications of allogeneic hematopoietic stem cell transplantation (allo-HCT) is acute Graft versus host disease (GVHD). It occurs almost exclusively in skin, liver, and gastrointestinal (GI) tract within the first 100 days after HCT. The first-line therapy is methylprednisolone, but only half of all patients respond and steroid-refractory is associated with a poor prognosis. Prognostic marker that predicts response to glucocorticoid therapy are still needed. In this retrospective study, we analysed the composition of T cells, innate lymphoid cells (ILCs) and tissue-resident memory T cells (TRMs) in acute GI GVHD samples and relate them to the response to glucocorticoid therapy.

Methods We searched for clinical data of allo-HCT patients with suitable stored samples of GI GVHD biopsies. We were able to obtain samples from 27 patients who have responded to glucocorticoid therapy (CR group) and 28 patients who developed glucocorticoid resistance (SR group). GVHD free GI biopsies taken from patients with bowel adenomas were included as control cohort. For Immunofluorescence staining, cells were labeled with the T cell markers, CD3, CD4 and CD8, and the TRM marker CD103. ILC3 were defined as CD3- RORgt+. Stained slides were imaged on a TissueFAXS imaging system and analyzed using TissueQuest software.

Results Our analysis showed that the percentage of ILC3s in the acute GI GVHD tends to be, but not significantly, higher than the SR group, but a significantly higher than the control group (p=0,008). In contrast, TRMs in the CR group were significantly reduced compared to the SR group (p=0,0261) and the control cohort (p=0,0014). In particular the TRMs with CD4 signature were lower in the CR cohort than in the SR cohort (p=0,0395).

Conclusion We have shown that TRMs and ILC3 are expressed differently in acute GI GVHD and might serve therefore as a useful clinical prognostic marker for response to glucocorticoid therapy.

Hameed Zobia

Abstract ID: 93425

Antibacterial effect of Lactobacillus casei rhamnosus (LCR 35) supernatant towards several clinical Helicobacter pylori and Clostridioides difficile isolates

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Helicobacter pylori is a Gram-negative, spiral-shaped, microaerophilic opportunistic pathogen that colonizes the mucus layer of human gastric epithelium. H. pylori is the major causative factor for peptic ulcer, gastric adenocarcinoma, and chronic gastritis in humans. C. difficile is a Gram-positive, drumstick-shaped, anaerobic spore forming opportunistic pathogen that colonizes the human colon and produces enterotoxins. C. difficile is the causative factor for pseudomembranous colitis, toxic megacolon, perforation of the colon and sepsis. For both, eradication therapy is losing efficiency due to the increasing microbial antibiotic resistance worldwide. Probiotics, in particular Lactobacillus strains, have been reported to show antimicrobial properties in vitro. Thus they are emerging as promising tool for the treatment of H. pylori and C. difficile infections.
The aim of this research is to study the antagonistic activity of Lactobacillus casei rhamnosus LCR 35 supernatant components against H. pylori and C. difficile. The results of our experiments are promising as LCR 35 supernatant drastically reduces cell viability of H. pylori and C. difficile immediately after exposure. Our study is a first, important step towards the potential application of probiotic lactobacilli in prophylaxis or treatment of H. pylori and C. difficile infections.

Javorova Pavlina

Abstract ID: 93429

Id3-KO mouse model of Primary Sjögren´s Syndrome shows mitochondrial alterations and signs of cellular senescence similar to pSS patients

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Primary Sjögren<U+0092>s Syndrome (pSS) is systemic autoimmune disorder characterised by lymphocytic infiltrations in exocrine glands.T-cells are considered major players in pathogenesis of pSS.Previously we showed,that peripheral lymphopenia-a frequent finding in pSS- affects mostly naïve CD4+ T-cells which show senescent phenotype. The aim of this study was to investigate T-cells phenotype in Id3-KO mouse model of pSS and compare obtained data with the data from human patients. Immune cells were isolated from peripheral blood,draining lymph nodes and spleen.Flow cytometry analysis was performed to characterize CD4 and CD8 subsets (naïve T-cells,central memory T-cells,effector memory T-cells and double negative T-cells).Further staining with mitochondrion?selective fluorescent dyes MitoTracker® Green,MitoTracker® Deep Red,MitoSox Red was performed to assess mitochondrial mass,membrane potential and superoxides production,respectively.Samples were analyzed on BD FACSLyric<U+0099> Flow Cytometer.Agilent Seahorse XFe96 Analyzer was used to measure oxygen consumption rate in isolated total CD4+ T-cells. In Id3-KO mice,there is significant decrease in naïve cells compartment in both CD4 and CD8 T-cells in all studied tissues (blood CD4 p=0,027,CD8 0,081;lymph nodes CD4 p=0,003,CD8 p<0,000001;spleen CD4 p<0,000001,CD8 p= 0,000006).These T-cells also have significantly decreased mitochondrial mass in the all phenotyped subsets (all p<0,01).Despite having reduced mitochondrial mass we observed that Id3 deficient T-cells produce higher amount of mitochondrial superoxides in the all phenotyped subsets,with most affected naïve CD4+ T-cells (p=0,021).Basal respiration was significantly decreased in Id3-KO total CD4+ T-cells (p=0,027). These preliminary data suggest that there are mitochondrial alterations in the immune cells of pSS mouse model. The mitochondrial alterations and significant decrease in naïve subsets indicate aged phenotype of T- cells,that is also present in pSS patients.

Krivic Denis

Abstract ID: 93364

TRPC6 Photopharmacology Allows for Dissociation of Mast Cell Degranulation and Transcription Factor Activation

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Besides a key role in allergic diseases, mast cells confer important modulatory functions in innate and adaptive immunity. The communication between mast cells and other immune cells is not only based on the acute or long-term release of pre-stored mediators involving distinct processes of degranulation but also on the expression of specific surface proteins. Activation of mast cells typically involves Ca2+ signalling, which evokes both transcriptional activation and degranulation. To dissect the complex functions of mast cells in human pathology and to explore new therapeutic strategies we aimed towards specific manipulation of mast cell phenotype. Characterization of an optochemogenetic protocol for activation RBL-2H3 mast cells revealed that repetitive photocycling of OptoBI-1 to generate oscillatory Ca2+ signals elicits rapid and efficient NFAT nuclear translocation without concomitant degranulation. By contrast, conventional pharmacological activation of overexpressed TRPC6 channel was strictly associated with CD63-associated secretion. Our results suggest that the specific modulation of mast cell phenotype as obtained by TRPC6-OptoBI-1-mediated activation, arises from introduction of a highly specific conformational and functional state of the TRPC6 channel, resulting in unique Ca2+signatures. Further understanding of these phenomena may lead to new approaches for (photo)pharmacological immunomodulation.

Nayak Barsha Baisakhi

Abstract ID: 93318

The role of JAK-STAT signaling in neutrophilic lung inflammation

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Background/aims Neutrophilic lung inflammation is a common feature of chronic inflammatory lung diseases like COPD and corticosteroid-resistant asthma resulting in exacerbation. The IL23-Th17 axis is a major contributor to neutrophilic inflammation. Exposure of the airway epithelium to triggering agents enables the release of IL23 from the antigen-presenting cells. IL23 binds to its receptors, associated with TYK2 and JAK2 on T naïve cells. This eventually leads to the phosphorylation of STAT4 and STAT3 and subsequently Th17 polarization. A major Th17 cytokine, IL17 binds to its receptor on structural and immune cells and results in the release of IL8 and GMCSF leading to neutrophil recruitment and activation. Thus, we aim to investigate the potential role of TYK2-JAK2 kinases and associated cytokines in the IL-23-Th17 axis of neutrophilic lung inflammation. Results Functional assays on neutrophils to study ROS production and migration were performed. IL17 and IL23 pretreatments significantly increased ROS production compared to control in neutrophils. IL17 significantly increased the migration of neutrophils and enhanced IL8-stimulated chemotaxis. The expression of TYK2 and JAK2 was studied in the various immune cell populations in whole blood of allergic and non-allergic donors using flow cytometry. Non-allergic donors showed significantly higher expression of TYK2 and JAK2 compared to allergic donors. Conclusions Our results indicate that IL17 and IL23 might be potential players in neutrophilic lung inflammation, affecting neutrophil migration and ROS production. TYK2 and JAK2 are differentially expressed in immune cell populations from allergic donors compared to healthy controls. From our preliminary data, we conclude that IL17 and IL23 directly affect neutrophil functions. In further experiments, we plan to evaluate neutrophil function and JAK-STAT expressions in samples from patients with COPD and non-allergic asthma.

Reiter Bernhard

Abstract ID: 92942

Immunoprofiling of secondary pulmonary hypertension in chronic lung diseases

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Chronic lung diseases (CLD), such as chronic obstructive pulmonary disease (COPD) and idiopathic pulmonary fibrosis (IPF) are severe progressive diseases and are associated with a large decrease in quality of life. Both diseases consists of several highly heterogeneous phenotypes, which can be distinguished by their degree of inflammation, pathology or treatment response. Patients with CLD can additionally develop an associated form of pulmonary hypertension (PH, WHO Class III). The presence of pulmonary hypertension in these diseases is associated with a worsened prognosis. Even though COPD, IPF and PH are very different in their appearance, vascular remodelling share common features, such as neointimal formation and medial hypertrophy. So far, there is no successful treatment against vascular remodelling, and usual treatments for PH are not recommended for PH in CLD. Previous work in our lab has shown an altered immune cell profile in arteries of idiopathic pulmonary arterial hypertension compared to healthy vessels. In this project, we will determine the inflammatory profile in arteries of PH associated with CLD and investigate how certain inflammatory cell subpopulations have an impact in the remodelling of pulmonary arteries. Human COPD-PH, IPF-PH and healthy donor pulmonary arteries are currently characterized using flow cytometry analysis. Additionally we are currently analysing an in-house database of already measured COPD, IPF, IPAH and healthy pulmonary arteries. To gain further insights in structural remodelling and cell localization image analysis of various fluorescence/ light microscopic stainings will be performed. Functional cell information will be gathered using cell culture techniques, such as direct and indirect co-cultures, and single cell RNA-seq. By combining this data with clinical parameters, we will be able to get a more complete and translational understanding of the impact from immune cells in the formation of PH in CLD.

Sinha Madhushri

Abstract ID: 93462

CLCA1/TMEM16 axis in chronic lung disease

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Our previous work identified the calcium-activated chloride channel TMEM16A, as a critical factor for the pathophysiology of pulmonary arterial hypertension. Recently, CLCA1, an endogenous activating factor, has been postulated as a regulator of TMEM16A. CLCA1 stabilizes TMEM16A and prevents its internalization leading to prolonged expression and perhaps function at the cell surface. However, this activation has been shown so far only in an artificial system in vitro. Thus, the current project aims to characterize the CLCA1/TMEM16A axis and its function in chronic lung diseases. Our investigations were carried out both in-vitro and in-vivo. In -vitro studies were performed on the epithelial cell line, A549, where CLCA1 was checked for a physiological role in two different settings. First by overexpressing CLCA1 in A549 cells and second by adding conditional CLCA1 medium to the A549 cells. Results show that CLCA1 causes cell proliferation when CLCA1 is overexpressed. For the first time, we show, that CLCA1 does not require to undergo cleavage inside the cell to get secreted outside the cell. For in-vivo studies, the Fra-2 chronic lung-diseased mouse model was used, where the active level of CLCA1 was first established through western blot. Quantifying the results revealed that the active CLCA1 levels are higher in Fra-2 compared to the WT mouse. Furthermore, TMEM16A is also present at comparable levels in both Fra-2 and WT mice. In conclusion, the CLCA1/TMEM16A axis was successfully established in our Fra-2 chronic disease model. Our new finding that CLCA1 itself causes cell proliferation warrants further investigation.

Teppan Julia

Abstract ID: 93182

The role of the molecular circadian clock in Asthma

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Asthma is a chronic inflammatory lung disease with a strong circadian signature. Nocturnal worsening of asthma is detected in 75% of all patients. At 4 a.m. most severe asthma attacks occur and the highest number of eosinophils, one of the main effector cells in asthma, is observed in the sputum. The molecular circadian clock produces oscillating expression patterns to time biological processes such as leukocyte trafficking. As disturbances in the circadian system can promote inflammatory diseases, this project aims to investigate the impact of the molecular circadian clock in asthma and clarify if exogenously applied ligands may represent a novel treatment approach in the future.

A comparison of the expression level of circadian nuclear receptors in leukocytes from asthmatic and healthy donors revealed significant differences of the expression level. Similar differences were observed by mimicking an inflammatory environment using sera from asthmatic patients or inflammatory mediators. The inverse ROR agonist SR1001 reduces the shape and migratory response, while decreasing respiratory burst and degranulation of human peripheral eosinophils. SR1001 treatment also affects nuclear circadian receptor expression and polarization of macrophages. Systemically applied SR1001 shows anti-inflammatory properties in vivo. Importantly, the treatment had no effect on rhythmic biological behaviour of the mice although the circadian clock is targeted.

We observed that circadian nuclear receptors are differentially expressed in leukocytes from asthmatic patients. Targeting the ROR receptor has an impact on the expression of nuclear circadian receptors and supresses effector cell functions of eosinophils, neutrophils and macrophages. Further SR1001 shows anti-inflammatory properties in vivo reducing the migratory responsiveness. Thus, exogenously applied inverse ROR agonists may represent a novel pharmacological approach for the treatment of allergic inflammation and asthma.

Waked Pamela

Abstract ID: 93520

Endotyping of Chronic Rhinosinusitis using proteomics approach

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Chronic Rhinosinusitis (CRS) is an inflammatory disease of the nose and paranasal sinuses and may affect the upper airways in severe cases. In the EPOS guidelines, CRS is diagnosed by at least two of the following major symptoms present for at least 12 weeks: nasal congestion, nasal discharge, pain or facial pressure or impaired sense of smell. There are two major types of CRS: presence of nasal polyps (CRSwNP) or without nasal polyps (CRSsNP). However, clinical classification by those two phenotypes does not reflect the variety of CRS endotypes which are related to different cytokine profiles and inflammatory responses and often lead to varying therapeutic response, surgical failures and recurrence, indicating that CRS is a hetegenous disease and proper pathophysiologic endotyping is necessary for advancement in patient management and treatments.

The aim of this project is to endotype CRS based on the proteomic analysis of the nasal mucus, Bronchoalveolar Lavage (BAL) and serum by profiling of inflammatory cytokines and immune cells and cluster analysis of CRS patients through untargeted proteomic analysis and targeted immunoassays and flow cytometry.

Difference in proteome of nasal mucus, BAL and peripheral blood of 200 samples will be studied and analysed. First, Proteomic analysis will be performed on TimsTOF mass spectrometry and protein abundances will be calculated as mean and standard deviation and statistically analysed. Second, FACS analysis will be performed and immune cell profile will be established. All these data from patient samples will be analysed and compared with clinical tests and data to cluster our patient cohort into relevant pathophysiologic subgroups.

This study gives us an insight into the different pathophysiological mechanisms that are involved in the disease and how it differs between individuals in the hopes of finding endotypes within this heterogeneous disease through analysing the protein profile and immune cells in CRS patients.

Wojcik Gabriela

Abstract ID: 93528

Loss of ANGPTL4 attenuates lung fibrosis

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Idiopathic pulmonary fibrosis (IPF) is a morbid disease of unknown origins. New findings suggest the influence of lipid metabolism in IPF progression. In a healthy lung, lipoprotein lipase (LPL) hydrolyses triglycerides (TG) to free fatty acids (FFA), which are necessary for processes such as surfactant production. Surfactant is produced by type II alveolar epithelial cells, which are supported by the adjacent lipofibroblasts in terms of lipid metabolism. As an inhibitor of LPL angiopoietin Like 4 (ANGPTL4) can, hence influence lipid metabolism and FFA availability. Therefore, we hypothesize that high levels of ANGPTL4 can alter FFA availability and hence lung fibrosis progression. ANGPTL4 KO mice exhibit higher levels of FFA at baseline condition, and lower collagen deposition upon bleomycin instillation. ANGPTL4 KO mice showed a decreased number of PDGFR? positive cells, suggesting that the decrease of collagen could be a consequence of the decreased number of collagen producing cells. In vitro experiments, confirmed that FFA stimulation of human parenchymal fibroblasts (hPF) reduced the collagen production, further suggesting an influence of FFA on collagen producing cells. Similar results were obtained with stimulation of hPF with Rosiglitazone and Metformin, two drugs whose efficacy in lung fibrosis mouse model has been already shown. These data suggest that ANGPTL4 via FFA availability can influence lung fibrosis development and progression. ANGPTL4 could serve as a potential target for lung fibrosis treatment, however further investigations are necessary to elucidate the underlying mechanism.

Zukauskaite Kristina

Abstract ID: 93347

PREOPERATIVE MECHANICAL BOWEL PREPARATION IMPACT ON THE INTESTINAL MICROBIOME IN PATIENTS UNDERGOING LEFT-SIDED COLORECTAL CANCER SURGERY

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Background Preoperative mechanical bowel preparation remains standard in left-sided colorectal cancer surgery. However, there is no consensus on the optimal way to do it, as oral bowel-cleansing agents and rectal enema are both widely used methods. Understanding of gut microbiome impact on postoperative outcomes is emerging, thus this study aimed to compare different bowel preparation techniques<U+0092> impact on the intestinal microbiome.

Methods Forty patients who underwent surgery for left-sided colorectal cancer at the National Cancer Institute, Vilnius, Lithuania were randomized at a 1:1 ratio for preoperative mechanical bowel preparation with oral agents (Fortrans; Ipsen Pharma, Paris, France) or rectal enema (2 L of 0.9% NaCl) (NCT04013841). Intestinal microbiome composition was analyzed in frozen stool samples collected at baseline, 6th, and 30th postoperative days using Illumina Miseq technology (Illumina, Eindhoven, the Netherlands). Raw sequencing data were processed using QIIME 2 tools on a local Galaxy instance (https://galaxy.medunigraz.at/).

Results Using an oral agent for mechanical bowel preparation significantly decreased ?-diversity parameters ($p<0.05$). In contrast, rectal enema had no such effect on the microbiome composition ($p>0.05$). Paired analysis revealed significant differences between patients in oral agents and rectal enema groups ($p<0.05$). On the 6th postoperative day, a significant increase in Enterococcus faecalis abundance was observed in the rectal enema group.

Conclusion This study showed that mechanical bowel preparation with oral agent results in more profound intestinal microbiome composition changes. Further investigations should explain these differences<U+0092> impact on clinical outcomes.

Föderl-Höbenreich Esther

Abstract ID: 92496

Different transcriptional responses of SARS-CoV-2 variants of concern revealed by comparative high-resolution spatial transcriptomics

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Recurrently emerging SARS-CoV-2 variants of concern (VOC) drive COVD-19 pandemic waves, as exemplified recently by the highly infective B.1.1.529 variant known as Omicron, which in general cause mild disease. Using spatial transcriptomic in Calu-3 cells infected with various VOC, we identified marked differences in the molecular host responses. We report decreased infection rates for the dominant VOC B.1.1.529 (Omicron) (27%) compared to B.1.617.2 (Delta) (63%) and the original SARS-CoV-2 strain (Wuhan-Hu-1) (43%) in Calu-3 cells. With a set of 84 immune-relevant genes, we discriminated transcriptional responses and compared immune strategies of these Omicron and Delta variants. By spatially dissecting highly virus replicating and bystander cells, we highlighted molecular signatures unique to individual VOCs. We detected a massive upregulation of NFKBIA in Wuhan-Hu-1 and B.1.617.2 infected cells, hinting at impaired interferon signalling which manifests in absent correlative gene expression in bystander cells. In contrast, NFKBIA levels in Omicron infected cultures were comparable to mock infected cells, and bystander cells accomplished correlative expression of antiviral genes, suggesting an increased cellular capability to launch a proper immune response against B.1.1.529 infection, which might prevent severe disease.

Balihodzic Amar

Abstract ID: 92574

Application of genome-wide CRISPR/Cas9 screen to identify synthetic lethality with MCL-1 inhibition in lung cancer

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Background/Aims Lung cancer is among the most diagnosed cancers and the leading cause of cancer-related deaths. Histologically, it is divided into small cell lung cancer and more prevalent non-small cell lung cancer (NSCLC). Recent reports demonstrated frequent amplifications of anti-apoptotic gene myeloid cell leukemia 1 (MCL-1) in NSCLC, contributing to tumor cell survival and treatment resistance. Pharmacologic inhibition of MCL-1 protein showed promising effects in pre-clinical models, but the malignant cells frequently exhibit resistance to this mono-therapy. The aim of this study is to uncover synthetic lethal interactions between MCL-1 inhibition and specific genes and thereby identify potential new targets for combination treatment approaches. Methods/Results To reveal synthetic lethal relationships with MCL-1 inhibitors in NSCLC, we employed a genome-wide pooled CRISPR/Cas9 screen. MCL-1 inhibitor-resistant A549 cells were lentivirally transduced with Cas9 and a whole-genome CRISPR knock-out guide RNA library, and treated for 12 days with MCL-1 inhibitor. At the end of the treatment period, genomic DNA was extracted and next generation sequencing applied to identify differences in guide RNA expression in control and MCL-1 inhibitor-treated cells. SgRNAs against co-essential genes should be lost in the treated cells during in the screening process. Bioinformatic analysis revealed that guide RNAs against 26 genes were significantly downregulated upon MCL-1 inhibition. One of the obtained hits was the BCL2L1 gene that encodes for another pro-survival protein named BCL-XL, a well-known resistance factor to a variety of therapies. Indeed, when we combined the MCL-1 inhibitor with specific inhibitors against BCL-XL, A549 cells were strongly sensitized and cell death induction was observed at very low concentrations of MCL-1 inhibitor. Conclusion Our data indicate the existence of a synthetic lethal relationship between MCL-1 inhibitor and multiple genes in NSCLC.

Cosic Mujkanovic Nejra

Abstract ID: 93496

Exploring the role of myeloperoxidase in non-small cell lung cancer

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Background/Aims: Despite the recent increase in number of treatments, Lung cancer remains the leading cause of cancer related deaths worldwide. Non-small cell lung cancer (NSCLC) is a very heterogeneous disease and represents ~85% of all lung cancer cases. It has been reported that NSCLC tumors have a high immune infiltration, where neutrophils represent an abundant immune cell type with an immunosuppressive function. Some of the cytoplasmic granule components of neutrophils, such as myeloperoxidase (MPO), are considered to contribute to tumor development. MPO is a heme containing peroxidase enzyme known for its host defence function against microbes. Some reports suggest that MPO might be able to influence cancer or immune cells and that way contribute to cancer development. We aim to investigate whether MPO can influence lung cancer cells cells in-vitro and tumor growth in-vivo.

Results: In-vivo data in our lab showed that MPO-/- mice developed smaller tumors and had prolongated survival when compared to MPO WT. Analysis of the TME revealed increased number of different T-cell populations as well as improved function (by measuring INF?) of T-cells in MPO KO mice vs WT. MPO was able to increase proliferation of human lung adenocarcinoma cells (A549 cells) in-vitro. Furthermore, MPO treated cells revealed a decreased number of apoptotic cells, suggesting a protective function of MPO towards apoptotic cell death. Besides the cytoplasmic uptake of MPO, for the first time we report a nuclear internalization of MPO in A549 cells.

Conclusion: MPO is able to regulate cancer cells and influence T-cells. Therefore, MPO may play a role in development of lung cancer.

Dey Saptaswa

Abstract ID: 93554

Blocking STAT3/5 through direct or upstream kinase targeting in leukemic cutaneous T-cell lymphoma

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Leukemic cutaneous T-cell lymphomas (L-CTCL) are lymphoproliferative disorders of skin-homing mature T-cells causing severe symptoms and high mortality through chronic inflammation, tissue destruction, and serious infections. Despite numerous genomic sequencing efforts, recurrent driver mutations have not been identified, but chromosomal losses and gains are frequent and dominant. We integrated genomic landscape analyses with innovative pharmacologic interference studies to identify key vulnerable nodes in L-CTCL. We detected copy number gains of loci containing the STAT3/5 oncogenes in 74% (n = 17/23) of L-CTCL, which correlated with the increased clonal T-cell count in the blood. Dual inhibition of STAT3/5 using small-molecule degraders and multi-kinase blockers abolished L-CTCL cell growth in vitro and ex vivo, whereby PAK kinase inhibition was specifically selective for L-CTCL patient cells carrying STAT3/5 gains. Importantly, the PAK inhibitor FRAx597 demonstrated encouraging anti-leukemic activity in vivo by inhibiting tumor growth and disease dissemination in intradermally xenografted mice. We conclude that STAT3/5 and PAK kinase interaction represents a new therapeutic node to be further explored in L-CTCL.

Geles Abidin

Abstract ID: 93559

Survival and Progression of Mucinous and Non-Mucinous Adenocarcinomas of the Lung

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Background There are over a million deaths from lung cancer each year due to its early spread and high invasiveness. Adenocarcinoma is the most common type, making up 42% of all lung cancers. Approximately one third of adenocarcinomas have a mucinous pathway. Aims This study aimed to compare the survival and progression of mucinous and non-mucinous adenocarcinomas of the lung. While it is thought that mucinous ACs are more aggressive, their cytomorphological and architectural features have not been evaluated in relation to prognosis and genetic changes. We will evaluate these features in 76 cases from a single institution. Methods We analyzed 335 surgically resected cases of mucinous and non-mucinous adenocarcinomas using clinical data collected retrospectively. We compared parameters such as gender, smoking status, pack-years, postoperative treatments, chemotherapy, radiotherapy, metastatic profile, and cause of death. We also studied 76 mucinous adenocarcinomas for their patterns, mucin storage and release, expression of certain proteins and genetic changes, and correlated this with survival. For comparison, 259 non-mucinous adenocarcinomas were selected. Results & Conclusion Mucinous ACs mostly showed acinar patterns (43/71), followed by papillary ones (10/71). Neither pattern nor mucin storage affected survival. Survival was also not affected by the presence or absence of signet ring cells. MUC1, MUC2, and MUC5AC proteins were expressed in 39, 7, and 50 ACs, respectively. There was a non-significant trend for loss of p16INK4A to be linked to worse prognosis, while p14ARF did not affect survival. High TTF1 expression trended towards better survival, but was not significant. There was no correlation between KRAS mutation and structural patterns or stage. All colloid ACs had KRAS mutations.

Godschachner Theresa Marie

Abstract ID: 93177

Morphological and genetic characterization of benign primary bone tumors and their correlation with clinico-pathological and radiological findings

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Background/Aim: Osteoid osteomas (OO) and osteoblastomas (OB) are common benign primary bone tumors separated by size (OO <2cm, OB >2cm). Both showing similar histological features and can mimic osteosarcoma. In contrast to OO, OB have a locally aggressive growth pattern and no pain relief after NSAID intake. Rearrangements involving FOS and FOSB were reported in both tumors. The aim of this study is to determine the diagnostic utility of epi-/genetic alterations in these tumors and to find genetic changes that can explain the different behavior of OB and OO. Methods: Retrospective analysis of OO and OB from 2000 until September 2022 was performed. Fusion analysis was done using next-generation sequencing (NGS) using Archer FusionPlex Sarcoma Panel (ArcherDX Analysis software). Methylation was performed using Illumina Infinium MethylationEPIC BeadChip technology. Our data will be compared with previously published data, including TCGA. Results: The cohort included 34 OO and 20 OB. OO occurred in the upper and lower extremity (12/34 and 16/34) and the spine (6/34). OB occurred in the spine and lower extremity equally often (each 9/20), one case in the upper extremity and the ribs. Curettage was performed in 28/34 OO and 14/20 OB, 8 (2/34 OO, 6/20 OB) underwent resection. After methylation analysis of 14 samples (8 OO, 6 OB) there is a tendency that the tumors cluster in different groups. In 10 samples fusion analysis was performed so far (9 OO, 1 OB), a FOS- or FOSB-rearrangement were detected with low confidence. In 7 cases, DNA quality was insufficient for further analysis. Discussion: Though OO and OB have the same macroscopic and histologic appearance, their behaviour is different. First data show a tendency, that OO and OB form two clusters with methylation analysis. FOS- and FOSB-rearrangement are difficult to discover by NGS, and further adjustment to the protocol is needed. As the sample size is small, further analysis will be performed to confirm our results

Jäger Vanessa

Abstract ID: 93192

Uncovering innovative molecular targets for lung cancer therapy on the highly recurrent chromosomal gain of 1q21

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Background/Aims Genotype directed/personalized therapies have revolutionized cancer treatment. Nevertheless, resistance development and treatment failure often limit its effectiveness. Somatic copy-number amplifications strongly contribute to carcinogenesis and treatment resistance. One such example located on chromosome 1q21 is the pro-survival gene MCL-1, which is frequently amplified/overexpressed in lung adenocarcinoma (LUAD). Of note, MCL-1 amplifications mostly co-occur with the amplification of a large genomic area on 1q21, resulting in the overexpression of MCL-1 and 315 additional genes, with mostly unknown function in tumorigenesis. Thus, this study aims to identify the role of potential critical cancer drivers affected by the chromosomal gain of 1q21 in LUAD.

Results/Methods Bioinformatic analysis evaluated the impact of increased expression of these amplified genes on lung cancer patient survival. Elevated expression of 1q21 genes, such as BCAN or NUF2, in lung cancer patients is clearly associated with reduced survival, even more significant than previously published MCL-1, suggesting their potential important role in LUAD carcinogenesis. To perform in-depth functional analysis of the role of these potential oncogenic drivers, different lung cancer cell lines and CRISPR/Cas9 genome editing technologies are used to induce overexpression/knockout of the respective genes and to assess the impact on cell proliferation, viability, migration, and sensitivity to commonly used therapies. Also, the most promising candidate genes are studied in a clinically-relevant Kras- and Tp53-loss-driven lung cancer mouse model allowing the rapid CRISPR/Cas9-mediated somatic modification of certain genes.

Conclusion This study will not only answer biological questions on the functional role of genes on the 1q21 amplicon, but also aid the urgently needed identification of molecular targets for the development of new treatment strategies in lung cancer.

Janisch Michael

Abstract ID: 92601

Non-contrast enhanced CT texture analysis of primary and metastatic pancreatic ductal adenocarcinomas: Value in assessment of histopathological grade and differences between primary and metastatic lesions.

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Background: Despite progress in therapeutic options, prognosis of pancreatic ductal adenocarcinoma (PDAC) remains poor. It is, among local and distant extension, influenced by histopathological grading, that is usually assessed post surgery. As neoadjuvant therapy may improve outcomes for patients with poorly differentiated PDAC, a non-invasive method to assess histopathological grade would be valuable. A non-invasive tool to assign liver lesions in patients with PDAC to draw conclusion to the primary tumor would also be clinically helpful. The aim was to evaluate the significance of CT-texture analysis (CTTA) in assessment of histopathological grade of PDAC and to compare CTTA texture features between primary and metastatic PDAC.

Method/Results: In this retrospective study with 120 patients and histopathologically confirmed PDAC, Sixty-five patients underwent CT-guided biopsy of primary PDAC, while 55 patients underwent CT-guided biopsy of hepatic PDAC metastasis. All lesions were segmented in non-contrast enhanced CT scans for CTTA based on histogram analysis, co-occurrence matrix and run-length matrix. Statistical analysis was conducted for 372 texture features using Mann-Whitney-U-test and ROC analysis. A p-value <0.05 was considered statistically significant. Three features were identified that differed significantly between histopathological G2 and G3 primary tumors. Of these, <U+0093>low gray-level zone emphasis<U+0094> yielded the largest AUC, reaching a sensitivity and specificity of 0.76 and 0.83, respectively (cut-off value of 0.482). Fifty-four features differed significantly between primary and hepatic metastatic PDAC (AUCs: 0.72-0.93).

Conlusion: CTTA of PDAC identified differences in texture features between primary G2 and G3 tumors that could be used for non-invasive tumor assessment. Differences between the features of primary and metastatic PDAC suggest that CTTA of metastatic lesions may not allow conclusions regarding the histology of the primary tumor.

Kindler Oliver

Abstract ID: 92909

Characterization of the tumor microenvironment in a cohort of KRAS- and EGFR mutant non-small cell lung cancer

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Introduction: Immune Checkpoint Blockade (ICB) led to better outcomes in non-small cell lung cancer (NSCLC) but only a subset of patients benefits from current treatment regimens. Different molecular subtypes show diverse treatment responses. It was reported that patients with KRAS-mutant tumors respond better to ICB therapy, in contrast, EGFR-mutant tumors show higher resistance to this type of therapy. In order to evaluate distinctions between these subtypes, a thorough characterization of the immune environment (IE) was performed in patients with untreated NSCLC. Methods: To characterize the immune environment, flow cytometry and multiplex immunohistochemistry was used. Additionally, TCR sequencing, and RNA sequencing was performed. The findings were validated in public datasets. Therapeutic blockage of CCL20 was tested in a murine in-vivo flank tumor model comparing CCL20-neutralizing antibody to its isotype control. Results: No apparent difference of immune cell composition was found between the molecular subgroups. Tumor Mutational Burden (TMB) and PDL1 expression on cancer cells was higher in KRAS-mutant NSCLC. Expression of CCL20 were upregulated in the same subgroup which could be validated in the TCGA lung adenocarcinoma cohort. Additionally, higher CCL20 expression was associated with lower survival probability. In-vivo experiments with a CCL20-blocking antibody showed reduced tumor growth in treatment group with reduced regulatory T cells and monocyte-derived dendritic cells. Conclusion: Higher TMB and PDL1 expression, both suggested biomarkers for the prognosis of ICB response, could explain better results in KRAS-mutant NSCLC. Targeting of CCL20 may be a new option for therapy in this subgroup. In-vivo experiments show promising results for therapeutic usage of blockade of CCL20 with minor modulation of the IE. In EGFR-mutated lung cancer, additional research is needed to assist therapy decisions.

Kühberger Stefan

Abstract ID: 92783

Correlation of tumor size and -biology with ctDNA release in early colorectal cancer

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Background/Aims

The analysis of circulating tumor DNA (ctDNA) from plasma has evolved into a promising clinical tool for monitoring advanced cancer patients. More recently, early detection of localized tumors is intensively investigated, but is highly challenged by minute amounts of ctDNA in plasma in these early stages. Except for tumor burden, biological factors contributing to the release of ctDNA are largely unknown. Since knowledge about the earliest time point in tumorigenesis, at which ctDNA becomes detectable in the circulation, is crucial for the development and assessment of noninvasive screening tests, we aim to shed light onto ctDNA release kinetics.

Methods/Results

In a cohort of 22 treatment naïve early stage (I-II) colorectal cancer patients, we performed matched targeted sequencing of tissue and plasma samples using a gene panel enriching for 523 cancer associated genes (TSO500, lllumina). Phenotypic properties of the tumor were determined using a novel spatial transcriptomics method called in situ sequencing (ISS), where a marker panel of 220 genes involved in common biological pathways was spatially resolved. To support the spatial analysis data, total RNA-Seq of the tissue samples is still ongoing. We were able to detect mutations identified in tumor tissues in 18/22 (~82%) of corresponding plasma samples. In addition, unique variants in plasma and tissue have been observed but with significantly lower variant allele frequencies. Preliminary phenotypic ISS data revealed that subsets of genes involved in apoptosis, autophagy, invasion, proliferation and stemness/differentiation were significantly upregulated in the ctDNA releasing- compared to the non-releasing patients.

Conclusion

Further analysis of ISS and RNA-Seq data will allow to extend our already established phenotypic correlation. The next steps will focus on targeting the distribution of immune cells within the tissue as well as looking at metabolic and stress related pathways.

Lara Gutierrez Ariadna

Abstract ID: 92463

OpenHRD - an open source platform for calculation of homologous recombination deficiency scores from OncoScan microarrays

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Homologous recombination deficiency is an important biomarker for PARP inhibitor therapy in ovarian cancer. Existing open-source tools lack standardized parameters and require IT expertise. This project aims to establish a web-based bioinformatic analysis system for robust assessment of genomic instability. OncoScan-CNV data was obtained from the DNA of ovarian cancer formalin fixed embedded specimens under the ethics approved project 33-113 ex 20/21. The cohort is composed of 20 training and 12 validation samples. The analysis platform uses raw OncoScan microarray images and applies the open source "Allele-specific copy number analysis of tumors" pipeline to perform window segmentation. Calculation of HRD was performed by combination of custom R-scripts, "scarHRD" and <U+0093>oncoscanR<U+0094> pipelines. Additionally commercial diagnostic HRD test by Myriad myChoice CDx was obtained. It was observed the penalty value affects the HRD score. Where at a higher penalty value, the correlation to Myriad myChoice CDx improved. For this reason penalty of 70 was selected as default for the analysis. Based on the results of the training cohort and considering the accepted HRD threshold of 42, a correction method based on the linear regression model was applied and tested in the validation cohort. Subsequently, the application of the fully automated OpenHRD pipeline with standardized parameters revealed a high correlation to results obtained with the commercial Myriad myChoiceCDx test. The OpenHRD web application runs with Django Python and Celery Task Queue web frameworks. It takes the OncoScan <U+0091>.CEL<U+0091> files as input and generates a zip archive with HRD results. Its is currently available under registration. Several studies have shown that HRD is a reliable marker for treatment decisions in PARPi therapy. In this study we provided a simple, standardized, web based system to analyse HRD, which is able to process the microarray scan raw image data and generate reliable HRD score values.

Lehner Christian

Abstract ID: 92962

Evaluation and diagnostic potential of pituitary adenoma derived extracellular vesicles

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Pituitary adenomas account for approximately 10-15 % of CNS tumors. According to their pathophysiology, size and endocrine activity, pituitary adenomas can be divided in distinct subgroups. Hormonal inactive tumours account for about 25 % of all pituitary adenomas (NFPA). In contrast to pituitary adenomas with endocrine activity, which cause symptoms by hormonal disturbances, NFPAs are usually diagnosed by symptoms such as ophthalmoplegia or visual field deficits through mechanical compression of cranial nerves in the sella region. In contrast to pituitary adenomas with hormonal activity there is no valid blood test available for the diagnosis or follow up procedures of NFPAs. The diagnostic method of choice in NFPAs is MRI. Extracellular vesicles(EVs) are endosome-derived membrane vesicels released by different cell types including tumor cells. It is expected that EVs influence tumor growth and development through connection with the tumor microenvironment. EVs in human plasma might be a valid tumour biomarker for patients with NFPA. There is no data available about the diagnostic potential of circulating EVs from NFPAs. We present our protocol for isolation and characterization of EVs in human plasma in a series of 10 patients with NFPA using the FOLR1 and EpCAM protein expression. Blood samples were collected preoperative and three months postoperative. Tumor samples were collected during surgery in all patients for histopathological analysis. For EV characterization we used the ExoView® from NanoView Biosciences. There was no significant difference between preoperative and postoperative FOLR1 and EpCAM protein expression on EVs in human plasma. According to our data no conclusion for the diagnosis or prognosis of NFPAs can be drawn. The research field of EVs is still very young with limited protocols and techniques for the isolation and characterization. Further trials using different antibodies or membrane proteins for the detection of EVs seems reasonable.

Schindlmaier Katharina

Abstract ID: 92584

Gluconeogenesis is activated in macrophages under glucose starvation

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Background/Aims: Cancer cells have a rewired metabolism and enhanced glucose consumption promotes growth and proliferation. As tumor cells outgrow their supply, steep gradients for glucose emerge and both, cancer and non-neoplastic cells need to adapt to glucose starvation. The key gluconeogenesis enzyme, PEPCK (PCK1/2), was shown to support tumor cell survival under low glucose in some cancers. PCK2 allows synthesis of glycolytic intermediates from non-carbohydrate precursors that are shunted towards various pathways. However, gluconeogenesis may support tumor-associated macrophages, one of the main tumor-infiltrating immune cells, as well. Whether PEPCK is functionally expressed in macrophages to promote gluconeogenesis is not known. Recently, high PCK2 expression was found in lung macrophages including tumor-associated macrophages in a study on human lung cancers.

Method/Results: PCK2 expression analysis in IFN-?/LPS (M1) or IL-4 (M2) polarized monocyte-derived macrophages revealed consistent expression of PCK2 on mRNA and protein level, irrespective of glucose abundance. Assessing cell metabolism with stable isotopic labelling and GC-MS, we verified functional expression of PCK2. Using 13C5-glutamine as tracer, we detected labelling of TCA cycle metabolites and phosphoenolpyruvate and glycerol-3-phosphate, showing that glutamine fuels initial steps of gluconeogenesis, primarily under low glucose. Thus, we found a trend towards higher gluconeogenesis activity in macrophages upon glucose starvation.

Conclusion: Macrophages functionally express PCK2 and generate glycolytic/gluconeogenic intermediates directing gluconeogenesis under low glucose. The data suggest a metabolic switch in macrophages facing a low glucose environment, however further work is needed to assess the role of gluconeogenesis in different macrophage functions. This study sheds light on macrophage metabolism upon unstable nutritional conditions, as present in the tumor microenvironment.

Szmyra Marta

Abstract ID: 93085

Brusatol induces apoptosis in aggressive lymphoma cells in vitro and synergizes with Venetoclax

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Aggressive lymphomas are the most common lymphoid malignancies in adults with an increasing incidence. Despite the available therapy, one third of DLBCL patients experiences treatment failure. One step forward seems to be Venetoclax, an FDA-approved Bcl-2 inhibitor. Here, we investigated the potential of Brusatol in the treatment of aggressive lymphoma cells as a single agent or in combination with Venetoclax. In ten cell lines representing different types of lymphomas, Brusatol induced cell growth inhibition in a concentration-dependent manner. Based on the results of apoptosis assays, they can be grouped into cell lines more and less sensitive to Brusatol. Cell cycle analysis showed an increased cell number in the subG1 phase in more sensitive cell lines. Western blot results of the more sensitive cell lines revealed reduced levels of Bcl-2, Bcl-XL, Mcl-1, p53 and Myc. Interestingly, the protein expression profile of untreated cells indicated that cell lines with higher Myc levels were more sensitive to Brusatol. mRNA expression analysis showed that the reduction of affected proteins occurred mainly at the protein level. Thus, we examined the effect of Brusatol on protein biosynthesis using click chemistry and observed inhibition of protein translation. Furthermore, co-treatment of Brusatol with Bcl-2, Bcl-XL and Mcl-1 inhibitors, respectively, revealed a higher apoptotic effect compared to these substances alone. Finally, the combination of Brusatol and Venetoclax synergistically increased lymphoma cell killing. Our data indicate that Brusatol efficiently induces cell death by reducing the expression of prosurvival proteins in aggressive lymphoma cells, especially in these with higher Myc levels. Additionally, the combination of Brusatol with Venetoclax results in enhanced induction of apoptosis. Thus, our study suggests that Brusatol, alone or in combination with Venetoclax, represents a very interesting agent for development of novel anti-lymphoma therapies.

Vlachos Georgios

Abstract ID: 92763

Investigating Homologous Recombination Deficiency in mCRPC through ctDNA

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Background Homologous Recombination Deficiency (HRD) refers to the impairment of accurate repair of double-strand breaks. This condition can be targeted with poly ADP ribose polymerase inhibitors (PARPi), through synthetic lethality. We investigate this condition in the context of metastatic castration-resistant prostate cancer (mCRPC), using circulating tumour DNA (ctDNA) as a tumour surrogate.

Methods/Results We analysed 366 plasma samples from mCRPC patients. To estimate the tumour fraction in plasma, we used aneuploidy screening (mFAST-SeqS, ichorCNA). Patients with elevated tumour fractions (>5%) were selected for further analysis (147 samples, 131 patients). A gene panel including genes involved in the HR repair pathway as well as other tumour suppressor genes (TSG) associated with an aggressive phenotype (PTEN, RB1, TP53) was designed and revealed a sensitivity 0.5%VAF, when tested with synthetic oligonucleotides. Longitudinal urine samples (n = 9) were available from 2 patients. After removing artefacts and germline variants, mutations in HR genes were identified in 66 patients (49.6%), with 26 (19.5%) patients carrying pathogenic or likely pathogenic mutations. The most frequently altered genes were BRCA2 (14.5%), ATM (13.0%), BRCA1 (9.3%) and CHEK2 (8.33%). At least one TSG was altered in 58 patients (43.6%) and in 42 (31.5%) patients no alterations was detected in cfDNA. The urine samples were concordant with the plasma samples, but exhibited a lower VAF. Next, the concordance of the identified variants in tissue will be assessed.

Conclusion Our data indicate that the presence of tumour-derived nucleic acids in plasma and urine can provide information about the HRD status of the tumour in mCRPC patients. The mutation rates in HR genes and other TSG align with publicly available data from tissue. Therefore, ctDNA and ucfDNA possibly hold promise in the clinical management of prostate cancer, in the context of HRD and PARPi treatments.

Talk Session III

Dey Saptaswa

Abstract ID: 93555

Modified microbiota by skin disinfection through topical triple antibiotic treatment delays tumor growth and increases survival in a cutaneous T-cell lymphoma mouse model

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Introduction: Cutaneous T-cell lymphomas (CTCL) are a heterogeneous group of lymphoproliferative disorders of skin-homing mature T-cells causing chronic inflammation, with an impairment of immune environment leading to severe infections and/or sepsis due to dysbiosis. Together, this promotes progression of disease, resulting in poor quality of life and high mortality. We were thus interested in microbiome characteristics of CTCL and if modulation of the skin microbiota could beneficially affect the course of CTCL. Methodology: Here we established a CTCL murine model by intradermally grafting murine EL4 T-cell lymphoma cells in C57BL/6 mice and treated them with conventional therapeutics such as psoralen plus UVA (PUVA) or UVB in the presence of normal microbiota or diminished microbiota achieved by disinfection with a topical triple antibiotic cream, containing neomycin, bacitracin and polymyxin B sulfate (Neosporin). Results: Our in vivo results indicated that skin disinfection significantly delayed tumor appearance and growth and prolonged survival of mice irrespective of allocation to therapeutic agents (PUVA, UVB or none). The effect of triple antibiotic cream on skin microbiota reduced Shannon diversity index and bacterial richness that correlated with diminished tumor growth. Moreover, it induced the growth of certain presumably beneficial staphylococcal species compared to vehicle treatment. Moreover, the effect of triple antibiotic cream on tumor growth was similar to the targeted therapy drugs, such as STAT3/5 blocker or multi-kinase inhibitor. Conclusion: In summary, we conclude that modifying the microbiota of the skin by disinfection through topical triple antibiotic treatment delays tumor growth and increases survival in a murine CTCL model. This observation opens up the avenues for the investigation of new therapeutic approaches in CTCL focusing on modification of the microbiota. Keywords: CTCL, Skin Microbiome, Tumor Growth

Rani Alankrita

Abstract ID: 93052

Synthetic HDL nanodiscs show potent anti-inflammatory properties.

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Background: High density lipoproteins (HDLs) are complex particles whose function and composition are critically altered in disease. Therefore, it is reasonable to replace or even increase the loss of function of HDL in inflammatory diseases. Apolipoprotein A-1 (apoA-I) is the most abundant protein of HDL and is primarily responsible for the its well documented anti-inflammatory effects. Artificially synthesized 18-37 amino acid long peptides, that mimic the activity of full length apoA-I, are being actively researched as HDL-mimics. Due to the ease of production and highly specific nature, peptidomimetics are a naturally preferable choice. However, there is a need to test the anti-inflammatory potential and toxicity to eliminate the limitations associated with the free peptides, which reduce the bioavailability and hence affect the bioactivity.

Results: We prepared differentially lipidated apoA-I mimetic nanoparticles using the NanoAssemblrTM platform. We characterized the size and confirmed the discoidal morphology of the nanodiscs using Transmission Electron microscopy and Native gel electrophoresis. We first tested the functionality of the nanodiscs compared with native HDL using an in- vitro cholesterol efflux capacity assay. We found that the nanodiscs mobilize cholesterol from mouse macrophages more effectively than native HDL. Moreover, the nanodiscs potently suppressed lipopolysaccharide induced human neutrophil activation and downmodulated human eosinophil migration response to eotaxin-1 and prostaglandin D2. We observed that the lipidation status of the nanodiscs strongly influenced their anti-inflammatory properties.

Conclusion: The apo A-1 mimetic based nanodiscs may have therapeutic potential, targeting hyper-inflammatory and hyper-eosinophilic conditions such as sepsis and asthma, where migration and activation of immune cells is the decisive point for disease exacerbation

Ristic Dusica

Abstract ID: 92559

G Protein-Coupled Receptor 55 in Pancreatic Cancer: An Immunomodulatory Role

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The G protein-coupled receptor 55 (GPR55) is a receptor that is considered to be part of an expanded endocannabinoid system (ES). It has been shown to have pro-tumorigenic effects in different cancer models, including models of pancreatic cancer. Most cells in the tumor microenvironment (TME) express cannabinoid receptors and thus can be influenced by the ES. However, the role of GPR55 in immune cells of the TME and its involvement in tumor growth is not well understood. Knowing that pancreatic cancer is characterized by low immune infiltration and poor treatment response, it is important to uncover the role of GPR55 in tumor immunity. KPCY cells (isolated from mouse pancreatic ductal adenocarcinoma with a high [T cell high; TCH] and low [T cell low; TCL] T cell response) were subcutaneously injected into GPR55 wild-type and knock-out mice. Flow cytometry and in situ hybridization were used to phenotype cells within the tumors, while cytokine array, ELISA, and qRT-PCR were used to determine the expression levels of proteins and cytokines. Functional in vitro assays were conducted on mouse and human neutrophils to elucidate their behavior in the TME. GPR55 knock-out mice injected with TCH KPCY cells had significantly smaller tumors than the wild-type mice. Additionally, they showed higher CD8+ T cell and dendritic cell infiltration with higher CCL21 expression, but lower infiltration of neutrophils when compared to wild-types. In the TCL model, tumor weight was significantly higher in the knock-out group compared to wild-types. The TCL GPR55 knock-outs also showed higher infiltration of neutrophils, suggesting that neutrophils could be important regulators of the TME in the KPCY tumor model. In summary, our data indicates that the knock-out of GPR55 in the TME of mouse pancreatic cancer models leads to differing immune cell infiltration, which could be important regarding future immuno-therapies of pancreatic cancer.

Talk Session IV

Mahdy Ali Kariem

Abstract ID: 93154

Brain Dissection Wizard <U+0096> An interactive, three-dimensional, photogrammetric fiber tract model

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Awake surgeries enable the intraoperative testing of neuronal functions with the aim to preserve them and to achieve the best possible oncological and functional result. The knowledge of cerebral fiber tracts is essential for these kind of surgeries. The study of these tracts can be tedious and time-consuming. Because the fiber tracts cannot be seen intraoperatively it is necessary to build a mental image that can be adapted at any time. The aim of this work is facilitate the development of a mental map with the help of an interactive, three-dimensional, photogrammetric model. 15 brain specimens were prepared and dissected using a modified Klingler technique. Every dissection step was recorded photographically. The images were transformed into a photogrammetric model. Three low grade gliomas that were localized in predefined regions (premotor region, insula, temporo-parieto-occipital junction) were segmented and transformed into the photogrammetric model. Relevant fiber tracts were tracked using fiber tractography and transformed into the photogrammetric model. These photogrammetric models display the dissection steps in a realistic and undistorted way. The localization of the tumors in the photogrammetric model correlates very well with the localization in the original MRI data set. The virtual fiber tracts match very well with the anatomically dissected tracts. All photogrammetric models display the dissection steps in a realistic and undistorted way. For the first time segmented tumors and virtually tracked fibers could be overlaid onto the photogrammetric brain dissection model. This interactive, three-dimensional model offers the possibility to study subcortical anatomy in the presence of a tumor. This facilitates the development of a mental image of the fiber tracts, improves the intraoperative orientation and should optimize the onocological as well as functional result of awake surgeries in patients harboring intrinsic brain tumors.

Nowakowska Marta

Abstract ID: 93007

Neuronal activation in organotypic hippocampal slice culture grown on photoactive material

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Background/Aims: Regenerative processes in neurons following injuries can be improved through electrical stimulation. Wireless, lightweight, organic photocapacitors (photocaps) pose an attractive alternative to standard stimulation procedures. Upon illumination, they charge within microseconds, creating electric field, which can activate excitable cells in their vicinity. In this study, we aimed to elicit molecular changes in cultivated hippocampal slices by means of photocapacitive stimulation. Methods/Results: Postnatal rat hippocampal slices were cultured on photocaps for 5 days. Then, the culture was exposed to red light pulses for 30 minutes with an LED red light source (3 W). Neuronal activation was assessed by c-fos immunoreactivity with fluorescent staining of paraffin-embedded serial transversal cuts of the slices. Double and triple immunostaining was performed to mark different brain cell populations. Light-stimulated slices showed increased cellular expression of c-fos protein compared to the controls without light treatment. Multiple immunostaining of c-fos with marker for neuronal nuclei revealed a predominantly neuronal expression of the protein. Cell activation was observed throughout the entire transversal cut of the slices. Conclusion: Single light treatment of organotypic hippocampal slice culture on organic photocaps resulted in an increased neuronal expression of c-fos as compared to the non-treated group, indicating photocapacitive stimulation leads to activation and molecular changes in excitable cells ex vivo. The appearance of c-fos signal in top layers of the cultivated slices implies bottom-up signal propagation from neurons located in the close contact with the device, meaning activation of wide neuronal networks.

Suljevic Omer

Abstract ID: 93546

Early post-operative exercise promotes bone healing kinetics. Preclinical evaluation of non-critical sized femur defect healing

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Department of Orthopedics and Traumatology, Medical University of Graz

Background/Aims: Physical exercise represents a well-known modality for maintaining healthy locomotor mechanism. On a preclinical level, studies demonstrated that treadmill training in rats is a reliable exercise protocol for in depth analysis of bone microstructural changes. Therefore, we decided to conduct a study that will investigate the early postoperative exercise effect on bone properties during the healing period of femoral defect in rats.

Method/Results: In twenty male Sprague Dawley rats a 1.6 mm bicortical defect was induced by drilling into both femoral diaphysis. Ten animals underwent continuous treadmill training (TR) over two weeks, while the other group were assigned as non-training (NT) control group. New bone formation labeling was performed by subcutaneous fluorochrome injections at day 5, 14 and week 5. In vivo µCT scans were performed after the surgery and then once a week during the 6-week postoperative period. Ten animals (five from each group) were euthanized at the 3rd week while the remaining animals were euthanized at the 6th week. Femur samples were extracted and underwent ex vivo µCT scanning and histological evaluation, while serum was used for evaluating ALP levels. In vivo µCT evaluation revealed already at one week post-surgery a significantly increased volume and surface of newly formed bone in the defect area of the TR group. BV/TV and number of osteocytes within the previous defect area were significantly increased in the TR group after 3 weeks. Fluorochrome distances demonstrated significantly increased distance between day 5 and 14 within the TR group. ALP levels were persistently increased in both groups over 3- and 6-week time points without statistical significance between TR and NT group.

Conclusion: This study demonstrated the positive effects of 2-week post-operative treadmill training in terms of increased bone healing kinetics, stimulation of new bone formation and the increase in osteocytes density.

Poster Session II

Haller Rosa

Abstract ID: 93454

Evidence of a clinically relevant relationship between intestinal permeability and microbiome composition in cirrhosis

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Background/Aims: Liver cirrhosis, the10th most common cause of death in the western world, is associated with increased intestinal permeability and alterations of the gut microbiome. However, it is not understood how intestinal permeability and the microbiome are related in cirrhosis. Therefore, we aim to investigate the potential of gut permeability biomarkers to predict mortality and their relation to microbiome composition. Methods/Results: We assessed intestinal permeability by zonulin ELISA in stool in two different study cohorts. In cohort 1 (78 cirrhotic patients) the zonulin dynamics over six months predicted a higher mortality after 24 months of patients whose zonulin levels worsened ($p = 0.048$). In cohort 2 (106 cirrhotic patients) zonulin levels were only available at baseline. In cohort 2 zonulin was able to predict mortality after 42 months ($p = 0.047$). In cohort 1, analysis of 16s rDNA sequencing data with ANCOM and LEfSe showed that Phascolarctobacterium was more abundant in patients with improved zonulin levels. Patients with a higher abundance of Phascolarctobacterium had a better liver function and a lower mortality. Metabolomics analysis showed in cohort 2 that stool acetate levels were lower in patients with high zonulin levels ($p = 0{,}026$). Microbiome analysis in cohort 2 is still pending. Conclusion: Zonulin as a marker of intestinal permeability is able to predict mortality in two different cohorts of cirrhotic patients. Cohort 1 suggests that the predictive power of zonulin might be increased by serial assessment and that microbiome composition and zonulin dynamics are related. The correlation of decreased levels of acetate, one of the most common SCFA produced by gut bacteria, with high zonulin levels further suggest a relationship between microbiome function.

Esparta Olaya

Abstract ID: 92704

The role of ??T and pDC cells in pulmonary arterial hypertension (PAH)

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Pulmonary arterial hypertension (PAH) is a cardiovascular disease characterised by a high mean pulmonary arterial pressure of ?20 mmHg at rest and it has an average survival rate of 6 years. It is associated with chronic inflammation, immune dysregulation, lung vascular remodelling and vasoconstriction that ultimately leads to heart failure and death. We hypothesise that by understanding of the inflammatory cell landscape and immune cell interaction will uncover new targetable pathologic pathways. We aim to investigate how gamma delta T (??T) and plasmacytoid dendritic cells (pDC) cell populations potentiate vascular remodelling by altering the local inflammatory environment in IPAH. We use several methods, such as cell isolations via magnetic beads, cell culture, flow cytometry, in combination with multicolour immunofluorescence and qPCR, to characterise the two cell populations and understand the cell-crosstalk between them and structural cells. Cell isolation protocols have been established to isolate ??T and pDC from buffy coats, resulting in an 84 and 89% purity, respectively. Using multi-colour flow cytometry, we characterised the isolated populations, which revealed two different subsets in ??T, V?2+ and V?2-. Co-culture of ??T and pDC with primary pulmonary arterial smooth muscle cells (PASMC), did not result in overt morphological changes, however, preliminary qPCR revealed changes in extracellular matrix components. The influence of PASMC on the two immune populations is yet to be shown by ongoing experiments. So far, these preliminary results are promising, however, additional experiments are needed to validate these findings and functional assays need to be performed to uncover the potential pathogenic role of ??T and pDC in PAH.

Guntur Divya

Abstract ID: 92254

The Role of BKCa channels in Pulmonary Artery Endothelial Cells

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Preliminary studies have suggested a differential regulation of the gene encoding Large/Big conductance calcium-activated potassium channels (BKCa) in idiopathic pulmonary arterial hypertension (IPAH) patient vs. healthy donor lungs, which could represent an important pathological mechanism of IPAH, as the pulmonary artery endothelium appears to control the vascular remodeling. The project aims to unravel the BKCA function in pulmonary artery endothelial cells (PAECs). The findings might lead to new drug targets to mitigate IPAH. qPCR of the RNA isolated from healthy donor PAECs revealed that the cells express the gene that encodes for BKCa channel and also that it is downregulated in PAECs from IPAH pulmonary arteries. Treatment of healthy donor PAECs with the BKCa activator x caused increased nitric oxide (NO) production and decreased cell proliferation. Healthy donor hPAECs where BKCa was silenced using siRNA, showed decreased NO production. Ex-vivo studies on pulmonary arteries from BKCa knockout (KO) mice showed lesser vasorelaxation when treated with acetylcholine compared to the BKCa wildtype (WT) mice. In addition, BKCa KO mice showed increased right heart hypertrophy and increased thickness of pulmonary arteries compared to BKCa WT mice as assessed by heart weight and lung tissue staining. Furthermore, electron microscopy images of mouse pulmonary arteries revealed that the BKCa KO mice have reduced endothelial surface caveolae compared to their WT counterparts. In conclusion, BKCa channels are present in PAECs and have an important protective function for remodeling of the small pulmonary arteries.

Kicker Eva

Abstract ID: 92836

Molecular interference with SARS-CoV-2 replication by the antiviral peptide TAT-I24

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The recent SARS-CoV-2 pandemic highlights the urgent medical need for antiviral therapeutics in addition to an immunisation with vaccines. At the moment, only a few safe and effective antivirals are available for the treatment of virus infections. Experimental peptides might be a future treatment option, as these peptides can act as entry-inhibitors that block or alter virus entry into the cell. The peptide TAT-I24, composed of I24, a 9-mer which inhibits gene expression from <U+0093>foreign<U+0094>- nucleic acids, might be such a candidate. This 9-mer sequence is linked to a TAT-peptide, which facilitates cell penetration and supports the transport of I24 into the cytosol in parallel with virus capsids. In previous studies, TAT-I24 has shown a broad range antiviral activity against DNA viruses, but also an inhibitory effect against the RNA virus SARS-CoV-2 could be observed in recent in-vitro neutralization experiments (unpublished data). We observed a reduction of virus particles in the presence of TAT-I24 in various cell lines when infected with SARS-CoV-2, although differences in the sensitivity to the peptide was observed for individual virus variants (Wuhan, Delta, Omicron). Considering differences on viral gene expression levels, preliminary data suggest a delayed assembly of virus particles and a reduced release, when cells are infected in the presence of TAT-I24. Further evaluation of the effects on host gene expression as well as intracellular localization and virus uptake studies (virus and peptide specific antibody staining) are currently ongoing. In addition, air-liquid interface cultures with human alveolar cells are planned as in-vitro models to mimic a possible future therapeutic target region.

Lins Thomas

Abstract ID: 92554

Endocan as contributor in pathogenic remodelling of pulmonary vasculature

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Systemic Sclerosis (SSc) is a severe chronic disease with a 50% chance of direct lung involvement; this can manifest as either pulmonary fibrosis, pulmonary arterial hypertension, or both. The Fos-related antigen 2 transgenic (Fra2 TG) mouse line has been established as functional model for SSc associated pulmonary fibrosis, recapitulating important features of SSc. Furthermore, this model suggests direct involvement of the pulmonary endothelium.

We have investigated changes in lungs of Fra2 TG mice at two different time-points (8 and 16 weeks) representing early-onset and severe disease progression, using: lung function and hemodynamic measurements, immunofluorescence and electron microscopy and RNA bulk sequencing of sorted endothelial cells. Further, in vitro silencing experiments utilizing human microvascular endothelial cells and GSE datasets from human patients suffering from lung fibrosis are being investigated.

Lung function measurements showed hampered respiratory capabilities and increased pulmonary arterial pressure in Fra2 TG mice. Gene expression analyses of lung homogenates suggested an imbalance of endothelial cell homeostasis. Electron microscopy visualized swelling of the endothelium in pulmonary arteries and capillaries increasing with age in TG subjects. Using RNAseq, we found Endocan as one of the most downregulated genes in the pulmonary endothelium of young Fra2 TG mice. Endocan is involved in extracellular matrix organization, cellular migration and proliferation, processes all linked to fibrosis. Similarly, endocan expression is decreased in human pulmonary fibrosis.

We conclude that endothelial cells have prominent contribution in early onset and development of the disease. Further studies are needed to elucidate pathomechanisms and investigate the role of Endocan.

Mohammadzadeh Rokhsareh

Abstract ID: 92517

Partnership between Methanobrevibacter smithii clades and different microbial taxa in the human gut

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Methanobrevibacter smithii is considered the predominant archaeal representative in the human gastrointestinal tract and recently, this archaeal species has been reported to comprise two species-level clades named M. smithii and Candidatus M. intestini. The high occurrence of M. smithii might be due to its ability to establish interactions with several gut bacteria. However, it is not yet clear if there is a unique partnership between these two M. smithii clades and different microbial taxa. To elucidate this, in this study, fecal samples from a total of 94 participants with the age range between 46 and 86 years old were recruited at the Medical University of Graz irrespective of their medical conditions. DNA was then extracted and sent for shotgun sequencing. Raw shotgun reads were processed using ATLAS for obtaining MAGs and also mapped against Unified Human Gastrointestinal Genome (UHGG) database using Kraken and then processed with Bracken. The correlation between the two M. smithii clades and top 800 most abundant bacterial taxa was calculated using Spearman correlation and bacterial species were chosen based on adjusted $p<0.01$. In total, it was possible to assemble high-quality MAGs (completeness>90%, contamination <1%) in 7 (6 M. smithii, and 1 Ca. M. intestini) out of 94 samples (7.4%). According to the co-occurrence network analysis, M. smithii has a strong negative correlation with Blautia spp. as well as Streptococcus spp. and a positive correlation with Bacteroidaceae members, while Candidatus M. intestini showed mostly unique positive correlations with specific members of Oscillospiraceae and Christensenellacea family, while also sharing positive correlations with different members of of these bacterial taxa with M. smithii. Notably, these two archaeal clades also strongly correlate to one another. According to these observations, these species might grow together and distinctive microbial taxa might support or hinder their presence and growth.

Myronenko Oleh

Abstract ID: 92516

Angiogenic proteome profiling of pulmonary hypertension in chronic obstructive pulmonary disease (COPD)

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Background. Pulmonary hypertension (PH) associated with COPD (COPD-PH) may lead to right heart failure with a very poor prognosis. However, reliable predictive/prognostic biomarkers of PH in COPD have not been established. In severe COPD-PH, vascular remodeling includes a distinctive loss of alveolar capillaries, and dysregulated angiogenesis may provide a clue for biomarker research. Therefore, we aimed to characterize the profiles of circulating angiogenesis-related factors (levels of pro- and anti-angiogenic proteins) in COPD patients with or without PH. Methods. Blood plasma samples from COPD patients were obtained from Graz biobank and divided into 3 groups: COPD (mPAP ? 24 mmHg, n=6), COPD-PHlow (mPAP = 25-34 mmHg, n=7) and COPD-PHhigh (mPAP ? 35 mmHg, n=7). Control group included 10 healthy donors. All groups were age- and gender-matched. Angiogenesis-associated proteins were measured with the Proteome Profiler Human Angiogenesis Array (ARY007, R&D Systems). Pixel densities of the membranes were analyzed by HLImage+ software (QuickSpots, R&D Systems). Group comparisons were performed with the Kruskal-Wallis H test. Correlations were calculated with the Spearman´s rank test. SPSS software (IBM SPSS Inc.) was used for statistical analysis. A p-value <0.05 was considered significant. Results. Thrombospondin-1 and prolactin may be suggested as candidate biomarkers of COPD, as their levels were significantly elevated in COPD groups (with and without PH), compared to healthy controls. We were not able to distinguish COPD-PH from COPD, although EGF and PDGF-AA levels were significantly decreased in COPD-PH compared to healthy controls. MMP-9 in COPD-PHhigh was significantly increased compared to COPD-PHlow, thus suggesting this enzyme to be a potential biomarker of severe PH. Among 55 angiogenesis-associated proteins, only IGFBP1 was correlated with age. Correlations with mPAP were not found. Further studies are required to validate the selected candidate biomarkers

Rajesh Rishi

Abstract ID: 93312

Succinate aggravates pulmonary fibrosis through the Succinate-GPR91 axis.

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Pulmonary fibrosis (PF) is a chronic, progressive, and restrictive pulmonary disorder estimated to affect 3 million people. It progresses with an increase in fibrotic tissue presenting as scarring of lung tissue, and deposition of extracellular matrix (ECM), obliterating the alveolar architecture. Consequently, there is loss of compliance, compromising the alveolar gas exchange capacity. Substantial findings from transcriptomic and metabolic profiling of fibrotic lungs point towards a dysregulation of metabolic pathways in the diseased state. The tricarboxylic acid (TCA) cycle, which is at the center of several metabolic pathways, has gained traction in fibrosis research. Intermediate members including succinate, acetyl CoA, and alpha ketoglutarate, have now been associated with non-metabolic functions such as cellular signaling, chromatin modification, and post-translational modification of proteins. In this study, we aim to understand the role of succinate, and its receptor GPR91 in regulating the outcomes of PF. METHODS Western blotting, qPCR, and FISH were employed to investigate the expression of GPR91 in human and mouse lung and in fibroblasts. In vitro assays were performed with IPF patient derived fibroblasts to evaluate the effect of succinate treatment on the expression of fibrotic markers. In vivo studies with the bleomycin mouse model of PF were used to evaluate the role of succinate in governing the outcomes of PF. RESULTS Several cell types in the lung express GPR91 including ATII cells, fibroblasts, and macrophages. In IPF patient derived fibroblasts, succinate treatment increased expression of markers associated with fibrosis such as alpha smooth muscle actin and collagen. In vivo, succinate significantly increased collagen accumulation and exaggerated weight loss in a model of pulmonary fibrosis. CONCLUSION The succinate-GPR91 axis appears to worsen PF. Deciphering the mechanisms involved will be key to investigating GPR91 as a therapeutic target.

Shinde Tejus

Abstract ID: 92941

A pan genome catalog of the human respiratory tract microbiome

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A dysbiotic airway microbiome is associated with various disorders of the human respiratory system, including chronic obstructive pulmonary disease (COPD) and chronic rhinosinusitis. The nature and dynamics of the airway microbiome are still not fully understood. This information is crucial for addressing the disease causality and potential mechanisms of disease progression with microbial dysbiosis.

To tackle this knowledge gap, we want to assemble a pan-genome catalog of microorganisms associated with the human respiratory tract. We can elucidate the complex microbial genomic inventory within an ecosystem with whole genome metagenomic sequencing. In particular, the reconstruction of metagenome-assembled genomes (MAGs) from these samples can provide species- and strain-specific insights into the functional and pathogenic potential of the human microbiome.

Therefore, we retrieved public metagenomic datasets from data archives like NCBI-SRA to perform a MAG de novo assembly, to compile a representative set of genomes specific to the human respiratory system. This catalog will be supplemented with the microbial genomes retrieved in-house after sequencing clinical samples and isolates.

We present some summary statistics from the public metagenomic datasets downloaded and analyzed, and some preliminary insights into the diversity of respiratory microbiome; after an initial screening of human lung explants with 16S rRNA gene amplicon sequencing. We get an initial estimate for the number of bacterial taxa and contaminant taxa present in the lung sample.

The final catalog of microbial genomes will enable us to leverage the whole gene content information for a multitude of purposes, including improved taxonomic classification, functional capacity prediction, or metabolic modeling of dysbiosis in the human respiratory system.

Syarif Ayu Hutami

Abstract ID: 92821

Compartment-specific Immunophenotyping in Chronic Obstructive Pulmonary Disease

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Chronic obstructive pulmonary disease (COPD) is the third leading cause of death worldwide and is pathologically marked by irreversible airway obstruction, chronic inflammation, and lung parenchyma destruction (emphysema). Its phenotypes encompass more emphysema and more airway limitation, with/without vascular changes. Increasing evidence showed specific immune infiltrates in lung compartments. However, how consistent the immune profile is between different compartments remains unknown. We hypothesize that specific immune profiles differentiate COPD phenotypes and that a specific subset of immune cells contributes to airway and/or vascular remodelling. Here we investigated compartment-specific (bronchi, pulmonary arteries (PA), and lung parenchyma) inflammatory profiles by applying multi-panel flow cytometry. In addition, we analyzed previously published COPD single-cell RNA (scRNA) datasets and performed receptor ligand analysis to infer cell-to-cell communications. The most abundant population is neutrophils in the lung parenchyma (25-30%), while T cells are in small PA (20-50%) and bronchi (30-55%). Across three compartments, macrophage and monocytes showed relatively similar proportions (20-25%), while NK cells showed the highest proportion in the lung parenchyma (10-15%), followed by small PA and bronchi (1-5%). Interaction analysis in COPD showed more communication between (1) cytotoxic T cells and antigen-presenting cells (APC) and between (2) cytotoxic T cells and endothelial cells. Receptor ligand analysis predicted MHC I genes such as B2M and HLA-E from APC and endothelial cells to be bound with T cell receptor complex genes such as CD3D and CD247. Overall, immune populations are differentially abundant in COPD lung compartments and potentially interact with structural cells. We plan to validate the interactions using functional assays and integrate current data with clinical and microbiome data from matched patients.

Weinberger Viktoria

Abstract ID: 92518

Characterization of cell surface structures of two major methanogenic archaea of the human gut

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Archaea, particularly Methanobrevibacter species, comprise more than 1% of the gastrointestinal microbiome but remain largely unexplored. These archaea are capable of producing methane (hence they are called methanogens) and interact widely with their environment as they can support bacterial fermentation and trigger immunogenic responses. To gain a better understanding of these archaeal residents and their interactive potential, we are performing detailed molecular and genome-based cell wall characterizations of Methanobrevibacter smithii <U+0093>ALI<U+0094>, M. smithii <U+0093>strain 2<U+0094>, and Candidatus M. intestini. Specifically, we are analyzing the capacity of the archaea to form extracellular vesicles and surface adhesins, for communication and attachment purposes, respectively. For that, we use a combination of genomics, proteomics and structural analyses (electron microscopy). In the genome-centric approach, the genomes of the above mentioned three Methanobrevibacter strains were analyzed with respect to the presence of adhesins. All strains contained a different number and composition of adhesin-related genes (four genes in M.smithii <U+0093>ali<U+0094>, 22 genes in M. smithii <U+0093>strain 2<U+0094>, and 24 genes in Candidatus M. intestini). Alignments were performed to identify the common adhesin gene clusters of the archaeal strains and to test whether they are inherently existing. Further, adhesin protein structures on the surface of the analyzed archaeal strains could indeed be visualized and identified in in a proteomics approach. Further, we were successful to isolate methanoarchaeal extracellular vesicles, a trait that had not been described for human-derived archaeal isolates so far. For a better characterization of the archaeal vesicles, multiomics analysis (Sequencing, Proteo-, Lipid-, and Metabolomics) are currently being performed. Our work is part of a detailed characterization of the human archaeome and provides the basis for further research on the immune system.

Babalola Joshua

Abstract ID: 93274

Combinational Effects of Type 2 Diabetes and Protein Post translationally Modification in the Pathophysiology and Progression of Alzheimer<U+0092>s disease

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Alzheimer<U+0092>s disease (AD) is a multi-factorial degenerative disease of the brain with an estimated prevalence of 1 in 9 age 65 and older and a global population burden projected to triple by 2050. Main pathological hallmarks of this disease are abnormal aggregation and misfolding of the amyloid beta peptide as well as tau protein. These are accompanied by pathophysiological events such as leaky blood brain barrier (BBB), synaptic degeneration, neuro-inflammation and neuronal degeneration. Main risk factor of this disease is aging, but obesity, Type 2 Diabetes, sleep disorders and stress also contribute to the progression of this neurodegenerative disorder. A? peptides are commonly subjected to post-translational modifications, including truncation and phosphorylation, which are shown to play a pivotal role in A? plaque aggregation. N-terminally truncated A? peptides containing pyroglutamatic acid (pGlu) catalyzed by glutaminyl cyclase (QC) e.g., pGlu3-A? (3-40/42) are the major A? peptide fragments within the core of the neuritic plaques and are shown to correlate with disease severity and progression. Type 2 diabetes (T2D) is one of the major risk factors associated with AD and compelling evidence supports the notion that insulin resistance, a key feature of T2D, is involved in AD-type neurodegeneration. Using APPxhQC transgenic mice expressing N-terminal modified pGlu A? peptides, we aim to understand what adding another risk factor (T2D besides hQC) could mean for the AD pathology in this model and if this could give a hint that more than one risk factor might affect AD in humans more significantly.

Bruneder Simon

Abstract ID: 92735

Expect the unexpected: The course of the inferior alveolar artery

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Introduction: The anatomical position of the inferior alveolar artery (IAA) within the mandibular canal and in relation to the substructures of the neurovascular mandibular bundle has been sparsely described to date. More detailed information on the exact IAA position would be beneficial for both dental and maxillofacial surgical procedures to minimize complications such as bleeding, nerve compression hematoma, and sensory deficiency. Material and methods: In 31 Thiel-preserved and fresh-frozen cadaver hemimandibles the position of the IAA in relation to the structures of the inferior alveolar neurovascular bundle and the mandible borders was analyzed anatomically and histologically. Results: In 77.4% of the cases, rotation of the IAA around the mental nerve was apparent, resulting in a typical site-dependent IAA position. While the IAA was situated buccally within the pterygomandibular space, buccal-inferior in the mandibular foramen, superior in the molar region, and lingually in the pre molar region. In 12.9% of the cases, a persistent lingual position of the IAA was observed for the entire mandibular canal. In one case, an additional mandibular canal and an accessory IAA were identified. Discussion: This study provides new and encompassing information on the complete course and position of the IAA. This course is of practical use for oral implantology and various surgical procedures in dental- and maxillofacial surgery. Variations in the typical IAA course and site-dependent positional changes may be referred to as mandible growth and functional adaption to occlusion anomalies. This report helps enhance the morphological and functional understanding of IAA relationship during mandible development

Demjaha Rina

Abstract ID: 88517

Serum neurofilament light levels in relation to the Brain-Age Paradigm in normal ageing

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Background: Serum neurofilament light (sNfL) is an easy accessible biomarker that increases upon neuro-axonal injury and neurodegeneration. Previous studies have shown that sNfL levels rise in normal ageing, which was further correlated to brain volume changes. The Brain-Age paradigm is a machine learning approach which predicts brain-age from neuroimaging data. We assessed whether brain-predicted age differences (brain-PAD) correlated with sNfL levels in a community-dwelling cohort.

Method: We included 328 neurologically normal individuals participating in a community-dwelling cohort study free of a history of previous stroke or dementia. There were 193 females. Age ranged from 38 to 85 years, with a median of 68.11 (IQR: 55.90 <U+0096> 73.18) years. Brain-PAD was measured using neuroimaging data attained from T1-weighted MRI, and sNfL was quantified by a single molecule array (Simoa) assay.

Results: sNfL correlated with chronological age ($r=0.73$, $p<0.001$) and brain-predicted age ($r=0.65$, $p<0.001$). However, sNfL was unrelated to brain-PAD ($r=0.038$, $p=0.50$). Further analyses revealed no differences in brain-PAD comparing individuals within the lowest and the highest sNfL quartile ($p=0.57$), with a mean brain-PAD of 0.79 ± 6.03 and 1.42 ± 7.97 years respectively.

Conclusion: Although sNfL correlated with chronological and brain predicted age, no correlation was found regarding brain-PAD. This could be due to a lower brain-PAD variation in our community-dwelling cohort. Moreover, factors apart from neurodegeneration such as reduced protein turn-over in higher age may be associated with the age-related increase in sNfL, which may have hampered to find associations between sNfL and brain-PAD.

Eder-Halbedl Michael

Abstract ID: 89726

Long term survival of Trabecular Metal Cones (TMC) for TKA revisions with severe bony defects

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Background During revision TKA with severe bony defects cones and sleeves are commonly used but long-term data are rare. Aim of this study is to present the 9-year survival analysis after implantation of TMC during revision TKA in a tertiary referral centre.

Materials and Methods 80 consecutive patients (44 female, average age 65,3 years) who underwent revision TKA surgery with 113 TMC (TMT©, Zimmer, USA) for tibia and/or femur were included in this retrospective study. The reasons for revision surgery were 64 (80%) aseptic and 16 (20%) septic failures. At the time of revision all knees showed large bony defects AORI Type 2a (18%), 2b (36%) and 3 (46%). Peri- and post OP complications, re-operations and re-revisions as well as pre- and post OP clinical outcome (ROM, KSS, VAS, WOMAC) were documented. Any loosening or osteolysis of the TMC were evaluated according to a modified Knee-Society Radiographic Evaluation system.

Results After an average FU of 6.1 years (5-9) all implanted TMC (n=113) except of two, showed no radiographic signs of loosening or osteolysis and were clinical stable. There were 2 (2.5%) periop. complications with wound healing problems and 11 (14%) post-operative complications including deep infections (n=4), periprosthetic fracture (n=2), aseptic loosening of components without TMC (n=2), instability (n=2) und one hinge dislocation (n=1). There were 5 reoperations and 8 re-revisions including 1 arthrodesis and 1 amputation. In 4 cases the well osteointegrated TMC had to be removed. The estimated 9-year Kaplan Meier survival rate for aseptic loosening was 95 %. All clinical parameters showed significant ($p<0,001$) improvement from pre- to post OP.

Conclusions In this study TMC showed an excellent metaphyseal fixation combined with hybrid stems for severe bony defects during revision TKA. The stable fixation could also be confirmed after an average of 6-year FU with a 9-year survival rate of 95 %.

Guggenberger Bernhard

Abstract ID: 92875

Do different walking strategies impact patella cartilage pressure in individuals with patellofemoral instability?

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Background: Patellofemoral instability (PFI) is a common orthopaedic condition in adolescence. Current studies used musculoskeletal simulations to investigate the influence of different morphological factors on patellofemoral joint loading. However, these studies were based on the gait pattern of one healthy individual and therefore, neglected the impact of different compensational walking strategies. Aim: This study aimed to investigate the influence of varying gait patterns on patella cartilage pressure (PCP) in individuals with PFI. Methods: We included 29 individuals (34 affected knees) with PFI. They were divided into a patellofemoral group 1 (PFG1, N = 12) and a patellofemoral group 2 (PFG2, N = 22), considering the sagittal knee moment in loading response phase according to Clark et al. (2016). The groups showed no differences in demographics, morphology and walking speed. Simulations were based on gait data and a musculoskeletal model with defined knee joint cartilage surfaces. PCPs were estimated using an elastic foundation model and the COMAK routine. For statistical analysis, alpha level was set to 0.05 and groups were compared using statistical parametric mapping. Results: Compared to PFG2, PFG1 showed increased knee extension, external rotation, hip extension and decreased dorsiflexion angles in stance phase. PFG1 showed lower peak and average cartilage pressure as well as cartilage contact area especially in the mid-stance phase compared to PFG2. Conclusion: Both groups walked with lower PCP compared to a typically developing group. The PFG1 walked with a more extended and externally rotated knee to achieve a higher reduction of the PCP. As simulations were based on a generic knee model, all differences of the simulations were related to the different walking strategies. Therefore, it can be concluded that it is essential to implement not only subject-specific geometry, but also individual gait pattern to investigate PCP in subjects with PFI.

Klivinyi Christoph

Abstract ID: 92938

Use of a capsaicin transdermal patch to induce central and peripheral sensitization: proposition of a human pain model

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Introduction Capsaicin is a natural occurring alkaloid and acts on transient receptor potential vanilloid type 1 (TRPV1) ion channels. It has been extensively used in human pain models to induce central and peripheral mechanisms of pain. When capsaicin is applied locally to the skin, it induces local neurogenic inflammation by stimulating TrpV1 receptors on dermal sensory nerve endings 3. In the past, capsaicin was preferably applied by intradermal injection, which is considered an invasive procedure but allowed for precise dosing. On the contrary, capsaicin-containing ointments and self-manufactured skin patches have lately been preferably used to circumvent invasive application but leading to imprecise dosing and standardization 1. Qutenza<U+0099> capsaicin skin patches contain a standardized amount of capsaicin (640µg/cm^2) and have been safely and effectively used to treat certain entities of pain in clinical practice. We propose Qutenza<U+0099> skin patches as an easy applicable, non-invasive and reliable human pain model, which could be used in future experimental studies. Methods We used Qutenza<U+0099> to induce pain and peripheral and central sensitization in ten healthy human subjects. Qutenza<U+0099> skin patches were applied on the volar side of both forearms for 60 minutes. Main concern of the study was the effect of low-level light therapy on sensitization processes, however basic characteristics of Qutenza<U+0099> as a human pain model were recorded. Quantitative sensory testing was used to evaluate its effects. Results Qutenza<U+0099> reliably induced pain (numeric rating scale, mean 2,15, 95% CI 1,006 - 3,294, p= <0,01) and increased wind-up as a marker of central sensitization (mean 4,4, 95% CI 3,631 - 5,169, p= <0,01). Discussion Qutenza<U+0099> capsaicin skin patches induced pain and increased a marker for central sensitization. Its use is furthermore a safe and standardized alternative to invasive application methods and should be evaluated and validated as a human pain model in detail.

Seifried Katharina Maria

Abstract ID: 91909

Redox state of human serum albumin in serum and cerebrospinal fluid of multiple sclerosis patients

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Background/Aim: Like in various neurodegenerative diseases, oxidative processes are involved in the pathophysiology of multiple sclerosis (MS). Human serum albumin (HSA) is the most abundant protein in blood and other body fluids and can serve as an indicator for oxidative stress. We aimed to analyze the redox state of HSA in serum and cerebrospinal fluid (CSF) of MS patients and controls, and to further find potential associations with disease activity and severity.

Methods/Results: We performed HPLC on serum and CSF of 20 MS patients and 21 controls to determine the redox state of HSA regarding cysteine-34 (Cys-34) in allocation to the fractions human mercaptalbumin (HMA), human nonmercaptalbumin 1 (HNA1) and human nonmercaptalbumin 2 (HNA2). HMA is the reduced form of the protein, with a free thiol group on Cys-34. In HNA1, HSA is reversibly oxidized with Cys-34 as disulfide together with another thiol, like another cysteine, homocysteine, or glutathione, whereas HNA2 is irreversibly oxidized with Cys-34 as sulfinic or sulfonic acid. In CSF, we found significantly higher fractions of HMA than in serum, and lower fractions of HNA1 and HNA2. There was no significant difference between patients and controls, although CSF of patients showed a trend to higher HNA2 fractions. However, we found an association between albumin redox state in serum and physical disability in remission, and between albumin redox state in CSF and disease activity.

Conclusion: In conclusion, our data affirm the involvement of oxidative stress in MS pathophysiology. This study should serve as a basis for further investigation of HSA in MS, particularly in larger cohorts and patients with more advanced disease stages. Additionally, HSA appears to be an interesting molecule to explore still poorly understood redox processes in CSF.

Vasilyeva Anna

Abstract ID: 92758

Features of microsurgical anastomoses in difficult cases of the condition of the recipient vessels (POAD, infection and trauma)

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Background/Aims: Microsurgical anastomoses of the atherosclerotic, inflammatory/postinflammatory, and traumatic/posttraumatic altered recipient vessels in the reconstruction area are difficult, have a high complication rate, and are associated with increased flap loss rates and limited alternatives of reconstruction. Method: The use of venous interponats (interposition vein graft) is an established technique in vascular surgery. Also, the end-to-end anastomoses of sclerotic vessels benefit from a venous interponate as a <U+0093>T-piece<U+0094>(<U+0093>T-shaped<U+0094> anastomosis to the connection vessel) or a venous patch with integrated end-to-side outlet of a vein. An alternative to venous interponate is an arterial "T-piece" of the flap artery or a "T-shaped" flap artery for anastomosis, which supports the terminal flow path and blood supply to the local and distal anatomical recipient regions. Results: Reduction/avoidance of the risk of thrombosis and revisions of microanastomoses, improvement of the patency of anastomosis in "problematic" arteries (POAD, infection, trauma) improvement of venous outflow with optimization of the blood circulation of the free flap and ensuring the success of the reconstructive microsurgical procedure in complex clinical cases. The use of a venous interponate (interpositional venous shunt) or a "T-shaped" flap artery and vein depends on the type of free flap. Conclusion: In patients, who suffer from systemic sclerosis, pronounced post-traumatic vascular changes, scarring and for whom no alternative possibilities of reconstruction are possible, it is essential to optimize a priori the often last possibility of reconstruction, which often means the preservation of a lower extremity. In such circumstances the loss of a microsurgical flap is usually equivalent to the loss of a limb.

Bergauer Andrej

Abstract ID: 93170

Can transorbital ultrasound scan of optic nerve sheath diameter (ONSD) detect ONSD increase in healthy young subjects after a 12-degree head down tilt maintained for 4 hours compared to baseline ONSD?

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Background: Increased volume and thus pressure may contribute to the structural and functional neuro-ophthalmic changes observed in spaceflight-associated neuro-ocular syndrome (SANS). ONSD was shown to increase with a 12-degree head-down tilt. Methods: The design of the study was a balanced crossover design with 2 test conditions with 14 days interval between test conditions. Test conditions were: head down tilt 0 degrees (supine) and head down tilt 12 degrees maintained for 4 hours (Table 1). The technique employed was a transorbital B mode scan of the optic nerve sheath. Results: the highest value of ONSD was measured in most instances at a 150-minute time point with 12 degree head-down tilt and at 60 minutes time point with a 0-degree head-down tilt. A paired-samples t-test was used to determine whether there was a statistically significant mean difference between the change in optic nerve sheath diameter when participants were put in 12 degrees head-down tilt position as opposed to a supine position. Head down tilt of 12 degrees produces a statistically significant increase of optic nerve sheath diameter change of 0.565 (95% CI, 0.491 to 0.639) mm as compared to the supine position (head down tilt 0 degrees), $t(7) = 17.996$, $p < .001$.A two-way repeated-measures ANOVA was used to understand the effects of the degree of head-down tilt (0 or 12 degrees) and time spent in each test condition (time) on optic nerve sheath diameter changes. Significant two-way interaction between time spent in specific test conditions and test condition, $F(8,24) = 32.93$, $p < .001$. Conclusion: In planning head-down tilt studies with 12 degrees HDT, the desired sample size to reach statistical significance would be around 12,7 to 18 subjects. Extending measuring time beyond 180 minutes does not seem to be reasonable; the maximal changes of ONSD should be detected by then. The 4-hour protocol envisioned at the conception of the pilot study can be shortened with further studies.

Honnef Gabriel

Abstract ID: 88533

Usefulness of Non-invasive Serum Hemoglobin Measurement in a Perioperative Setting: Prospective Observational Study

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Patient Blood Management (PBM) programmes seek to reduce the number of missed anaemic patients in the run-up to surgery. The aim of this study was to evaluate the usefulness of haemoglobin (Hb) measured non-invasively (SpHb) in preoperative screening for anaemia. We conducted a prospective observational study in a preoperative clinic. Adult patients undergoing examination for surgery who had their Hb measured by laboratory means also had their Hb measured non-invasively by a trained health care provider. 1216 patients were recruited. A total of 109 (9.3%) patients (53 men and 56 women) was found to be anaemic by standard laboratory Hb measurement. Sensitivity for SpHb to detect anaemic patients was 0.50 (95% CI 0.37<U+0096>0.63) in women and 0.30 (95% CI 0.18<U+0096>0.43) in men. Specificity was 0.97 (95% CI 0.95<U+0096>0.98) in men and 0.93 (95% CI 0.84<U+0096>1.0) in women. The rate of correctly classified patients was 84.7% for men and 89.4% for women. Positive predictive value for SpHb was 0.50 (95% CI 0.35<U+0096>0.65) in men and 0.40 (95% CI 0.31<U+0096>0.50) in women; negative predictive value was 0.93 (95% CI 0.92<U+0096>0.94) in men and 0.95 (95% CI 0.94<U+0096>0.96) in women. We conclude that due to low sensitivity, SpHb is poorly suitable for detecting preoperative anaemia in both sexes under standard of care conditions.

Huemer Matthias

Abstract ID: 88828

Gender differences in using complementary and alternative medicine in cancer patients: a cross-sectional study

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Background/aims: Complementary and alternative medicine (CAM) is widely used by cancer patients. Previous studies indicate that women female patients show more affinity to CAM than men. Therefore, we aimed to assess gender differences in the types of used CAM to develop individualized guidance during oncological care for health care practitioners. Methods/Results: We conducted a survey asking cancer patients about their use of CAM. We grouped the different modalities in five categories according to published literature: Whole medical systems (WMS), Body-Mind-Interventions (BMI), Biological treatments (BIO), Manipulative Treatments (MAN), and other treatments (OTH). Additionally, we assessed the symptom burden using the Edmonton Symptom Assessment Scale (ESAS). We then calculated odds ratios to analyze gender differences in patterns of use and patient characteristics. In total, 199 patients completed the survey of which 109 patients reported to use at least one CAM intervention. However, only 48% of CAM users discussed their use with a clinical oncologist. 53% of CAM users were women, which were more likely to use WMS (OR 2.99, CI 95% 1.17, 8.50) and BMI (OR 3.48, CI 95% 1.37, 9.83) interventions compared to men. However, men tended to use more biological interventions (OR 1.54, CI 95% 0.72, 3.35, ref.=women) but this trend was not significant. Women also reported significant higher levels of fatigue (4.38±2.56 vs. 3.18±2.55, p=0.019). MAN and OTH interventions as well as all other patient demographics and clinical characteristics were evenly distributed among genders. Conclusions: Women are more likely to use WMS and BMI therapies and reported greater fatigue than men. The results may indicate a gender bias in the treatment of cancer-related fatigue, leading to a higher chance of women these CAM therapies. Understanding the factors associated with CAM use can provide the healthcare personnel with the theoretical basis for professional guidance of the patients.

Liebhauser Martin

Abstract ID: 89975

Implant Breakage after Shoulder Arthroplasty: A systematic review of worldwide arthroplasty registries and clinical data

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Purpose: Implant breakage after shoulder arthroplasty is a rare complication resulting in malfunction or loosening of the components requiring revision surgery. This correlates with a high burden for the patient and steadily increasing costs for the health care system. Specific data of implant breakage are available in detailed arthroplasty registries, but rarely described in published studies. The aim of this systematic review and meta-analysis was to point out the frequency of implant breakage after shoulder arthroplasty. We hypothesized that rates of implant breakage don<U+0092>t differ between registry datasets and clinical studies. Methods: The breakage rate per 100,000 observed component years was used to compare data from national arthroplasty registries with data from clinical studies, published in peer-reviewed journals. All relevant different types of shoulder prosthetics were analyzed and considered in this investigation. Results: Data of 5 registries and 13 studies were included. Rates of implant breakage after shoulder arthroplasty were reported with 0.06-0.86% in registries versus 0.01-6.65% in clinical studies. The breakage rate per 100,000 observed component years in clinical studies and registries was 10. Conclusion: Clinical studies revealed a similar incidence of implant failure compared to data of worldwide arthroplasty registries. These complications arise mainly due to breakage of implanted screws and glenosphere and there seems to be a direct correlation between loosening/disengagement and implant breakage. We believe that this analysis can help physicians to advise patients on potential risks after shoulder arthroplasty.

Paal Katarzyna

Abstract ID: 92448

Association of age-related parameters with acute toxicity in patients treated with radiation therapy for prostate cancer.

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Background: Radiation therapy (RT) for prostate cancer (PCa) is a highly effective therapy modality, also for patients, who are ineligible for surgical treatment of PCa. Geriatric assessment (GA) comprises a number of tools, which can be used to measure age-associated parameters, and to identify frail patients who are at risk of higher radiation toxicity. The aim of this study was to investigate the association between age-related characteristics and the development of higher acute radiogenic side effects. Patients and methods: A total of 314 patients who received primary curative RT for PCa were enrolled into our prospective study. A GA consisting of the Mini-Mental State Examination (MMSE), Mini Nutritional Assessment (MNA), Nikolaus Scale for Social Situation (SOS), Geriatric Depression Scale, Activities of Daily Living (ADL), Instrumental Activities of Daily Living (IADL), Timed Up and Go (TUG), Charlson Comorbidity Index (CCI) and survey of polypharmacy (P) was performed before start of irradiation. Reported genitourinary (GU) and gastrointestinal (GI) side effects was classified according to EORTC/RTOG scale. High-grade acute radiogenic toxicity was defined as GU and/or GI toxicity grade ?2. Results: Radiation induced side effects grade ?2 were reported in 40 patients (12.7%), GU side effects ?2 in 37 patients (11.8%), and GI side effects grade ?2 in 8 patients (2,5%), respectively. The initial CKI, ADL, and GDS significantly correlated with GI and/or GU toxicity grade ?2 (p=0.029, p=0.050, and p=0.043, respectively). There was also a significant association of ADL with GU toxicity grade ?2 (p=0.046) and of TUG with GI toxicity grade ?2 (p=0.032). Conclusion: Our results show a significant association of comorbidities, mood state, reduced functionality, and mobility with the risk of high-grade acute toxicity in patients treated with RT for PCa. The tools of GA can help in detecting unidentified health problems that influence the risk of radiogenic toxicity.

Pilz Rene

Abstract ID: 90793

The efficiency of Eye Movement Desensitization and Reprocessing (EMDR) for the treatment of substance use disorders: A randomized controlled trial

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INTRODUCTION. Substance use disorders (SUD) can be described as a chronic and relapsing condition that is strongly associated with changes in emotion, motivation and cognition. Emotion regulation is associated with a variety of mental health and well-being parameters (e.g., having satisfying employment, healthy relationships) - long-term substance misuse negatively affects these parameters, so emotion regulation can be severely disrupted. Eye Movement Desensitisation and Reprocessing (EMDR) is a therapeutic method whose effectiveness has been demonstrated primarily in the treatment of traumatic disorders. EMDR is based on the idea of dysfunctional memories that can lead to maladaptive behaviour. At the latest since the assumption of a specific addiction memory, the EMDR method is also considered to have its own field of application for substance use disorders. The aim of this study is to explore the effectiveness of EMDR for the regulation of emotional processes in the context of long-term drug therapy in a therapeutic community. METHODS. Male SUD patients receive a standardised EMDR intervention in three weekly sessions. As a control, there is a randomly selected group of patients who continue to receive “treatment as usual” and, as a sham intervention, cognitive training to the same extent as the EMDR intervention. In addition to the pre- and post-tests, there are two follow-up surveys (one and three months later). At all testing times, emotion regulation ability, impulsivity, general symptom burden and psychotherapy motivation are assessed by means of questionnaires. RESULTS/CONCLUSION. Emotion regulation: Compared to the control group with sham intervention (CogPack group), the EMDR group showed significant improvement from baseline to the end of the intervention and a trend towards until the first follow-up. Impulsivity: The statistical analysis showed no significant results. The study show that the EMDR method can have a positive effect on emotion regulation.

Pivec Vid

Abstract ID: 93199

The effect of operative stress in abdominal surgery on the hepatovisceral circulation - preliminary results

Vid Pivec[1]

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We will present the preliminary result of the ongoing study with the tiitle: "The effect of operative stress in abdominal surgery on the hepatovisceral circulation"

Objective #1: To assess the impact of abdominal surgery on visceral arterial and portal venous flow. Objective #2: To assess how different types of resections influence the postoperative visceral arterial and portal venous flow. Objective #3: To determine the effect of comorbidity on the hepatovisceral circulatory response on surgery.

Expected results: The hepatovisceral blood flow after abdominal resections will probably be increased for the first few days after surgery. The increment will probably be lower in polymorbid patients. The whole body tolerance to volume depletion and falls in systemic pressure will probably be diminished.

What will be measured: The observed parameter is the estimed hepatovisceral BF in the first few (4-7) days after surgery measured with Dopper ultrasound. We will measure BF at the level of the suprarenal aorta (SRA), t. Coeliacus (TC), a. Mesenterica superior (AMS) and Portal vein (PV). In the two separate study groups we will enroll patients scheduled for right hemicolectomy and radical gastrectomy. The control group will be composed of patients with incisional hernias. By comparing this two groups we will be able to eliminate the effect of general anesthesia and laparotomy on the changes in the hepatovisceral BF. Also of interest are the differences in the hepatovisceral BF distribution among polymorbid and otherwise healthy patients.

We will discuss and present the actuall preliminary results in regard to the expected results.

Schmid Paul

Abstract ID: 88423

Stability, growth and decrease of bacteria in fiber-based food packaging materials

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Microorganisms can be found ubiquitously and were previously shown to be present also in various types of packaging materials. Although the microbial community of fiber-based packaging materials has been studied extensively for years, the interaction of bacteria with the surrounding packaging matrix still needs more research.

Therefore, this study investigated the survival of food relevant species e.g. E. coli, S. aureus, B. cereus as well as the environmental strains Cytobacillus firmus and Niallia circulans (directly isolated from packaging material) and bacterial spores of B. cereus and B. subtilis. All strains were separately spiked into sterilized and homogenized packaging material samples of different fiber types. The stability of the added bacteria differed between the packaging material samples. Respecting on the different fiber types, some packaging samples appeared to supply more nutrients for bacteria than others resulting in prolonged bacterial stability, higher growth rates and spore germination. Furthermore, none of the strains could grow in all included samples. Rather, the use of fiber based packaging materials as a nutrient is no universally applicable strain characteristic, but depends on the bacterial strain and the type of packaging material. Moreover, one sample strongly reduced all added bacteria within 24h suggesting antimicrobial activity. Bacterial spores were able to germinate in only one out of four sample, while spores remained dormant in the other three samples.

Although further investigations are needed, this knowledge is essential for the development of packaging materials and their application as food packaging to understand and control bacterial growth in packaging material.

Smolle Christian

Abstract ID: 93558

Design and Preliminary Evaluation of a Newly Designed Patient-Friendly Discharge Letter <U+0096> A Randomized, Controlled Participant-Blind Trial

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Introduction: Low health literacy has been associated with poor health outcome and impaired use of healthcare services. The hospital discharge letter represents a key source of medical information for patients and can be used to address the problem of low health literacy. The aim of this project was to develop and evaluate a new, patient-directed, version of the discharge letter. Methods: Based upon two conventional discharge letters (CDL; one surgical and one medical letter), two new, patient-friendly discharge letters (PFDL) were designed following 5 key principles: short sentences, few abbreviations, large font size, avoidance of technical terms and no more than 4 pages length. Medical undergraduates were randomized into two blinded groups (CDL, PFDL) and asked to assess the assigned letter for the 3 domains structure, content and patient-friendliness. Subsections were rated on a 6-point Likert scale (1=completely agree, 6=completely disagree), the results of the survey were compared using the Mann-Whitney-U-Test with a $p < 0.05$ being the level of significance. Results: In total, 74 undergraduates participated in the study. PFDL (35 participants) were rated significantly better than CDL (39 participants) regarding structure (median 1 vs. 2, $p=0.005$), content (1 vs. 3, $p<0.001$) and patient-friendliness (2 vs. 6, $p<0.001$). Of all 17 subsections, PFDL were rated significantly better in 12 cases, and never worse than CDL. Conclusion: PFDL were rated significantly better than their CDL counterparts. Medical undergraduates were considered the ideal cohort, not being medical lays and yet unbiased regarding everyday clinical practice procedures. Further tests evaluating the impact of the PFDL on patient comprehension and health literacy are necessary.

Steyer Gerhard Ernst

Abstract ID: 90465

Colorectal anastomosis leakless

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Background: Anastomotic leakage (AL) in intestinal surgery is a worldwide, since decades well known problem [Schardey 2021] and recently P. Aeruginosa and E. faecalis are detected [Alverdy 2017] to cause leaks via activating matrix metallo protease 9 that can cut leaks into tissues. Aim: To reduce AL means to reduce the hospital bug P. aeruginosa in the microbiota of the gut. If we make assumptions (or in equal wise words <U+0093>sea pocks<U+0094> [Anschober 2020]) such a reduction is theoretically possible in two different ways: one is competition via probiotics, but in literature there is no evidence for a multispecies probiotic (in a <U+0093>study<U+0094> [Zhang 2012] the <U+0093>multispecies probiotic<U+0094> contains E. faecalis!) and on the other hand P. aeruginosa is a strong bacteria that itself suppresses other bacteria [Bullen Sept. 2022]; an other, but promising way, is to kill P. aeruginosa via special antibiotics that act local in the gut and are not resorbable. Method: To prevent AL we compared a prospective treatment group of 150 patients, who recieved a mixture of 3 antibiotics [Rx by Schardey 2017] with a retrospective control group (n=150). Result: According to our protocol clinically we did not find a single case of AL in the treatment group; that result is highly significant (p<0,01) in the Fisher exact test. Conclusion: The triple antibiotic mixture from our study is a very promising method to prevent / reduce AL after colorectal surgery (AL is reported in the literature to be over 10%). Dose finding studies are necessary. It is also worth to investigate, if AL occures periodically (P. aeruginosa is a hospital bug and Hippocrates said <U+0096> of course in ancient Greek <U+0096> <U+0084>do not harm<U+0093>!).
Acknowledgement: Without a sponsor we interpreted results without any conflict of interest. We want to thank Prof. HM Schardey, Clinic Agatharied, Germany, for his input regarding the magistral preparation of the antibiotic mixture used in the study.

Stijic Marko

Abstract ID: 92867

Parents´ thoughts, needs and worries concerning their children<U+0092>s surgery <U+0096> a pilot study

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Background: Surgical procedures can represent a common source of stress and preoperative anxiety both for children and their parents. Since parental perioperative anxiety (PPA) can affect children´s anxiety and their perception of postoperative pain in a negative way, it is important to investigate factors, which further explain PPA.

Aim: To better understand parents' thoughts, needs and concerns about their children's surgery and to identify their perceptions about the most effective strategy to reduce their perioperative anxiety.

Methods: A qualitative study with parents of children who underwent outpatient surgery at the University Department of Pediatric and Adolescent Surgery at the Medical University of Graz was conducted. Open-ended questions were derived from from a literature review. The verbatim text of 30 semi-structured interviews was analysed by content analysis. A combination of deductive and inductive approach was used to categorize the parents´ responses.

Results: Most of the 22 mothers and 8 fathers (age range: 24 - 54 years) stated that they felt comfortable before their child's operation. Most of the parents felt (very) well looked after. However, some parents felt anxious and had concerns, especially about their child's post-operative health and the anesthetic used. Some parents were also concerned about infection and did not know what to do postoperatively. Pre-operative information about the surgery procedure was reported as the most desirable coping mechanism.

Conslusion: Anxiety and concerns about the postoperative health status of their child and the anesthetics / narcosis used have been well documented in the literature. Concerns about infection and parental postoperative helplessness were not reported previously. Based on these results, we decided to include the Amsterdam Preoperative Anxiety and Information Scale (APAIS), a validated tool for our main survey study on PPA.

Verheyen Sarah

Abstract ID: 93552

Diagnostic Database Rare Diseases at the D&R Institute of Human Genetics Graz <U+0096> a single center registry

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Background/Aims Increasing use of whole exome sequencing has dramatically improved the diagnostic rate in the field of rare diseases. However, most of detected variants and their functional consequences have not yet been described and investigated and are therefore classified as variant of unknown significance (VUS). Gene-associated phenotypes are often not documented in literature in their entire range. Due to these challenges, recognizable causative variants can still be overlooked during the analysis process. A <U+0093>Diagnostic Database Rare Diseases<U+0094> was created to achieve an overview of diagnostic cases and the diagnostic rates depending on evaluation strategies and the patients<U+0092> phenotypes.

Methods/Results The database was created using the RDA platform based on a detailed questionnaire for patient referrals. Between 2020 and 2022 phenotypic information according to the questionnaire, terms of the Human Phenotype Ontology (HPO) used for genetic analysis, testing strategies and detected genetic variants were documented. For the present study, 314 cases were analyzed of which 45 (14%) were prenatal. 65/314 (20.7%) cases were solved, 30/314 (9.3%) were probably/partially solved. 602 different HPO terms were used for description of the phenotypes. The most common terms were global developmental delay (n=72), seizures (n=45), muscular hypotonia (n=30), microcephaly (n=29) and delayed speech and language development (n=28). Solved cases were characterized by assignment of significantly more HPO terms than unsolved cases (median [interquartile range] 5 [3-7] versus 3 [2-6], P=0.019). Two cases included in the database were published as reports of novel gene-phenotype descriptions for the genes ARSK and ATP9A.

Conclusion Number of HPO terms was associated with a better diagnostic rate. Detailed phenotyping in rare disease cases is essential to exploit the diagnostic potential of exome analysis.

Weiss Veronika

Abstract ID: 93083

Non-invasive methods in pediatric radiology - a focus on neuroradiology

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B/A:Non-invasive procedures in radiology include sonography, native cross-sectional imaging as well as preceding clinical and physical examinations. Focusing on pediatric neuoradiology a special meaning lies in MRI. MRI itself is a safe procedure if contraindications for implants is obeyed, but additional injection of intravenous contrastmedia, taking blood samples or the use of anaesthesia represent invasive procedures. The aim of this study is to validate the comparability of non-invasive methods to those invasive examinations in current use. M/R:In a retrospective study design, archived data of pediatric MRI images are analyzed to compare validity of scans based on conventional acquisition to a faster image assessment using the software SyMRI NEURO which was acquired by the department of pediatric radiology, Medical University Hospital Graz, in 2021 and has been in use since then. This method is fast, which helps to be less invasive, and rich in results. The software obtains within a few minutes not only “usual” weighted sequences but also gives a qualitative and quantitive analysis of certain intracranial tissues, in particular brain parenchyma and its myelination and also cerebrospinal fluid. The further advantage of this primarily non-invasive method is the output of comparison to standard values in the patient’s age group. Comparison of conventional to synthetic MRI and evaluation of the software output is the result of the first acquired data. C:The use of MRI in fields of neuroradiology is currently gold standard. Due to long acquisition times and additional invasive procedures, the call for non-invasive and short acquisition timed methods is getting louder, especially in pediatric radiology as long-term effects of invasive methods are not yet fully explored. A future goal is to achieve the practical applicability of renunciation of invasive MRI examinations so that their short and long term physical effects and possible complications can be prevented.

Woltsche Nora

Abstract ID: 88701

The ocular surface microbiome in Dupilumab associated ocular surface disease

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Background/Aim: In real-world analyses, ocular surface adverse events have been reported in as many as 60% of atopic dermatitis (AD) patients treated with the monoclonal antibody Dupilumab targeting the IL-4 receptor-? subunit of IL-4 and IL-13. The aim of this study was to clarify the pathogenesis behind Dupilumab associated ocular surface disease (DAOSD).

Methods: Twenty moderate to severe AD patients receiving Dupilumab underwent thorough ophthalmological slit lamp examinations, dermatological examinations and conjunctival smears and swabs before Dupilumab initiation, four weeks after initiation, at the time point of developing DAOSD and sixteen weeks after initiation. Ten healthy controls underwent these examinations, except the dermatological whole-body examination, at a single time point. 16S sequencing was carried out for microbiome analyses.

Results: Six of the twenty patients receiving Dupilumab developed DAOSD. An increased granulocytic infiltrate was observed in histological stainings from conjunctival smears in these patients. An unaltered microbial diversity and a unique colonization of certain microbial species in patients with DAOSD was shown, whereas AD patients not developing DAOSD showed a decrease in microbial diversity after Dupilumab treatment.

Conclusion: Our results strongly indicate an important role of the ocular surface microbiome in the pathogenesis of DAOSD.

Talk Session V

Lehner György

Abstract ID: 92961

Incidence and predictors of postoperative complications in breast oncological surgery

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Background Postoperative mortality and morbidity are important quality markers of surgical care. The operative therapy of breast cancer has generally low mortality and morbidity, however, little is known about the predictors of postoperative complications (PCs). Aims To evaluate the type and incidence of PC and relevant risk predictors in women who underwent breast cancer surgery. Methods This retrospective study included all women with a histological diagnosis of invasive breast cancer or ductal carcinoma in situ who underwent surgery between 1997 and 2019 at the Department of Gynecology at the Medical University of Graz. PCs were defined as adverse events within 8 weeks after breast cancer surgery. These were categorized by the Clavien-Dindo classification; uni- and multivariable analysis were performed to identify the predictors of risk for PCs. Results N=326 patients were included (median age=59, IQR:50-70); most of the women were postmenopausal (72.1%). In 18.4% of patients more than 3 comorbidities were present while 56.1% had only one to three comorbidities. The most common concomitant disease was arterial hypertension (39.3%), followed by endocrinopathies (27.6%). 35.3% of the patients received neoadjuvant chemotherapy. Out of 458 surgical procedures, 232 had any postoperative complications. There were no life-threatening events or deaths. Clavien-Dindo Grade I complications occurred in 58%, Grade II-IIIb in 42% of cases. Basic demographics, TNM classification or hormone receptor status of the tumor were no predictors of PCs. However, pathological N0 status was predictive in both univariate and multivariate analysis ($p<0.001$, OR:3.69 (2.11 <U+0096> 6.45)); neoadjuvant chemotherapy was predictive in univariate ($p<0.001$) but not in multivariate analysis. Conclusion Although the overall incidence of the PCs was high, most of them were minor. Neoadjuvant chemotherapy and N0 status were predictors of postoperative adverse events after breast oncologic surgery.

Mairinger Marco

Abstract ID: 92040

Gut Microbiome Composition and Its Association with Sleep in Major Psychiatric Disorders

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Introduction: Sleep disturbances are highly prevalent across most major psychiatric disorders. Alterations in the hypothalamic-pituitary-adrenal axis, neuroimmune mechanisms and circadian rhythm disturbances partially explain this connection. The gut microbiome is also suspected to play a role in sleep regulation and recent studies suggest that certain probiotics can improve sleep quality.

Methods: We aimed to assess the relationship between gut-microbiota-composition, psychiatric disorders and sleep quality, in this cross-sectional, cross-disorder study. We recruited 103 participants, 63 patients with psychiatric disorders (Major Depressive Disorder n=31, Bipolar Disorder n=13, Psychotic Disorder n=19) along with 40 healthy controls. Sleep quality was assessed with the Pittsburgh Sleep Quality Index (PSQI). The fecal microbiome was analyzed using 16S rRNA sequencing, and groups were compared based on alpha- and beta-diversity metrics as well as differentially abundant species and genera.

Results: A transdiagnostic decrease in alpha diversity and differences in beta diversity indices were observed in psychiatric patients, compared to controls. Correlation analysis of diversity metrics and PSQI score showed no significance in the patient and control groups. However, three species, Ellagibacter isourolithinifaciens, Senegalimassilia faecalis and uncultured Blautia sp. and two genera, Senegalimassilia and uncultured Muribaculaceae genus were differentially abundant in psychiatric patients with good sleep quality (PSQI > 8), compared to poor sleep quality patients (PSQI ? 8).

Conclusion: In conclusion, this study raises important questions about the interconnection of the gut microbiome and sleep disturbances.

Schönthaler Elena

Abstract ID: 89111

Associations between Metabolic Syndrome and Dark Triad Traits in Affective Disorders

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Background: Previous studies have shown an association between metabolic syndrome (MetS) and affective disorders (AD). Nevertheless, personality traits that could further explain this relation have rarely been studied in this context. The Dark Triad of personality (DT; e.g., Narcissism, Machiavellianism, Psychopathy) has been highlighted regarding both somatic health and AD. Objectives: This study examined the association between MetS and DT in AD to identify the effect of these traits. Further, the role of sex in this context and the relations between the MetS parameters, Body-Mass-Index (BMI) and DT traits were analyzed. Methods: Parameters of MetS (triglycerides, high-density lipoprotein cholesterol, fasting glucose, blood pressure, waist circumference) and BMI were collected in 112 inpatients with AD. Additionally, the <U+0084>Short Dark Triad<U+0093> questionnaire was used to asses DT traits. Results: Results indicated no significant association between MetS and DT traits. Moreover, we found that sex, but not MetS, significantly influenced the DT and significantly interacted with MetS in their influence on DT traits. Thus, further analyses revealed that men with or without MetS demonstrated significantly higher DT scores than women with or without MetS. An examination of the MetS parameters showed that only Machiavellianism was significant positively correlated with BMI. Conclusion: Our findings indicate that DT traits are not a risk factor for developing MetS in AD. However, Machiavellianism seems to worsen health parameters, possibly contributing to MetS or other somatic comorbidities. Other factors (e.g. lifestyle) might influence the relationship between MetS and AD, however, these are also affected by the DT traits.

Talk Session VI

Lenard Aneta

Abstract ID: 92458

Deciphering structural and functional effects of protein citrullination

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Background: Arginine-glycine(-glycine) (RG/RGG) regions are highly abundant in RNA-binding proteins (RBPs) and play roles in numerous physiological processes. Aberrant liquid-liquid phase separation (LLPS) and recruitment into membraneless organelles of RG/RGG regions have been implicated in the onset of several neurodegenerative disorders. Both, LLPS and the association with biomolecular condensates of RG/RGG proteins, can be regulated by the interaction with nuclear import receptors, such as transportin-1, and by post-translational modifications, such as phosphorylation or arginine methylation. Furthermore, arginine residues within RG/RGG regions harbour potential sites for the conversion to a citrulline that is catalyzed by calcium-dependent protein arginine deiminases (PADs). The increased levels of protein citrullination have been observed in patients suffering from autoimmune, inflammatory, and neurodegenerative diseases. Here, we hypothesize that RG/RGG-rich RBPs constitute substrates for PADs-mediated citrullination, and this modification regulate their function, structure, and subcellular localization. Aims: The aim of this project is to uncover whether and how citrullination regulates structural and functional properties of RNA-binding proteins enriched with RG/RGG regions. Method/Results: Here, by applying solution NMR spectroscopy we show that arginines within RG/RGG regions of different RBPs constitute sites for citrullination in vitro. With the use of biophysical techniques, we demonstrate that citrullination suppresses in vitro phase separation and RNA binding of RG/RGG-rich RBPs. Our data furthermore reveal that citrullination of RG/RGG-rich RBPs impairs binding to the nuclear import receptor transportin-1. Conclusion: In conclusion, our findings indicate that citrullination regulates the function and structural properties of RNA-binding proteins.

Usluer Sinem

Abstract ID: 92885

p53 Transactivation Domain Mediates Binding and Phase Separation with Poly-PR/GR

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Background/Aims: The most common genetic cause of amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD) is the presence of poly-PR/GR dipeptide repeats (DPRs) which are encoded by the chromosome 9 open reading frame 72 (C9orf72) gene. Recently, it was shown that poly-PR/GR alters chromatin accessibility which results in stabilization and enhancement of transcriptional activity of the tumor suppressor p53 in several neurodegenerative disease models. Reduction of p53 protein levels in cell and model organisms protects against neurotoxicity of poly-PR, and partially protects against neurotoxicity of poly-GR. The mechanistic details leading to poly-PR mediated stabilization and activation of p53 remain enigmatic. Here, we aimed to study the detailed molecular mechanisms how p53 contributes to poly-PR/GR mediated neurodegeneration. Our findings might help to understand the mechanistic role of p53 in poly-PR/GR - associated neurodegeneration. Method/Results: Using a combination of biophysical techniques such as nuclear magnetic resonance (NMR) spectroscopy, fluorescence polarization, turbidity assays and differential interference contrast (DIC) microscopy, we found that p53 physically interacts with poly-PR/GR and triggers liquid-liquid phase separation of p53. We identified p53 transactivation domain 2 (TAD2) as the main binding site for PR25/GR25 and show that binding of poly-PR/GR to p53 is mediated by a network of electrostatic and/or hydrophobic interactions. We demonstrated that binding of PR25/GR25 to p531-94 enhances rigidity and formation of ?-helical propensity. Conclusion: Poly-PR/GR mediated LLPS of p53 observed here might be of general importance in the regulation of transcriptional condensates. In the future it will be interesting to reveal if poly-PR/GR modulates formation of p53 transcriptional condensates and through this regulates expression of p53 target genes.

Zhou Qishun

Abstract ID: 93135

Nucleotides modulate RNA-driven phase separation of CIRBP

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Background: Intrinsically disordered regions (IDRs) promote formation of protein condensates via liquid-liquid phase separation (LLPS). It could further develop into aggregations under pathological conditions. Within a cell, proteins with IDRs are exposed to high concentration of metabolites, which could be up to millimolar range and form an environment diverged from buffer used in biochemical studies. This rendered difficult to predict the behavior of IDRs in cell from purified proteins.

Aims: We used cold inducible RNA binding protein (CIRBP), containing an arginine<U+0096>glycine/arginine<U+0096>gl (RG/RGG)-rich region, as a paradigm to investigate in detail of the interaction between condensate-forming IDRs and cellular metabolites. In the meanwhile, we aimed to characterize the dynamics of interactions within a protein condensate.

Method: We used solution nuclear magnetic resonance (NMR) spectroscopy to characterize titrations of purified CIRBP in its full length or individual regions by various metabolites. In addition, we performed molecular dynamic (MD) simulations to study the condensate driven by RG/RGG regions.

Results: We found that CIRBP forms direct interactions with nucleotides and dinucleotides, including ATP, ADP, and AMP as well as NAD+, NADH, NADP+, and NADPH. These interactions are through the RNA recognition motif (RRM) and the disordered RG/RGG region, with the latter modulates RNA-driven CIRBP phase separation. From MD simulation, we observed that aromatic cycles can effectively prolong the time of peptide intra- and interchain contact formed between amino acid side chains.

Conclusion: Given that proteins harboring RG/RGG regions are highly abundant in the human proteome, their phase separation in the cellular environment could be commonly modulated by (di)nucleotides. This could be an important factor regulating the development of cancers or neurodegenerative diseases.

Poster Session III

Braun Celine Kaja

Abstract ID: 93066

Impact of Low Social Support on Patients with Acute Myocardial Infarction

Celine Kaja Braun[1] , Albrecht Schmidt[2] , Ewald Kolesnik[2] , Hanna Pagitz[2] , Lara Keding[1] , Andreas Baranyi[1]

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Psychological stress burden and anxiety are often associated with a worse prognosis of cardiac disease and increased risk of adverse cardiac outcomes. Especially social support and the social environment have a further impact on people's mental health, their health-related quality of life and the development of diseases. During the COVID-19 pandemic, infection rate minimization measures ans self-isolation were widely implemented, that might cause uncertainty and may result in psychological and physical negative consequences. However, it remains questionable to what extent different levels of social support have an additional impact on different facets of anxiety in AMI patients.

AMI patients were divided into low, medium and high social support groups using the Social Support Questionnaire. These groups were compared in terms of anxiety and depression symptoms, measured with State-Trait-Anxiety Inventory (STAI), Hospital Anxiety and Depression Scale (HADS), Illness Attitude Scale (IAS) and Beck Anxiety Inventory (BAI), using one-way analyses of variances.

112 patients were assessed for mood and anxiety symptoms after admission to the hospital due to acute myocardial infarction. Statistical analyses revealed that AMI patients with low social support showed significantly more state and trait anxiety, more physical symptoms of anxiety, increased health related anxiety, as well more symptoms of depression than the group with high social support. In addition, AMI patients with high social support showed significantly less trait anxiety and depression in comparison with AMI patients with medium social support.

This may support the hypothesis that patients with low social support are more likely to experience negative psychological facets to a greater extent and, in this regard, might be in a higher risk to experience a negative outcome of AMI. Future studies should investigate potential opportunities for improvement in social support.

Fuchs-Neuhold Bianca

Abstract ID: 92603

A qualitative study examining the impact of pregnancy and childbirth on women's health-related attitudes and beliefs one year after delivery

Bianca Fuchs-Neuhold[1]

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Background/Aim: Motherhood is perceived as a time of transition into a new role that brings health changes that affect women's lifestyle like diet and physical activity behaviors. There is limited information on how women experience lifestyle changes due to pregnancy and the transition to motherhood, with a focus on diet and physical activity. The purpose of this qualitative study was to examine women's health-related attitudes and beliefs one year after delivery, focusing on pregnancy- and childbirth-related changes in dietary behaviors and physical activity.

Methods/Results: As part of a prospective longitudinal study, 24 pregnant women were selected from the HPL study population and interviewed face-to-face one year after delivery. Semistructured interview topics included (i) subjective concept of health, (ii) feelings about lifestyle and health behavior changes focusing diet and physical behavior, and (iii) stresses and resources that influence personal lifestyle/health behaviors. After obtaining informed consent, all interviews were recorded. A systematic thematic content analysis was conducted to identify recurring themes. Among the well-educated participants aged 23-42 years, 17 were first-time mothers. Analysis of the data revealed overarching themes such as conscious reflection on one's own health needs and its implementation in everyday life through family and external support services. The study was approved by the Ethics Committee of the Medical University of Graz (EC No. 26-066 ex 13/14), and all participants gave informed consent.

Conclusion: Analysis of mostly well-educated Austrian mothers living in couples revealed that the transition to motherhood represents a turning point for lifestyle change. Furthermore, the results indicate that individual health support is needed to address self-care, physiological changes, stress, and negative emotions of motherhood.

Glatz Marlene

Abstract ID: 93557

Blindness and Low Vision in Austria

Marlene Glatz[1] , Rupert Strauß[1] , Regina Riedl[2] , Andreas Wedrich[1]

Department of Ophthalmology, Medical University of Graz, Austria;Institute for Medical Informatics, Statistics and Documentations, Medical University of Graz, Austria

Background/Aims To assess the prevalence and causes of visual impairment and blindness in Austria. The findings may have implications for the planning of further research and development of therapies in order to prevent blindness. Methods/Results The database of the Main Confederation of Austrian Social Insurances was searched for patients with visual impairment, legal blindness or deaf-blindness. To determine the prevalence of these conditions, the number of all entries recorded in February 2019 was evaluated. Additionally, all new entries between (January 1st,) 2017, and (December 31st,) 2018, were analysed for distinct characteristics, such as sex, the cause of blindness/visual impairment, and age. On February 2nd, 2019, 17,730 patients with visual impairments, blindness or deaf-blindness were registered in Austria, resulting in a prevalence of these diagnoses of 0.2% in the country. During the observational period from 2017 to 2018, 4040 persons met the inclusion criteria. Of these, 2877 were female (65.3%), and 1527 were male (34.7%). The mean age was 75.7 ± 18.0 years (median 82). Most patients (n = 3675, 83.4%) were of retirement age, while 729 (16.6%) were working-age adults or minors. In total, an incidence of 25.0 (95% confidence limit (CL) 24.3<U+0096>25.8) per 100,000 person-years was observed from 2017 to 2018. In total, the most frequent diagnoses were macular degeneration (1075 persons, 24.4%), other retinal disorders (493 persons, 11.2%) and inherited retinal and choroidal diseases (IRDs) (186 persons, 4.2%). In contrast, among working-aged adults and children, IRDs were the leading cause of visual impairment and blindness (103 persons, 14.1%). Conclusion These data show that IRDs are the leading cause of blindness and visual impairment in working-aged persons and children in Austria. Thus, these findings suggest to draw attention to enhance further research in the fields of emerging therapies for IRDs.

Großpötzl Manuel

Abstract ID: 90454

Progression of Stargardt disease type 4 as measured by spectral-domain optical coherence tomography (SD-OCT) in the ProgStar-4 Study

Manuel Großpötzl[1]

Department of Ophthalmology

Purpose: To evaluate progression of loss in various retinal layers in patients with Stargardt disease type 4 (STGD4) over 24 months follow-up of SD-OCT findings Setting/Venue: Five centers in the U.S.A., United Kingdom and Germany.

Methods: Fifteen patients with molecularly confirmed STGD4 (PROM1 disease causing variants) were enrolled at five sites in the United States, United Kingdom and Germany. SD-OCT scans were obtained at baseline from a 20° x 20° scan area centered on the fovea and was repeated after 24 months using the built-in follow-up mode with gradable images available for 26 eyes; right eyes were chosen for analysis. The mean thicknesses (MT) of individual layers and the proportions of damage/tissue loss relative to the scanned area were calculated by a custom software within the central subfield (CS; 0.5mm radius) and the inner ring (IR; 0.5-1.5mm). The outer ring (OR; 1.5-3mm) was only analysed in eyes with adequate coverage of the posterior pole.

Results: There was a statistically significant change (all $p<0.05$) over 2 years in the segmented central subfield of the RPE in estimated trajectory of MT -3.87 (±6,1) µm, the HFC -6.22 (± 6.95) µm, the inner ring of the RPE -3.88 (±3,68) µm/, the HFC -5.49 (±6.39) µm/, the outer ring of the ONL -3.1 (±2.5) µm, the IS -1,43 (±1,26) µm, the total mean of the ONL -2.5 (±2.97) µm, the IS -1.7 (±1.45) µm, the intact area of the RPE of the inner ring -0,79 (±0,95) mm2, the PIS -0,23 (±0.36) mm2 and the outer ring of the ONL -0.12 (±0.09) mm2.

Conclusions: Significant loss could be detected in outer retinal layers by SD-OCT over a 24 months period in patients with STGD4. Loss of thickness and/or intact area of RPE, IS, OS and ONL may serve as potential endpoints for clinical trials that aim to slow down the disease progression of STGD4.

Häussl Alfred

Abstract ID: 92076

Use of a socially assisting robot "Pepper" in psychoeducational training sessions for patients in acute psychiatric treatment - a project description of a feasibility study

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Background: The development of new technologies, such as robots, is gradually accelerating and transforming healthcare. At a time when human resources are becoming increasingly scarce, robots should be considered to support existing treatment options. Therapies in psychiatric settings should always have a multimodal approach, they should be cost-effective and readily accepted. Therefore, it is highly in demand to include new technologies (e.g., a socially assistive robots (SAR)) in psychiatric treatment. Currently, the use of new technologies in the psychiatric therapy is largely limited to mobile apps and online websites. However, SAR have the potential to support traditional psychiatric therapy, for example, through psychoeducational training. Nevertheless, the use of robots in psychiatry is almost non-existent. The aim of this feasibility-study is to find out whether patients undergoing acute psychiatric inpatient treatment are satisfied with psychoeducation training sessions with the SAR named Pepper. Method: The study will be conducted at the Clinical Department of Psychiatry and Psychotherapeutic Medicine at the Medical University Graz between March 2023 and August 2023. Patients are given the opportunity to complete psychoeducational training sessions with SAR Pepper during their inpatient stay in addition to their psychotherapy and medication. Standardized and non-standardized questionnaires, which are completed before the first (t1) and after the last (t2) training session, as well as focus group interviews are used to check acceptance criteria and individual beliefs. Results: We present the study plan, outcome variables, and the questionnaires to measure acceptance in SAR. Conclusion: Due to the rapid development of robotic programs and the variety of potential opportunities in therapy support for psychiatric patients, it is important to scientifically evaluate the use of SAR in the psychiatric setting.

John Nikolaus

Abstract ID: 93556

Predictive value of t-cell activity and change in perfusion weighted imaging for the response of immune-oncologic therapy in NSCLC <U+0096> a pilot study

Nikolaus John[1] , Christoph Wohlkönig[1] , Robert Wurm[1] , Gudrun Absenger[2] , Angelika Terbuch[2] , Martin Zacharias[3] , Luca Brcic[3] , Martin Stradner[5] , Michael Fuchsjäger[4] , Horst Olschewski[1]

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Background and aims PD-L1 (programmed death ligand) is the most important checkpoint in oncology and the percentage of PD-L1 expression in tumour cells is a predictor of treatment response in patients on PD-L1 inhibitor therapy. However, there is an unmet need for a better biomarker of response to ICIs. Furthermore, in some cases so called pseudo progression complicates evaluation of treatment response. Hence, we aimed to investigate if CD 8 pos. T-cell immune response may be a better biomarker for patients<U+0092> response to ICIs and if perfusion imaging of cancer lesions may help to distinguish tumour mass increase by progression from pseudo progression. Methods We are enrolling 21 patients with metastatic NSCLC and indication for mono PD-L1 / PD-1 checkpoint inhibitors. For T-cell immune response we used Interferon-Analysis with mitogen vial of the <U+0093>QuantiFERON TB-GOLD plus<U+0094> test kit. To exclude undiagnosed immune defects, we additionally performed FACS analysis including CD 8 pos. cell count and CD 8 to Mitogen ratios. Patients underwent perfusion CT-angiography of the tumour before and after 6 weeks of therapy. This perfusion CT-scan was performed following a newly developed protocol designed by a senior radiologist and performed by a specialised radiology technical assistant. Results Nine patients (n=9, age: 70(66-77)ys, (male: n=7) were enrolled prospectively. In n=6 patients all study examinations have been concluded. All patients were ex-or active smokers (5/3) with 39(28-43)py. Median Interferon-? levels were 8.21 (4.75-9.22) U/L. Those patients with the lowest Interferon-? values dropped out of the study due cancer progression and clinical worsening. Radiology analysis has not been completed yet. Conclusion First results of this pilot project suggest a correlation between lower Interferon-? and a worse outcome in patients with NSCLC treated with ICIs. We are curious about upcoming data of perfusion imaging that will be presented in January.

Mileder Lukas

Abstract ID: 93113

Quality of neonatal resuscitation and impact of interdisciplinary and interprofessional in situ simulation training

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Background/Aims: Resuscitation of newly born infants after birth is a high risk, low occurrence event. In situ simulation training, i.e. training in the real healthcare environment, allows to analyse and improve healthcare delivery as well as to identify latent safety threats. However, it has only been shown for a few medical disciplines if, and to what extent, in situ simulation training improves healthcare delivery and patient outcome. Therefore, we investigated in this prospective monocentric observational study (ethics committee number 27-014 ex 14/15) if in situ simulation training was associated with improved postnatal management and superior neonatal outcome.

Method/Results: We delivered a total of 41 in situ simulation trainings, each involving two to five physicians and/or neonatal nurses, over a four-month-period at the Neonatal Intensive Care Unit, Medical University of Graz. These trainings targeted both technical and non-technical skills, such as communication, leader-/followership, decision making, situational awareness, and task management. Two months before and after these trainings, actual neonatal resuscitations were video-recorded. For the primary study outcome of teamwork during postnatal stabilization and resuscitation, an independent, blinded neonatologist rated all available videos (12 before and 13 after the training intervention, respectively) in random order using the Anaesthetists' Non-Technical Skills (ANTS) score (Br J Anaesth 2003;90(5):580-8). When comparing the pre- and post-training period, there were no differences in the four major ANTS categories Task Management (p=0.769), Team Working (p=0.252), Situation Awareness (p=0.608), and Decision Making (p=0.813).

Conclusion: Based on the analysis of the first video assessor, in situ simulation training was not associated with an improvement in the non-technical skills domain. A potential reason could be the already high level of non-technical skills during the pre-training period.

Posch-Pertl Laura

Abstract ID: 93183

Thrombophilia and atherosclerosis related gene polymorphism in retinal vein occlusion

Laura Posch-Pertl[1] , Martin Weger[1] , Andreas Wedrich[1] , Wilfried Renner[2] , Wolfgang List[1]

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Introduction Retinal vein occlusion is the second most prevalent blinding retinal vascular disorder with a prevalence of around 0.4% in the population. Risk factors for RVO largely coincide with risk factors for cardiovascular disease (CVD). However, its exact pathomechanism is still unknown. In order to explore more on its pathomechanism, our study aim is to investigate thrombophilia and atherosclerosis related gene polymorphism associated with CVD in patients with RVO.

Methods We included 485 RVO patients and 295 control subjects who were recruited in this case-control study. We determined genetic polymorphisms by polymerase chain reaction.

Results In this study, we could not find an association between the presence of RVO and the ABO blood group (p=0.693), the HO-1 associated polymorphism at rs2071746 genotype (p=0.443), the two PON-1 associated polymorphism at rs854560 (p=0.451) and at rs662 (p=0.466). The allele frequencies in the adiponectin polymorphism at rs1501299 were significantly different distributed in RVO patients and controls (p=0.023). Patients with at least one T allele had an OR of 0.74 (0.55-0.99, p=0.041) for being an RVO patient. This association remained after multivariable adjustment.

Discussion In this study, we could only find an association between the adiponectin polymorphism and RVO. The T allele at the rs1501299 polymorphism of the adiponectin gene was shown to be protective for cardiovascular disease. The proposed mechanism is the association of the T allele with higher plasma adiponectin levels and lower concentrations of triglycerides and small dense LDL.

Rohrer Ursula

Abstract ID: 89309

Prevention of early sudden cardiac death after myocardial infarction using the wearable cardioverter defibrillator <U+0096> Results from a real-world cohort

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Background and Aims After acute myocardial infarction (AMI), patients are at elevated risk of sudden cardiac death. The VEST trial failed to show a significant reduction in arrhythmic mortality in patients prescribed with a wearable converter-defibrillator (WCD), having a lower than expected wearing compliance. The aim was to investigate the incidence of WCD treatments and outcomes of all patients with acute myocardial infarction and left ventricular ejection fraction (LVEF) ?35% in a real world and well-compliant national cohort in Austria.

Methods A retrospective analysis of all Austrian WCD patients meeting the in- and exclusion criteria of the original VEST trial between 2010 and 2020 was performed.

Results 105/896 Austrian patients (12%) with an average age of 64±11 years (12% female; LVEF 28±6%) met the VEST in- and exclusion criteria. 104/105 patients were revascularized, one patient did not receive a coronary intervention. All received a WCD for a median of 69 (1;277) days. The wearing duration was 23.5 (0;24) hours/day. Within 90 days after prescription 4/105 (3.8%) patients received 9 appropriate shocks (median of 2 (1;5) shocks). No inappropriate shocks were delivered. 3/105 (2.9%) patients died: two patients received shocks and died in ventricular storm; one patient died due to asystole. Arrhythmic mortality (1.9% Austria vs. 1.6% VEST, p=n.s.), as well as all-cause mortality (2.9% vs. 3.1%, p=n.s.) was comparable in both cohorts.

Conclusion The WCD is a safe treatment option in a highly selected cohort of patients with LVEF ?35% after AMI. However, despite excellent WCD wearing duration, as opposed to the VEST study, only 3.8% of patients received appropriate WCD shocks and the arrhythmic mortality rate was not significantly different from the VEST study.

Schließleder Gernot

Abstract ID: 90360

DEEP PHENOTYPING OF PROM1-ASSOCIATED RETINAL DEGENERATION

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Background/Aims: The purpose of this study was to investigate retinal structure in detail of subjects with autosomal-dominant (ad) and autosomal-recessive (ar) PROM1-associated retinal degeneration (PROM1-RD), study design: institutional, cross-sectional study

Methods: Four eyes from four subjects (three with ad and one with ar) PROM1-RD were investigated by ophthalmic examination including best-corrected visual acuity (BCVA) and multimodal retinal imaging: fundus autofluorescence (FAF), spectral-domain optical coherence tomography (SD-OCT) and adaptive optics scanning light ophthalmoscopy (AOSLO). Quantitative assessment of atrophic lesions determined by FAF, thickness of individual retinal layers and cone photoreceptor quantification was performed.

Results: BCVA ranged from 20/16 to 20/200. Initial pathologic changes included the presence of hyperautofluorescent spots on FAF imaging, while later stages demonstrated discrete areas of atrophy. In all patients, thinning of the outer retinal layers on SD-OCT with varying degrees of atrophy could be detected depending on disease-causing variants and age. Cone density was quantified both in central and/or at different eccentricities from the fovea. Longitudinal assessments were possible in two patients.

Conclusions: PROM1-RD comprises a wide range of clinical phenotypes. Depending on the stage of disease, the cone mosaic in PROM1-RD is relatively preserved and can potentially be targetted by cone-directed interventions.

Vötsch Andreas

Abstract ID: 89836

Bleeding in patients undergoing urgent cardiac surgery during dual anti platelet therapy

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Background Despite recommendations for standardized preoperative waiting of at least 3, 5, and 7 days for ticagrelor, clopidogrel, and prasugrel, respectively, there is still substantial inter-institutional variation in preoperative discontinuation of dual antiplatelet therapy in patients needing coronary artery bypass grafting (CABG). Methods In 299 patients undergoing CABG ± valve intervention <7 days after last P2Y12 receptor inhibition, we evaluated calculated red blood cell loss. and Bleeding Academic Research Consortium (BARC)-4 bleeding. Results 83% of patients underwent CABG within <48 hours of last drug intake. Calculated blood loss was lower in patients on clopidogrel as compared to prasugrel or ticagrelor [1063 (690-1394) vs. 1351 (876-1829) vs. 1330 (994-1691) ml, p<0.001]. Overall, 135 (45%) patients sustained BARC-4 bleeding; incidence differed between groups (p=0.015) and was significantly higher in prasugrel-, as compared to clopidogrel-treated patients. In multivariable linear regression analysis, EuroSCORE II, aspirin dose, cardio-pulmonary-bypass time, drug withdrawal time, and type of P2Y12 receptor inhibitor were significantly associated with RBC loss. Compared to 0-24, >48 hours preoperative discontinuation substantially reduced calculated blood loss by 37-48% und BARC-4 bleeding by 58- 71%, depending on P2Y12 receptor inhibitor. Conclusions Exposure to prasugrel and ticagrelor within 24 hours before CABG increases both calculated blood loss and BARC-4 bleeding as compared to clopidogrel. Although a >48 hours discontinuation substantially reduced calculated blood loss and BARC-4 bleeding across all P2Y12 receptor inhibitors, our single center data further support strict adherence to the 2017 guidelines whenever justified by stable hemodynamics and non-jeopardized myocardium.

Balandin Denys

Abstract ID: 92759

Structural and functional insights into intramolecular interactions within T-cell factor/lymphoid enhancer-binding factor transcription factors

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The Wnt/?-catenin signaling pathway is evolutionary conserved and regulates cellular apoptosis, stem cell renewal, migration, proliferation, and genetic stability. Aberrant activation of this pathway is a hallmark of numerous cancers as well as non-cancerous diseases. The protein ?-catenin acts as co-activator of Wnt signaling; upon pathway activation, ?-catenin becomes stabilized, translocates to the nucleus, binds members of the T cell factor/lymphoid enhancer factor transcription factor (TCF/LEF) family, and activates transcription. The molecular mechanisms of how TCF/LEFs are inactivated in the absence of ?-catenin and become activated upon its nuclear translocation remain elusive. Based on recent data obtained in the Madl lab, I hypothesize that inactivation of TCF/LEFs is governed by an auto-inhibitory interaction of the TCF/LEF N-terminal region with the highly conserved DNA-binding high-mobility group (HMG) domain and that ?-catenin activates TCF/LEF proteins by competing with the auto-inhibition. Aims: I aim to use a combination of in vitro biophysical, structural, computational, and cell-based techniques to: characterize the molecular details of the LEF1 auto-inhibition; define the similarities and differences between TCF/LEF family members; study regulation of the auto-inhibition by post-translational modifications and disease-specific mutations; validate in vitro data in cells and develop peptide-based compounds targeting TCF/LEFs. Results: The intrinsically disordered regions of TCF/LEF transcription factors (LEF1, TCF1, TCF4) and respective DNA binding domains were expressed and purified. The IDR-HMG interaction was studied by NMR and isothermal titration calorimetry (ITC). Conclusion: Here we obtained first molecular insight into TCF/LEF transcription factor regulation through auto-inhibition. The identified molecular mechanism can be used as the template for the further development of peptide-based compounds targeting protein-protein interactions.

Bergmann Martina

Abstract ID: 93167

A novel platform for testing synergistic effects of anti-aging drugs

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Background/Aims:

Aging is the primary risk factor for several chronic diseases including cardiovascular and metabolic-syndrome related problems such as atherosclerosis, hypertension, type II diabetes, stroke and myocardial infarction as well as cancer and neurodegenerative diseases. Through the application of pharmacological geroprotectors, substances that slow or delay aging and repair age-associated damage, it may be possible to extend the period of our lives when we are free from severe disease and frailty. The goal is to find an optimal composition of low dose caloric restriction (CR) mimetics that reduces the prevalence of toxic side effects without compromising the conveyed extension of life expectancy.

Method/Results:

We developed a novel platform that serves as a potent health span indicator since it integrates the measurement of various aspects of age-associated dysfunction through the precise determination of climbing activity, which declines with age. To explore synergistic effects of CR mimetics we use wild type fruit flies (Drosophila melanogaster). Through combination of different anti-aging compounds tested in wild type fruit flies we could identify different combinatorial effects: e.g., healthspan improving, boosting and even toxic effects.

Conclusion:

The new semi-automated climbing platform is less time consuming and shows a better reproducibility. The climbing distance measurement is more accurate and there is an option to analyze additional parameters like climbing direction, speed, or acceleration. Most importantly, in contrast to previously used methods, we can analyze single fly performance and classify the animals into different performance categories.

Habersack Andreas

Abstract ID: 88767

Validity and reliability of a novel 3D ultrasound approach to assess static lengths and the lengthening behavior of the gastrocnemius medialis muscle and the Achilles tendon in vivo

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Human muscle<U+0096>tendon units (MTUs) are highly plastic and undergo changes in response to specific diseases and disorders. To investigate the pathological changes and the effects of therapeutic treatments, the use of valid and reliable examination methods is of crucial importance. Therefore, in this study, a simple 3D ultrasound approach was developed and evaluated with regard to: (1) its validity in comparison to magnetic resonance imaging (MRI) for the assessment of the gastrocnemius medialis (GM) MTU, muscle belly, and Achilles tendon lengths; and (2) its reliability for static and dynamic length measurements. Sixteen participants were included in the study. To evaluate the validity and reliability of the novel 3D ultrasound approach, two ultrasound measurement sessions and one MRI assessment were performed. By combining 2D ultrasound and 3D motion capture, the tissue lengths were assessed at a fixed ankle joint position and compared to the MRI measurements using Bland<U+0096>Altman plots. The intra-rater and inter-rater reliability for the static and dynamic length assessments was determined using the coefficient of variation, standard error of measurement (SEM), minimal detectable change (MDC95), and intraclass correlation coefficient (ICC). The 3D ultrasound approach slightly underestimated the length when compared with MRI by 0.7%, 1.5%, and 1.1% for the GM muscle belly, Achilles tendon, and MTU, respectively. The approach showed excellent intra-rater as well as inter-rater reliability, with high ICC (? 0.94), small SEM (? 1.3 mm), and good MDC 95 (? 3.6 mm) values, with even better reliability found for the static length measurements. The proposed 3D ultrasound approach was found to be valid and reliable for the assessment of the GM MTU, muscle belly, and Achilles tendon lengths, as well as the tissue lengthening behavior, confirming its potential as a useful tool for investigating the effects of training interventions or therapeutic treatments (e.g. orthotics).

Khanna Yukti

Abstract ID: 93079

Biophysical characterization of p53 regulation by the mRNA export factor-THOC4

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Background: The p53 tumour suppressor protein is a transcription factor (TF) and regulates the transcription of target genes involved in cellular processes like DNA damage repair, cell cycle arrest, apoptosis, and senescence. Its activity is regulated by cofactors including certain RNA-binding proteins (RBPs). Examples involve, RBPs like, SUMOylated hnRNP K, which regulates the transcriptional activity of p53 via direct interaction and CIRBP, which regulates p53 activity and to inhibit DNA damage-induced apoptosis. Previously, our lab found that the RG/RGG repeat region of CIRPB directly interacts with the transactivation domain (TAD) of p53. The RG/RGG region containing RBP, THOC4/ALYREF which is responsible for nuclear export of mRNA in the THO complex, is also known to interact with, and regulate the activity of multiple TFs like MYCN, E2F2, LEF-1 AND AML-1. Considering the proclivity of THOC4 towards regulation of TF activity, we aim to: 1) Unravel the molecular determinants and functional implications of the interaction between p53 and THOC4 2) Study the implications of post-translational modifications on this interaction Results/Methods: 1) NMR titration show that the N- and C-terminal RG/RGG regions of THOC4 and the TAD of p53 directly bind with each other. ITC experiments reveal that the THOC4 N-terminal RG/RGG region is the strongest binding site for p53 TAD. 2) Since RG/RGG regions are regulated by arginine methylation, we in-vitro arginine methylated both RG/RGG regions of THOC4 with PRMT1. Strikingly, and in contrast to the impact of arginine methylation in the context of RNA-binding, methylation of the THOC4 N-terminal region enhances binding to p53 TAD Conclusions: 1) Both RG/RGG regions of THOC4 bind to p53 TAD and arginine methylation enhances this interaction 2) As the TAD of p53 is essential for the transcriptional activation activity of p53, its interaction with THOC4 might affect the transcription of p53 targets in cells and in turn p53 function

Krappinger Julian

Abstract ID: 93322

Non-coding Natural Antisense Transcripts: Large-Scale Analysis of Expression Patterns in Human Cancers

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Although a large part of the transcriptome does not encode proteins, research on long non-coding cis natural antisense transcripts (ncNATs) lags far behind the investigation of their protein-coding counterparts. These transcripts are overlapping with their partner transcripts but are transcribed in opposite orientation to their partners. They are involved in gene regulation through multiple functional processes, and it has been suggested that many could affect the expression of their sense partners. In an effort to present a detailed overview of ncNATs and their expression patterns, we investigated a large collection of human RNA-seq samples in cancerous and normal conditions by making use of public repositories as an immense resource.

Strand-specific RNA-seq samples of cancerous and healthy tissues were curated (>4000 samples). Subsequently, we applied our high-throughput RNA-seq analysis pipeline on the curated data. After rigorous quality assessment, we mapped the data to the human reference genome and further determined new transcript models using a reference-guided assembly approach. Novel transcripts were refined by a custom transcript selection approach, which combined novel transcripts with known lncRNA resources and filtered artifacts. Finally, transcripts were filtered depending on their coding potential assessment and classified based on their genomic location. After computing the expression levels, we determined the differential gene expression of ncNATs in large patient cohorts and conducted diverse statistical analyses.

We explored our large collection of normal and cancerous tissue samples in an effort to establish a detailed overview of ncNAT expression, including the evaluation of ncNAT dysregulation, expression heterogeneity in cancerous conditions and their tissue/cancer-specificity. Furthermore, our analyses shed light on correlations of ncNATs with their partners and focused further on immune-related and germline-specific ncNATs.

Kuppassery Abdulnazar Akhila Naz

Abstract ID: 91679

SAP-BERT based Token N-Gram Normalization using SNOMED-CT

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Normalization, i.e., context-aware mapping of medical terms to semantic identifiers of a terminology standard such as SNOMED CT, is of immense importance in making clinical data interoperable. Known obstacles are idiosyncratic, compact, ambiguous and often faulty language in clinical narratives. This work aims at improving the normalization of token n-grams (words and word sequences) for n = 1 (<U+0093>cholecystectomy<U+0094>) up to n = 5 (<U+0093>removal of gallbladder by laparoscopy<U+0094>) from a clinical document. These n-grams are mapped to their corresponding SNOMED CT codes using embeddings, i.e., representations in a low-dimensional vector space. Different normalization variants such as contextualized, non-contextualized and fine-tuned ones were compared, using the SAP-BERT model. Evaluation on N2C2 data revealed that non-contextualized normalization with SAP-BERT performed best with an accuracy of 0.85 on validation data and 0.819 on test data. N-gram based normalization yields a still acceptable accuracy of 0.787 accuracy, due to a large number of false positives. Future work will optimize filtering during normalization and fine-tuning of term recognition.

Mandl Spela

Abstract ID: 92939

Discovery of positive allosteric modulators for the treatment of mannosidosis

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Alpha-mannosidosis (MANSA) is a human genetic disease characterized by a deficiency in the lysosomal ?-mannosidase. Three major clinical subtypes exist from a mild form showing skeletal abnormalities, myopathy, and slow progression to severe form manifesting as prenatal loss or early death. MANSA belongs to the lysosomal storage disorders (LSDs) and there is no cure for it. The two available treatments so far are bone marrow transplantation (BMT) and enzyme replacement therapy (ERT). However, they are not without drawbacks and can cause serious complications. Thus, novel therapeutic options are urgently needed. MANSA is caused due to the expression of defective human lysosomal ? -mannosidase (hLAMAN) variants. hLAMAN catalyzes the cleavage of end-terminal mannosidic linkages ?(1?2), ?(1?3), and ?(1?6) from glycoproteins. The main goal of my PhD project is the discovery of small molecules (positive allosteric modulators) to rescue the activity of defective hLAMAN variants within the lysosome as a novel therapeutic approach. I will present the first work package of my PhD project, where the wild-type hLAMAN and the three MANSA-relevant defective variants D159N, R229W, and E402K will be expressed in Origami E. coli 2(DE3) and Komagataella phaffii (aka. Pichia pastoris) X33 cells and purified using liquid chromatography. One of the main open scientific questions is the impact of the glycosylation in the expressed hLAMAN variants on the standardized enzymatic assay as well as the setup of the in vitro screening platform. Further workpackages will involve the screening of chemical libraries previously identified in silico on the in vitro activity of hLAMAN variants and on the binding to the enzyme.. Additionally, we will collaborate to determine the 3D structure of hLAMAN in complex the best candidates. Overall, this project is committed to discover for the first time positive allosteric modulators of hLAMAN to rescue the enzymatic activity in defective variants.

Plass Markus

Abstract ID: 91300

The role of explainable AI in regulatory practices

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Introduction: In the context of digital pathology and in-vitro diagnostics, Artificial Intelligence (AI) must empower (bio)medical professionals to take responsibility for their decision-making, raising the demand for explainable AI. The US Food and Drug Administration (FDA) and the European In-Vitro Diagnostics Regulation (IVDR) address explainability in their recommendations and documents. However, to achieve efficient and effective explanations in AI systems, it is essential to know who uses which type of AI-solution for what purpose and how the human-AI interface is designed.

Material and methods: We propose definitions for AI solutions in the field of digital pathology, including the classes of algorithms involved and how these may be applied. We identify the stakeholders using such applications,their aims and potential requirements. We define a taxonomy describing the interface between the AI solutions and their stakeholders, as well as varieties of explanations and metrics for their quality.

Results: Usability encompasses measurements for the quality of use, and causability encompasses measurements for the quality of explanations produced by explainable AI methods. We describe both concepts and give examples of how both are essential for demonstrating scientific validity, as well as analytical and clinical performance in digital pathology.

Conclusion: Explainable AI methods provide answers to important questions in scientific validation and the evaluation of analytical and clinical performance of AI solutions in digital pathology: <U+0093>Why does an AI solution generate reliable results for an intended purpose?<U+0094>, <U+0093>Why did it produce a specific result?<U+0094>, <U+0093>Was the explanation satisfactory for the user?<U+0094>.

Platero Rochart Daniel

Abstract ID: 92778

Computer aided (re)-design of enzymes for therapeutics: First steps on energy barriers prediction

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Enzyme manipulation has proven to be an effective way to modulate properties such as catalytic activity, substrate specificity, and (thermo)stability. Nowadays, advances in cloning and expression have allowed the use of experimental techniques such as directed evolution (DE) for protein engineering campaigns. Nevertheless, DE has unpredictable outcomes and can be an expensive process. Therefore, in-silico methods like steered QM/MM molecular dynamics (sMD) for calculating free energy barriers, constitute an alternative for a rational design. However, the use of sMD for comprehensive mutation libraries is still a time- and resource-demanding approach. In the present work, we explored the possibility of using Machine Learning approaches to overcome the sMD limitations for the exploration of the mutational landscape in enzyme engineering.

Employing two kernel regression methods (kernel ridge regression and ElasticNet) and different chemical representations, we were able to obtain good regression scores for the prediction of the pulling work for each sMD frame. We also demonstrate that using initial snapshots (directly from molecular dynamics (MD) simulations) the predicting power is remarkably low, being needed structures closer to the transition state.

On one hand, our results show that kernel regression methods along with a simple representation such as the Coulomb matrix, are capable of learning and predicting pulling work from an entire sMD trajectory. On the other hand, while using only a single structure, representation can play an important role. Moreover, results strongly depend on the selected snapshot for the training process. We also show that the inherent variability of the sMD makes the delivery of predictive models a challenging task.

Rakhimbekova Anastasia

Abstract ID: 92881

Structural and Functional Studies of FOXM1 Regulation by ?-catenin

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Background/Aims Forkhead box protein M1 (FOXM1) is a proliferation-related transcription factor (TF) exhibiting increased levels of expression in a variety of human cancers. A link between FOXM1 and Wnt signalling has been reported recently. Aberrant oncogenic activation of the Wnt pathway is characterized by increased concentration of nuclear ?-catenin. FOXM1 and ?-catenin seem to mutually depend on each other for recruitment to Wnt target-gene promoters. Given that parts of FOXM1 share similar structural features with TFs for which we recently revealed the ?-catenin binding sites, I hypothesize that ?-catenin regulates transcriptional activity of FOXM1 by binding a mixed acidic/hydrophobic transactivation domain (TAD). I aim to explore the interaction of FOXM1 with ?-catenin in vitro, its regulation by post-translational modifications, solve the structure of the FOXM1/?-catenin complex, and corroborate my findings with cell-based assays in a defined pathophysiological context Method/Results I will present first results characterizing the FOXM1 TAD/?-catenin interaction by using biophysical techniques such as NMR spectroscopy. I discovered that the C-terminal part of FOXM1 TAD adopts a transient ?-helical structure in the absence of binding partners and that this region directly binds the Armadillo repeat region of ?-catenin. This part of FOXM1 TAD sequence overlaps with the binding site for the FOXM1 negative regulatory domain (NRD) and is involved in interactions with DNA-binding domains of other TFs Conclusion I found that FOXM1 TAD is recognized by ?-catenin, and that the binding site for ?-catenin overlaps with the one for NRD. Assuming that this leads to FOXM1 activation, our data could provide a first molecular explanation for the pro-oncogenic role of the ?-catenin/FOXM1 axis. In the next steps, we aim to proceed with the in vitro characterization, cellular validation, and development of molecules interfering with the ?-catenin-dependent FOXM1 activation

Toledo Marcos Juan

Abstract ID: 93545

Development of peptidomimetics to target CRAC channel complex

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Calcium ion is the most ubiquitous intracellular messenger in eukaryotic cells and is responsible for different biological processes including cell proliferation, growth, muscle contraction, exocytosis, immune cell activation, and apoptosis. The main plasma-membrane Ca2+ entry route in non-excitable cells involves calcium-release activated Ca2+ channels (CRAC), localized in the endoplasmic reticulum membrane junctions. CRAC has two molecular key components, the plasma membrane highly selective Ca2+ channel Orai1 and the endoplasmic reticulum resided Ca2+ sensor protein, stromal interaction molecule 1 (STIM1), which binds to Orai1 in response to ER Ca2+ depletion. The STIM1 binding site to Orai1 C-terminus is crucial upon the activation of store-operated Ca2+ entry (SOCE). This channel complex is involved in several diseases and channelopathies, therefore it is an attractive therapeutic target. The aim of this PhD project pursues the design, the synthesis and the biophysical characterization of peptidomimetics to target Stim1-Orai1 interaction. Here I will describe first achievements that are currently conducted. Based upon the available NMR complex between STIM1-Orai1, different peptidomimetics have been proposed by modifying the chain length and the linker for macrocyclization. Then, the potential binding of the STIM1-derived -helical macrocycles (MCXs) proposed against Orai1 have been assessed by means of Molecular Dynamics simulations. The MCXs were obtained by solid phase peptide-synthesis and protein expression. As a result, some MCXs have been already obtained and their activities are under investigation. Overall, these abstract aims to show the rationalize behind the proposed strategy on the design of MCXs and on the early obtained results.

Avdili Afrim

Abstract ID: 92443

Molecular regulation of emerging hematopoiesis from human induced pluripotent stem cells (iPSCs)

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Our group recently reported enhanced erythroid differentiation from human iPSC by closer mimicry of physiological cell contacts in a three dimensional network, termed <U+0084>haematopoietic cell forming complex<U+0093> (HCFC). From this HCFC, CD43+ hematopoietic cells (purity >95 %) were continuously released into the culture supernatant. Characterization of the HCFC in more detail is currently limited by accompanying destruction of the complex, hindering further haematopoietic cell fate tracking. To overcome this limitation, we developed a CD43 fluorescence reporter hiPSC line that allows live-cell imaging of haematopoietic cells as they emerge in a spatiotemporal manner from the HCFC.We have successfully reprogrammed human peripheral blood (PB) CD34+ derived erythroblasts to iPSC (PEB-AL#6). Thereafter, we have successfully performed gene knock-in of a CD43 promoter region tagged with GFP using CRISPR/Cas9-mediated homology-directed repair (HDR) mechanisms in the AAVS1 safe harbor locus. Adeno-associated viral vectors (AAV) were used for efficient DNA donor delivery into hiPSCs. Following HDR-mediated donor integration, clones with on-target seamless DNA integration were isolated, which was confirmed by Sanger sequencing and in-out PCR. To confirm the functionality of the CD43 fluorescent reporter, haematopoietic and erythroid differentiation of the CD43R-iPSC was induced. GFP-expression of CD43+ hematopoietic cells inside the HCFC was monitored by fluorescence microscopy and live cell imaging. Haematopoietic nature of released GFP+ cells was confirmed by flow cytometry (CD43, CD34, CD45) and colony formation assay. The established CD43 fluorescent reporter system allows to track hematopoietic development from hiPSCs within three-dimensional structures like organoids. Therefore, the system represents an attractive tool to investigate the hematopoietic development from hiPSCs.The established system can be used for different hiPSC lines or other primary cells.

Greimel Theresa

Abstract ID: 90405

Bile acid-induced tissue factor activity in hepatocytes correlates with activation of farnesoid X receptor

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Background Bile acids (BA) have been shown to affect intrahepatic coagulation processes via enhanced tissue factor (TF) decryption and thrombin generation. The exact mechanism of TF decryption within the liver parenchyma and the role of farnesoid X receptor (FXR), a nuclear BA receptor, remain unclear. In this study, the impact of various BA on TF activity and thrombin generation in hepatocytes was investigated and the effects were correlated with activation of FXR-dependent signaling and apoptosis. Methods HepG2 cells and primary human hepatocytes were incubated with chenodeoxycholic acid (CDCA), glycochenodeoxycholic acid (GCDCA), ursodeoxycholic acid (UCDA), the synthetic FXR agonist GW4064 and the FXR antagonist DY268 for 12 and 24 hours respectively. MTT tests were used to determine cell viability. TF activity was tested via factor Xa generation and thrombin generation was measured by calibrated automated thrombography. Quantitative polymerase chain reaction (qPCR) was used to determine FXR activation. Western blotting was used to determine TF protein levels and fluorescence microscopy of stained HepG2 cells to denote upregulation of TF. Cleaved caspase-3 and increased Annexin V binding were tested as apoptotic markers. Results Increased TF activity alongside enhanced thrombin generation was observed with CDCA and GW4064 and in human hepatocytes also with GCDCA. UDCA showed no effect. TF activity was reduced when FXR activation was blocked with DY268. qPCR revealed upregulation of FXR targets only by CDCA and GW4064. Western blot analysis and fluorescence microscopy showed no TF overexpression, arguing for TF decryption. No signs of apoptosis were denoted. Conclusion Exposure of hepatocytes to BA may cause intracellular FXR overstimulation, triggering TF decryption irrespective of the amphiphilic properties of BA. The effect of BA on TF activation correlates with the molecule's ability to activate FXR. TF decryption occurs independently of apoptotic mechanisms.

Heininger Christoph

Abstract ID: 93548

Hepatic enhancer activities and transcription factor networks in response to fasting

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The liver is a first-line-responder to fluctuations in food intake, adapting gene expression (GE) programs by recruitment of specific transcription factors (TFs) to promoter and enhancer regions. Transcriptionally engaged TFs have been described to bind enhancers, resulting in the generation of enhancer RNAs (eRNAs). To investigate fasting-selective eRNAs we established qPRO-seq for complex tissues and applied it to mouse livers sampled at several time points over a 24-hour fasting period. Various bioinformatics tools and custom scripts were used. Differential GE analysis from qPRO-seq time series delivered dynamics of known fasting-regulated genes, such as induction of gluconeogenesis (Pck1) as early as 3 and peaking after 24 hours and repression <U+0096> and, paradoxically, induction after 3 hours <U+0096> of de novo fatty acid synthesis (Fasn) already 1 hour after food withdrawal, showing the strongest repression after 12 hours. GO annotation of fasting-activated enhancers<U+0092> (from around 140 up to 1300, depending on time point) neighboring genes showed an overrepresentation of metabolic processes regarding various metabolites, protein transport and cell signaling processes after 1, 12 and 24 hours respectively. This stark temporal difference suggests dynamic regulation of numerous pathways throughout fasting. Finally, enriched TF motifs within each set of fasting-activated enhancers were compiled. This revealed regulatory dynamics of known nuclear receptor TFs, like PPAR? and forkhead TFs, such as FOXA1, as well as less described TFs like NR2F1, NR2F6 and Rfx6. Currently our results suggest a timely coordination of fasting GE by specific TF clusters a genome-wide enhancer activation in fasted liver. In future analyses, we will focus on individual (super-) enhancer clusters and TF networks to reveal novel regulatory mechanisms in the fasting context.

Lyssy Freya

Abstract ID: 92241

THE EFFECT OF MATERNAL PLATELETS ON THE DEVELOPMENT OF EXTRAVILLOUS TROPHOBLASTS USING A 3D CELL CULTURE MODEL

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During human placentation extravillous trophoblasts (EVTs) migrate into the maternal endometrium and form plugs within the lumen of uterine spiral arteries preventing maternal blood cells from entering the intervillous space. However, by the middle of first trimester, cells within trophoblast plugs become loosely cohesive leading to the formation of narrow capillary-sized channels. Due to their small size, maternal platelets may be the first amongst maternal blood cells which pass such narrow intercellular gaps of trophoblast plugs. We aimed to establish a proper 3D cell culture model to address the hypothesis if release of platelet-derived factors into the intercellular space of EVTs plays a significant role in their differentiation and behavior. The human trophoblast cell line ACH-3P was used to create spheroids which were either co-cultured with platelets directly or treated with released factors from activated platelets. The difference in the morphology of spheroids was visualized by immunofluorescence staining and transmission electron microscopy. Their diversity on the molecular level was measured using qualitative real-time PCR and western blotting as well as standard RNA sequencing analysis. Upon spheroid formation in presence of maternal platelets, the latter were enclosed into a cavity of the trophoblast spheroids. However, some platelets were also found between the trophoblasts, mirroring in vivo circumstances. Factors released by platelets showed a deregulation of certain genes of the trophoblast spheroids. In this study we aimed to establish a unique 3D cell culture model using the human trophoblast cell line ACH-3P. We pursued to show if platelets and their derived factors have an effect on the development of extravillous trophoblasts in early gestation. We could indicate that the morphology of the spheroids will change upon co-culture with platelets but equally important the release of the content of platelets will affect them on the molecular level.

Niedrist Tobias

Abstract ID: 93466

Peritoneal interleukin-6 - The trueness of an automated method

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Background/Aims Interleukin-6 (IL-6) measured in ascitic fluid (AF) or peritoneal dialysis effluent (PDE) is known to be a surrogate marker for inflammatory damage to the peritoneal mesothelium. While there is no lack of laboratory methods which determine IL-6 in blood plasma or serum, these assays are not validated for the use in diagnostic peritoneal fluids. This study compares the analytical trueness of peritoneal IL-6 values determined using a routine IL-6 blood assay. Besides the precision, the trueness of a laboratory test is the main criterion in order to gain high accuracy.

Method/Results Each 25 samples of AF and PDE were analyzed with an automated high-throughput electrochemiluminiscence immunoassay (ECLIA) which is validated for measuring IL-6 in human blood between 1.5 to 5000 pg/mL. Consecutively, we analyzed these samples with an enzyme-linked sandwich immunoassay (ELISA) which served as the reference method. Both, the ECLIA and the ELISA were calibrated against the international reference standard for IL-6 (NIBSC 89/548). Passing Bablok regression analysis and Bland Altman Plotting revealed a slight constant bias comparing the PDE results. A good agreement between the ECLIA and the ELISA was seen in the AF group. Furthermore, the IL-6 values were significantly higher ($p<0.001$, Mann-Whitney U test) in the AF than in the PDE group.

Conclusion The results of this method comparison indicate that the ECLIA are safely applicable to peritoneal fluid samples. Presuming an equally good analytical precision (for which further studies are needed), this method would certainly enrich the routine laboratory marker panel for the monitoring of peritoneal integrity in patients with ascites or undergoing peritoneal dialysis.

Pignet Anna-Lisa

Abstract ID: 92491

The impact of age on wound healing progression

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Background. Increasing incidences of wound healing disorders represent a growing problem in our aging society. Although poor wound healing occurs more frequently in older individuals, the factor of age has often been neglected in previous in vivo models. Methods. For this study, 54 Wistar rats from 3 different age groups (11; 27; and 56 weeks) were divided into 3 subgroups (burns, full-thickness wounds, unwounded controls). Animals in each of the experimental groups had four 2b3rd-degree contact burns or full-thickness excisional wounds placed. During the observation period of 7 days, regular wound documentation by laser speckle, thermography, and photography was performed, blood was drawn and daily food-intake and animal weights were recorded. Tissue biopsies obtained from the wounds and control areas on the final day (day 7) were analyzed at the histological and gene expression levels. Inflammatory markers (interleukins, TGFb, TNFa, etc.) and markers for tissue perfusion (VEGFa, HIF1a) were quantified by qPCR. Results. Measurements of skin thickness from the histologic sections revealed significantly reduced thickness of the epidermis but not the dermis in the old animals. In addition, the 14-month- and 7-month-old animals had significantly poorer tissue perfusion of the affected areas on the fourth day after burning than did the young animals. Local immune cell infiltration, especially by leukocytes, was also delayed in the aged animals. Nevertheless, there were no significant differences between the age groups in either wound sizes or weight loss after burns. Conclusion. Although there appear to be age-related differences in epidermal thickness, angiogenic potential, and immune response, these do not appear to have a significant effect on wound healing of small wounds. It is reasonable to assume that it is only an additional trigger, such as diabetes, that causes the often observed poorer wound healing in the elderly.

Stadler Julia

Abstract ID: 92924

Alterations in high-density lipoprotein-related parameters and mortality risk among hospitalized COVID-19 patients.

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Background: Cholesterol in the plasma membrane is required for proper trafficking of receptors which facilitate severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection. High-density lipoproteins (HDL) mobilize cholesterol from the plasma membrane, and low HDL cholesterol levels are associated with COVID-19 disease severity and mortality. However, HDL cholesterol levels alone poorly reflect the function of this complex family of particles, and a detailed assessment of COVID-19-associated changes in HDL functionality and its prognostic value is lacking.

Methods: In the present study, we assessed HDL cholesterol efflux capacity, HDL anti-inflammatory and antioxidant properties, and changes in HDL composition and metabolism in COVID-19 (n = 48) and non-COVID pneumonia patients (n = 32).

Results: COVID-19 infection markedly reduced the activity of lecithin-cholesteryl-acyltransferase and functional parameters of HDL, such as the cholesterol efflux capacity, arylesterase activity of paraoxonase-1, and anti-oxidative capacity of apoB-depleted serum when compared to non-COVID pneumonia at baseline, paralleled by markedly reduced levels of HDL-cholesterol. Of particular interest, low HDL cholesterol efflux capacity was associated with increased mortality risk in COVID-19 patients, independent of HDL-C levels.

Conclusion: Our findings highlight profound effects of COVID-19 infection on HDL function, metabolism, and composition. Low HDL cholesterol efflux capacity indicates a fatal course of COVID-19, independent of HDL-cholesterol levels.

Tawfik Ines

Abstract ID: 92590

T3-induced enhancement of mitochondrial Ca2+ uptake as a boost for cellular metabolism

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Ph.D

Thyroid hormones are the main regulators of cellular metabolism, conveying their action via regulation of expression changes. Thereby, the biologically active triiodothyronine (T3) induces the expression of genes to enhance mitochondrial metabolic function. Notably, mitochondrial Ca2+ is essential to the function of Ca2+-dependent matrix dehydrogenases and, thus, mitochondrial respiration. However, just few genes are controlled in their expression by thyroid hormones, among others the uncoupling proteins 2 and 3 (UCP2/3). The biologically T3 induces upregulation of UCP2/3 in various cell types. In the current study, we studied the impact of T3 on [Ca2+]mito homeostasis. T3 induced a significant upregulation in mRNA expression of UCP2 and UCP3 and of protein arginine methyltransferase 1 (PRMT1) in HeLa cells after 3 h. Live-cell imaging in HeLa cells expressing mitochondrial-targeted Ca2+ biosensors revealed that short-time incubation (3 h) with T3 elevates basal [Ca2+]mito and causes increased [Ca2+]mito uptake upon Ca2+ depletion of the endoplasmic reticulum (ER), while cytosolic Ca2+ levels remained unchanged. Also T3-induced enhancement of mitochondrial Ca2+ uptake depends on the mitochondrial Ca2+ uniporter (MCU), UCP2, and PRMT1 that are essential for increased mitochondrial ATP ([ATP]mito) production after T3 treatment. T3's impact on [Ca2+]mito correlates with the expression and activity of UCP2, MCU und PRMT1 and translates into increased [ATP]mito. Increases in mitochondrial ATP and [Ca2+]mito supply the production of reactive oxygen species (ROS). We revealed that enhanced mitochondrial Ca2+ uptake is essential to elevate mitochondrial ROS production after 3 h of T3 incubation. These results suggest that mitochondrial Ca2+ homeostasis is essential for the role of T3 in controlling metabolic activity.