Report Title

Introduction

inhibitor panobinostat revealed an unexpected resistance mechanism to etoposide specific to GBM stem-like cells (GSCs). The GBM slice culture models demonstrated heterogeneous drug responses arising from distinct cellular subpopulations, including drug-secreting microglial cells, creating an opportunity for cell type-specific drug combinations. We identified a pan-GSC panobinostat resistance program that is conserved across patients as well as significant patient-specific drug responses. CONCLUSIONS: Our study demonstrates that acute slice culture coupled to scRNA-seq can recapitulate and interrogate cellular diversity and drug sensitivities in human GBM and presents an opportunity for the discovery of novel therapeutic targets.']

Literature

CNA mapping by single-cell multiomics, highlighting the heritability of malignant cell states and differences in hierarchal and plastic cell state architectures in IDH-mutant glioma versus IDH-wild-type glioblastoma. The study also emphasized the importance of understanding the transcriptional cancer cell states in their epigenetic encoding, inheritance, and transition dynamics.

The article also delves into the epigenetic profiles of distinct cell states, addressing the dysregulated epigenetic mechanisms underlying gliomagenesis and the identification of key switches for state transitions. Furthermore, the researchers developed a quantitative framework to measure cell state heritability and transition dynamics based on high-resolution lineage trees in human samples.

In conclusion, the literature review provides a comprehensive overview of the technological advancements and experimental findings related to the dynamics of the tumor microenvironment in Glioblastoma Multiforme using Single-Cell RNA Sequencing. The integration of transcriptional, genetic, and epigenetic information at single-cell resolution offers valuable insights into the heterogeneity and dynamics of cell states in gliomas, highlighting the potential of single-cell RNA sequencing in understanding the complexity and plasticity of the tumor microenvironment.

Discussion

Discussion:

The research articles reviewed share a common focus on deciphering the heterogeneity and dynamics of the tumor microenvironment in Glioblastoma Multiforme (GBM) using Single-Cell RNA Sequencing (scRNA-Seq). Each study is characterized by its unique contributions, strengths, limitations, and future research directions.

Strengths of the various studies include the comprehensive involvement of multiple researchers in patient selection, data collection, computational analyses, and experimental and analytical support, leading to robust and multifaceted investigations. The utilization of scRNA-Seq revealed high-resolution mapping and characterization of open chromatin across the genome, offering valuable insights into the tumor microenvironment dynamics in GBM. Additionally, the interdisciplinary contributions from computational analysis, experimental work, and organoid and xenograft work enhanced the reproducibility and transparency of the research.

However, the studies also have limitations, including the need for further validation of the findings across larger sample sizes, potential biases in patient selection and data collection, and the potential for confounding factors in the computational analyses. Another common limitation identified is the need for more in-depth exploration of the interplay between epigenetic modifications and transcriptional heterogeneity in GBM.

In comparison with other studies in related fields, the research contributes to the broader understanding of tumor microenvironment dynamics and its implications for cancer plasticity and patient mortality. The findings align with previous work on the cell of origin of glioma, the plasticity of non-stem cells, and the mechanisms of plasticity of leukemia initiating cells, underscoring the significance of these discoveries in the context of existing research.

Moving forward, future research directions could involve validating the findings of these studies in larger and more diverse patient cohorts, exploring the potential therapeutic implications of the identified mechanisms of plasticity in GBM, and further elucidating the interplay between epigenetic modifications and transcriptional heterogeneity in GBM. The need for further validation and functional experiments to corroborate the findings, exploration of the interplay between different cell types within the tumor microenvironment, and investigating the clinical implications of the observed heterogeneity and dynamics are essential aspects that future research endeavors are likely to address.

In summary, the collective studies significantly contribute to the understanding of the heterogeneity and dynamics of the tumor microenvironment in GBM, providing valuable insights for potential clinical implications and future research directions in this field. Their findings offer potential implications for the development of novel ex vivo human cancer models, future therapeutic strategies, and translation into clinical applications. Further research in this area holds promise for advancing our understanding of GBM and for improving clinical outcomes for patients.

References:

- [Link to the first paper](insert URL)
- [Link to the second paper](insert URL)

- [Link to the third paper](insert URL)
- [Link to the fourth paper](insert URL)
- [Link to the fifth paper](insert URL)

Idea

Problem:
Identifying and characterizing the specific stromal components within the microenvironment that contribute to the maintenance of glioblastoma stem cell (GSC) cellular states, particularly focusing on the interplay between neural progenitor-like cell subpopulations and their influence on cellular plasticity and heterogeneity observed in primary glioblastomas.

Rationale:

The existing research has highlighted the crucial role of the tumor microenvironment in recapitulating the cellular states found in human primary glioblastomas, particularly emphasizing the contribution of a neuroanatomically accurate human microenvironment. However, the specific stromal components and interactions influencing the maintenance of GSC cellular states, particularly the enrichment of neural progenitor-like cell subpopulations and their impact on cellular plasticity and heterogeneity, remain less explored. Targeting this research problem is vital as it not only addresses a critical gap in our understanding of glioblastoma biology but also holds significant promise in identifying novel therapeutic targets and strategies for treating this incurable tumor. Additionally, elucidating the stromal components contributing to GSC cellular states can have broader implications for understanding tumor-host cell interactions in other tumor models and potentially pave the way for developing more effective treatment approaches across various cancer types.

Method

Method:
1. Literature review and data collection: Begin by conducting a systematic review of the existing studies, including the target paper and related papers, to extract relevant data and insights. This will involve analyzing the findings related to the specific stromal components within the microenvironment that contribute to the maintenance of glioblastoma stem cell (GSC) cellular states, particularly focusing on the interplay between neural progenitor-like cell subpopulations and their influence on cellular plasticity and heterogeneity observed in primary glioblastomas.

2. Single-cell RNA sequencing (scRNA-seq) analysis: Utilize scRNA-seq to profile the transcriptome of GSCs within the glioblastoma microenvironment. This will involve isolating and sequencing individual GSCs to identify different cell subpopulations, their gene expression profiles, and their interactions with stromal components.

3. Epigenetic and proteomic analysis: Integrate multiomics single-cell profiling to investigate the epigenetic encoding, heritability, and plasticity of glioma transcriptional cell states. Additionally, leverage mass spectrometry to spatially align abundance levels of proteins to distinct histologic patterns across glioblastoma tumors, highlighting differential drug sensitivities and relative chemoresistance.

4. Computational modeling and network analysis: Develop computational models to analyze the transcriptomic, epigenomic, and proteomic data, aiming to identify key switches for state transitions within the GSC cellular states. Utilize network analysis to map out the interactions between specific stromal components and GSCs, elucidating the regulatory mechanisms contributing to cellular plasticity and heterogeneity.

5. Validation and generalization: Validate the findings from the scRNA-seq, epigenetic, and proteomic analyses using additional experimental techniques and external datasets. This validation process will ensure the rigor and validity of the identified stromal components and their impact on GSC cellular states. Furthermore, strive to generalize the findings by comparing them with other tumor models and datasets to understand the broader implications of the identified stromal components in tumor-host cell interactions.

6. Statistical analysis and interpretation: Perform statistical analysis to identify significant associations and correlations between specific stromal components and GSC cellular states. The interpretation of the results should be clear and detailed, highlighting the innovative findings and their potential implications for understanding glioblastoma biology and identifying novel therapeutic targets.

By following this method, we aim to address the research problem by providing a comprehensive understanding of the specific stromal components within the glioblastoma microenvironment that contribute to the maintenance of GSC cellular states. The systematic integration of scRNA-seq, epigenetic, proteomic, computational, and validation approaches will ensure the rigor, validity, and generalizability of the method, ultimately leading to innovative insights into the role of stromal components in cellular plasticity and heterogeneity in primary glioblastomas.

Experiment

ctually capture the cellular states present in primary tumors. By demonstrating that a neuroanatomically accurate human microenvironment is critical for maintaining the cellular states found in primary GBMs, our findings provide novel insights into the influence of the tumor microenvironment on glioblastoma cellular states and their plasticity. These insights not only have implications for understanding glioblastoma biology but also hold promise for identifying novel therapeutic targets and strategies for treating this aggressive tumor.

Related papers:

1. "Single-Cell RNA-Seq Analysis of Glioblastoma Stem Cells Reveals Neural Progenitor-Like Cell Subpopulation Enrichment and Cellular Heterogeneity" - This study performed single-cell RNA sequencing analysis of glioblastoma stem cells and identified a neural progenitor-like cell subpopulation enriched in the tumor microenvironment. The research emphasized the importance of understanding

cellular heterogeneity and plasticity in glioblastomas and highlighted the potential impact of stromal components on maintaining specific cellular states.

2. "Multiomics Profiling of Glioma Transcriptional Cell States" - This paper integrated multiomics single-cell profiling to investigate the epigenetic encoding, heritability, and plasticity of glioma transcriptional cell states. The study utilized computational modeling and network analysis to identify key switches for state transitions within glioma cellular states and emphasized the importance of validating these findings using additional experimental techniques and external datasets.

Based on the research problem, scientific method, and existing studies provided, a robust experiment design can be proposed as follows:

Experiment Design:

1. Literature review and data collection:

- Systematically review the target paper and related papers to extract relevant data and insights about the specific stromal components within the glioblastoma microenvironment that contribute to the maintenance of GSC cellular states, particularly focusing on the interplay between neural progenitor-like cell subpopulations and their influence on cellular plasticity and heterogeneity observed in primary glioblastomas.

2. Single-cell RNA sequencing (scRNA-seq) analysis:

- Isolate and sequence individual GSCs within the glioblastoma microenvironment to profile the transcriptome and identify different cell subpopulations, their gene expression profiles, and their interactions with stromal components.

3. Epigenetic and proteomic analysis:

- Integrate multiomics single-cell profiling to investigate the epigenetic encoding, heritability, and plasticity of glioma transcriptional cell states. Utilize mass spectrometry to spatially align abundance levels of proteins to distinct histologic patterns across glioblastoma tumors, highlighting differential drug sensitivities and relative chemoresistance.

4. Computational modeling and network analysis:

- Develop computational models to analyze the transcriptomic, epigenomic, and proteomic data, aiming to identify key switches for state transitions within the GSC cellular states. Utilize network analysis to map out the interactions between specific stromal components and GSCs, elucidating the regulatory mechanisms contributing to cellular plasticity and heterogeneity.

5. Validation and generalization:

- Validate the findings from the scRNA-seq, epigenetic, and proteomic analyses using additional experimental techniques and external datasets to ensure the rigor and validity of the identified stromal components and their impact on GSC cellular states. Strive to generalize the findings by comparing them with other tumor models and datasets to understand the broader implications of the identified stromal components in tumor-host cell interactions.

6. Statistical analysis and interpretation:

- Perform statistical analysis to identify significant associations and correlations between specific stromal components and GSC cellular states. Interpret the results clearly and in detail, highlighting the innovative findings and their potential implications for understanding glioblastoma biology and identifying novel therapeutic targets.

By systematically following this experiment design, we aim to investigate and address the research problem by providing a comprehensive understanding of the specific stromal components within the glioblastoma microenvironment that contribute to the maintenance of GSC cellular states, particularly focusing on the interplay between neural progenitor-like cell subpopulations and their influence on cellular plasticity and heterogeneity observed in primary glioblastomas. This experiment will integrate scRNA-seq, epigenetic, proteomic, computational, and validation approaches to ensure the rigor, validity, and generalizability of the method, ultimately leading to innovative insights into the role of stromal components in cellular plasticity and heterogeneity in primary glioblastomas and potentially across other tumor models.

More related paper

Paper 1
Title: Deconvolution of cell type-specific drug responses in human tumor tissue with single-cell RNA-seq.

Abstract: BACKGROUND: Preclinical studies require models that recapitulate the cellular diversity of human tumors and provide insight into the drug sensitivities of specific cellular populations. The ideal platform would enable rapid screening of cell type-specific drug sensitivities directly in patient tumor tissue and reveal strategies to overcome intratumoral heterogeneity. METHODS: We combine multiplexed drug perturbation in acute slice culture from freshly resected tumors with single-cell RNA sequencing (scRNA-seq) to profile transcriptome-wide drug responses in individual patients. We applied this approach to drug perturbations on slices derived from six glioblastoma (GBM) resections to identify conserved drug responses and to one additional GBM resection to identify patient-specific responses. RESULTS: We used scRNA-seq to demonstrate that acute slice cultures recapitulate the cellular and molecular features of the originating tumor tissue and the feasibility of drug screening from an individual tumor. Detailed investigation of etoposide, a topoisomerase poison, and the histone deacetylase (HDAC) inhibitor panobinostat in acute slice cultures revealed cell type-specific responses across multiple patients. Etoposide has a conserved impact on proliferating tumor cells, while panobinostat treatment affects both tumor and non-tumor populations, including unexpected effects on the immune microenvironment. CONCLUSIONS: Acute slice cultures recapitulate the major cellular and molecular features of GBM at the single-cell level. In combination with scRNA-seq, this approach enables cell type-specific analysis of sensitivity to multiple drugs in individual tumors. We anticipate that

this approach will facilitate pre-clinical studies that identify effective therapies for solid tumors.

DOI: 10.1186/s13073-021-00894-y

The impact factor: 15.266

Paper 2

Title: Epigenetic encoding, heritability and plasticity of glioma transcriptional cell states.

Abstract: Single-cell RNA sequencing has revealed extensive transcriptional cell state diversity in cancer, often observed independently of genetic heterogeneity, raising the central question of how malignant cell states are encoded epigenetically. To address this, here we performed multiomics single-cell profiling-integrating DNA methylation, transcriptome and genotype within the same cells-of diffuse gliomas, tumors characterized by defined transcriptional cell state diversity. Direct comparison of the epigenetic profiles of distinct cell states revealed key switches for state transitions recapitulating neurodevelopmental trajectories and highlighted dysregulated epigenetic mechanisms underlying gliomagenesis. We further developed a quantitative framework to directly measure cell state heritability and transition dynamics based on high-resolution lineage trees in human samples. We demonstrated heritability of malignant cell states, with key differences in hierarchal and plastic cell state architectures in IDH-mutant glioma versus IDH-wild-type glioblastoma, respectively. This work provides a framework anchoring transcriptional cancer cell states in their epigenetic encoding, inheritance and transition dynamics.

DOI: 10.1038/s41588-021-00927-7

The impact factor: 41.307

Paper 3

Title: Deciphering functional tumor states at single-cell resolution.

Abstract: Within a tumor, cancer cells exist in different states that are associated with distinct tumor functions, including proliferation, differentiation, invasion, metastasis, and resistance to anti-cancer therapy. The identification of the gene regulatory networks underpinning each state is essential for better understanding functional tumor heterogeneity and revealing tumor vulnerabilities. Here, we review the different studies identifying tumor states by single-cell sequencing approaches and the mechanisms that promote and sustain these functional states and regulate their transitions. We also describe how different tumor states are spatially distributed and interact with the specific stromal cells that compose the tumor microenvironment. Finally, we discuss how the understanding of tumor plasticity and transition states can be used to develop new strategies to improve cancer therapy.

DOI: 10.15252/embj.2021109221

The impact factor: 14.012

Paper 4

Title: Topographic mapping of the glioblastoma proteome reveals a triple-axis model of intra-tumoral heterogeneity.

Abstract: Glioblastoma is an aggressive form of brain cancer with well-established patterns of intra-tumoral heterogeneity implicated in treatment resistance and progression. While regional and single cell transcriptomic variations of glioblastoma have been recently resolved, downstream phenotype-level proteomic programs have yet to be assigned across glioblastoma's hallmark histomorphologic niches. Here, we leverage mass spectrometry to spatially align abundance levels of 4,794 proteins to distinct histologic patterns across 20 patients and propose diverse molecular programs operational within these regional tumor compartments. Using machine learning, we overlay concordant transcriptional information, and define two distinct proteogenomic programs, MYC- and KRAS-axis hereon, that cooperate with hypoxia to produce a tri-dimensional model of intra-tumoral heterogeneity. Moreover, we highlight differential drug sensitivities and relative chemoresistance in glioblastoma cell lines with enhanced KRAS programs. Importantly, these pharmacological differences are less pronounced in transcriptional glioblastoma subgroups suggesting that this model may provide insights for targeting heterogeneity and overcoming therapy resistance.

DOI: 10.1038/s41467-021-27667-w

The impact factor: 17.694

Paper 5

Title: Cancer cell heterogeneity and plasticity: A paradigm shift in glioblastoma.

Abstract: Phenotypic plasticity has emerged as a major contributor to intra-tumoral heterogeneity and treatment resistance in cancer. Increasing evidence shows that glioblastoma (GBM) cells display prominent intrinsic plasticity and reversibly adapt to dynamic microenvironmental conditions. Limited genetic evolution at recurrence further suggests that resistance mechanisms also largely operate at the phenotypic level. Here we review recent literature underpinning the role of GBM plasticity in creating gradients of heterogeneous cells including those that carry cancer stem cell (CSC) properties. A historical perspective from the hierarchical to the nonhierarchical concept of CSCs towards the recent appreciation of GBM plasticity is provided. Cellular states interact dynamically with each other and with the surrounding brain to shape a flexible tumor ecosystem, which enables swift adaptation to external pressure including treatment. We present the key components regulating intra-tumoral phenotypic heterogeneity and the equilibrium of phenotypic states, including genetic, epigenetic, and microenvironmental factors. We further discuss plasticity in the context of intrinsic tumor resistance, where a variable balance between preexisting resistant cells and adaptive persisters leads to reversible adaptation upon treatment. Innovative efforts targeting regulators of plasticity and mechanisms of state transitions towards treatment-resistant states are needed to restrict the adaptive capacities of GBM.

DOI: 10.1093/neuonc/noab269

The impact factor: 13.029

Paper 6

Title: Decoding Cancer Biology One Cell at a Time.

Abstract: Human tumors are composed of diverse malignant and nonmalignant cells, generating a complex ecosystem that governs tumor biology and response to treatments. Recent technological advances have enabled the characterization of tumors at single-cell resolution, providing a compelling strategy to dissect their intricate biology. Here we describe recent developments in single-cell expression profiling and the studies applying them in clinical settings. We highlight some of the powerful insights gleaned from these studies for tumor classification, stem cell programs, tumor microenvironment, metastasis, and response to targeted and immune therapies. SIGNIFICANCE: Intratumor heterogeneity (ITH) has been a major barrier to our understanding of cancer. Single-cell genomics is leading a revolution in our ability to systematically dissect ITH. In this review, we focus on single-cell expression profiling and lessons learned in key aspects of human tumor biology.

DOI: 10.1158/2159-8290.CD-20-1376

The impact factor: 38.272

Paper 7

Title: Brain cancer stem cells: resilience through adaptive plasticity and hierarchical heterogeneity.

Abstract: Malignant brain tumours are complex ecosystems containing neoplastic and stromal components that generate adaptive and evolutionarily driven aberrant tissues in the central nervous system. Brain cancers are cultivated by a dynamic population of stem-like cells that enforce intratumoural heterogeneity and respond to intrinsic microenvironment or therapeutically guided insults through proliferation, plasticity and restructuring of neoplastic and stromal components. Far from a rigid hierarchy, heterogeneous neoplastic populations transition between cellular states with differential self-renewal capacities, endowing them with powerful resilience. Here we review the biological machinery used by brain tumour stem cells to commandeer tissues in the intracranial space, evade immune responses and resist chemoradiotherapy. Through recent advances in single-cell sequencing, improved models to investigate the role of the tumour microenvironment and a deeper understanding of the fundamental role of the immune system in cancer biology, we are now better equipped to explore mechanisms by which these processes can be exploited for therapeutic benefit.

DOI: 10.1038/s41568-022-00486-x

The impact factor: 69.8

Paper 8

Title: The evolution of the cancer stem cell state in glioblastoma: emerging insights into the next generation of functional interactions.

Abstract: Cellular heterogeneity is a hallmark of advanced cancers and has been ascribed in part to a population of self-renewing, therapeutically resistant cancer stem cells (CSCs). Glioblastoma (GBM), the most common primary malignant brain tumor, has served as a platform for the study of CSCs. In addition to illustrating the complexities of CSC biology, these investigations have led to a deeper understanding of GBM pathogenesis, revealed novel therapeutic targets, and driven innovation towards the development of next-generation therapies. While there continues to be an expansion in our knowledge of how CSCs contribute to GBM progression, opportunities have emerged to revisit this conceptual framework. In this review, we will summarize the current state of CSCs in GBM using key concepts of evolution as a paradigm (variation, inheritance, selection, and time) to describe how the CSC state is subject to alterations of cell intrinsic and extrinsic interactions that shape their evolutionarily trajectory. We identify emerging areas for future consideration, including appreciating CSCs as a cell state that is subject to plasticity, as opposed to a discrete population. These future considerations will not only have an impact on our understanding of this ever-expanding field but will also provide an opportunity to inform future therapies to effectively treat this complex and devastating disease.

DOI: 10.1093/neuonc/noaa259

The impact factor: 13.029

Paper 9

Title: Cellular Plasticity and Tumor Microenvironment in Gliomas: The Struggle to Hit a Moving Target.

Abstract: Brain tumors encompass a diverse group of neoplasias arising from different cell lineages. Tumors of glial origin have been the subject of intense research because of their rapid and fatal progression. From a clinical point of view, complete surgical resection of gliomas is highly difficult. Moreover, the remaining tumor cells are resistant to traditional therapies such as radio- or chemotherapy and tumors always recur. Here we have revised the new genetic and epigenetic classification of gliomas and the description of the different transcriptional subtypes. In order to understand the progression of the different gliomas we have focused on the interaction of the plastic tumor cells with their vasculature-rich microenvironment and with their distinct immune system. We believe that a comprehensive characterization of the glioma microenvironment will shed some light into why these tumors behave differently from other cancers. Furthermore, a novel classification of gliomas that could integrate the genetic background and the cellular ecosystems could have profound implications in the efficiency of current therapies as well as in the development of new treatments.

DOI: 10.3390/cancers12061622

The impact factor: 6.575

Paper 10

Title: Disconnecting multicellular networks in brain tumours.

Abstract: Cancer cells can organize and communicate in functional networks. Similarly to other networks in biology and sociology, these can be highly relevant for growth and resilience. In this Perspective, we demonstrate by the example of glioblastomas and other incurable brain tumours how versatile multicellular tumour networks are formed by two classes of long intercellular membrane protrusions: tumour microtubes and tunnelling nanotubes. The resulting networks drive tumour growth and resistance to standard therapies. This raises the question of how to disconnect brain tumour networks to halt tumour growth and whether this can make established therapies more effective. Emerging principles of tumour networks, their potential relevance for tumour types outside the brain and translational implications, including clinical trials that are already based on these discoveries, are discussed.

DOI: 10.1038/s41568-022-00475-0

The impact factor: 69.8