# Report Title

Introduction

Introduction:
Glioblastoma multiforme (GBM) is a highly aggressive and heterogeneous brain tumor, characterized by complex cellular diversity and plasticity. Understanding the dynamics of the tumor microenvironment in GBM is crucial for disease prognosis and the development of effective treatment strategies. Recent advancements in single-cell RNA sequencing (scRNA-Seq) have provided unprecedented insights into the heterogeneity and dynamics of the tumor microenvironment, offering a comprehensive understanding of the cellular and molecular landscape of GBM.

The importance of studying the tumor microenvironment in GBM is underscored by its potential impact on disease progression, therapeutic resistance, and clinical outcomes. Identifying distinct cell states, their epigenetic regulation, and dynamic transitions has significant implications for understanding the underlying mechanisms of gliomagenesis and for developing targeted therapies. Moreover, the discovery of heritable malignant cell states and their transition dynamics provides critical insights into the evolution and progression of GBM, shedding light on the development of therapeutic resistance and disease recurrence.

This research addresses the necessity of unraveling the complex cellular and molecular interactions within the tumor microenvironment of GBM. By integrating multiomics single-cell profiling, including DNA methylation, transcriptome, and genotype analyses, this study aims to elucidate the epigenetic encoding, inheritance, and transition dynamics of malignant cell states in GBM. The findings from this research are expected to contribute to a deeper understanding of the pathobiology of GBM and may have implications for the development of novel therapeutic interventions targeting the tumor microenvironment. The use of multimodality single-cell RNA sequencing in primary diffuse gliomas provides insights into the epigenetic encoding, heritability, and plasticity of cell states in glioma, offering potential for improved treatment strategies.

The significance of this research lies in its potential to enhance our understanding of GBM at the single-cell level and to uncover novel insights into the dynamics of cell state transitions and epigenetic mechanisms underlying gliomagenesis. It also sets new standards for rigorous and transparent scientific inquiry in the field of oncology research. The use of scRNA-Seq has revealed extensive transcriptional cell state diversity in cancer, often independently of genetic heterogeneity, emphasizing the importance of understanding the intratumoral heterogeneity in GBM. Moreover, the use of multimodality single-cell sequencing, including DNA methylation, transcriptome, and genotype profiling, has enabled the exploration of methylation–transcription relationships at the single-cell level, revealing aberrant epigenetic patterning in IDH-MUT gliomas.

Conclusively, the research on the heterogeneity and dynamics of the tumor microenvironment in GBM through scRNA-Seq not only enhances our understanding of the disease at the genetic and epigenetic levels but also holds promise for the development of more effective treatments and targeted therapeutic strategies.

Literature

, the study applies joint capture of transcriptional, genetic, and epigenetic information at single-cell resolution to primary diffuse gliomas.

The experimental methods used in the article involve the profiling of viable cells enriched for CD45- cells from GBM and IDH-MUT glioma primary patient samples using multimodality single-cell sequencing of DNA methylation (scDNAme). This approach increases the resolution of single-cell identification of copy number alterations (CNAs), demonstrates significant DNA methylation intratumoral heterogeneity (ITH), and reveals the epigenetic encoding, heritability, and plasticity of cell states in glioma.

The experimental results highlight high-resolution CNA mapping by single-cell multiomics, direct comparison of epigenetic profiles of distinct cell states, and the development of a quantitative framework to measure cell state heritability and transition dynamics based on high-resolution lineage trees in human samples. The study demonstrates 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 methods used to verify the topic include single-cell RNA sequencing (scRNA-seq) and the development of a classifier to assign samples to their expected DNA methylation subtypes based on pseudo-bulk DNA methylation profiles. The proposed method is essential for increasing the resolution of single-cell identification, demonstrating DNA methylation heterogeneity, and understanding the epigenetic encoding, heritability, and plasticity of cell states in glioma.

In conclusion, the literature review provides a comprehensive overview of the experimental methods, processes, and results related to deciphering the heterogeneity and dynamics of the tumor microenvironment in glioblastoma multiforme using single-cell RNA sequencing. It offers valuable insights into the complexities of glioma and the potential for advancements in understanding and targeting this disease.

References:

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8114529/pdf/
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8675181/pdf/
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8762559/pdf/

Discussion

protein interactions. The use of scRNA-Seq in this study provides a comprehensive analysis of the heterogeneity and dynamics of the GBM microenvironment, contributing to the broader understanding of this complex disease.

However, there are limitations that should be considered, such as the need for further validation of the findings and potential biases in patient selection. Future research directions could involve validation studies in larger patient cohorts, exploring the translational potential of the identified molecular signatures for targeted therapy, and investigating the functional roles of specific cell types identified in the tumor microenvironment.

In summary, the research makes a significant contribution to the field by providing valuable insights into the heterogeneity and dynamics of the tumor microenvironment in GBM using scRNA-Seq. The findings have both academic and practical implications, paving the way for future research aimed at improving the understanding and treatment of GBM. The collaborative and multi-disciplinary approach taken in this study sets a strong foundation for continued research in this area, with the potential to impact clinical practice and patient outcomes.

In conclusion, the research report on deciphering the heterogeneity and dynamics of the tumor microenvironment in Glioblastoma Multiforme using single-cell RNA sequencing (scRNA-Seq) has provided valuable insights into the complexities of the tumor microenvironment, offering potential implications for therapeutic interventions and personalized treatment approaches. The study's findings significantly contribute to the understanding of the mechanisms of plasticity in cancer cells and pave the way for future investigations into personalized therapeutic approaches for this aggressive form of brain cancer.

Idea

Problem:
Identifying and targeting the specific cellular states within the glioblastoma tumor microenvironment that contribute to treatment resistance and disease progression, leveraging insights from single-cell RNA sequencing (scRNA-seq) data and the associated proteogenomic and epigenetic mechanisms.

Rationale:

The research problem aims to address the critical challenge of understanding and characterizing the cellular states within the glioblastoma tumor microenvironment, which are known to underpin treatment resistance and intra-tumoral heterogeneity. Building upon the findings of the target paper and related studies utilizing scRNA-seq, epigenetic profiling, and proteogenomic analysis, this research problem seeks to delve deeper into unraveling the functional diversity of glioblastoma cells at a single-cell level. By elucidating the molecular programs and epigenetic mechanisms regulating these states, it becomes feasible to identify potential vulnerabilities and develop targeted therapeutic strategies to combat

glioblastoma heterogeneity and improve treatment outcomes.

Method

1. **Literature Review and Data Collection:**
- Start by collecting and reviewing existing scRNA-seq data from glioblastoma tumor samples, focusing on the specific cellular states identified and their associated proteogenomic and epigenetic mechanisms, as highlighted in the target paper and related studies.
- Additionally, gather information on drug responses and treatment strategies that have been explored in relation to these cellular states, as well as any relevant spatial and functional heterogeneity within the tumor microenvironment.

2. **Integration and Analysis of scRNA-seq Data:**

- Integrate the collected scRNA-seq data from glioblastoma samples to identify and characterize the specific cellular states contributing to treatment resistance and disease progression.
- Utilize advanced computational methods such as clustering algorithms and pathway analysis to elucidate the functional diversity and associated molecular programs of these cellular states.

3. **Multi-omics Profiling and Comparative Analysis:**

- Perform multi-omics profiling, incorporating epigenetic and proteogenomic data from the same cellular samples, to further understand the regulatory mechanisms and molecular programs governing the identified cellular states.
- Conduct comparative analysis between different patient-derived models and primary tumors to assess the recapitulation of cellular states and their plasticity within diverse tumor microenvironments.

4. **Validation and Functional Characterization:**

- Validate the findings from the integrated analysis using additional experimental techniques, such as in vitro and in vivo assays, to functionally characterize the identified cellular states and their contributions to treatment resistance.
- Investigate the potential vulnerabilities and therapeutic targets associated with specific cellular states, taking into consideration the spatial and functional heterogeneity observed within the tumor microenvironment.

5. **Generalization and Clinical Relevance:**

- Generate a comprehensive framework that provides insights into the heterogeneity of cellular states within the glioblastoma tumor microenvironment, with the aim of generalizing these findings to other tumor types and clinical settings.
- Translate the research findings into potential clinical applications, including the development of personalized therapeutic strategies targeting specific cellular states to improve treatment outcomes for glioblastoma patients.

By combining data integration, advanced computational analysis, multi-omics profiling, experimental validation, and translational implications, the proposed method aims to address the research problem

by systematically unraveling the complexities of cellular states within the glioblastoma tumor microenvironment, with the potential for broader applicability in understanding treatment resistance and disease progression in cancer.

Experiment

responses in a spatially and phenotypically resolved manner. RESULTS: We deconvolve cell type-specific drug responses within tumor tissue, including identification of drug-sensitive and -resistant tumor and immune cell states. Pathway-level analyses reveal candidate drug targets and intriguing cell communication responses. Finally, we show that the approach is compatible with patient-derived xenograft and dissociated tissue cultures. CONCLUSIONS: We demonstrate that the scRNA-seq drug profiling pipeline can serve as a powerful platform for characterizing cell type-specific drug responses in preclinical models, potentially guiding individualized therapeutic strategies.', 'BACKGROUND: Epigenomic modifications play a crucial role in tumor cell state transitions and treatment responses. METHODS: We integrate chromatin accessibility (ATAC-seq) with the single-cell RNA sequencing (scRNA-seq) to map cell type-specific epigenomes across a large collection of human gliomas. RESULTS: Using this multimodal profiling approach, we identify epigenetically regulated glioma cell populations, hub genes and regulatory elements involved in cell state transitions. We also reconstruct the clonal evolution and quantify the heritability of the chromatin landscape in gliomas. CONCLUSIONS: Our study highlights the importance of integrating single-cell epigenomics with transcriptomics for in-depth dissection of the complex regulatory networks and epigenetic basis of glioma cell states.', 'The diverse phenotypic and functional states of tumor cells present a major challenge in understanding and targeting cancer. The limited knowledge on the spectrum and functional roles of these states has hindered the development of effective therapeutic strategies. Recent advances in single-cell genomic and transcriptomic technologies now enable the high-throughput characterization of cellular phenotypes across tumor types. Here, we review the current state of single-cell resolution analyses of cancer cell states, emphasizing the methodologies and applications for dissecting the functional tumor heterogeneity.', 'BACKGROUND: Glioblastoma multiforme (GBM) is one of the most aggressive and heterogeneous cancers. Tumor heterogeneity can occur at the genetic, phenotypic, and spatial levels within the tumor, presenting a challenge for effective therapeutic intervention. METHODS: Here, we utilized high-resolution mass spectrometry imaging (HR-MSI) to interrogate the proteomic landscape of GBM at the single-cell level. We discovered that single-cell proteomic data align along three axes: the distance from the tumor margin, the presence of immune infiltration, and cellular heterogeneity. We demonstrate that proteomic data combined with information on tumor topography can reveal distinct cellular states in GBM that are associated with unique biological and clinical features. CONCLUSIONS: These findings provide molecular insight into the intra-tumoral heterogeneity of GBM and illustrate the utility of multi-dimensional proteomic data for mapping functional tumor states.']Based on the information provided, your experiment design will involve the following key steps:

1. Collect and review existing scRNA-seq data from glioblastoma tumor samples, focusing on the specific cellular states identified and their associated proteogenomic and epigenetic mechanisms, as highlighted in the target paper and related studies.

2. Integrate the collected scRNA-seq data from glioblastoma samples to identify and characterize the specific cellular states contributing to treatment resistance and disease progression. Utilize advanced computational methods such as clustering algorithms and pathway analysis to elucidate the functional diversity and associated molecular programs of these cellular states.
3. Perform multi-omics profiling, incorporating epigenetic and proteogenomic data from the same

cellular samples, to further understand the regulatory mechanisms and molecular programs governing the identified cellular states. Conduct comparative analysis between different patient-derived models and primary tumors to assess the recapitulation of cellular states and their plasticity within diverse tumor microenvironments.
4. Validate the findings from the integrated analysis using additional experimental techniques, such as in vitro and in vivo assays, to functionally characterize the identified cellular states and their contributions to treatment resistance. Investigate the potential vulnerabilities and therapeutic targets associated with specific cellular states, taking into consideration the spatial and functional heterogeneity observed within the tumor microenvironment.
5. Generate a comprehensive framework that provides insights into the heterogeneity of cellular states within the glioblastoma tumor microenvironment, with the aim of generalizing these findings to other tumor types and clinical settings. Translate the research findings into potential clinical applications, including the development of personalized therapeutic strategies targeting specific cellular states to improve treatment outcomes for glioblastoma patients.

Your experimental design should be backed by robust data collection, computational analysis, multi-omics profiling, and experimental validation methods to address the research problem effectively. At each stage, it is key to refer back to the insights gained from the existing studies, the proposed method, and the primary focus of the research problem to ensure that your approach aligns with the scientific methodology. Finally, it is essential to ensure that your experimentation is designed to be reproducible, valid, and feasible to yield meaningful results in the study of glioblastoma tumor microenvironment and treatment resistance.

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