

Current and Future Perspectives of Multi-omics in Gliomas: From Basic Research to Translational Medicine

Abstract

Keywords

Multi-omics, glioma, tumor microenvironment, biomarker, subtype, therapy

Abbreviations

1 Introduction

Gliomas represent the most significant threat among human brain tumors, with glioblastoma, the most malignant form, having a 5-year survival rate of only 5%^[1]. This formidable cancer casts a remarkable shadow over clinical practice, as effective precision diagnosis, classification, and targeted therapies remain elusive due to its considerable heterogeneity, plasticity, and resistance^[2, 3].

Recent advancements in omics technologies have helped unveil the landscape of glioma at the tissue, cellular, and molecular levels, thereby offering new insights for addressing these challenges. Various bulk single-omics approaches, including genomics, transcriptomics, proteomics, and metabolomics, have generated a vast amount of “population-level” monomodal data, successfully elucidating the genetic origins, transcriptional regulation, dynamic drivers, and functional metabolism of gliomas, thereby bridging the upstream and downstream aspects of the glioma “gene-phenotype” axis^[4-7]. Crucially, the glioma microenvironment exhibits cellular diversity, where malignant and non-malignant cells undergo proliferation, evolution, and death, forming an interactive network. This diversity is particularly important when attempting to understand the microecology and identify key therapeutic targets. Therefore, capturing and analyzing various microenvironmental cell types is essential for unraveling the heterogeneity of this tumor. To address this challenge, single-cell omics has provided insights at the cellular level within the glioma microenvironment, aiming to uncover intra- and inter-cellular heterogeneity, cell lineage trajectories, and adaptive mechanisms driven by the environment, thus completing the transition from a “population” to an “individual” perspective^[8]. However, bulk and single-cell omics still rely on tissue slicing, resulting in the loss of spatial coordinates of tissues and cells^[9]. There is an urgent need to decode the internal genetic selection and the external spatial drivers of gliomas, as the microenvironment possesses specific cellular spatial maps and interactive effects. Recently, spatial omics—a fascinating technology—has enabled the representation of spatial information at the tissue or cellular level, including the spatial molecular maps of malignant cells (abundance, gradients, and distribution) and the interactions among various cells. This technology has filled the final piece of the puzzle in glioma research^[10].

In glioma biology and translational research, there is an urgent need for a more comprehensive understanding of genetic alterations, functional states, cellular interactions, and spatial transitions of various cells in specific pathological and disturbed contexts. The integration of multi-omics technologies at the bulk, single-cell, and spatial levels has led to continuous breakthroughs in throughput, accuracy, and resolution, revolutionizing our ability to understand and utilize the relationship between malignant glioma cells and the non-malignant components of the microecosystem^[11]. These technologies have ushered glioma research into a transformative era, giving us greater confidence in deciphering the complex molecular evolution of gliomas, developing more precise subtype classifications and management strategies, and attempting to overcome the therapeutic challenges posed by tumor plasticity.

In this review, we discuss the current state of various single omics approaches in the glioma field, tools for multi-omics data processing and integration, and the biological and clinical implications of these technologies, offering an outlook for the field.

2 Landscape of Single Omics in Glioma

2.1 Genomics

The individual susceptibility to diseases is influenced by genetic factors, and the human genome provides a crucial framework for genetic research in disease^[12, 13]. Genomics, through whole-genome sequencing (WGS), targeted sequencing, and genotyping microarrays^[14], enables comprehensive observation of genetic sequences and variations within tissues, including base substitutions and copy number variations^[15, 16]. Consequently, these technologies offer insights into genetic information at the nucleotide and chromosomal levels, marking a revolutionary advancement in elucidating the upstream mechanisms of glioma's malignant biological behavior^[17].

Firstly, genomics reveals the temporal genetic heterogeneity and subtype-specific copy number variations in gliomas by analyzing the mutation spectra of different glioma subtypes (LGG and GBM) and at various stages of glioma progression (initial diagnosis and subsequent surgery)^[18, 19]. Furthermore, genomics uncovers how single-nucleotide polymorphisms in non-coding regions of tumors activate regulatory gene factors, thereby driving the malignant biological behavior of gliomas^[20]. Interestingly, beyond nucleotide-level analysis, genomics also investigates chromosomal structural variations in tumors at different stages of progression (primary and recurrent), revealing how changes in chromosomal aneuploidy and structural heterogeneity may contribute to glioma recurrence and resistance^[21, 22]. Overall, genomics provides the first high-throughput exploration of the comprehensive genetic landscape of gliomas, serving as a pivotal tool in explaining the upstream mechanisms within the "gene-phenotype" axis.

2.2 Transcriptomics

Through high-throughput analysis of cDNA from reverse-transcribed libraries, RNA sequencing-based transcriptomics enables comprehensive mapping of transcripts and regulatory factors, nearly eliminating the background noise typical of traditional microarrays^[23]. Transcripts can more precisely locate aberrantly active genes, rather than focusing on static copy number variations, which holds unique significance for gliomas with complex heterogeneity^[24].

A significant breakthrough in transcriptomics is the integration of causal analysis and gene editing to capture potential driving molecules (including mRNA and non-coding RNA) and signaling pathways that shape specific glioma biological behaviors and tumor microenvironments along the "gene-phenotype" axis^[25-28]. Importantly, benefiting from interdisciplinary integration, transcriptomics provides three key pathways for clinical translation. First, by combining clinical data with artificial intelligence, key molecular factors at the mechanistic level can become biomarkers for clinical diagnosis and prognosis^[29-31]. Second, transcriptomics can create disruptive subtype classifications that transcend the anatomical origins of gliomas, enabling the prediction of treatment benefits^[32]. Finally, molecular docking techniques can be used to identify novel targeted drugs aimed at driving genes^[33].

Additionally, the post-transcriptional level, including translation and post-translational modifications, is so complex that simply expressing genes does not accurately explain the diverse phenotypes and functions of gliomas. Therefore, exploring omics technologies at the functional level is essential for truly mapping the comprehensive landscape of the "gene-phenotype" axis.

2.3 Proteomics

The abundance of proteins in human cells spans 7-8 orders of magnitude more than that of transcripts^[34], and alternative splicing and protein modifications generate numerous functional protein isoforms from specific mRNA or gene loci^[35]. Therefore, the complexity of disease phenotypes cannot be fully explained by transcriptional data alone. To address this, mass spectrometry-based proteomics enables comprehensive detection of the entire

process of protein translation and post-translational modifications^[36].

This technology, capable of describing the complex phenotypes and key molecules of an organism at a given time, has ushered glioma biological research from the "genomic era" into the "post-genomic era." At the protein level, our research on glioma driving factors has been summarized into five key areas: (1) How driving factors promote glioma progression at the protein level, highlighting the broader picture often overlooked by genetic perspectives^[37-42]; (2) How driving factors interact with molecules such as RNA, proteins, and metabolites to influence glioma^[43, 44]; (3) How post-translational modifications alter driving factors^[45-47]; (4) The dynamics and heterogeneity of driving factors in host system changes^[48-50]; (5) How driving factors are described at the phenotypic level through population data analysis, focusing on clinical translation^[51-54]. Overall, proteomics bridges the upstream and downstream of the glioma "gene-phenotype" axis, providing new perspectives to uncover glioma heterogeneity and biological functions.

2.4 Metabonomics

The abundance of metabolites is 3-4 orders of magnitude lower than that of proteins, thereby concentrating functional information, making them the ultimate downstream products of gene transcription and protein translation^[55, 56]. Unlike genomics, which is influenced by epigenetic regulation, and proteomics, which is affected by post-translational modifications, metabolomics provides a more direct and accurate means of explaining how external environmental and internal physiological or pathological stimuli impact downstream metabolic states^[57, 58]. This enables the characterization of metabolic heterogeneity in gliomas.

First, metabolomics reveals the metabolic heterogeneity of glioma tissues across different subtypes (IDH-mutant and IDH-wildtype) and identifies subtype-specific metabolic profiles that drive the development of glioma metabolic subtyping^[59]. However, the metabolic reprogramming mechanisms underlying these subtype-specific profiles remain to be further elucidated. By analyzing the metabolic differences between IDH1-mutant and wild-type tumor tissues, metabolomics supports the hypothesis that lipid reprogramming associated with IDH1 mutations drives the differences in patient prognosis^[60]. In addition to the metabolic heterogeneity driven by genetic and histological differences, metabolomics also captures the metabolic heterogeneity driven by spatial and cell-line differences. By analyzing the metabolic differences between distinct tumor regions (such as the glioma core and adjacent normal tissue) and between glioma cell lines of varying malignancy, metabolomics supports the notion that metabolic pathway heterogeneity drives tumor progression, invasion, and other malignant biological behaviors^[61, 62]. Overall, as the final piece in the comprehensive "gene-phenotype" map, metabolomics provides a novel perspective for understanding glioma heterogeneity.

2.5 Single-cell Omics

Bulk sequencing alone cannot reflect the subgroups, states, and evolution at the cellular resolution^[23, 63, 64]. In this context, single-cell omics provide insights at the cellular level, including single-cell transcriptomics^[8], single-cell proteomics^[65], and single-cell metabolomics^[66]. The technological advancements and applications of these approaches have been comprehensively reviewed^[67]. Our focus is on how these fascinating technologies complement and refine our understanding of glioma's spatiotemporal heterogeneity and plastic resistance.

First, single-cell transcriptomics has the most extensive application in gliomas, revealing: (1) The inter- and intracellular heterogeneity of the glioma microenvironment, including genetic and transcriptional heterogeneity^[68], cell type-level heterogeneity^[69-71], and host-level heterogeneity^[72]; (2) The cell trajectories, maturation programs, and lineage evolution of glioma cells^[73], immune cells^[74], and vascular cells^[75] within the microenvironment; (3) Based on disturbances in the microecosystem, hypotheses have been proposed regarding the dynamic heterogeneity and resistance of gliomas: this may not result from genetic inheritance, but rather from adaptive changes driven by the microenvironment^[76]. Furthermore, single-cell proteomics has corroborated the remodeling of cellular protein signaling networks that lead to environment-driven glioma resistance^[77]. Currently, single-cell metabolomics supports the metabolic heterogeneity between glioma cells^[78]. In summary, single-cell omics have facilitated a pivotal revolution, advancing glioma research from the low-resolution "cellular population" to the high-resolution "individual" level.

2.6 Spatial Omics

Due to the disruptive nature of bulk and single-cell omics, we lose the spatial relationship information between cell populations^[79]. Clinical practice in gliomas has revealed a fundamental truth: to fully understand the malignant biological behavior of gliomas, it is essential to decode the combined drivers of internal genetic selection and the external microenvironment. This includes the cellular landscape, spatial distribution, and interactions. Therefore, spatial omics—including spatial transcriptomics^[80], spatial proteomics^[10], and spatial metabolomics^[81]—has become the final piece of the puzzle in decoding gliomas, as it allows for molecular characterization of tumor regions or individual cells from a spatial perspective.

First, spatial transcriptomics preserves the spatial coordinates of transcriptomic data, enabling the characterization of transcriptional states and subgroup differences in various cell types within different glioma regions (tumor core, infiltrative boundary) and microenvironments, thereby helping identify the mechanisms and targets underlying biological behaviors such as invasion and resistance^[82-84]. Second, spatial proteomics emphasizes the use of spatial protein information to reveal the molecular features, localized regions, and spatial reorganization of the glioma cell landscape^[85-87]. Finally, spatial metabolomics is used to map the abundance, gradients, and distribution of metabolites across different glioma regions and microenvironments under multifactorial regulation, highlighting the role of environment-driven processes in shaping heterogeneity and resistance^[88-91]. Overall, spatial omics provides a previously unknown spatial map of gliomas.

3 Integrated Methods of Multi-omics in Glioma

3.1 Multi-Omics Data Preprocessing Methods

3.1.1 Cleaning Methods for Raw Multi-Omics Data

Missing Data Handling A common issue in omics data analysis is missing values, which can arise due to various reasons, such as poor tissue quality, insufficient sample size, and limitations of detection technologies. These missing values hinder downstream analyses and can be caused by factors like insufficient sample size, technical limitations, and human errors^[92]. The most common method for handling missing values is imputation, which can be categorized into mean imputation, hot deck imputation, multiple imputation, and artificial intelligence-based methods. The choice of imputation method depends on the characteristics of the specific omics data. For example, metabolomics data obtained by LC-MS often results in a substantial proportion (30-50%) of missing values. Using simple methods like mean imputation in such cases would introduce considerable noise, so a more robust approach, such as using random forest algorithms for imputation, is preferred. This method has a lower normalized root mean square error (NRMSE) compared to other approaches and maintains high robustness even when the missing value proportion increases^[93]. However, excessive imputation may introduce significant systematic biases. In 2022, the SUMO framework, based on non-negative matrix factorization, directly used existing data for molecular subtype identification through patient similarity networks, avoiding the need for imputation. This approach successfully identified prognostic differences in the IDH wild-type subgroup in glioma data^[94]. Overall, with the growing use of artificial intelligence, the handling of missing values in omics data is no longer limited to traditional computational and statistical methods but increasingly relies on machine learning approaches.

Scaling Adjustment of Data Different omics analyses may have different quantification workflows, but they often involve the issue of data scaling adjustment. This step is crucial for eliminating systematic biases caused by experimental variations and enabling comparisons or processing of data from different features at the same scale. Common data scaling methods include normalization and standardization. In transcriptomics and proteomics, the CONSTANd normalization method, which uses iterative proportional fitting (IPF), effectively corrects for systematic biases in the experiment. For other types of quantitative omics data obtained under similar experimental conditions, CONSTANd can still function independently for each dataset^[95]. For genomics data, a normalization method based on internal relative gene expression ranking is used to convert data into a standard normal distribution (SND). When combined with convolutional deep neural networks (CDNN) for analysis, this method has shown superior classification performance compared to the raw data^[96].

3.1.2 Dimensionality Reduction in Multi-Omics Data

Multi-omics data are typically characterized by a large number of features and relatively few samples, a phenomenon known as the $n \ll P$ problem. This can lead to issues such as overfitting and a lack of generalization ability in subsequent training models^[97], making early feature selection crucial. Depending on the availability of data labels, feature selection methods can be categorized into supervised and unsupervised types^[98]. Supervised methods select features based on the target variable, whereas unsupervised methods do not rely on the target variable and instead remove irrelevant variables based on correlations. In recent years, many studies have opted for unsupervised methods, such as autoencoders, for dimensionality reduction^[99-102].

Autoencoders are unsupervised neural networks consisting of an encoder and a decoder. The core function of an autoencoder is to compress and reconstruct data, capturing key features in the input data and replicating the input variables to the output variables with minimal information loss. Compared to other dimensionality reduction methods, autoencoders, which are derived from neural networks, can capture nonlinear relationships in omics data, thereby preserving more of the essential information^[103]. A framework based on autoencoders, *DeepAutoGlioma*, first encodes the gene expression and methylation data of LGG and GBM into 4314-dimensional and 715-dimensional feature vectors, respectively, using the encoder. The bottleneck layer dimensions for LGG and GBM were set to 400 and 100 dimensions, respectively. Mean square error (MSE) was used to assess the dimensionality reduction performance, and the results showed that the MSE for both LGG and GBM was 0.04. This demonstrated that, despite effective dimensionality reduction, the autoencoder still captured the inherent relational features in the genomic and methylation data^[104].

3.2 Integration Methods for Glioma Across Different Omics

Machine learning models typically perform better on large training datasets, making them particularly well-suited for multi-omics data integration studies^[97]. This section introduces various machine learning methods used for integrating multi-omics data in gliomas.

3.2.1 Genomic and Transcriptomic Data

Mutations at the genomic level are often associated with changes in transcriptomic expression patterns or the differential expression of specific genes^[105]. For example, in a mouse glioma model, researchers discovered that different mutation combinations of tumor suppressor genes (such as Ink4a/Arf, PTEN, p53) and oncogenes (such as EGFRvIII, BrafV600E) could activate the expression of Sox2, Olig2, and Zeb1, which in turn initiates tumor formation^[106]. The synergistic analysis of genomic and transcriptomic data enables more precise pathway analysis of glioma samples, thereby improving the accuracy of predictive models. Feng et al. established a biologically meaningful simplified deep learning model, *DeepSigSurvNet*, which replaces traditional fully connected layers with signaling pathway layer connections at the input layer. This model connects copy number variations (CNVs) and gene expression data to their respective KEGG pathway nodes. They then introduced the Inception module to reduce model complexity and enhance biological interpretability. Finally, by combining genomic data with clinical variables and using the MSE loss function to predict survival time, they achieved a higher c-index value in test datasets than the random forest model, demonstrating the dual benefits of genomic and transcriptomic integration for survival prediction and pathway mechanism explanation^[107].

3.2.2 Epigenomic and Transcriptomic Data

Epigenetics dynamically regulates gene transcription activity through mechanisms such as DNA methylation and histone modification^[108]. For instance, in IDH-mutant gliomas, over-methylation of the CTCF insulator can lead to the abnormal activation of the oncogene PDGFRA (with average methylation levels reaching 77%)^[109]. Importantly, there is overlapping biological information between epigenomic and transcriptomic data, and integration is necessary to maximize the efficiency of the analysis. *iGlioSub* integrates the epigenomic and transcriptomic data from glioma patients, using the random forest (RF) algorithm to select subtype-specific features. It applies the nearest shrunken centroid (NSC) method to optimize the feature set, while ensuring that each subtype retains five key features (at least two positively correlated and two negatively correlated). This

integrated classifier was validated through 10-fold cross-validation and independent cohort validation, showing that the integration strategy outperformed single-omics models in terms of accuracy and achieved a higher kappa agreement coefficient than other machine learning strategies (accuracy = 0.9)^[110]. Therefore, the simultaneous integration of epigenomic and transcriptomic data captures the functional connections between epigenetic modifications and gene transcription, effectively improving the performance of predictive models.

3.2.3 Transcriptomic and Proteomic Data

Integrating transcriptomic and proteomic data can reveal the dynamic relationships between gene expression and protein function, thereby addressing the limitations of single-omics approaches in regulatory mechanism analysis^[111]. Y-box binding protein 1 (YB-1), a core member of the cold shock domain (CSD) protein family, participates in nearly all RNA-related biological processes by specifically binding RNA^[112-114]. In a knockdown experiment targeting YB-1 in glioblastoma cell lines U251 and U87, genes that did not exhibit significant changes at the mRNA level showed a notable downregulation at the protein level. This indicates that YB-1 promotes glioma growth through translational regulation^[115], highlighting the necessity of integrated transcriptomic and proteomic analysis. To identify molecular signatures that predict the survival time and quality of life (Karnofsky Performance Scores, KPS) of glioblastoma (GBM) patients, Nassani et al. used a multiview iterative random forest (MiRF) model to independently train mRNA expression data and proteomic data from the TCGA database. Clinical data from the cBioPortal platform were also incorporated to identify differentially expressed genes and proteins associated with survival and KPS scores. The two datasets were then integrated into a multi-omics dataset, and the MiRF model was applied for joint analysis. Stable features were selected through three rounds of bootstrapping, yielding 35 non-redundant molecular features (19 differentially expressed genes and 16 proteins). The model's high-quality stability was validated using AUPR (≥ 0.6 for all training datasets, with significant 95% CIs) and AUROC ($\geq 70\%$ for all training datasets, with significant 95% CIs) curves, providing potential targets for formulating GBM treatment plans that balance both survival time and quality of life^[116].

3.2.4 Metabolomics and Proteomics Data

Metabolites reflect the true state of cells and tissues^[117], while proteomics elucidates protein products and their post-translational modifications^[118]. The core goal of these two omics technologies is to uncover the intrinsic relationship between altered metabolites or proteins and pathogenic pathological changes. To more efficiently reveal the protein functional pathways in gliomas, Lagache et al. proposed a "dry proteomics" approach based on spatial lipidomics. Specifically, researchers first obtained raw MALDI MSI data of lipids, peptides, and proteins from glioma samples. They then used singular value decomposition (SVD) to reduce computational demands and extract principal components, followed by improved clustering using the k-means++ algorithm. Subsequently, LightGBM and RidgeClassifier were independently used to construct high-precision classification models from the lipid spatial distribution and protein pathway dimensions. The reliability of using spatial lipidomics to predict proteomic pathways was cross-validated, bypassing the need for tedious wet-lab experiments and providing a rapid, low-cost molecular profiling tool for clinical use^[119].

4 The Research and Application of Multi-omics in Glioma

Humanity has uncovered many mysteries of glioma through single omics. However, the limitations of data from a single layer have led to the integration of multiple omics sequencing data, aiming to approach and address glioma from a systemic perspective. We have summarized the multi-omics approach to glioma research in Figure 1.

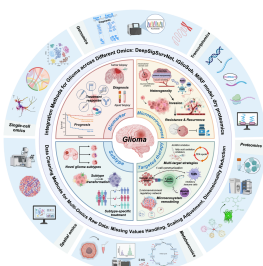


Figure 1. The research and application of multi-omics. Through data processing and integration methods, genomics, transcriptomics, proteomics, and metabolomics at bulk, single-cell, and spatial levels are integrated to study the glioma tumor microenvironment, subtypes, biomarkers, and therapeutic targets.

胶质瘤基础研究与临床转化研究中的多组学研究。多组学分析是一种综合性的研究方法，可整合多个层面单组学数据。展现胶质瘤“分子-表型”轴中的分子动态变化与调控机制提高胶质瘤亚型分类与管理策略。大规模的单组学方法，主要包括基因组学、转录组学、蛋白组学、代谢组学、单细胞组学与空间组学。基因组学通过下一代测序技术等高通量测序方法对揭示胶质瘤核苷酸水平与染色体结构异质性。Interestingly，转录组学同样通过高通量测序技术揭示胶质瘤转录调控因子、转录本异质性与核糖体异质性景观。Apart from转录组学，蛋白组学反映了基因组的最终功能表达情况。蛋白组学通过质谱技术分析经过制备的胶质瘤样品，揭露蛋白质水平结构变异与相互作用驱动胶质瘤异质性的全面景观。Besides，代谢组学同样通过液相色谱法等质谱技术分析肿瘤样本，揭示胶质瘤代谢物异质性与代谢重编程景观，为“基因-表型”图谱补上最后一块拼图。Significantly，单细胞组学揭示胶质瘤细胞层面谱系分化、相互作用与细胞轨迹的全面景观，将组学研究从“细胞群体层面”推向了具有更高分辨率的“个体”水平。More profoundly，空间组学通过数据分析与激光捕获显微切割测序（LCM-seq）等技术构建可视化空间基因表达与细胞空间分布景观，弥补了其他组学丢失空间关系信息的缺点。测序获得的原始组学数据需要经过数据清洗与多组学数据整合，才能阐明上游下游因子的相互作用机制。原始组学的数据清洗包括对缺失数据进行处理、数据缩放调整，才能用于下一步的多组学数据整合分析。而多组学数据整合方法包括整合基因组与转录组数据的深度学习模型DeepSigSurvNet、整合表观基因组与转录组数据的整合分类器iGlioSub、整合转录组与蛋白组数据的多视角迭代随机森林（MiRF）模型与整合代谢组与蛋白组数据的“干蛋白质组学”方法。多组学数据能够通过整合多个层面分子数据，用于胶质瘤生物标志物、亚型特异性胶质瘤异质性，细胞微环境异质性与治疗策略研究。多组学生物标志物研究聚焦于胶质瘤液体活检早期诊断、组织活检、治疗反应预测、预后分析的标志物发现。除此以外，多组学技术对于胶质瘤亚型的研究聚焦于补充原有亚型异质性与新亚型的发现、动态亚型的发展与亚型特异性治疗策略。Additionally，胶质瘤对于微环境的研究目前聚焦于异质性、侵袭、耐药性与复发方面。多组学技术揭示肿瘤坏死核心与侵袭性外围的异质性，表征不同来源细胞促进肿瘤微环境发生间充质分化、代谢重编程与血管生成。除此之外，多组学技术证明了肿瘤微环境通过代谢重编程与细胞通讯重塑局部环境，从而诱导胶质瘤发生增殖与侵袭。Last，微环境的可塑性诱导了化疗与放疗耐药性的产生。More profoundly，多组学研究能够为胶质瘤治疗靶点提供新视角，包括多靶点治疗策略与胶质瘤微环境重塑。例如，多靶点治疗通过AURKA抑制剂与脂肪酸氧化抑制剂通过阻断TCA循环延长胶质瘤异种移植模型生存期。胶质瘤微环境重塑方法包括重新构建胶质瘤细胞微环境中的细胞通讯与免疫微环境调控网络。例如，抑制 JAK-STAT 通路通过重塑胶质瘤微环境中的细胞通讯，阻断髓样细胞与T细胞通讯，恢复T细胞功能使肿瘤细胞裂解死亡。Moreover，多组学技术揭示胶质瘤微环境调控网络，通过三联疗法抑制纤维化微环境的作用，提高疗效。

4.1 Tumor Microenvironment

Glioblastoma remains difficult to cure due to its aggressive invasiveness, resistance to treatment, and high probability of recurrence^[2]. A single perspective, particularly one limited to clonal selection or population-level changes in functional molecules, confined to individual cells or mechanisms, and ignoring microenvironmental interactions, is insufficient to fully understand the comprehensive driving factors behind glioma invasion, resistance, and recurrence. To address these challenges, integrated multi-omics strategies have enabled the comprehensive observation of glioma's macro trends from genetic states to protein metabolism within defined spatial patterns, while distinguishing these trends at single-cell resolution. Therefore, we believe that discussions based on multi-omics will provide new insights and impetus for understanding the glioma tumor microenvironment.

4.11 Heterogeneity and Plasticity

Previous studies have only revealed the heterogeneity of the glioma microenvironment, including molecular state heterogeneity^[50], cell type and density variation^[120], and differences in nutrition and angiogenesis^[121], without explaining the corresponding functional characteristics. To overcome these limitations, multi-omics has provided a paired map of glioma heterogeneity and functionality, emphasizing the necrotic core of the tumor and its invasive periphery. Firstly, multi-omics has supplemented the detailed information regarding glioma microenvironment heterogeneity. Spatial transcriptomics/proteomics, combined with single-cell transcriptomics, has characterized the regional distribution of tumor cells, endothelial cells, and immune cells within gliomas^[122]. However, these

studies have not yet uncovered the anatomical, functional, and phenotypic significance of these regions. Notably, three further multi-omics studies have addressed these issues. Ren et al used spatial transcriptomics and single-cell transcriptomics to observe that the tumor core was enriched with oligodendrocyte precursor-like cells. Meanwhile, the invasive microenvironment, rich in neurons, showed a dense accumulation of invasive growth, resembling glioma stem cells^[123]. Single-cell transcriptomics and spatial transcriptomics demonstrated that the mesenchymal-like state was predominantly present in the tumor core, enriched in regions with hypoxia and microvascular proliferation, and expressed corresponding genes. In contrast, malignant cells in the infiltrative regions exhibited increased expression of genes associated with AC-like, NPC-like, and OPC-like malignant states^[83]. Mathur et al, combining genomics, transcriptomics, and single-nucleus ATAC-seq, revealed: (1) that cells from different origins within the core collectively promote mesenchymal differentiation, with AP-1 as a key mediator; (2) that neuronal hijacking occurs in the periphery, forming a glioma-neuron and microtubule network^[124]. Therefore, multi-omics divides gliomas functionally into two major internal regions: the mesenchymal necrotic core generated to adapt to hypoxia, and the neuronal infiltrative periphery driven by invasion. However, the driving factors behind this heterogeneity remain unclear.

At this point, multi-omics technologies, particularly spatial and single-cell omics, have played a crucial role. Liu et al^[125] used spatial and single-cell omics to uncover the association between mesenchymal features and age, suggesting that non-coding mutations and/or non-genetic factors may play a role in the observed age-specificity, with extracellular/environmental factors potentially being the key drivers. The complementary nature of spatial transcriptomics and proteomics has, for the first time, proposed how the gradual changes in angiogenesis and oxygen transport shape the characteristic five-layer tissue and cell states of gliomas^[126]. Notably, while environmental factors drive glioma's heterogeneity, the mechanisms still require further exploration. To address this, Ravi et al integrated single-cell transcriptomics, spatial transcriptomics, spatial proteomics, and spatial metabolomics to reveal how the stress environment reshapes the spatial heterogeneity of gliomas: The glioblastoma cells, characterized by a "developmental" basal transcriptional program, continuously proliferate and grow, creating a hypoxic environment through the consumption of large amounts of oxygen. This hypoxia leads to downregulation of cell cycle programs, causing S-phase arrest. Most of these cells subsequently undergo apoptosis, contributing to the typical necrosis observed in glioblastoma^[127]. Overall, the driving factors composed of environmental stress and survival pressures promote active clonal evolution, referred to as the plasticity of gliomas. Environment-driven plasticity aids in understanding the heterogeneity of gliomas, though the mechanisms behind further malignant biological behaviors remain unclear.

4.12 Survival, Proliferation, and Invasion

The importance of multi-omics in glioma research lies in its ability to comprehensively investigate the "gene-phenotype" axis within a precise spatiotemporal context. This approach enables the identification of key microenvironmental mechanisms driving glioma cell survival, proliferation, and invasion.

In the rapidly proliferating regions of gliomas (i.e., the necrotic core), multi-omics centered on metabolomics suggests that continuous cell activity consumes large amounts of oxygen and nutrients. Thus, gliomas are forced to adapt through metabolic reprogramming to survive and proliferate in this environment. For example, bulk transcriptomics, metabolomics, and spatial transcriptomics reveal that glioblastoma stem cells (GSCs) with limited carbon supply utilize lactate as an alternative energy source to maintain the TCA cycle, supporting both proliferation and invasion^[128]. As previously noted, hypoxic regions typically exhibit characteristics of mesenchymal transformation, a mechanism also validated by multi-omics. Spatial transcriptomics and single-cell omics reveal that BGN, through LRP6 binding, invades tumor-initiating cells, activating the Wnt/ β -catenin pathway, promoting glioma's MES-like transformation and enhancing invasiveness^[129]. Interestingly, metabolic reprogramming in gliomas often occurs simultaneously with mesenchymal transformation. He et al^[130] used single-cell transcriptomics and spatial transcriptomics to discover that oxidative stress in high-grade gliomas activates the AP1 transcription factor, inducing a shift from oxidative phosphorylation to glycolysis, thereby preventing tumor cell apoptosis and promoting proliferation, alongside epithelial-mesenchymal transition. Notably, this study, like that of Mathur et al^[124], highlights the critical role of AP1 in mesenchymal transformation. Multi-omics has also revealed that in mesenchymal glioblastomas, macrophages with a lipid-rich phenotype transfer

lipids to tumor cells via an LXR/Abca1-dependent mechanism, thereby meeting the high metabolic demands of mesenchymal cancer cells^[131]. In summary, mesenchymal transformation represents a glioma cell state that maintains invasiveness under oxygen and nutrient-deficient conditions, requiring corresponding metabolic reprogramming strategies to sustain it. Therefore, both metabolic reprogramming and mesenchymal transformation serve as mechanisms by which gliomas maintain their survival, proliferation, and invasiveness in harsh environments.

In the same hypoxic regions, gliomas not only undergo metabolic reprogramming and mesenchymal transformation but also recruit specific immune cells to form an immunosuppressive microenvironment. For example, multi-omics observations reveal that glioma cells in hypoxic/mesenchymal/necrotic regions secrete chemokines to recruit numerous myeloid cells with immunosuppressive features^[132]. Single-cell transcriptomics and spatial transcriptomics further identified downregulation of TREM2 in tumor-infiltrating myeloid-derived suppressor cells (MDSCs) and tumor-associated macrophages (TAMs), with the degree of downregulation gradually decreasing from the tumor core to the peripheral regions, thereby shaping an immunosuppressive state^[133]. Notably, the immunosuppressive state in these regions is not a result of clonal selection by the immune cells themselves but rather is reshaped by glioma cells through cell communication to achieve immune evasion. For instance, single-cell transcriptomics and spatial omics revealed that GSCs and MDSCs are spatially adjacent, with GSCs secreting MIF to activate MDSCs and suppress immune responses^[134]. Single-cell and spatial transcriptomics analyses indicate that in tumor regions enriched with mesenchymal transcriptional signatures, HMOX1+ myeloid cells activate the JAK-STAT pathway through IL-10, leading to dysfunction in effector T cells and shaping the immunosuppressive microenvironment^[135]. Furthermore, the MDSCs recruited by glioma cells to the core region maintain a non-exhausted state, not solely for immune evasion, as the latter only requires the induction of immune cell exhaustion, such as T cell exhaustion. Supporting this, multi-omics have revealed a positive feedback loop between glioma cells and MDSCs that promotes mutual proliferation. Single-cell and spatial transcriptomics have revealed that glioma cells with mesenchymal stem-like features recruit early precursor MDSCs through chemokines, upregulating their metabolic and antioxidant programs, which promotes active proliferation in the nutrient-deprived microenvironment. In turn, these MDSCs, through ligand-receptor signals such as FGF11-FGFR1, facilitate the conversion of tumor cells to a mesenchymal and stem-like state, supporting their growth and proliferation^[135]. In addition to MDSCs, gliomas also recruit TAMs through cytokine secretion^[136]. Interestingly, while there is overlap between these cell types and MDSCs, they are still discussed separately. Multi-omics have demonstrated that TAMs, once recruited, are induced by the microenvironment to undergo M2 polarization, a state considered to be immunosuppressive^[137, 138]. Glioma cells and TAMs also engage in a bidirectional positive feedback loop, continuously promoting the mesenchymal transformation of glioma cells and the M2 polarization of macrophages^[139]. Notably, multi-omics have confirmed that M2-type TAMs not only facilitate immune evasion in gliomas but also promote tumor cell invasion. Bulk, single-cell, and spatial transcriptomics have shown that tumor cells and stromal cells in the hypoxic regions of glioblastomas upregulate C3, which, through the C3aR-dependent pathway, induces M2 polarization of microglia and macrophages, enhancing tumor invasiveness^[140]. Single-cell transcriptomics and spatial transcriptomics have also identified that microglia expressing BRMS1 may lead to M2 polarization of TAMs, activating the SPP1/CD44 pathway in glioblastoma cells, thereby inhibiting apoptosis and promoting invasion^[141]. Recently, multi-omics technologies have revealed that plasma cells in the B lineage can also promote glioma cell proliferation, a previously unrecognized finding^[142]. Furthermore, multi-omics have uncovered the immune-metabolic axis interaction that promotes glioma proliferation. Bulk RNA sequencing, single-cell RNA sequencing, and spatial transcriptomics suggest that when glioblastoma is in a hypoxic environment, VASN promotes NFκB nuclear translocation, increasing tumor cell glycolysis and lactate production, which in turn suppresses TNFα secretion from tumor-associated macrophages. Low levels of TNFα create an immunosuppressive environment and promote glioma cell proliferation and invasion^[143].

However, despite an increasingly profound understanding of the glioma immune microenvironment, some gaps remain: (1) There is no clear consensus on the status and lineage formation of immune cells; (2) Overlapping cell types complicate the clear assessment of their functions. To address these issues, Gupta et al^[144] used single-

cell and bulk transcriptomics to define TAMs into three polarization states: a broad pro-inflammatory state, a chronic inflammation-stimulated state, and an anti-inflammatory state, thus overcoming the limitations of the M1/M2 polarization model that fails to describe heterogeneity and plasticity. Miller et al^[145] also used single-cell RNA sequencing, chromatin analysis, and spatial transcriptomics to identify four immune-regulatory expression programs in myeloid cells, which do not depend on cell type, developmental lineage, or tumor gene state. Therefore, both studies suggest that our understanding of the glioma immune microenvironment should move beyond cell types and lineages and focus on expression programs and patterns for differentiation and study.

Overall, multi-omics has revealed the triadic interactions between tumor biology, metabolism, and immunity, forming positive feedback loops through communication networks that ultimately induce glioma mesenchymal transformation in harsh survival environments to sustain survival, proliferation, and invasion.

As previously described, in the core region, which is relatively deficient in oxygen and nutrients, glioma cells tend to favor glycolysis and lactate as energy sources^[128, 130]. In contrast, in the periphery, a region with relatively higher oxygen levels and lower glioma cell density, gliomas are more likely to utilize non-glucose alternative energy sources. For example, genomics, proteomics, and spatial multi-omics have revealed that in the aerobic regions of glioblastomas, tumor cells undergo metabolic remodeling through an AMPK-PGC-1 α axis-dependent adaptive program, shifting toward non-glucose nutrient utilization^[146]. Spatial metabolomics has also shown a gradient of fatty acid metabolism heterogeneity in gliomas, with the intensity of long-chain acylcarnitine molecules significantly increased at the tumor-normal tissue interface^[89]. Interestingly, considering the neural connections and invasiveness at the boundary^[124], fatty acid metabolism may be crucial for meeting the high energy demands of invasiveness and promoting microtubule network formation. On one hand, this suggests that carbohydrates may be the true metabolic essential for gliomas, as alternative energy sources are prioritized in favorable environments to preserve essential survival resources. On the other hand, the new metabolic reprogramming is associated with neuro-tumor interactions and microtubule network formation.

Notably, multi-omics suggest that in the peripheral or marginal regions, which are more accessible for communication with the external environment, gliomas interact with neurons. This area of study is referred to as cancer neuroscience^[147]. A combination of single-cell and bulk transcriptomics revealed that approximately 41.3% of cells in IDH-mutant gliomas exhibit transcriptional features of both neurons and oligodendrocyte precursor cells, which receive GABA-mediated inputs from neurons and are capable of firing single, brief action potentials^[148]. This suggests that a specific tumor cell population may communicate with neurons, promoting glioma progression. Cell-specific proteomics revealed that when glioblastoma initiating cells near the lateral ventricles interact with neural progenitor cells, cathepsin B is upregulated, promoting glioma proliferation and inhibiting neural progenitor cell maturation^[149]. This confirms the existence of a tumor-neuron axis. Furthermore, multi-omics observations indicate that glioma cells facilitate both tumor-neuron and neuron-neuron synaptic connections. For instance, combining single-cell transcriptomics, bulk transcriptomics, and proteomics, it was demonstrated that glioblastoma induces synapse formation between neurons and glioma cells both in vitro and in vivo, with glioma cells showing strong neuronal lineage characteristics^[150]. Another study, using bulk and single-cell transcriptomics, revealed that glioma cells from high-functioning brain regions remodel and promote the growth of neuron-to-neuron and neuron-to-glioma synapses through platelet-derived growth factor-1^[151]. This effect was less pronounced in tumor regions with fewer functional connections. Importantly, these synapses, as confirmed by multi-omics, are involved in enhancing glioma invasion. Single-cell transcriptomics revealed that neuron-glioma synapses mediate membrane depolarization through GABAergic input, which increases chloride ion concentration, promoting glioma proliferation^[152]. Therefore, in the more invasive peripheral regions, gliomas interact with neurons via synaptic connections, with a bidirectional positive feedback loop that ultimately promotes tumor invasion. Interestingly, multi-omics found that glioma growth and invasion involve a division of labor among distinct cell populations. A type of long axonal projection, known as microtubules, connects tumor cells, forming a multicellular network with increased resistance to therapy and greater invasiveness^[153]. Initially, single-cell transcriptomics, bulk transcriptomics, and proteomics indicated that astrocytes and mesenchymal glioblastoma cells exhibited the most microtubule connections, regulated upstream by the CH13L1 gene^[154]. Further, using single-cell omics and other experimental techniques, Venkataramani et al demonstrated that glioma cells split into

relatively stationary cell populations connected by microtubules and scattered, active cells that lack such connections. These scattered cells lead glioma invasion, migrating into the brain tissue without microtubule networks. There, neuronal activity induces the formation of new, relatively stationary microtubule structures^[155]. Thus, these studies suggest that glioma invasion is primarily driven by scattered, peripheral cells influenced by neurons, whose goal is to establish new colonies and form microtubule networks, continuing biological processes such as survival, proliferation, and invasion.

In summary, within the context of survival, proliferation, and invasion, the differences in glioma cell density and energy consumption shape distinct microenvironments. These cells exhibit remarkable adaptability, altering themselves (through metabolic reprogramming and cellular state transitions) and communicating with other cells (immune cells, neurons, endothelial cells) to remodel the local environment in a way that supports their survival, much like their host—humans.

4.13 Dormancy and Drug Resistance

If hypoxia and nutrient deprivation are considered environmental stresses driving glioma remodeling, we can similarly view various treatment modalities (radiotherapy, chemotherapy, immunotherapy) as environmental pressures applied to gliomas. Multi-omics has similarly revealed how the plasticity of the microenvironment and its specific mechanisms are shaped under treatment-induced environmental stress.

First, gliomas can acquire chemotherapy resistance through metabolic reprogramming. Single-cell omics, spatial transcriptomics, and metabolomics, through reciprocal validation, have shown that during temozolomide treatment, the expression of the nucleotide reductase subunit M2 increases, which regulates the metabolism of specific dNTPs, thereby shaping temozolomide-specific (rather than other alkylating drugs) resistance^[156]. This study revealed how glioma cells remodel themselves to acquire resistance when facing DNA-targeting treatments.

Second, mesenchymal transition associated with metabolic changes also confers resistance to radiotherapy and chemotherapy in glioma cells. Interestingly, the process of mesenchymal transition itself already exhibits resistance, without the need for a complete shift to a fully mesenchymal state. Combining bulk transcriptomics, proteomics, single-cell transcriptomics, and spatial transcriptomics, Pichol-Thievent et al^[157] revealed that after radiochemotherapy, the brain vasculature induces glioma cells to adopt a VC-Resist state through the FGFR1-YAP1 axis. This state is characterized by therapy resistance, slow cell cycle, stemness, and senescence-like features, situated between the neurogenic-mesenchymal transition. However, these studies did not identify which components of the vascular microenvironment induce the acquisition of resistance. Another study, using single-nucleus RNA sequencing, single-cell open chromatin analysis, DNA, and spatial transcriptomics/proteomics, demonstrated that after radiation therapy, AP1 regulates glioma cell mesenchymal transition via cis-regulatory enhancers, positively modulating mesenchymal phenotypic traits such as inflammation and radiation resistance^[158]. This study further affirms the critical role of AP1 in mesenchymal transition and explains that the mesenchymal state is a universal adaptation to harsh environments, including hypoxia, nutrient deprivation, and radiation-induced stress.

Additionally, immune-vascular interactions in different regions present distinct resistance mechanisms but all serve to hinder drug delivery to the tumor interior. In peripheral regions, single-cell and spatial transcriptomics revealed that TAMs, derived from monocytes and accumulated in the perinecrotic area, disrupt endothelial cell adhesion by activating paracrine signals from adrenal medullary cells, leading to vascular hyperpermeability and subsequent drug leakage^[159]. In more central regions, single-cell spatial transcriptomics confirmed that the perivascular microenvironment in recurrent glioblastomas is enriched with macrophage populations exhibiting unique transcriptional features, which tightly localize to prevent the leakage of other immune cells and blood components, potentially hindering drug delivery^[160]. Finally, Cheng et al^[161] used proteomics and transcriptomics to reveal the dual drug resistance role of myeloid cells in glioblastoma: by upregulating humanin, these cells stimulate the GP130-EPK pathway, driving temozolomide resistance in glioma cells while accelerating blood-brain barrier formation and reducing drug delivery.

Metabolic reprogramming, mesenchymal transformation, and vascular remodeling enable gliomas to resist

chemotherapy and radiotherapy, but these mechanisms do not apply to immune therapies that primarily activate T cells. Recently, multi-omics studies have identified T cell exhaustion as a key factor in the failure of immune therapies. Single-cell transcriptomics indicates that T cell populations activated by anti-PD-1 treatment recruit new T cells from peripheral blood through chemokines. However, TAMs still dominate the myeloid cell population and, through the activation of immune checkpoints such as TIGIT and CTLA-4, drive the expansion of T cells into an exhausted state, ultimately leading to immune therapy failure^[162].

Overall, the plasticity and adaptability of gliomas to both them and their environment contribute to drug resistance. This should be understood as a strategy for glioma adaptation to the environment, rather than simply a result of clonal selection. However, it remains unclear how glioma cells will transform when the environment changes (i.e., when stress levels decrease). To address this, Watson et al used proteomics, single-cell transcriptomics, and spatial transcriptomics to reveal, during the window period between 7 and 14 days after BLZ945 treatment, that fibroblast-like cells derived from the vasculature mediate the generation of fibrotic areas by producing extracellular matrix and recruiting immune cells. These regions shape immunosuppression by exhausting T cells, thereby protecting the remaining dormant glioma cells from immune attack. Recurrent growth is driven by tumor cells escaping from the fibrotic structure into a local environment that promotes growth and migration^[163]. Therefore, this study highlights an important point: gliomas undergo "protective dormancy" and "proliferative awakening" in response to environmental stress, with these being two distinct phases.

4.14 Awakening and Recurrence

Multi-omics has provided greater insight into the mechanisms underlying glioma recurrence by enabling microscopic resolution in a defined spatiotemporal context.

First, after recurrence, glioma cells transition from a dormant state to an invasive state, specifically a neuronal state. A comprehensive analysis combining genomics, transcriptomics, proteomics, and single-cell omics in 123 longitudinal glioblastoma cases suggests that recurrent tumors may induce molecular transitions of tumor cells to a neuronal state via the WNT/PCP pathway and BRAF kinase, promoting synapse formation^[164]. Moreover, following recurrence, mesenchymal transformation and neural connectivity are reactivated. Integration of single-cell RNA sequencing, single-nucleus ATAC-seq, and longitudinal glioblastoma spatial transcriptomics revealed: (1) the vascular microenvironment in recurrent patients promotes mesenchymal transformation of glioma cells through the AP1 and TNF signaling pathways; (2) neurons accumulate during synaptic organization, suggesting extensive neural network reorganization and synapse formation in recurrent glioblastomas^[165]. Finally, immune evasion continues to occur through immunosuppression. Single-cell transcriptomics and proteomics revealed significant enrichment of M2-polarized TAMs in recurrent malignant gliomas, which activate the PI3K/Akt/HIF-1 α /CA9 pathway in malignant glioma cells through the SPP1/CD44 interaction, suppressing immune responses and promoting proliferation and recurrence^[166].

In conclusion, from a biological perspective, recurrent gliomas appear to return to a state of uncontrolled proliferation, while simultaneously readapting to the environmental changes induced by this proliferation, such as hypoxia, nutrient deprivation, immune stress, and neural communication.

4.2 Novel Subtypes

Gene expression-based clustering and the well-established three GBM transcriptomic subtypes (proneural, classical, and mesenchymal) are widely used due to their consistency and stability^[167, 168]. However, the complexity and inconsistency of glioma omics data call for the involvement of multi-omics in classification^[169-172]. From upstream genetic alterations to downstream functional features, the integration of multi-omics in glioma classification can address the heterogeneity at different layers that is often overlooked between the known subtypes. For example, previous studies have shown that the methylation status of the MGMT promoter in glioblastoma is correlated with patient prognosis in temozolomide treatment^[173]. However, these studies didn't explain the metabolic and functional differences between the two subtypes. To tackle this, Chen et al.^[174] conducted a parallel profiling of metabolomics and proteomics and found that ketone body synthesis and degradation, glycerophospholipid metabolism, and fatty acid degradation were key differentiating pathways

between the two methylation subtypes. Furthermore, combining transcriptomic, methylomic, radiomic, histological, and clinical features has been applied to diffuse midline glioma (DMG) studies. The resulting DMG_K27-BRAF/FGFR1 subtype exhibited features such as microcalcification, senescence programs, and preferential mutations in H3.3-K27M. This supplemented the previous descriptions of “DMG, H3 K27-altered,” which did not capture the full disease diversity^[175]. Studies conducted in patient-derived glioma cell lines demonstrate the broad applicability of multi-omics. Wu et al.^[176] characterized three molecular subtypes using transcriptomics, genomics, and drug response data, revealing that the OXPHOS subtype, which shows alterations in RB1, MYC/MYCN, is sensitive to histone deacetylase inhibitors, oxidative phosphorylation inhibitors, and HMG-CoA reductase inhibitors. Importantly, the OXPHOS subtype was distributed across the subtypes identified by Wang et al.^[168]. This suggests that multi-omics can uncover additional internal features within new subtypes that can offer new avenues for clustering. Overall, these studies not only bolster that previous subtype classifications are reliable but also unravel previously unrecognized heterogeneity within these subtypes. They further characterize the multi-omics features of newly classified subgroups, which provide a valuable guidance for clinical trial interpretation and patient management based on these new subtypes.

In addition to complementing known subtypes, the coupling of omics data has led to the identification of new glioma subtypes, which reflect multi-layered heterogeneity and possess prominent molecular features and clinical relevance. These findings are summarized in Table 1.

Various algorithms and scoring methods, such as K-means^[177], Non-negative Matrix Factorization (NMF)^[171], Similarity Network Fusion (SNF)^[178], ssGESA^[179], and sciBiPaD^[180], have been used for data processing to identify differences and similarities that can aid in classification. NMF and consensus clustering are two commonly used clustering methods. NMF, based on non-negative matrix factorization, has lower uniqueness in its solutions but is more interpretable^[181]. In contrast, consensus clustering is a resampling-based ensemble method that enhances robustness while reducing interpretability^[182]. A recent study employed MOVICS to integrate multiple omics data, yielding four LGG integrative clusters (ICs) by ten integrated clustering algorithms, which were able to independently predict patient survival prognosis^[183].

In addition to varying algorithms, strategies for omics-based classification also differ. One common approach is single-omics-based classification, typically using transcriptomics, to provide new classification standards, which are systematically characterized by the heterogeneity between subtypes using other omics data. For example, transcriptomic subtypes classification related to tumor^[179], mitochondrial^[184], and lysine acetylation regulators^[185] have exhibited robust prognostic capabilities through K-M survival analysis. Leveraging genomic alterations and tumor microenvironment features, differences between subtypes were further depicted. Therefore, a “transcriptome and genome” combined approach can merge genetic mutations with their potentially mediated transcriptomic changes, revealing potential synergistic drivers of tumor progression. Moreover, scRNA-seq and spatial transcriptomics can extend glioma tissue heterogeneity to cellular and spatial levels. Neurons are distributed in the peripheral areas of gliomas and depend on dopamine breakdown, which explains the clinical outcomes of metabolic subtypes^[186]. Pathway-based GBM classification in tandem with multi-omics revealed an MTC subtype characterized by the deletion of SLC 45 A1^[180]. The team subsequently characterized four functional subtype-specific multi-omics features and identified the primary kinases driving GPM and PPR subtypes, such as PKC δ and DNA-PKcs. Additionally, they developed a GBM probability functional classifier based on RNA-seq, which demonstrated high AUROC, specificity, sensitivity, and accuracy^[187].

Another classification strategy takes account of multi-omics data to derive new subtypes, thus maximizing the use of data from multiple layers^[177, 178, 183, 188]. This strategy can involve direct incorporation of multi-omics data clustering or clustering across multiple single-omics clusters, known as “clustering of clusters”^[189, 190]. These studies have employed a variety of omics types and different combinations for multi-omics clustering, with transcriptomics and DNA methylation often being selected together. Building on this, Yang et al. included SNPs in their clustering approach, which identified unsupervised GBM subtypes along with three methylation sites and 15 gene mutation patterns that can differentiate the subtypes^[188]. Notably, the HX-2 subtype exhibited more gene co-occurrence events and poorer survival prognosis. In contrast, Picard et al. integrated transcriptomics and

proteomics because of the known inconsistency between mRNA and protein expression, and they identified two subgroups of pilocytic astrocytoma (PA)^[178]. Group 1 was characterized by the overexpression of immune response pathways, such as "interferon signaling" and "T-cell receptor signaling." Given the known or unknown discrepancies between the genomic, transcriptomic, and translational levels, multi-omics integrated analysis may reveal the dominant factors influencing these differences. From this perspective, this classification strategy could avoid the potential flaws associated with single-omics-driven multi-omics classifications, which might resemble single-omics classifications.

Additionally, the composition of the tumor immune microenvironment has been identified as a critical factor in tumor-immune interactions. Immune subtypes based on multi-omics reflect glioma's immune heterogeneity, such as subtype-specific fibroblast^[191], lymphocyte infiltration differences^[191], and variations in the activation levels of immune responses and metabolic processes^[192]. These differences often impact the effectiveness of immunotherapy in glioma patients, necessitating multi-omics analysis to explore the correlations between immune heterogeneity and patient treatment responses, which genetic alterations, pathway enrichment, and metabolic changes might define. Wang et al.^[171] performed NMF clustering on the proteogenomic features of GBM and unearthed three multi-omics subtypes in IDH-WT tumors: nmf1 (enriched in neuronal activity-related pathways), nmf2 (enriched in immune response pathways), and nmf3 (enriched in cell cycle pathways). They then used the xCell method, transcriptomic data, and protein deconvolution to obtain and validate four GBM immune subtypes (im1-4), with the multi-omics subtype nmf2 being enriched in immune subtypes im1 and im2. This illustrates that while multi-omics subtypes may not completely distinguish finer immune heterogeneity, they can still reflect the characteristics of immune subtypes. Other studies based on glioblastoma stem cells (GSCs)^[193], epithelial-to-mesenchymal transition (EMT)^[194], and liquid-liquid phase separation^[195] further demonstrate that multi-omics can leverage the heterogeneity of glioma origin and biological behavior to distinguish new glioma subtypes.

Importantly, it should be noted that only a few studies explain the rationale behind selecting specific omics data for clustering^[178], and how to choose multiple omics datasets for integration remains an important consideration. Picard et al. found that integrating transcriptomics and proteomics data yielded better separation compared to single-omics methods, but further integration with DNA methylation data disrupted the original subtype separation^[178]. Therefore, it is inappropriate to stack more omics data for glioma classification without considering the separation effectiveness of integrated subtypes. Additionally, in some cases, single-omics features can effectively represent multi-omics classifications^[178, 190]. Moreover, integrated multi-omics classification requires datasets with multiple overlapping samples, and clinical translation must consider limitations such as sample quality or funding constraints. In future practice, optimizing the "omics combinations" and integration methods will be essential to handle the consistency and inconsistency between omics datasets, thereby enhancing the performance of multi-omics classification.

Building on molecular feature heterogeneity, multi-omics subtype classification offers new perspectives for subtype-based glioma treatment. Conventional treatment strategies for glioma, including surgery, radiation, and chemotherapy, often fail to achieve the expected efficacy or result in tumor recurrence due to the intrinsic heterogeneity of gliomas^[167, 168]. Currently, glioma classification based on single-omics data is insufficient to indicate which subtypes are more responsive to specific treatments. For instance, transcriptomic classification has not effectively predicted GBM prognosis and pharmacological vulnerabilities. Therefore, integrating multi-omics data has the potential to provide subtype-specific therapeutic strategies. Combining GBM gene expression and scRNA-seq data, Alhalabi et al. discovered that MES GSCs are sensitive to the SRC inhibitor dasatinib, while PN GSCs are particularly resistant, with this treatment response being associated with SERPINH1^[196]. They further confirmed, through proteomics, shRNA drop-out screening, and transcriptomics, that WEE1 is a target for overcoming dasatinib resistance in PN GSCs, pointing toward combination therapies as a strategy to combat subtype-specific drug resistance^[197]. Additionally, the new subtypes suitable for treatment with temozolomide^[191], bevacizumab^[198], specific inhibitors^[176], or differentiation therapies^[190] have been identified in various studies.

On the other hand, multi-omics subtype classification studies have also suggested potential treatment strategies

specific to each subtype. Oligo1 tumors may be more suitable for therapeutic strategies targeting Wnt, EGFR, and signaling pathways, whereas Oligo2 tumors have a highly immunosuppressive tumor microenvironment (TME) and may be candidates for checkpoint inhibition immunotherapy^[192]. Other studies have also compared potential druggable gene targets^[183], immune scores, and responses to immunotherapy^[184, 198] across subtypes. Ultimately, the goal of multi-omics integration for glioma subtype classification at multiple levels is to provide personalized treatment plans, improve clinical outcomes for patients, and avoid unnecessary economic and physical burdens from ineffective treatments for certain subtypes.

Interestingly, due to the characteristic abnormalities of each subtype, some researchers believe that subtype conversion does not occur at different stages of glioma^[167]. However, the possibility of "subtype development" or "dynamic subtypes" should not be overlooked. Binder et al. performed similarity tree analysis on the molecular tumor landscapes of methylation and transcriptomic LGG subtypes and proposed the "subtype development tree" model^[199]. Cellular dynamic changes and plasticity also provide a potential for glioma subtype conversion. Garofano et al.^[180] untangled that four GBM cell states converge on two biological axes, reflecting changes in cell composition in recurrent GBM, thus illustrating dynamic changes within gliomas. Transcriptomic studies have elucidated that SPI1-mediated transcription of MIR222HG promotes the proneural-to-mesenchymal transition of glioma stem cells.^[200] Similarly, it has been found that individual cells within GBM can contain two different subtypes, and this plasticity has been extensively reviewed^[68, 201]. Therefore, future multi-omics subtype studies could further explore the potential and trends of subtype conversion and design strategies aimed at reducing the malignancy of gliomas. Such therapeutic strategies have already been explored in other cancers, such as pancreatic cancer.

Table 1. A summary of multi-omics for glioma subtypes

Glioma types (data set)	Muti-omics involved	Clustering strategy	Subtypes	Main characters	Reference
GBM (data from oncopression, TCGA, and REMBRANDT; samples from 52 non-recurrent GBM patients (severance cohort))	Microarray; WES, CNA and G-CIMP status	Prognosis scores (ssGESa)	Invasive (poor), mitotic (favorable), and intermediate	Invasive subtype: increased invasiveness; Mitotic subtype: enriched MGMT methylation	Park et al ^[179]
GBM (n = 117 samples from GDC)	SNP, CNV, DNA methylation, mRNA expression	Unsupervised clustering and CoCA	HX-1 and HX-2	HX-2: higher gene co-occurrence events, tumor mutation burden (TBM), and poor median overall survival	Yuan et al ^[188]
GBM (n = 99 treatment-naive samples)	CNV, bulk RNA, and LC-MS/MS (protein and phosphoprotein expression); Lipid composition and metabolomic features	NMF	Three IDHwt subtypes: proneural-like (nmf1), mesenchymal-like (nmf2), and classical-like (nmf3)	Nmf1: pathways enrichment of neuronal activity-related nmf2: pathways enrichment of immune response nmf3: pathways enrichment of cell cycle	Wang et al ^[171]
	immune enrichment; confirmed by snRNA-seq data and protein deconvolution (TCGA cohort)	Single-sample GSEA by xCell	Four immune subtypes (im1-4)	Im3: a relative depletion of macrophage-microglial; significantly enriched T lymphocytes and natural killer (NK) cells.	

IDHwt GBM (Three separate sc-RNA data sets; TCGA (n = 534); external validation: TCGA (n = 146), CGGA (n = 183), GSE13041 (n = 219))	scRNA-seq, CNV, SNV, DNA methylation, mi-RNA expression, reverse-phase protein array, Metabolic assays	scBiPaD and Consensus clustering	Glycolytic/plurimetabolic (GPM), mitochondrial (MTC), neuronal (NEU), and proliferative/progenitor (PPR)	MTC: the most favorable clinical outcomes; completely dependent on oxidative phosphorylation for energy production; marked vulnerability to inhibitors of oxidative phosphorylation	Garofano et al ^[180]
GBM (multi-omics datasets from TCGA and GSE4272)	Genomic statistics of CNV, MET, and RNA expressions	iClusterPlus package and NMF	iC1-3	iC2: increased percentage of neutrophils; higher methylation rate of DEG; MYBP6 and IGFBP6 were markedly advanced with the wickedest prognosis	Ma et al ^[189]
LGG (four multi-omics cohorts from CGGA, TCGA)	Somatic mutation, CAN, and RNA-seq data	Consensus clustering	Three immune subtypes: Im1, Im2, and Im3	Im2: worst outcome; elevated cytolytic activity; exhausted CD8+ T cells; macrophages; higher levels of aneuploidy; tumor mutation burden	Wu et al ^[202]
GBM (multi-omics datasets from TCGA and GSE4272)	Genomic statistics of CNV, MET, and RNA expressions	iClusterPlus package and NMF	iC1-3	iC2: increased percentage of neutrophils; higher methylation rate of DEG; MYBP6 and IGFBP6 were markedly advanced with the wickedest prognosis	Ma et al ^[189]
LGG (n = 500 samples from TCGA)	CNV, methylation, and expression profile data of CNV-miRs and DNAm-miRs, SNV	MOVICS	Four molecular subtypes reflected the multi-layered pattern of the CNV-miRs and DNAm-miRs: IC1-4	IC2: the worst prognosis; 1p/19q co-deletion; bearing nearly all the mutations for EGFR; highest TMB; higher enrichment of all immune signatures	Yang et al ^[195]
LGG (n = 423 samples from TCGA)	Somatic variations, CNA	NMF	Subtypes based on liquid-liquid phase separation: LS1-4	Four subtypes differ in prognosis, clinicopathological features, cancer markers, genomic alterations, TIME patterns, and immune responses	Zheng et al ^[195]
ADHGG (multi-omics from CGGA, TCGA, and Rembrandt cohort)	scRNA-seq, stRNA-seq, bulk RNA-seq, and CNV	Consensus clustering	Two cancer-associated fibroblasts (CAF) subtypes: A and B	Subtype A: higher CAF infiltration rate; worse prognosis as well as responsiveness to immunotherapy and radiotherapy; higher sensitivity to temozolomide	Zhang et al ^[191]
OG (n = 141 from POLA)	miRNA-seq, mRNA expression and DNA methylation array	Consensus clustering	C1-C5; C1, C4 and C5 are three new subtypes of 1p/19q co-deletion gliomas (O1-3)	O1: higher expression of oligodendrocyte precursor cell markers, especially GPR17 and CCND1; frequent loss of Chromosomes 9p and 14; poorer outcome associated with high MYC activity	Kamoun et al ^[190]

OG (discovery cohort: n = 137 samples from TCGA; validation cohort: n = 124 samples from CGGA and n = 94 samples from POLA)	Somatic variations, DNA methylation, Transcriptome analysis, Metabolic profiling	Consensus clustering	Two immune subtypes: Oligo1 and Oligo2	Oligo1: enriched cell proliferation, cell cycle, and Wnt signaling pathways Oligo2: increased activation of immune response and metabolic process; Higher T cell/APC co-inhibition and inhibitory checkpoint levels	Wu et al ^[192]
PA (n = 28 tumor tissue samples; validation cohorts from ICGC (n = 73), CPTAC (n = 65), and Kool et al (n = 191))	RNA-seq and Mass spectrometry (proteomic profiling)	SNF	Group1 and Group 2	G1: Younger; more supratentorial location; poor prognosis; enriched immune response pathway and T cell-related genes. G2: Older; more subtentorial location; better prognosis; enriched nerve signaling pathways and cell respiration related genes	Picard et al ^[178]
Glioma (discovery cohorts: n = 563 samples from TCGA; validation cohort: n = 970 samples from CGGA)	RNA-seq and DNA methylation	K-means clustering and SVM	Two prognostic subtypes: G1-2	G1: better prognosis; enriched gene in glutathione metabolic pathway G2: worse prognosis; enriched gene in herpes simplex virus 1 infection pathway	Tian et al ^[177]

4.3 Promising Biomarkers

4.3.1 Early Diagnosis

Magnetic Resonance Imaging (MRI) faces significant challenges in diagnosing minimal residual disease (MRD) and is prone to misdiagnosis and missed diagnoses due to tissue inflammation^[203]. In addition to the challenges faced in MRI diagnostics, tissue biopsies also have limitations in detecting tumor progression and assessing treatment response in real time, and they may cause complications. As a result, the clinical practice of gliomas urgently requires novel methods for early diagnosis and dynamic monitoring.

Public databases have significantly reduced the difficulty in obtaining glioma omics data, overcoming the limitations of geographic and temporal constraints^[204]. Integrating cross-omics data of gliomas can compensate for the narrow biological perspective of single-omics approaches, offering a more comprehensive view. Such integrations have already been utilized to uncover diagnostic biomarkers (summarized in Table 2). A notable example is the study conducted by Ren et al., who integrated single-omics data, transcriptomic data, and clinical information to find that the Area Under the Curve (AUC) score for SIRPB1 in gliomas was 0.674^[205], suggesting its potential as a diagnostic marker for gliomas. To address the lack of peripheral detectability in this study, another research team integrated transcriptomics, genomics, epigenomics, and proteomics data to detect CSF-specific brain protein PRKCG, which could serve as a potential diagnostic biomarker for gliomas^[206]. However, due to the spatiotemporal heterogeneity of gliomas, diagnostic biomarkers based on tissue samples not only provide localized information with a risk of misinterpretation but also fail to reflect the dynamic internal changes of gliomas in real-time. Therefore, we focus more on diagnostic approaches that can perform dynamic monitoring and provide comprehensive information, such as peripheral, repeatable liquid biopsy techniques to guide early screening and diagnosis of gliomas (summarized in Table 2).

Compared to tissue-based detection, using body fluids such as blood for biomarker detection involves less invasiveness, higher reproducibility, and easier collection. Therefore, serum biomarkers for GBM are more feasible for clinical use. For instance, a study used transcriptomic and epigenomic data to define a set of serum

features related to cfDNA methylation (GeLB), achieving 98% accuracy in distinguishing primary glioma patients from metastatic glioma patients^[207]. Notably, through comprehensive analysis of serum protein and metabolite levels, it was found that a combination of ten omics features served as a biomarker, achieving significantly higher diagnostic performance ($AUC = 1$)^[208]. Additionally, Zhou et al. screened 11 lipid markers in plasma using metabolomics and transcriptomics methods to develop a diagnostic model that also showed promising diagnostic accuracy for MBG patients. In the test cohort, the accuracy was 94.67%, specificity was 95.34%, sensitivity was 94.30%, and AUC was 0.9877^[209]. Significantly, the LC-MRM-MS-based assay developed in their study reduced the average sample analysis time, making it more suitable for clinical application.

The contents released from local tumor sites are easily diluted by the bloodstream, whereas changes in cerebrospinal fluid (CSF) can more sensitively reflect the pathological processes of the central nervous system. A notable example is the study conducted by Yoko Fujita, which utilized genomic and metabolomic analyses of CSF samples obtained through four different methods. This study revealed that CSF D-2HG levels could distinguish IDH mutant from IDH wild-type gliomas, with an AUC of 0.7376^[210]. However, liquid biopsy based on CSF still faces challenges. First, physiological and hormonal differences related to gender can influence the metabolite levels in the CSF of GBM patients. Therefore, when using CSF metabolite-associated GBM diagnostic markers, gender-specific reference values need to be applied^[211]. Second, previous studies have shown that CSF biomarkers are closely related to the methods of sample collection^[212]. To accelerate clinical translation, it is necessary to popularize sequencing technologies, reduce costs, and standardize collection procedures.

Urine, compared to CSF, is less invasive and allows for repeat sampling, enabling the accumulation of systemic physiological and pathological changes and providing access to abundant uEVs (urinary extracellular vesicles)^[213]. However, the accuracy of urine testing is still influenced by kidney function and blood biochemical characteristics^[214]. Current research on urinary biomarkers for glioma diagnosis primarily employs single-omics techniques, and more multi-omics studies focusing on urine are needed in the future.

Saliva, another non-invasive liquid sample, can also be used for detection. However, current research has mainly focused on single-omics studies of saliva, and more multi-omics investigations are necessary.

Single-sample biopsies may not fully represent the complexity and heterogeneity of a patient's tumor^[215]. Furthermore, single samples are prone to interference from external factors, which can reduce diagnostic accuracy. Therefore, the use of multiple liquid samples for cross-validation is essential. Integrating multiple bodily fluids allows for the detection of a broader range of genetic material or metabolites flowing into these fluids. Combining these liquid media and their respective biomarkers can provide a more holistic approach to cancer management. This multi-fluid integration enhances diagnostic accuracy and treatment effectiveness, ultimately improving the prognosis and personalized care of oncology patients^[216]. Figueroa et al. used genomic and transcriptomic analyses to examine extracellular vesicles in tumor and CSF samples, showing that CSF-derived EVs could be used to diagnose EGFRvIII-positive GBM with 61% sensitivity and 98% specificity. Non-invasive diagnosis of EGFRvIII-positive GBM facilitates precision treatment for patients^[217]. In addition to EGFR mutations, preoperative liquid biopsy for diagnosing the presence of IDH mutations can also impact intraoperative decisions and patient prognosis. To address the issue of intraoperative liquid biopsy for diagnosing IDH mutation status, several studies have performed integrated genomic and metabolomic analyses. Although 2-HG is pivotal for IDH1/2 mutations and is also abundant in urine samples, it shows less accuracy in distinguishing IDH1/2 mutations ($AUC = 0.684$)^[218]. A study identified an alternative diagnostic biomarker, plasma rDL, which showed improved diagnostic performance for distinguishing IDH-mutant from wild-type gliomas ($AUC = 0.805$)^[219]. Notably, the ratio of 2-HG exhibits highly diagnostic performance ($AUC = 0.83$)^[220], suggesting that measuring the ratio of 2-HG levels in plasma and urine is a better diagnostic method for distinguishing wild-type from mutant IDH1 patients.

There are still some limitations in the clinical application of diagnostic biomarkers: databases like TCGA do not encompass data from multiple populations around the world, and the selection of biomarkers still requires further clinical validation. Moreover, the detection rates of circulating tumor cells (CTC) and DNA in blood are relatively low^[221], and the analysis of proteins or metabolites demands specialized techniques and equipment, making it

difficult to meet clinical diagnostic needs^[209]. Plasma-derived extracellular vesicles (EVs) have demonstrated 95% sensitivity and 90% specificity in detecting various cancers^[222]; however, there is still a lack of standardized methods for isolating extracellular vesicles^[223].

We hypothesize that low-invasive, repeatable liquid biopsy could fill the gap in early screening and diagnosis within traditional medical models, and its integration with artificial intelligence for data analysis may become an essential tool for real-time monitoring of molecular changes in gliomas. Therefore, a feasible clinical translation pathway should focus on reducing costs through technological advancements and developing standardized liquid sampling procedures. Ultimately, an integrated multi-fluid, multi-omics “screening-diagnosis-decision” analytical platform may become the cornerstone of precision medicine.

Table 2. A summary of multi-omics for glioma diagnosis

Author (year)	Biomarker	Data sources	Source material	Major omics approaches	AUC
Ren et al ^[205] (2024)	SIRPB1	670 glioma samples ; 1152 normal samples	Glioma samples	Transcriptomics, single-cell transcriptomics	0.674
Liu et al ^[206] (2020)	PRKCG	CGGA Chinese Glioma Genome Atlas ; GEO Gene Expression Omnibus ; GTEx Genotype-Tissue Expression ; TCGA The Cancer Genome Atlas ; Ivy GAP Ivy Glioblastoma Atlas Project	Glioma samples	Transcriptomics, proteomics, epigenomics	N/A
Sabedot et al ^[207] (2021)	GeLB signature (476 CpG sites)	208 serum samples	Serum samples, glioma samples	Genomics, transcriptomics, epigenomics	N/A
Fontanilles et al ^[208] (2024)	5 metabolites: (Pimeloylcarnitine, Leucine, Asparagine, LysoPC a C18:0, PC ae C40:3,) and 5 proteins (NPY, KLK13, SCLY, S100A4, CXCL17)	99 serum samples from control samples and 102 serum samples from unresected glioblastoma patients	Serum samples	Metabolomics, proteomics	1.000
Zhou et al ^[209] (2022)	11 non-redundant lipids (LPC 16:0, LPC 18:0, LPC 18:2, PC 16:0-18:1, PC 16:0-18:2, PC 16:0-20:4, PC 16:0-22:6, PC 18:0-18:1, PC 18:0-18:2, PC 18:0-22:4 and TG 18:1-18:2-18:3)	2,002 participants including healthy controls (n=1079) and MBGs patients(n=923)	Serum samples	Transcriptomics, lipidomics	0.988
Fujita et al ^[210] (2023)	D-2HG	57 CSF samples (48 from the patients with IDH-wildtype glioma , 9 from the patients with IDH-mutant glioma)	CSF sample	Metabolomics, genomics	0.738
Figueroa et al ^[217] (2017)	CSF-derived EVs	23 EGFRvIII tissue-positive GBM patients and 48 EGFRvIII tissue-negative patients	Tumor samples, CSF samples	Transcriptomics, genomics	N/A
Fathi et al ^[218] (2016)	Urinary 2-HG	60 patients with glioma : 16 patients with IDH-WT glioma , 44 patients had IDH1/2-mutated glial tumors	CSF samples, serum, urine	Metabolomics, genomics	0.684
Tuna et al ^[219] (2022)	RDL in plasma	49 GBM patients	CSF samples, serum urine	Metabolomics, genomics	0.805

Lombardi et al ^[220] (2015)	Plasma and urine 2HG (normalized by creatinine) concentration (Ratio_2HG)	84 GBM patients (38 patients with mutant IDH1 , 46 patients with wild-type IDH1)	Serum, urine	Metabolomics, genomics	0.830
--	---	--	--------------	------------------------	-------

4.3.2 Treatment Response

Glioma treatment responses exhibit significant heterogeneity, which may be attributed to various factors such as genetic mutations, epigenetic modifications, and immunosuppressive microenvironment features. Classic biomarkers, including IDH mutations^[224, 225], MGMT promoter methylation^[226], and 1p/19q co-deletion^[227], have facilitated patient stratification and treatment selection. However, their predictive power is limited. Therefore, the application of multi-omics has the potential to enhance treatment response prediction or identify new biomarkers that traditional methods may fail to uncover (summarized in Table 3).

Lewis et al. integrated genomic, transcriptomic, metabolite prediction and clinical datasets to develop a multi-omics machine learning classifier. This classifier demonstrated superior performance compared to previous machine learning classifiers used for radiation sensitivity prediction (AUC: 0.906±0.004)^[228]. Additionally, compared to single-molecule models (AUROC: 0.65, 0.76, 0.7), a model based on chr 1 : 179325742_SOAT1_miR-485- 5p triplet (AUC: 0.91) showed enhanced performance in predicting LGG patients' response to temozolomide^[229].

In terms of drug sensitivity, researchers have utilized new biomarkers to stratify patients into risk groups. The low-risk group typically exhibits higher or more widespread drug sensitivity. These differences may arise from the genetic landscape, functional pathways, metabolic status, immune status, and communication patterns distinguished by the biomarkers, although the specific mechanisms require further exploration^[230, 231]. Additionally, specific “biomarker-drug” pairing predictions have been identified: overexpression of IER3 is linked to high sensitivity to dasatinib^[232]; expression of DNMBP-AS1 is positively correlated with the IC50 of mitoxantrone^[231]; and expression of WTAP correlates with sensitivity to five anticancer drugs^[233]. Interestingly, in predicting glioma responses to immunotherapy, multi-omics has identified cell subtype biomarkers in addition to new molecular biomarkers such as NOTCH4^[234], CREB5^[235], and SCLs (solute carrier proteins)^[230]. FN1+ tumor-associated macrophages (FN1+ TAM) have increased abundance in recurrent gliomas, with scores associated with the activation of immune signaling pathways like IFN-γ, PDL1 signaling, leukocyte migration, and T-cell dysfunction^[236]. These macrophages may serve as potential biomarkers for predicting patient responses to immunotherapy.

The novel findings from multi-omics have provided new directions for treatment response stratification, but there are still limitations regarding the distance between discoveries and clinical application: (1) The predictive performance of most biomarkers is based on scoring rather than animal or clinical trials, resulting in relatively insufficient evidence quality; (2) Quantitative indicators for biomarkers have yet to be established; (3) Further validation is needed in larger data cohorts.

Table 3. A summary of multi-omics for predicting glioma treatment response

Author (year)	Biomarker	Data source	Major omics approache	Treatment	Performance
Zhou et al ^[229] (2024)	Chr 1: 179325742_SOAT1_miR-485-5p triplet	LGG samples from TCGA	Genomics, transcriptomics	Temozolomide	AUC = 0.910

Wu et al ^[230] (2024)	Risk score model based on seven solute carrier protein genes (SLC43A3, SLC2A10, SLC25A43, SLC7A7, SLC47A1, SLC1A6 and SLC24A4)	TCGA pan-glioma (152 GBM samples and 451 LGG samples), CGGA693 (237 GBM samples and 420 LGG samples), and CGGA325 (137 GBM samples and 176 LGG samples)	Genomics, epigenomics, transcriptomics	Immunotherapy	AUC = 0.802
Yang et al ^[231] (2023)	Four Ψ -related lncRNAs (DNAJC27-AS1, GDNF-AS1, ZBTB20-AS4 and DNMBP-AS1)	Glioma samples from TCGA	Genomics, transcriptomics	Radiotherapy	Kaplan-Meier survival curve: P = 0.026 and P = 0.00042
Wang et al ^[232] (2024)	IER 3	Glioma samples from TCGA, CGGA, and GSE16011 cohorts	Genomics, epigenomics, transcriptomics , single-cell transcriptomics , spatial transcriptomics	Dasatinib	Sensitivity prediction analysis: p < 0.001
Long et al ^[234] (2021)	NOTCH4	117 glioma samples from Samstein et al (an independent ICI-treated cohort)	Genomics , transcriptomics	Immune checkpoint inhibitor therapy	Kaplan-Meier survival curve: P = 0.328
Chen et al ^[234] (2024)	SLFN12	Over 3000 patients (GBM and glioma)	Bulk transcriptomics, single-cell transcriptomics	Anti-PD-1 immunotherapy	Submap analysis: adjust P = 0.024

4.3.3 Clinical Prognosis

Glioma prognosis exhibits significant heterogeneity across different molecular subtypes, which is associated with traditional biomarkers such as 1p/19q codeletion, MGMT promoter methylation, and IDH mutations^[237, 238]. However, the accuracy of some traditional genetic markers in prognostic prediction may be limited^[239]. Therefore, there is a need to leverage multi-omics integration to uncover new glioma prognostic biomarkers, aiding in patient stratification and treatment (summarized in Table 4).

The malignant biological behavior and invasiveness of gliomas are major contributors to poor prognosis^[240]. Increasingly, studies are utilizing multi-omics approaches to identify prognostic biomarkers related to tumor malignancy, thereby improving predictive accuracy. The EGFR pathway is closely associated with GBM proliferation, invasion, and resistance, leading to poor prognosis^[241]. One study revealed, through genomics and transcriptomics, that the expression of PomGnT1 is negatively correlated with patient survival and is linked to the regulation of the EGFR/ERK signaling pathway^[242]. In addition to the EGFR pathway, angiogenesis-related genes also influence glioma progression and prognosis^[243, 244]. Notably, Wan used genomics and transcriptomics to identify 12 prognostic features related to angiogenesis genes and developed a prognostic model, which achieved an AUC of 0.938 in the test cohort^[245]. Furthermore, multi-omics biomarkers can predict hypermutation at glioma recurrence, providing additional insights for prognostic information and clinical decision-making^[246]. In summary, multi-omics studies have introduced new perspectives in understanding prognostic biomarkers related to glioma recurrence and malignant biological behavior, advancing our knowledge of the mechanisms underlying poor prognosis and glioma progression.

As previously mentioned, current research on gliomas has shifted beyond the abnormal behavior of tumor cells themselves, with the complex interactions within the glioma microenvironment now proven to profoundly affect the progression of the disease. In terms of prognosis, an immunosuppressive microenvironment influences glioma heterogeneity and immune therapy resistance, ultimately leading to poor prognosis. Multi-omics exploration of

prognostic biomarkers associated with tumor-infiltrating immune cells (TIICs) reveals the impact of immune responses on glioma prognosis. A notable example is the study conducted by Ganghua Zhang, who utilized spatial transcriptomics and single-cell transcriptomics to identify key genes related to cancer-associated fibroblasts (CAFs) that have prognostic significance^[191]. The study found that the expression levels of these CAF-related genes were negatively correlated with patient survival, although a predictive model was not constructed. Furthermore, another study employed transcriptomics and epigenomics to identify MD2, a prognostic marker closely associated with tumor-associated macrophage (TAM) infiltration, which predicted long-term survival with an AUC of 0.675^[247]. Notably, single-cell transcriptomics and proteomics identified prognostic markers that regulate mitochondrial metabolism, thereby influencing macrophage polarization, with an AUC of 0.725 in predicting short-term survival^[248]. Another study integrated epigenomics and single-cell transcriptomics to develop a glioma prognosis model associated with the lymphotoxin- β receptor (LT β R) gene, demonstrating superior predictive efficacy (AUC = 0.82)^[249]. In addition to immune components, the neuronal activity within the glioma microenvironment is also linked to malignant tumor behavior, as tumor-induced functional connectivity may lead to poorer prognosis^[151]. Interestingly, another study, using spatial transcriptomics, proteomics, and epigenomics, revealed that glioblastoma patients with high neural characteristics exhibited upregulated synaptic gene expression, correlating with poor prognosis.^[250] Notably, high-grade gliomas are known to impair cognitive function^[151], and multi-omics approaches can detect glioma prognostic biomarkers associated with neurodegenerative diseases^[251].

The application of multi-omics provides new directions for precisely predicting glioma prognosis; however, several challenges remain: (1) there is a lack of prospective studies to validate the reliability of prognostic prediction models; (2) the clinical information used in model construction comes from various databases, and the impact of data collection methods and demographic differences on the accuracy of prognostic models should be considered.

CONFIDENTIAL CONTENT REDACTED

稿件尚未接收。为保护在审项目，仅对这一处核心内容作集中遮挡，敬请谅解。

Protected scope: A key evidence-synthesis table

4.4 Therapeutic Targets

Undoubtedly, the era of omics has enabled a deeper and more comprehensive understanding of the spatiotemporal dynamics of gliomas. However, an important underlying question remains: How can we develop and select drug targets based on this dynamic nature—should we target glioma cells themselves or the microecosystem?

From the perspective of glioma cells, as mentioned earlier, when faced with environmental stress and emergencies, they undergo remodeling to adapt, with a typical example being metabolic reprogramming. In the past, two challenges have weakened the effectiveness of targeting metabolism in treatment: (1) After blocking a single metabolic pathway, plasticity leads to new metabolic reprogramming that sustains survival; (2) After blocking multiple pathways, glioma cells enter a low-metabolic, drug-resistant dormant state and promote a fibrotic, immunosuppressive microenvironment. The first challenge can be addressed by multi-target strategies. ChIP-seq, ATAC-seq, transcriptomics, proteomics, and metabolomics studies have shown that AURKA inhibition of glycolysis reactivates fatty acid oxidation through c-MYC and PGC1 α to maintain survival^[252]. Combining AURKA inhibition with fatty acid oxidation inhibitors can extend the overall survival of glioblastoma xenograft models (PDX). Untargeted metabolomics studies have found that the small molecule inhibitor EPIC-0412 targets the glioma TCA metabolism^[253]. Simultaneously using phospholipid and glycolysis inhibitors can block the TCA-

phospholipid glycolysis metabolic axis, inhibiting glioma cell proliferation and growth. These dual or multiple strategies can disrupt the key energy supply chains of gliomas to inhibit proliferation and growth. However, targeting the metabolism of glioma cells themselves may be ineffective against dormant malignant cells in an immunosuppressive, drug-resistant microenvironment.

Another strategy, which focuses on remodeling the microecosystem, targets the relatively stable cellular components of the microenvironment, aiming to prevent malignant cell growth by artificially reshaping it. Lee et al.^[162] proposed through single-cell transcriptomics that the dominant suppressive cells within the immune microenvironment led to T cell exhaustion, which inhibits anti-glioma T cell activity and may be a direct cause of the ineffectiveness of anti-PD-1 therapy. Therefore, we propose two approaches to targeting the immune microenvironment: (1) enhancing the effector T cell population; and (2) intervening in suppressive immune cells. Due to intercellular communication among immune cells, these two strategies could function synergistically. Utilizing single-cell and spatial transcriptomics, it was demonstrated that inhibiting the JAK-STAT pathway could block communication between myeloid cells and T cells by reducing IL-10 release, thus restoring T cell function and reshaping a normal immune microenvironment^[135]. Moreover, immune restoration strategies not only maintain immune surveillance of glioma cells but also synergistically enhance the efficacy of immunotherapy. Targeted deletion of immune-suppressive genes highly expressed in TAMs, identified by single-cell and spatial transcriptomics, can activate T cells and improve the efficacy of anti-PD-1/PD-L1 therapy^[254]. AAV-mediated TREM2 overexpression, identified through single-cell transcriptomics, spatial transcriptomics, and metabolomics, inhibits GBM progression and enhances anti-PD-1 therapy efficacy^[255]. The study by Goswami et al. further supports the idea of synergistic enhancement of treatment efficacy^[256]. Interestingly, HEXB, identified through RNA sequencing and single-cell RNA sequencing, not only directly promotes glioma cell glycolysis associated with HIF1 α and YAP1 but also recruits M2-polarized TAMs, indirectly enhancing tumor cell glycolysis^[257]. The use of the HEXB inhibitor Gal-PUGNAc simultaneously blocks metabolic suppression of glioma growth and enhances the effects of immune checkpoint inhibition. Therefore, theoretically, targeting both glioma metabolism and the immune microenvironment simultaneously should provide better therapeutic outcomes, though no clinical studies have yet verified this hypothesis.

However, remodeling the microecosystem presents a significant challenge: we may not be able to remodel the local glioma microenvironment, including the immune microenvironment mentioned above, in isolation because the microecosystem is a multi-layered, interactive regulatory network. Watson et al.^[258] used proteomics, single-cell transcriptomics, and spatial transcriptomics to reveal a complex, interrelated fibrotic and immunosuppressive microenvironment, consisting of blood vessels, fibroblasts, immune cells, extracellular matrix, and glioma cells. This study employed a triple therapy combining BLZ945, the steroid dexamethasone, and the TGF- β RI inhibitor Galunisertib, which inhibited pathways associated with this fibrotic microenvironment and significantly improved the efficacy of CSF-1R inhibition therapy. Therefore, we believe that utilizing multi-omics to understand the complex regulatory network of the glioma microenvironment and precisely cutting key links, rather than being limited to targeting a single class of targets or pathways, will achieve the maximum therapeutic efficacy.

Overall, multi-omics, as a groundbreaking technology, has gradually unveiled the secrets of glioma's spatiotemporal changes. This insight suggests important considerations for the future development of glioma treatment targets: (1) Due to heterogeneity and plasticity, targeting both glioma cells and the remodeling of the microenvironment may offer greater advantages in maintaining immune surveillance and enhancing treatment efficacy; (2) When only a small number of drug-resistant dormant cells remain, the key is to precisely select targets from the complex regulatory network of the microenvironment to disrupt the ecosystem that gliomas create to support their survival.

5 Current Shortcomings and Future Directions

The specific challenges related to multi-omics research in gliomas have been previously described. Here, we present an essential common issue: the differential associations of multi-omics data. In July 2024, Liu et al.^[259] reported that the genomic expression levels in glioblastoma do not align with those in transcriptomics and proteomics. In some cases, genes were expressed at high levels, but their corresponding transcript and protein

le [REDACTED]
re [REDACTED]
it comes to abundance changes. Transcriptomics and proteomics are the two omics layers most closely related to the gene-phenotype axis; however, transcript levels sometimes conflict with protein expression, which is referred to as false positives or false negatives in transcriptomics. On one hand, it is currently unclear whether these discrepancies arise due to data contamination, processing methods, or regulatory influences such as post-transcriptional modifications, post-translational modifications, and non-coding RNAs. On the other hand, such differential associations could lead to erroneous predictions in the development of clinical biomarkers or therapies based on these data, potentially reducing their efficacy and delaying clinical translation. Cross-disciplinary collaboration between bioinformatics and neuro-oncology experts may help address this issue.

Importantly, similar interdisciplinary collaboration could bring new developments to multi-omics research in gliomas. Multi-omics has progressed from population-based or single-cell studies to spatial analyses. However, it must be acknowledged that current spatial omics technologies still rely on tissue slicing from pre-selected regions, limiting the ability to identify rare features, such as the detection of subtle early lesions or metastatic foci. This limitation arises from the inability to perform slice sequencing based on assumed exact locations. By combining tissue clearing techniques with multi-omics focused on spatial omics, we can integrate spatial context with molecular data^[260]. Multi-omics sequencing and spatial characterization in cleared tissues have already been shown to be feasible^[261-263]. For gliomas, the pathways and mechanisms of invasion, metastasis, and recurrence remain unclear. Integrating tissue clearing with multi-omics sequencing could lead to the development of dynamic "4D" methods. By observing the spatial mapping of tumor cells and their interactions with other cells over time in large, intact brain tissues or live transparent animal models, we can gain new insights into this heterogeneous tumor.

Currently, multi-omics technologies are limited to observing current or past cellular changes and are unable to predict future cellular trends. This limitation is crucial in glioblastoma, a tumor with substantial plasticity, for therapy development and strategy formulation. Perhaps, true precision medicine for glioblastoma will only be achievable when our ability to predict cellular behavior and proactively adjust treatment strategies is advanced enough to counteract the tumor's adaptability. Interestingly, Bunne et al.^[264] proposed that by integrating large-scale multi-omics data with microscopic imaging data, we can construct AI virtual cells to predict future cellular changes and states. These virtual cells could predict the future status of the tumor microenvironment and how tumor cells will respond to environmental changes, thus aiding in target selection, therapy optimization, and strategy development. We believe that in the future, using AI virtual cells, we may develop the "final omics"—temporal omics—specifically designed to study and address the heterogeneity and plasticity of tumors, particularly gliomas. Based on extensive sequencing data, "temporal omics" could be used to predict internal and microenvironmental changes in glioblastomas after treatment, enabling timely or preemptive adjustments to treatment targets or the selection of alternative immunotherapies, ensuring that glioblastoma cells remain in a detrimental environment, ultimately controlling this highly threatening tumor.

6 Conclusion

Multi-omics has revolutionized glioma research and clinical applications by revealing previously undescribed molecular profiles and dynamic evolution, thereby advancing clinical diagnosis, risk stratification, and the identification of new therapeutic targets. However, many enigmas remain, such as how to develop adaptive precision therapies to combat the environment-driven plasticity and drug resistance of gliomas. We believe that basic and translational research driven by technological innovation will continuously bring new hope to glioma patients.

References

[1] DELGADO-LOPEZ P D, CORRALES-GARCIA E M. Survival in glioblastoma: a review on the impact of treatment modalities [J]. Clin Transl Oncol, 2016, 18(11): 1062-71.

- [2] KOREBY V, YANG J, SARAF P, et al. Evolutionary Trajectories of IDHWT Glioblastomas Reveal a Common Path of Early Tumorigenesis Initiated Years ahead of Initial Diagnosis [J]. *Cancer Cell*, 2019, 35(7): 692-705. e12.
- [3] RANA M, LIOU K C, THAKUR A, et al. Advancing glioblastoma therapy: Learning from the past and innovations for the future [J]. *Cancer Lett*, 2025, 617: 217601.
- [4] HAN H, DU A, LI J, et al. Transitioning from molecular methods to therapeutic methods: An in-depth analysis of glioblastoma (Review) [J]. *Oncol Rep*, 2025, 53(4).
- [5] DAKAL T C, KAKDE G S, MAURYA P K. Genomic, epigenomic and transcriptomic landscape of glioblastoma [J]. *Metab Brain Dis*, 2024, 39(8): 1591-611.
- [6] GIULIANI P, DE SIMONE C, FEBBO G, et al. Proteomics Studies on Extracellular Vesicles Derived from Glioblastoma: Where Do We Stand? [J]. *Int J Mol Sci*, 2024, 25(18).
- [7] YAN X, LI J, ZHANG Y, et al. Alterations in cellular metabolism under different grades of glioma staging identified based on a multi-omics analysis strategy [J]. *Front Endocrinol (Lausanne)*, 2023, 14: 1292944.
- [8] YABO Y A, HEILAND D H. Understanding glioblastoma at the single-cell level: Recent advances and future challenges [J]. *PLoS Biol*, 2024, 22(5): e3002640.
- [9] PIWECKA M, RAJEWSKY N, RYBAK-WOLF A. Single-cell and spatial transcriptomics: deciphering brain complexity in health and disease [J]. *Nat Rev Neurol*, 2023, 19(6): 346-62.
- [10] WHITTLE J R, KRIEL J, FATUNLA O E, et al. Spatial omics shed light on the tumour organisation of glioblastoma [J]. *Semin Cell Dev Biol*, 2025, 167: 1-9.
- [11] TAMBI R, ZEHR A B, VIJAYAKUMAR A, et al. Artificial intelligence and omics in malignant gliomas [J]. *Physiol Genomics*, 2024, 56(12): 876-95.
- [12] XUAN J, YU Y, QING T, et al. Next-generation sequencing in the clinic: promises and challenges [J]. *Cancer Lett*, 2013, 340(2): 284-95.
- [13] CLAUSSNITZER M, CHO J H, COLLINS R, et al. A brief history of human disease genetics [J]. *Nature*, 2020, 577(7789): 179-89.
- [14] HAO X, CHENG S, JIANG B, et al. Applying multi-omics techniques to the discovery of biomarkers for acute aortic dissection [J]. *Front Cardiovasc Med*, 2022, 9: 961991.
- [15] TRAN B, DANCEY J E, KAMEL-REID S, et al. Cancer genomics: technology, discovery, and translation [J]. *J Clin Oncol*, 2012, 30(6): 647-60.
- [16] HAN G, SUN J, WANG J, et al. Genomics in neurological disorders [J]. *Genomics Proteomics Bioinformatics*, 2014, 12(4): 156-63.
- [17] HENG H H, BREMER S W, STEVENS J B, et al. Genetic and epigenetic heterogeneity in cancer: a genome-centric perspective [J]. *J Cell Physiol*, 2009, 220(3): 538-47.
- [18] LAZOW M A, HOFFMAN L, SCHAFER A, et al. Characterizing temporal genomic heterogeneity in pediatric low-grade gliomas [J]. *Acta Neuropathol Commun*, 2020, 8(1): 182.
- [19] SUN X, JIA Q, LI K, et al. Comparative genomic landscape of lower-grade glioma and glioblastoma [J]. *PLoS One*, 2024, 19(8): e0309536.
- [20] YANCHUS C, DRUCKER K L, KOLLMEYER T M, et al. A noncoding single-nucleotide polymorphism at 8q24 drives IDH1-mutant glioma formation [J]. *Science*, 2022, 378(6615): 68-78.
- [21] XIE T, DANIELI-MACKAY A, BUCCARELLI M, et al. Pervasive structural heterogeneity rewires glioblastoma

- [22] ZHANG J, FENG Y, LI G, et al. Distinct aneuploid evolution of astrocytoma and glioblastoma during recurrence [J]. NPJ Precis Oncol, 2023, 7(1): 97.
- [23] SOON W W, HARIHARAN M, SNYDER M P. High-throughput sequencing for biology and medicine [J]. Mol Syst Biol, 2013, 9: 640.
- [24] JIANG Z, ZHOU X, LI R, et al. Whole transcriptome analysis with sequencing: methods, challenges and potential solutions [J]. Cell Mol Life Sci, 2015, 72(18): 3425-39.
- [25] KASTENDIEK N, COLETTI R, GROSS T, et al. Exploring glioma heterogeneity through omics networks: from gene network discovery to causal insights and patient stratification [J]. BioData Min, 2024, 17(1): 56.
- [26] HE Y, YUAN Y, JI L, et al. Integrative Analysis Identifies NSUN2 as an Essential Coordinator for Glioma Malignancy and Glucose Metabolism [J]. J Gene Med, 2024, 26(12): e70004.
- [27] CÓMITRE-MARIANO B, SEGURA-COLLAR B, VELLILA-ALONSO G, et al. S100A proteins show a spatial distribution of inflammation associated with the glioblastoma microenvironment architecture [J]. Theranostics, 2025, 15(2): 726-44.
- [28] GRIGORE I A, RAJAGOPAL A, CHOW J T, et al. Discovery of miRNA-mRNA regulatory networks in glioblastoma reveals novel insights into tumor microenvironment remodeling [J]. Sci Rep, 2024, 14(1): 27493.
- [29] WU T, DU M, ZENG L, et al. Increased UBD Is a Potential Diagnostic and Prognostic Biomarker in Glioma [J]. Environ Toxicol, 2024, 39(12): 5250-63.
- [30] ZHANG W, XIE M, HUANG Q, et al. STEAP3 is a potential preliminary prognostic biomarker of glioblastoma [J]. Sci Rep, 2024, 14(1): 30994.
- [31] YANG Z, ZHANG T, ZHU X, et al. Ferroptosis-Related Transcriptional Level Changes and the Role of CIRBP in Glioblastoma Cells Ferroptosis [J]. Biomedicines, 2024, 13(1).
- [32] ZHANG J, LI L, TANG A, et al. Pan-cancer analysis of the transcriptional expression of histone acetylation enzymes in solid tumors defines a new classification scheme for gliomas [J]. Front Immunol, 2024, 15: 1523034.
- [33] CAO R, LIU Y, WEI K, et al. Genes related to neural tube defects and glioblastoma [J]. Sci Rep, 2025, 15(1): 3777.
- [34] ANDERSON N L, ANDERSON N G. The human plasma proteome: history, character, and diagnostic prospects [J]. Mol Cell Proteomics, 2002, 1(11): 845-67.
- [35] NAGARAJ N, WISNIEWSKI J R, GEIGER T, et al. Deep proteome and transcriptome mapping of a human cancer cell line [J]. Mol Syst Biol, 2011, 7: 548.
- [36] UZOZIE A C, AEBERSOLD R. Advancing translational research and precision medicine with targeted proteomics [J]. J Proteomics, 2018, 189: 1-10.
- [37] WANG W, SHI Y, YAN B, et al. The dual role of calnexin on malignant progression and tumor microenvironment in glioma [J]. Sci Rep, 2024, 14(1): 30796.
- [38] ZHANG J, SUN R, LYU Y, et al. Proteomic profiling of gliomas unveils immune and metabolism-driven subtypes with implications for anti-nucleotide metabolism therapy [J]. Nat Commun, 2024, 15(1): 10005.
- [39] YANG S, ZHENG Y, ZHOU C, et al. Multidimensional Proteomic Landscape Reveals Distinct Activated Pathways Between Human Brain Tumors [J]. Adv Sci (Weinh), 2025, 12(7): e2410142.
- [40] ZHANG M, WANG X, CHEN X, et al. Novel Immune-Related Gene Signature for Risk Stratification and Prognosis of Survival in Lower-Grade Glioma [J]. Front Genet, 2020, 11: 363.

- [41] YANG W, CHAHAD B, OFEK P, YEINIE E, et al. Proteogenomics of glioblastoma associates molecular patterns with survival [J]. *Cell Rep*, 2021, 34(5): 109737.
- [42] GONZALEZ H, MEI W, ROBLES I, et al. Cellular architecture of human brain metastases [J]. *Cell*, 2022, 185(4): 729-45.e20.
- [43] THANASUPAWAT T, MEJIA Y P, ANANDHAN S S, et al. Proteomic and cytokine profiling of a CTRP8-RXFP1 glioma mouse model [J]. *Biochem Pharmacol*, 2025, 232: 116722.
- [44] WANG S, CASTRO B A, KATZ J L, et al. B cell-based therapy produces antibodies that inhibit glioblastoma growth [J]. *J Clin Invest*, 2024, 134(20).
- [45] HAYDO A, SCHMIDT J, CRIDER A, et al. BRAT1 - a new therapeutic target for glioblastoma [J]. *Cell Mol Life Sci*, 2025, 82(1): 52.
- [46] WANG B, YU R Z, ZHANG X Y, et al. Polo-like kinase 4 accelerates glioma malignant progression and vasculogenic mimicry by phosphorylating EphA2 [J]. *Cancer Lett*, 2024, 611: 217397.
- [47] LI G, WANG D, ZHAI Y, et al. Glycometabolic reprogramming-induced XRCC1 lactylation confers therapeutic resistance in ALDH1A3-overexpressing glioblastoma [J]. *Cell Metab*, 2024, 36(8): 1696-710.e10.
- [48] CHEN H, KOUL D, ZHANG Y, et al. Pulsed focused ultrasound alters the proteomic profile of the tumor microenvironment in a syngeneic mouse model of glioblastoma [J]. *J Neurooncol*, 2024, 170(2): 347-61.
- [49] SHEN L, ZHANG Z, WU P, et al. Mechanistic insight into glioma through spatially multidimensional proteomics [J]. *Sci Adv*, 2024, 10(7): eadk1721.
- [50] RIVIERE-CAZAUX C, GRASER C J, WARRINGTON A E, et al. A field resource for the glioma cerebrospinal fluid proteome: impacts of resection and location on biomarker discovery [J]. *Neuro Oncol*, 2024.
- [51] HALLAL S M, TÚZESI Á, SIDA L A, et al. Glioblastoma biomarkers in urinary extracellular vesicles reveal the potential for a 'liquid gold' biopsy [J]. *Br J Cancer*, 2024, 130(5): 836-51.
- [52] MONTIU A, MORESI F, VINCENZONI F, et al. Proteomic Profiling of Pre- and Post-Surgery Saliva of Glioblastoma Patients: A Pilot Investigation [J]. *Int J Mol Sci*, 2024, 25(23).
- [53] CLAVREUL A, GUETTE C, LASLA H, et al. Proteomics of tumor and serum samples from isocitrate dehydrogenase-wildtype glioblastoma patients: is the detoxification of reactive oxygen species associated with shorter survival? [J]. *Mol Oncol*, 2024, 18(11): 2783-800.
- [54] LERTSUMITKUL L, ILIOPOULOS M, WANG S S, et al. EphA3-targeted chimeric antigen receptor T cells are effective in glioma and generate curative memory T cell responses [J]. *J Immunother Cancer*, 2024, 12(8).
- [55] QIU S, CAI Y, YAO H, et al. Small molecule metabolites: discovery of biomarkers and therapeutic targets [J]. *Signal Transduct Target Ther*, 2023, 8(1): 132.
- [56] VIGNOLI A, GHINI V, MEONI G, et al. High-Throughput Metabolomics by 1D NMR [J]. *Angew Chem Int Ed Engl*, 2019, 58(4): 968-94.
- [57] PATTI G J, YANES O, SIUZDAK G. Innovation: Metabolomics: the apogee of the omics trilogy [J]. *Nat Rev Mol Cell Biol*, 2012, 13(4): 263-9.
- [58] FUHRER T, ZAMBONI N. High-throughput discovery metabolomics [J]. *Curr Opin Biotechnol*, 2015, 31: 73-8.
- [59] BJÖRKBLOM B, WIBOM C, ERIKSSON M, et al. Distinct metabolic hallmarks of WHO classified adult glioma subtypes [J]. *Neuro Oncol*, 2022, 24(9): 1454-68.
- [60] ZHOU L, WANG Z, HU C, et al. Integrated Metabolomics and Lipidomics Analyses Reveal Metabolic Reprogramming in Human Glioma with IDH1 Mutation [J]. *J Proteome Res*, 2019, 18(3): 960-9.

- [61] SHAO W, GU J, HUANG C, et al. Metabolomic differentiation of tumor core versus edge in glioma [J]. *Neurooncol Focus*, 2023, 31(5): E1.
- [62] SHAO W, GU J, HUANG C, et al. Malignancy-associated metabolic profiling of human glioma cell lines using ¹H NMR spectroscopy [J]. *Mol Cancer*, 2014, 13: 197.
- [63] SUSSMAN J H, XU J, AMANKULOR N, et al. Dissecting the tumor microenvironment of epigenetically driven gliomas: Opportunities for single-cell and spatial multiomics [J]. *Neurooncol Adv*, 2023, 5(1): vdad101.
- [64] KAMINSKA B, OCHOCKA N, SEGIT P. Single-Cell Omics in Dissecting Immune Microenvironment of Malignant Gliomas-Challenges and Perspectives [J]. *Cells*, 2021, 10(9).
- [65] XUE M, WEI W, SU Y, et al. Chemical methods for the simultaneous quantitation of metabolites and proteins from single cells [J]. *J Am Chem Soc*, 2015, 137(12): 4066-9.
- [66] GUO S, ZHANG C, LE A. The limitless applications of single-cell metabolomics [J]. *Curr Opin Biotechnol*, 2021, 71: 115-22.
- [67] BAYSOY A, BAI Z, SATIJA R, et al. The technological landscape and applications of single-cell multi-omics [J]. *Nat Rev Mol Cell Biol*, 2023, 24(10): 695-713.
- [68] PATEL A P, TIROSH I, TROMBETTA J J, et al. Single-cell RNA-seq highlights intratumoral heterogeneity in primary glioblastoma [J]. *Science*, 2014, 344(6190): 1396-401.
- [69] XIE X P, GANBOLD M, LI J, et al. Glioblastoma functional heterogeneity and enrichment of cancer stem cells with tumor recurrence [J]. *Neuron*, 2024, 112(24): 4017-32.e6.
- [70] YIN W, PING Y F, LI F, et al. A map of the spatial distribution and tumour-associated macrophage states in glioblastoma and grade 4 IDH-mutant astrocytoma [J]. *J Pathol*, 2022, 258(2): 121-35.
- [71] FINOTTO L, COLE B, GIESE W, et al. Single-cell profiling and zebrafish avatars reveal LGALS1 as immunomodulating target in glioblastoma [J]. *EMBO Mol Med*, 2023, 15(11): e18144.
- [72] HUANG Y, SHAN Y, ZHANG W, et al. Sex differences in the molecular profile of adult diffuse glioma are shaped by IDH status and tumor microenvironment [J]. *Neuro Oncol*, 2025, 27(2): 430-44.
- [73] RICHARDS L M, WHITLEY O K N, MACLEOD G, et al. Gradient of Developmental and Injury Response transcriptional states defines functional vulnerabilities underpinning glioblastoma heterogeneity [J]. *Nat Cancer*, 2021, 2(2): 157-73.
- [74] YEO A T, RAWAL S, DELCUZE B, et al. Single-cell RNA sequencing reveals evolution of immune landscape during glioblastoma progression [J]. *Nat Immunol*, 2022, 23(6): 971-84.
- [75] XIE Y, YANG F, HE L, et al. Single-cell dissection of the human blood-brain barrier and glioma blood-tumor barrier [J]. *Neuron*, 2024, 112(18): 3089-105.e7.
- [76] LIU Y, WU J, NAJEM H, et al. Dual targeting macrophages and microglia is a therapeutic vulnerability in models of PTEN-deficient glioblastoma [J]. *J Clin Invest*, 2024, 134(22).
- [77] WEI W, SHIN Y S, XUE M, et al. Single-Cell Phosphoproteomics Resolves Adaptive Signaling Dynamics and Informs Targeted Combination Therapy in Glioblastoma [J]. *Cancer Cell*, 2016, 29(4): 563-73.
- [78] CHENG K W, SU P R, FELLER K J, et al. Investigating the Metabolic Heterogeneity of Cancer Cells Using Functional Single-Cell Selection and nLC Combined with Multinozzle Emitter Mass Spectrometry [J]. *Anal Chem*, 2024, 96(2): 624-9.
- [79] RAO A, BARKLEY D, FRANÇA G S, et al. Exploring tissue architecture using spatial transcriptomics [J]. *Nature*, 2021, 596(7871): 211-20.

- [80] STANCLIFFE E, SALMEN F, VICKOVIC S, et al. Spatial visualization and analysis of gene expression in tissue sections by spatial transcriptomics [J]. *Science*, 2016, 353(6291): 73-78.
- [81] RONČEVIĆ A, KORUGA N, SOLDI KORUGA A, et al. MALDI Imaging Mass Spectrometry of High-Grade Gliomas: A Review of Recent Progress and Future Perspective [J]. *Curr Issues Mol Biol*, 2023, 45(2): 838-51.
- [82] FU R, NORRIS G A, WILLARD N, et al. Spatial transcriptomic analysis delineates epithelial and mesenchymal subpopulations and transition stages in childhood ependymoma [J]. *Neuro Oncol*, 2023, 25(4): 786-98.
- [83] HARWOOD D S L, PEDERSEN V, BAGER N S, et al. Glioblastoma cells increase expression of notch signaling and synaptic genes within infiltrated brain tissue [J]. *Nat Commun*, 2024, 15(1): 7857.
- [84] AL-HOLOU W N, WANG H, RAVIKUMAR V, et al. Subclonal evolution and expansion of spatially distinct THY1-positive cells is associated with recurrence in glioblastoma [J]. *Neoplasia*, 2023, 36: 100872.
- [85] SLOAN L, SEN R, LIU C, et al. Radiation immunodynamics in patients with glioblastoma receiving chemoradiation [J]. *Front Immunol*, 2024, 15: 1438044.
- [86] DUHAMEL M, DRELICH L, WISZTORSKI M, et al. Spatial analysis of the glioblastoma proteome reveals specific molecular signatures and markers of survival [J]. *Nat Commun*, 2022, 13(1): 6665.
- [87] WATSON S S, DUC B, KANG Z, et al. Microenvironmental reorganization in brain tumors following radiotherapy and recurrence revealed by hyperplexed immunofluorescence imaging [J]. *Nat Commun*, 2024, 15(1): 3226.
- [88] BASU S S, MCMINN M H, GIMÉNEZ-CASSINA LOPÉZ B, et al. Metal Oxide Laser Ionization Mass Spectrometry Imaging (MOLI MSI) Using Cerium(IV) Oxide [J]. *Anal Chem*, 2019, 91(10): 6800-7.
- [89] RANDALL E C, LOPEZ B G C, PENG S, et al. Localized Metabolomic Gradients in Patient-Derived Xenograft Models of Glioblastoma [J]. *Cancer Res*, 2020, 80(6): 1258-67.
- [90] LU T, FREYTAG L, NARAYANA V K, et al. Matrix Selection for the Visualization of Small Molecules and Lipids in Brain Tumors Using Untargeted MALDI-TOF Mass Spectrometry Imaging [J]. *Metabolites*, 2023, 13(11).
- [91] SCHWAIGER-HABER M, STANCLIFFE E, ANBUKUMAR D S, et al. Using mass spectrometry imaging to map fluxes quantitatively in the tumor ecosystem [J]. *Nat Commun*, 2023, 14(1): 2876.
- [92] QIU Y L, ZHENG H, GEVAERT O. Genomic data imputation with variational auto-encoders [J]. *Gigascience*, 2020, 9(8).
- [93] KOKLA M, VIRTANEN J, KOLEHMAINEN M, et al. Random forest-based imputation outperforms other methods for imputing LC-MS metabolomics data: a comparative study [J]. *BMC Bioinformatics*, 2019, 20(1): 492.
- [94] SIENKIEWICZ K, CHEN J, CHATRATH A, et al. Detecting molecular subtypes from multi-omics datasets using SUMO [J]. *Cell Rep Methods*, 2022, 2(1).
- [95] VAN HOUTVEN J, HOOYBERGHS J, LAUKENS K, et al. CONSTANd: An Efficient Normalization Method for Relative Quantification in Small- and Large-Scale Omics Experiments in R BioConductor and Python [J]. *J Proteome Res*, 2021, 20(4): 2151-6.
- [96] MAO X G, XUE X Y, WANG L, et al. Deep learning identified glioblastoma subtypes based on internal genomic expression ranks [J]. *BMC Cancer*, 2022, 22(1): 86.
- [97] FELDNER-BUSZTIN D, FIRBAS NISANTZIS P, EDMUNDS S J, et al. Dealing with dimensionality: the application of machine learning to multi-omics data [J]. *Bioinformatics*, 2023, 39(2).
- [98] KANG M, KO E, MERSHA T B. A roadmap for multi-omics data integration using deep learning [J]. *Brief Bioinform*, 2022, 23(1).

- [9]  learning models for prognosis prediction using multi-omics data [J]. *Genomics*, 2021, 103(1): 1-12.
- [100] CHAI H, ZHOU X, ZHANG Z, et al. Integrating multi-omics data through deep learning for accurate cancer prognosis prediction [J]. *Comput Biol Med*, 2021, 134: 104481.
- [101] LIU C, WANG L, LIU Z. Single-cell multi-omics integration for unpaired data by a siamese network with graph-based contrastive loss [J]. *BMC Bioinformatics*, 2023, 24(1): 5.
- [102] WEI Z, HAN D, ZHANG C, et al. Deep Learning-Based Multi-Omics Integration Robustly Predicts Relapse in Prostate Cancer [J]. *Front Oncol*, 2022, 12: 893424.
- [103] CHAUDHARY K, POIRION O B, LU L, et al. Deep Learning-Based Multi-Omics Integration Robustly Predicts Survival in Liver Cancer [J]. *Clin Cancer Res*, 2018, 24(6): 1248-59.
- [104] MUNQUAD S, DAS A B. DeepAutoGlioma: a deep learning autoencoder-based multi-omics data integration and classification tools for glioma subtyping [J]. *BioData Min*, 2023, 16(1): 32.
- [105] YANG Q, XIONG Y, JIANG N, et al. Integrating Genomic Data with Transcriptomic Data for Improved Survival Prediction for Adult Diffuse Glioma [J]. *J Cancer*, 2020, 11(13): 3794-802.
- [106] SINGH D K, KOLLIPARA R K, VEMIREDDY V, et al. Oncogenes Activate an Autonomous Transcriptional Regulatory Circuit That Drives Glioblastoma [J]. *Cell Rep*, 2017, 18(4): 961-76.
- [107] FENG J, ZHANG H, LI F. Investigating the relevance of major signaling pathways in cancer survival using a biologically meaningful deep learning model [J]. *BMC Bioinformatics*, 2021, 22(1): 47.
- [108] TAMMEN S A, FRISO S, CHOI S W. Epigenetics: the link between nature and nurture [J]. *Mol Aspects Med*, 2013, 34(4): 753-64.
- [109] RAHME G J, JAVED N M, PUORRO K L, et al. Modeling epigenetic lesions that cause gliomas [J]. *Cell*, 2023, 186(17): 3674-85.e14.
- [110] ESENAT-MENDEZ M, ÍÑIGUEZ-MUÑOZ S, SESÉ B, et al. iGlioSub: an integrative transcriptomic and epigenomic classifier for glioblastoma molecular subtypes [J]. *BioData Min*, 2021, 14(1): 42.
- [111] WANG H, DIAZ A K, SHAW T I, et al. Deep multiomics profiling of brain tumors identifies signaling networks downstream of cancer driver genes [J]. *Nat Commun*, 2019, 10(1): 3718.
- [112] MORDOVKINA D, LYABIN D N, SMOLIN E A, et al. Y-Box Binding Proteins in mRNP Assembly, Translation, and Stability Control [J]. *Biomolecules*, 2020, 10(4).
- [113] LINDQUIST J A, MERTENS P R. Cold shock proteins: from cellular mechanisms to pathophysiology and disease [J]. *Cell Commun Signal*, 2018, 16(1): 63.
- [114] JAYAVELU A K, SCHNÖDER T M, PERNER F, et al. Splicing factor YBX1 mediates persistence of JAK2-mutated neoplasms [J]. *Nature*, 2020, 588(7836): 157-63.
- [115] WANG J Z, ZHU H, YOU P, et al. Upregulated YB-1 protein promotes glioblastoma growth through a YB-1/CCT4/mLST8/mTOR pathway [J]. *J Clin Invest*, 2022, 132(8).
- [116] NASSANI R, BOKHARI Y, ALRFAEI B M. Molecular signature to predict quality of life and survival with glioblastoma using Multiview omics model [J]. *PLoS One*, 2023, 18(11): e0287448.
- [117] BI J, CHOWDHRY S, WU S, et al. Altered cellular metabolism in gliomas - an emerging landscape of actionable co-dependency targets [J]. *Nat Rev Cancer*, 2020, 20(1): 57-70.
- [118] KIM K H, MIGLIOZZI S, KOO H, et al. Integrated proteogenomic characterization of glioblastoma evolution [J]. *Cancer Cell*, 2024, 42(3): 358-77.e8.

[119] LAGACHE L, ZIREMY Y, LE RHON E, et al. Predicting Protein Pathways Associated to Tumor Heterogeneity by Considering Spatial Epigenetics and Proteomics: The Dry Proteomic Concept [J]. Mol Cell Proteomics, 2024, 24(1): 100891.

[120] XIE X P, GANBOLD M, LI J, et al. Glioblastoma functional heterogeneity and enrichment of cancer stem cells with tumor recurrence [J]. Neuron, 2024, 112(24): 4017-32 e6.

[121] XIE Y, YANG F, HE L, et al. Single-cell dissection of the human blood-brain barrier and glioma blood-tumor barrier [J]. Neuron, 2024, 112(18): 3089-105 e7.

[122] KIM Y, DANAHER P, CIMINO P J, et al. Highly Multiplexed Spatially Resolved Proteomic and Transcriptional Profiling of the Glioblastoma Microenvironment Using Archived Formalin-Fixed Paraffin-Embedded Specimens [J]. Mod Pathol, 2023, 36(1): 100034.

[123] REN Y, HUANG Z, ZHOU L, et al. Spatial transcriptomics reveals niche-specific enrichment and vulnerabilities of radial glial stem-like cells in malignant gliomas [J]. Nat Commun, 2023, 14(1): 1028.

[124] MATHUR R, WANG Q, SCHUPP P G, et al. Glioblastoma evolution and heterogeneity from a 3D whole-tumor perspective [J]. Cell, 2024, 187(2): 446-63 e16.

[125] LIU I, JIANG L, SAMUELSSON E R, et al. The landscape of tumor cell states and spatial organization in H3-K27M mutant diffuse midline glioma across age and location [J]. Nat Genet, 2022, 54(12): 1881-94.

[126] GREENWALD A C, DARNELL N G, HOEFFLIN R, et al. Integrative spatial analysis reveals a multi-layered organization of glioblastoma [J]. Cell, 2024, 187(10): 2485-501 e26.

[127] RAVI V M, WILL P, KUECKELHAUS J, et al. Spatially resolved multi-omics deciphers bidirectional tumor-host interdependence in glioblastoma [J]. Cancer Cell, 2022, 40(6): 639-55 e13.

[128] GUYON J, FERNANDEZ-MONCADA I, LARRIEU C M, et al. Lactate dehydrogenases promote glioblastoma growth and invasion via a metabolic symbiosis [J]. EMBO Mol Med, 2022, 14(12): e15343.

[129] MIRZAEI R, D'MELLO C, LIU M, et al. Single-Cell Spatial Analysis Identifies Regulators of Brain Tumor-Initiating Cells [J]. Cancer Res, 2023, 83(10): 1725-41.

[130] HE Z, LIU Z, WANG Q, et al. Single-cell and spatial transcriptome assays reveal heterogeneity in gliomas through stress responses and pathway alterations [J]. Front Immunol, 2024, 15: 1452172.

[131] KLOOSTERMAN D J, ERBANI J, BOON M, et al. Macrophage-mediated myelin recycling fuels brain cancer malignancy [J]. Cell, 2024, 187(19): 5336-56 e30.

[132] SANKOWSKI R, SUSS P, BENKENDORFF A, et al. Multiomic spatial landscape of innate immune cells at human central nervous system borders [J]. Nat Med, 2024, 30(1): 186-98.

[133] ZHONG J, XING X, GAO Y, et al. Distinct roles of TREM2 in central nervous system cancers and peripheral cancers [J]. Cancer Cell, 2024, 42(6): 968-84 e9.

[134] CAO L, LU X, WANG X, et al. From single-cell to spatial transcriptomics: decoding the glioma stem cell niche and its clinical implications [J]. Front Immunol, 2024, 15: 1475235.

[135] RAVI V M, NEIDERT N, WILL P, et al. T-cell dysfunction in the glioblastoma microenvironment is mediated by myeloid cells releasing interleukin-10 [J]. Nat Commun, 2022, 13(1): 925.

[136] SHAH N, HALLUR P M, GANESH R A, et al. Gelatin methacrylate hydrogels culture model for glioblastoma cells enriches for mesenchymal-like state and models interactions with immune cells [J]. Sci Rep, 2021, 11(1): 17727.

[137] YANG Y, JIN X, XIE Y, et al. The CEBPB(+) glioblastoma subcluster specifically drives the formation of M2 tumor-associated macrophages to promote malignancy growth [J]. Theranostics, 2024, 14(10): 4107-26.

- [138] ZHAO S, ZHAO R, WANG C, et al. HDAC7 drives glioblastoma to a mesenchymal-like state via LGALS3-mediated crosstalk between cancer cells and macrophages [J]. *Theranostics*, 2024, 14(18): 7072-87.
- [139] ZHAO S, ZHAO R, WANG C, et al. HDAC7 drives glioblastoma to a mesenchymal-like state via LGALS3-mediated crosstalk between cancer cells and macrophages [J]. *Theranostics*, 2024, 14(18): 7072-87.
- [140] ROSBERG R, SMOLAG K I, SJOLUND J, et al. Hypoxia-induced complement component 3 promotes aggressive tumor growth in the glioblastoma microenvironment [J]. *JCI Insight*, 2024, 9(19).
- [141] ZHAO S, NI K, XIE J, et al. Exploring the prognostic value of BRMS1 + microglia based on single-cell anoikis regulator patterns in the immunologic microenvironment of GBM [J]. *J Neurooncol*, 2024, 170(1): 101-17.
- [142] GAO J, GU D, YANG K, et al. Infiltrating plasma cells maintain glioblastoma stem cells through IgG-Tumor binding [J]. *Cancer Cell*, 2025, 43(1): 122-43 e8.
- [143] ZHANG Y, KANG T, WANG Y, et al. A low level of tumor necrosis factor alpha in tumor microenvironment maintains the self-renewal of glioma stem cells by Vasorin-mediated glycolysis [J]. *Neuro Oncol*, 2024, 26(12): 2256-71.
- [144] GUPTA P, DANG M, OBERAI S, et al. Immune landscape of isocitrate dehydrogenase-stratified primary and recurrent human gliomas [J]. *Neuro Oncol*, 2024, 26(12): 2239-55.
- [145] MILLER T E, EL FARRAN C A, COUTURIER C P, et al. Programs, origins and immunomodulatory functions of myeloid cells in glioma [J]. *Nature*, 2025.
- [146] SAUER B, KUECKELHAUS J, LORENZ N I, et al. An AMP-activated protein kinase-PGC-1alpha axis mediates metabolic plasticity in glioblastoma [J]. *Clin Transl Med*, 2024, 14(11): e70030.
- [147] LI N, TONG H, HOU W, et al. Neural-cancer crosstalk: Reciprocal molecular circuits driving gastric tumorigenesis and emerging therapeutic opportunities [J]. *Cancer Lett*, 2025, 616: 217589.
- [148] CURRY R N, MA Q, MCDONALD M F, et al. Integrated electrophysiological and genomic profiles of single cells reveal spiking tumor cells in human glioma [J]. *Cancer Cell*, 2024, 42(10): 1713-28 e6.
- [149] NORTON E S, WHALEY L A, JONES V K, et al. Cell-specific cross-talk proteomics reveals cathepsin B signaling as a driver of glioblastoma malignancy near the subventricular zone [J]. *Sci Adv*, 2024, 10(32): eadn1607.
- [150] DREXLER R, KHATRI R, SAUVIGNY T, et al. A prognostic neural epigenetic signature in high-grade glioma [J]. *Nat Med*, 2024, 30(6): 1622-35.
- [151] KRISHNA S, CHOUDHURY A, KEOUGH M B, et al. Glioblastoma remodelling of human neural circuits decreases survival [J]. *Nature*, 2023, 617(7961): 599-607.
- [152] BARRON T, YALCIN B, SU M, et al. GABAergic neuron-to-glioma synapses in diffuse midline gliomas [J]. *Nature*, 2025.
- [153] WEIL S, OSSWALD M, SOLECKI G, et al. Tumor microtubules convey resistance to surgical lesions and chemotherapy in gliomas [J]. *Neuro Oncol*, 2017, 19(10): 1316-26.
- [154] HAI L, HOFFMANN D C, WAGENER R J, et al. A clinically applicable connectivity signature for glioblastoma includes the tumor network driver CHI3L1 [J]. *Nat Commun*, 2024, 15(1): 968.
- [155] VENKATARAMANI V, YANG Y, SCHUBERT M C, et al. Glioblastoma hijacks neuronal mechanisms for brain invasion [J]. *Cell*, 2022, 185(16): 2899-917 e31.
- [156] PERRAULT E N, SHIREMAN J M, ALI E S, et al. Ribonucleotide reductase regulatory subunit M2 drives glioblastoma TMZ resistance through modulation of dNTP production [J]. *Sci Adv*, 2023, 9(20): eade7236.

- [158] WANG L, JUNG J, BABIKIR H, et al. A single-cell atlas of glioblastoma evolution under therapy reveals cell-intrinsic and cell-extrinsic therapeutic targets [J]. *Nat Cancer*, 2022, 3(12): 1534-52.
- [159] WANG W, LI T, CHENG Y, et al. Identification of hypoxic macrophages in glioblastoma with therapeutic potential for vasculature normalization [J]. *Cancer Cell*, 2024, 42(5): 815-32 e12.
- [160] ONUBOGU U, GATENBEE C D, PRABHAKARAN S, et al. Spatial analysis of recurrent glioblastoma reveals perivascular niche organization [J]. *JCI Insight*, 2024, 9(12).
- [161] CHENG J, LI M, MOTTA E, et al. Myeloid cells coordinately induce glioma cell-intrinsic and cell-extrinsic pathways for chemoresistance via GP130 signaling [J]. *Cell Rep Med*, 2024, 5(8): 101658.
- [162] LEE A H, SUN L, MOCHIZUKI A Y, et al. Neoadjuvant PD-1 blockade induces T cell and cDC1 activation but fails to overcome the immunosuppressive tumor associated macrophages in recurrent glioblastoma [J]. *Nat Commun*, 2021, 12(1): 6938.
- [163] WATSON S S, ZOMER A, FOURNIER N, et al. Fibrotic response to anti-CSF-1R therapy potentiates glioblastoma recurrence [J]. *Cancer Cell*, 2024, 42(9): 1507-27 e11.
- [164] KIM K H, MIGLIOZZI S, KOO H, et al. Integrated proteogenomic characterization of glioblastoma evolution [J]. *Cancer Cell*, 2024, 42(3): 358-77 e8.
- [165] WANG X, SUN Q, WANG W, et al. Decoding key cell sub-populations and molecular alterations in glioblastoma at recurrence by single-cell analysis [J]. *Acta Neuropathol Commun*, 2023, 11(1): 125.
- [166] YOU G, ZHENG Z, HUANG Y, et al. scRNA-seq and proteomics reveal the distinction of M2-like macrophages between primary and recurrent malignant glioma and its critical role in the recurrence [J]. *CNS Neurosci Ther*, 2023, 29(11): 3391-405.
- [167] VERHAAK R G, HOADLEY K A, PURDOM E, et al. Integrated genomic analysis identifies clinically relevant subtypes of glioblastoma characterized by abnormalities in PDGFRA, IDH1, EGFR, and NF1 [J]. *Cancer Cell*, 2010, 17(1): 98-110.
- [168] WANG Q, HU B, HU X, et al. Tumor Evolution of Glioma-Intrinsic Gene Expression Subtypes Associates with Immunological Changes in the Microenvironment [J]. *Cancer Cell*, 2017, 32(1): 42-56 e6.
- [169] THOMAS A, TANAKA M, TREPEL J, et al. Temozolomide in the Era of Precision Medicine [J]. *Cancer Res*, 2017, 77(4): 823-6.
- [170] CAMPOS B, ZENG L, DAOTRONG P H, et al. Expression and regulation of AC133 and CD133 in glioblastoma [J]. *Glia*, 2011, 59(12): 1974-86.
- [171] WANG L B, KARPOVA A, GRITSENKO M A, et al. Proteogenomic and metabolomic characterization of human glioblastoma [J]. *Cancer Cell*, 2021, 39(4): 509-28 e20.
- [172] DAMODHARAN S, SHIREMAN J M, XIE E, et al. Transcriptomic and proteomic spatial profiling of pediatric and adult diffuse midline glioma H3 K27-Altered [J]. *Sci Rep*, 2024, 14(1): 22668.
- [173] HEGI M E, DISERENS A C, GORLIA T, et al. MGMT gene silencing and benefit from temozolomide in glioblastoma [J]. *N Engl J Med*, 2005, 352(10): 997-1003.
- [174] CHEN X, SUN J, LI Y, et al. Proteomic and metabolomic analyses illustrate the mechanisms of expression of the O(6) -methylguanine-DNA methyltransferase gene in glioblastoma [J]. *CNS Neurosci Ther*, 2024, 30(2): e14415.
- [175] AUFFRET L, AJLIL Y, TAUZIEDE-ESPARIAT A, et al. A new subtype of diffuse midline glioma, H3 K27 and

- [176] WU M, WANG T, JI N, et al. Multi-omics and pharmacological characterization of patient-derived glioma cell lines [J]. *Nat Commun*, 2024, 15(1): 6740.
- [177] TIAN J, ZHU M, REN Z, et al. Deep learning algorithm reveals two prognostic subtypes in patients with gliomas [J]. *BMC Bioinformatics*, 2022, 23(1): 417.
- [178] PICARD D, FELSBURG J, LANGINI M, et al. Integrative multi-omics reveals two biologically distinct groups of pilocytic astrocytoma [J]. *Acta Neuropathol*, 2023, 146(4): 551-64.
- [179] PARK J, SHIM J K, YOON S J, et al. Transcriptome profiling-based identification of prognostic subtypes and multi-omics signatures of glioblastoma [J]. *Sci Rep*, 2019, 9(1): 10555.
- [180] GAROFANO L, MIGLIOZZI S, OH Y T, et al. Pathway-based classification of glioblastoma uncovers a mitochondrial subtype with therapeutic vulnerabilities [J]. *Nat Cancer*, 2021, 2(2): 141-56.
- [181] YANG Z, MICHAELIDIS G. A non-negative matrix factorization method for detecting modules in heterogeneous omics multi-modal data [J]. *Bioinformatics*, 2016, 32(1): 1-8.
- [182] MANGANARO L, BIANCO S, BIRONZO P, et al. Consensus clustering methodology to improve molecular stratification of non-small cell lung cancer [J]. *Sci Rep*, 2023, 13(1): 7759.
- [183] YANG Z, LIU X, XU H, et al. Integrative analysis of genomic and epigenomic regulation reveals miRNA mediated tumor heterogeneity and immune evasion in lower grade glioma [J]. *Commun Biol*, 2024, 7(1): 824.
- [184] JIANG C, WU W, JIANG X, et al. Integrative multi-omics analysis unveils the connection between transcriptomic characteristics associated with mitochondria and the tumor immune microenvironment in lower-grade gliomas [J]. *Sci Rep*, 2024, 14(1): 23675.
- [185] TU Z, WU L, LUO H, et al. Systematic and Multi-Omics Prognostic Analysis of Lysine Acetylation Regulators in Glioma [J]. *Front Mol Biosci*, 2021, 8: 587516.
- [186] XIAO Y, ZHAO M, WANG R, et al. Bulk and single-cell transcriptome revealed the metabolic heterogeneity in human glioma [J]. *Heliyon*, 2025, 11(1): e41241.
- [187] MIGLIOZZI S, OH Y T, HASANAIN M, et al. Integrative multi-omics networks identify PKCdelta and DNA-PK as master kinases of glioblastoma subtypes and guide targeted cancer therapy [J]. *Nat Cancer*, 2023, 4(2): 181-202.
- [188] YUAN Y, QI P, XIANG W, et al. Multi-Omics Analysis Reveals Novel Subtypes and Driver Genes in Glioblastoma [J]. *Front Genet*, 2020, 11: 565341.
- [189] MA Y, XI Z. Integrated Analysis of Multiomics Data Identified Molecular Subtypes and Oxidative Stress-Related Prognostic Biomarkers in Glioblastoma Multiforme [J]. *Oxid Med Cell Longev*, 2022, 2022: 9993319.
- [190] KAMOUN A, IDBAIH A, DEHAIS C, et al. Integrated multi-omics analysis of oligodendroglial tumours identifies three subgroups of 1p/19q co-deleted gliomas [J]. *Nat Commun*, 2016, 7: 11263.
- [191] ZHANG G, TAI P, FANG J, et al. Multi-omics reveals the impact of cancer-associated fibroblasts on the prognosis and treatment response of adult diffuse highest-grade gliomas [J]. *Heliyon*, 2024, 10(15): e34526.
- [192] WU F, YIN Y Y, FAN W H, et al. Immunological profiles of human oligodendrogliomas define two distinct molecular subtypes [J]. *EBioMedicine*, 2023, 87: 104410.
- [193] VISIOLI A, TRIVIERI N, MENCARELLI G, et al. Different states of stemness of glioblastoma stem cells sustain glioblastoma subtypes indicating novel clinical biomarkers and high-efficacy customized therapies [J]. *J Exp Clin Cancer Res*, 2023, 42(1): 244.

- [194] ZHANG Y, ZHANG Y, ZHANG Y, et al. Integrative multi-omics analysis identifies novel glioma subtypes and prognostic signatures [J]. *EBioMedicine*, 2024, 10(10): 104978.
- [195] ZHENG J, WU Z, QIU Y, et al. An integrative multi-omics analysis based on liquid-liquid phase separation delineates distinct subtypes of lower-grade glioma and identifies a prognostic signature [J]. *J Transl Med*, 2022, 20(1): 55.
- [196] ALHALABI O T, FLETCHER M N C, HIELSCHER T, et al. A novel patient stratification strategy to enhance the therapeutic efficacy of dasatinib in glioblastoma [J]. *Neuro Oncol*, 2022, 24(1): 39-51.
- [197] ALHALABI O T, GOTTMANN M, GOLD M P, et al. Integration of transcriptomics, proteomics and loss-of-function screening reveals WEE1 as a target for combination with dasatinib against proneural glioblastoma [J]. *Cancer Lett*, 2024, 605: 217265.
- [198] LUO D, LUO A, HU S, et al. Genomics and proteomics to determine novel molecular subtypes and predict the response to immunotherapy and the effect of bevacizumab in glioblastoma [J]. *Sci Rep*, 2024, 14(1): 17630.
- [199] BINDER H, WILLSCHEER E, LOEFFLER-WIRTH H, et al. DNA methylation, transcriptome and genetic copy number signatures of diffuse cerebral WHO grade II/III gliomas resolve cancer heterogeneity and development [J]. *Acta Neuropathol Commun*, 2019, 7(1): 59.
- [200] FAN Y, GAO Z, XU J, et al. SPI1-mediated MIR222HG transcription promotes proneural-to-mesenchymal transition of glioma stem cells and immunosuppressive polarization of macrophages [J]. *Theranostics*, 2023, 13(10): 3310-29.
- [201] YABO Y A, NICLOU S P, GOLEBIEWSKA A. Cancer cell heterogeneity and plasticity: A paradigm shift in glioblastoma [J]. *Neuro Oncol*, 2022, 24(5): 669-82.
- [202] WU F, WANG Z L, WANG K Y, et al. Classification of diffuse lower-grade glioma based on immunological profiling [J]. *Mol Oncol*, 2020, 14(9): 2081-95.
- [203] WELLER M, VAN DEN BENT M, PREUSSER M, et al. EANO guidelines on the diagnosis and treatment of diffuse gliomas of adulthood [J]. *Nat Rev Clin Oncol*, 2021, 18(3): 170-86.
- [204] ZHAO Z, ZHANG K N, WANG Q, et al. Chinese Glioma Genome Atlas (CGGA): A Comprehensive Resource with Functional Genomic Data from Chinese Glioma Patients [J]. *Genomics Proteomics Bioinformatics*, 2021, 19(1): 1-12.
- [205] GENG R, ZHAO Y, XU W, et al. SIRPB1 regulates inflammatory factor expression in the glioma microenvironment via SYK: functional and bioinformatics insights [J]. *J Transl Med*, 2024, 22(1): 338.
- [206] LIU L, WANG G, WANG L, et al. Computational identification and characterization of glioma candidate biomarkers through multi-omics integrative profiling [J]. *Biol Direct*, 2020, 15(1): 10.
- [207] SABEDOT T S, MALTA T M, SNYDER J, et al. A serum-based DNA methylation assay provides accurate detection of glioma [J]. *Neuro Oncol*, 2021, 23(9): 1494-508.
- [208] FONTANILLES M, HEISBOURG J D, DABAN A, et al. Metabolic remodeling in glioblastoma: a longitudinal multi-omics study [J]. *Acta Neuropathol Commun*, 2024, 12(1): 162.
- [209] ZHOU J, JI N, WANG G, et al. Metabolic detection of malignant brain gliomas through plasma lipidomic analysis and support vector machine-based machine learning [J]. *EBioMedicine*, 2022, 81: 104097.
- [210] FUJITA Y, NUNEZ-RUBIANO L, DONO A, et al. IDH1 p.R132H ctDNA and D-2-hydroxyglutarate as CSF biomarkers in patients with IDH-mutant gliomas [J]. *J Neurooncol*, 2022, 159(2): 261-70.
- [211] WANG D H, FUJITA Y, DONO A, et al. The genomic alterations in glioblastoma influence the levels of CSF metabolites [J]. *Acta Neuropathol Commun*, 2024, 12(1): 13.

- [212] ZHANG Y, ZHANG Y, ZHANG Y, et al. Multi-omics analysis of cerebrospinal fluid metabolites in patients with primary olivocerebellar central nervous system tumors [J]. *Acta Neuropathol Commun*, 2018, 6(1): 33.
- [213] WU J, ZHANG J, WEI J, et al. Urinary biomarker discovery in gliomas using mass spectrometry-based clinical proteomics [J]. *Chin Neurosurg J*, 2020, 6: 11.
- [214] NAKADE Y, KINOSHITA M, NAKADA M, et al. Urinary D-asparagine level is decreased by the presence of glioblastoma [J]. *Acta Neuropathol Commun*, 2024, 12(1): 122.
- [215] TU H Y, LI Y S, BAI X Y, et al. Genetic Profiling of Cell-Free DNA From Pleural Effusion in Advanced Lung Cancer as a Surrogate for Tumor Tissue and Revealed Additional Clinical Actionable Targets [J]. *Clin Lung Cancer*, 2022, 23(2): 135-42.
- [216] BAO Y, ZHANG D, GUO H, et al. Beyond blood: Advancing the frontiers of liquid biopsy in oncology and personalized medicine [J]. *Cancer Sci*, 2024, 115(4): 1060-72.
- [217] FIGUEROA J M, SKOG J, AKERS J, et al. Detection of wild-type EGFR amplification and EGFRvIII mutation in CSF-derived extracellular vesicles of glioblastoma patients [J]. *Neuro Oncol*, 2017, 19(11): 1494-502.
- [218] FATHI A T, NAHED B V, WANDER S A, et al. Elevation of Urinary 2-Hydroxyglutarate in IDH-Mutant Glioma [J]. *Oncologist*, 2016, 21(2): 214-9.
- [219] TUNA G, DAL-BEKAR N E, AKAY A, et al. Minimally Invasive Detection of IDH1 Mutation With Cell-Free Circulating Tumor DNA and D-2-Hydroxyglutarate, D/L-2-Hydroxyglutarate Ratio in Gliomas [J]. *J Neuropathol Exp Neurol*, 2022, 81(7): 502-10.
- [220] LOMBARDI G, CORONA G, BELLU L, et al. Diagnostic value of plasma and urinary 2-hydroxyglutarate to identify patients with isocitrate dehydrogenase-mutated glioma [J]. *Oncologist*, 2015, 20(5): 562-7.
- [221] RUSSANO M, NAPOLITANO A, RIBELLI G, et al. Liquid biopsy and tumor heterogeneity in metastatic solid tumors: the potentiality of blood samples [J]. *J Exp Clin Cancer Res*, 2020, 39(1): 95.
- [222] HOSHINO A, KIM H S, BOJMAR L, et al. Extracellular Vesicle and Particle Biomarkers Define Multiple Human Cancers [J]. *Cell*, 2020, 182(4): 1044-61.e18.
- [223] SEYHAN A A. Circulating Liquid Biopsy Biomarkers in Glioblastoma: Advances and Challenges [J]. *Int J Mol Sci*, 2024, 25(14).
- [224] MILLER J J. Targeting IDH-Mutant Glioma [J]. *Neurotherapeutics*, 2022, 19(6): 1724-32.
- [225] MELLINGHOFF I K, VAN DEN BENT M J, BLUMENTHAL D T, et al. Vorasidenib in IDH1- or IDH2-Mutant Low-Grade Glioma [J]. *N Engl J Med*, 2023, 389(7): 589-601.
- [226] BUTLER M, PONGOR L, SU Y T, et al. MGMT Status as a Clinical Biomarker in Glioblastoma [J]. *Trends Cancer*, 2020, 6(5): 380-91.
- [227] CAIRNCROSS G, WANG M, SHAW E, et al. Phase III trial of chemoradiotherapy for anaplastic oligodendroglioma: long-term results of RTOG 9402 [J]. *J Clin Oncol*, 2013, 31(3): 337-43.
- [228] LEWIS J E, KEMP M L. Integration of machine learning and genome-scale metabolic modeling identifies multi-omics biomarkers for radiation resistance [J]. *Nat Commun*, 2021, 12(1): 2700.
- [229] ZHOU X, LIU H, HOU F, et al. REMR: Identification of RNA Editing-mediated MiRNA Regulation in Cancers [J]. *Comput Struct Biotechnol J*, 2024, 23: 3418-29.
- [230] WU W, JIANG C, ZHU W, et al. Multi-omics analysis reveals the association between specific solute carrier proteins gene expression patterns and the immune suppressive microenvironment in glioma [J]. *J Cell Mol Med*, 2024, 28(9): e18339.

[231] WANG Q, WANG C, PENG H, et al. Integrative analysis of multi-omics data reveals a pseudotime-related immune signature for prediction of glioma prognosis and chemoradiotherapy sensitivity [J]. Comput Biol Med, 2023, 166: 107428.

[232] WANG Q, ZHANG C, PANG Y, et al. Comprehensive analysis of bulk, single-cell RNA sequencing, and spatial transcriptomics revealed IER3 for predicting malignant progression and immunotherapy efficacy in glioma [J]. Cancer Cell Int, 2024, 24(1): 332.

[233] ZHAO K, LI W, YANG Y, et al. Comprehensive analysis of m(6)A/m(5)C/m(1)A-related gene expression, immune infiltration, and sensitivity of antineoplastic drugs in glioma [J]. Front Immunol, 2022, 13: 955848.

[234] LONG J, WANG D, YANG X, et al. Identification of NOTCH4 mutation as a response biomarker for immune checkpoint inhibitor therapy [J]. BMC Med, 2021, 19(1): 154.

[235] WU Z, WANG X, WU H, et al. Identification of CREB5 as a prognostic and immunotherapeutic biomarker in glioma through multi-omics pan-cancer analysis [J]. Comput Biol Med, 2024, 173: 108307.

[236] XU H, CHAI H, CHEN M, et al. Single-cell RNA sequencing identifies a subtype of FN1 + tumor-associated macrophages associated with glioma recurrence and as a biomarker for immunotherapy [J]. Biomark Res, 2024, 12(1): 114.

[237] ALENTORN A, VAN THUIJL H F, MARIE Y, et al. Clinical value of chromosome arms 19q and 11p losses in low-grade gliomas [J]. Neuro Oncol, 2014, 16(3): 400-8.

[238] BARZEGAR BEHROOZ A, DARZI RAMANDI H, LATIFI-NAVID H, et al. Genetic Prognostic Factors in Adult Diffuse Gliomas: A 10-Year Experience at a Single Institution [J]. Cancers (Basel), 2024, 16(11).

[239] DELLA PUPPA A, PERSANO L, MASI G, et al. MGMT expression and promoter methylation status may depend on the site of surgical sample collection within glioblastoma: a possible pitfall in stratification of patients? [J]. J Neurooncol, 2012, 106(1): 33-41.

[240] ZHANG Y, YU H, LI Y, et al. Raman spectroscopy: A prospective intraoperative visualization technique for gliomas [J]. Front Oncol, 2022, 12: 1086643.

[241] FAN Q W, CHENG C K, GUSTAFSON W C, et al. EGFR phosphorylates tumor-derived EGFRvIII driving STAT3/5 and progression in glioblastoma [J]. Cancer Cell, 2013, 24(4): 438-49.

[242] LAN J, GUO P, LIN Y, et al. Role of glycosyltransferase PomGnT1 in glioblastoma progression [J]. Neuro Oncol, 2015, 17(2): 211-22.

[243] YAO X, PING Y, LIU Y, et al. Vascular endothelial growth factor receptor 2 (VEGFR-2) plays a key role in vasculogenic mimicry formation, neovascularization and tumor initiation by Glioma stem-like cells [J]. PLoS One, 2013, 8(3): e57188.

[244] CHEN L, XIE X, WANG T, et al. ARL13B promotes angiogenesis and glioma growth by activating VEGFA-VEGFR2 signaling [J]. Neuro Oncol, 2023, 25(5): 871-85.

[245] WAN Z, ZUO X, WANG S, et al. Identification of angiogenesis-related genes signature for predicting survival and its regulatory network in glioblastoma [J]. Cancer Med, 2023, 12(16): 17445-67.

[246] MATHUR R, ZHANG Y, GRIMMER M R, et al. MGMT promoter methylation level in newly diagnosed low-grade glioma is a predictor of hypermutation at recurrence [J]. Neuro Oncol, 2020, 22(11): 1580-90.

[247] ZHAO M, LI X, CHEN Y, et al. MD2 Is a Potential Biomarker Associated with Immune Cell Infiltration in Gliomas [J]. Front Oncol, 2022, 12: 854598.

[248] CHEN P, WANG H, ZHANG Y, et al. Construction of a Prognostic Model for Mitochondria and Macrophage Polarization Correlation in Glioma Based on Single-Cell and Transcriptome Sequencing [J]. CNS Neurosci Ther,

- [249] TANG Q, YUAN Y, LI L, et al. Comprehensive analysis reveals that LTBR is a immune-related biomarker for glioma [J]. *Comput Biol Med*, 2024, 174: 108457.
- [250] DREXLER R, KHATRI R, SAUVIGNY T, et al. A prognostic neural epigenetic signature in high-grade glioma [J]. *Nat Med*, 2024, 30(6): 1622-35.
- [251] REYES-GONZÁLEZ J, BARAJAS-OLMOS F, GARCÍA-ORTIZ H, et al. Brain radiotoxicity-related 15CAcBRT gene expression signature predicts survival prognosis of glioblastoma patients [J]. *Neuro Oncol*, 2023, 25(2): 303-14.
- [252] NGUYEN T T T, SHANG E, SHU C, et al. Aurora kinase A inhibition reverses the Warburg effect and elicits unique metabolic vulnerabilities in glioblastoma [J]. *Nat Commun*, 2021, 12(1): 5203.
- [253] YANG S, ZHAO J, CUI X, et al. TCA-phospholipid-glycolysis targeted triple therapy effectively suppresses ATP production and tumor growth in glioblastoma [J]. *Theranostics*, 2022, 12(16): 7032-50.
- [254] MEI Y, WANG X, ZHANG J, et al. Siglec-9 acts as an immune-checkpoint molecule on macrophages in glioblastoma, restricting T-cell priming and immunotherapy response [J]. *Nat Cancer*, 2023, 4(9): 1273-91.
- [255] ZHONG J, XING X, GAO Y, et al. Distinct roles of TREM2 in central nervous system cancers and peripheral cancers [J]. *Cancer Cell*, 2024, 42(6): 968-84.e9.
- [256] GOSWAMI S, RAYCHAUDHURI D, SINGH P, et al. Myeloid-specific KDM6B inhibition sensitizes glioblastoma to PD1 blockade [J]. *Nat Cancer*, 2023, 4(10): 1455-73.
- [257] ZHU C, CHEN X, LIU T Q, et al. Hexosaminidase B-driven cancer cell-macrophage co-dependency promotes glycolysis addiction and tumorigenesis in glioblastoma [J]. *Nat Commun*, 2024, 15(1): 8506.
- [258] WATSON S S, ZOMER A, FOURNIER N, et al. Fibrotic response to anti-CSF-1R therapy potentiates glioblastoma recurrence [J]. *Cancer Cell*, 2024, 42(9): 1507-27.e11.
- [259] LIU J, CAO S, IMBACH K J, et al. Multi-scale signaling and tumor evolution in high-grade gliomas [J]. *Cancer Cell*, 2024, 42(7): 1217-38 e19.
- [260] ERTURK A. Deep 3D histology powered by tissue clearing, omics and AI [J]. *Nat Methods*, 2024, 21(7): 1153-65.
- [261] BHATIA H S, BRUNNER A D, OZTURK F, et al. Spatial proteomics in three-dimensional intact specimens [J]. *Cell*, 2022, 185(26): 5040-58 e19.
- [262] SYLWESTRAK E L, RAJASETHUPATHY P, WRIGHT M A, et al. Multiplexed Intact-Tissue Transcriptional Analysis at Cellular Resolution [J]. *Cell*, 2016, 164(4): 792-804.
- [263] MURAKAMI T C, HEINTZ N. Multiplexed and scalable cellular phenotyping toward the standardized three-dimensional human neuroanatomy [J]. *bioRxiv*, 2022: 2022.11.23.517711.
- [264] BUNNE C, ROOHANI Y, ROSEN Y, et al. How to build the virtual cell with artificial intelligence: Priorities and opportunities [J]. *Cell*, 2024, 187(25): 7045-63.