A Mini-review on Spatiotemporal Evolution of Glioma Under Treatment

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Abstract Glioblastoma is the most malignant form of brain tumors in adults. Therapeutic development has been stagnant for decades until recent years. With the advent of precision medicine and next generation sequencing, it is crucial to examine the complex mechanisms underlying this deadly disease for accurate prognostic prediction. Secondary or recurrent glioblastomas with matched initial tumors are invaluable cases to study, as they allow us to understand glioma progression over time and space with resistance to treatment. Here we review the complexities within glioblastomas, including a wide array of driver alterations, spatial heterogeneity and diverging evolutionary trajectories over time, and how these knowledge can facilitate prognostic prediction and therapeutic translation.

Key words glioblastoma, molecular features, clonal evolution

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1 Glioblastoma overview - epidemiology and treatment

Glioma is the most common brain tumor in adults. At the most malignant end of its spectrum is glioblastoma multiforme (GBM). With a median survival of 14.6 months\(^1\), GBM, classified as WHO grade IV, is both more deadly and more prevalent than its low-grade counterparts of diffuse glioma including oligodendroglioma and diffuse astrocytoma\(^2\). In the population-wide study in the US, between 2000 and 2014, GBM accounted for over 60% of all glioma incidence, and had the lowest 5-year relative survival after diagnosis at 5.4% compared with oligodendroglioma (70.1%) and astrocytoma (44.4%)\(^3\). More interestingly, there is a significantly lower incidence of GBM in Asian populations compared to Hispanic or non-Hispanic Whites with better median survival statistics\(^2-3\).

The short survival of GBM could be attributed to the difficulty in surgery and the limited choice of chemotherapy drugs. Currently, standard treatment of newly diagnosed GBM begins with maximal safe surgical resection, followed by concurrent radiotherapy with temozolomide (TMZ), and then adjuvant chemotherapy with TMZ\(^4\). Better outcomes are for those with IDH1 mutation or MGMT promoter methylation, for those with greater extent of resection\(^5-9\), and for those with concomitant TMZ chemotherapy than radiotherapy alone\(^10\). Unfortunately, almost all GBM inevitably recur, and there has not been any standard therapeutic strategy for recurrent GBM.

Recurring low-grade gliomas that progressed to a more malignant grade IV are termed secondary GBM (sGBM), which accounts for <10% of all GBM. Its age-adjusted survival is comparable to primary GBM (pGBM), although sGBMs are diagnosed at a younger mean age of 45 compared to 62 for pGBM\(^11-13\). Because of its low-grade glioma precondition and the futility of treatment leading to recurrence, sGBM has unique molecular features that differ from pGBM, and its evolutionary trajectory can help us better understand vulnerabilities of the cancer, unlocking new treatment options.

2 Molecular features of primary and secondary GBMs

Tumor cells of GBM commonly harbor driver mutations in TP53, EGFR, and in the PI3K pathway PIK3CA, PIK3R1, PTEN. In parallel, focal copy-number alterations could alter expression levels of critical receptor tyrosine kinases such as MET, EGFR, PDGFR\(^\alpha\)\(^{11-12}\). In addition, we often observe whole chromosome arm deletion or amplifications, particularly chromosome 7 gain (containing MET, EGFR and PDGFA), and chromosome 10 loss (where PTEN tumor suppressor gene is located) in GBM\(^{13}\).

In sGBM but less common in pGBM, we observe more frequent mutations in TP53, CIC, FUBP1 and ATRX\(^{14-15}\), likely carried over from low-grade glioma together with IDH1 mutations shown by Johnson et al.\(^{16}\), while pGBM are mostly IDH1 wildtype. Also, higher levels of MET alterations, including point mutations, copy number amplifications, exon 14-skipping and fusion with PTPZR1 have been observed\(^{17}\). On the methylation spectrum, sGBM patients carried high levels of MGMT and TIMP-3 promoter methylations (75% and 71% respectively, compared to 36% and 28% in pGBM), the former particularly associated with better responses to TMZ therapy\(^3,17-18\).

The genomic landscape of sGBM is often altered. Most notable are the changes in mutational signatures, as the alkylating drug induces DNA damage with a unique mutational signature: CpC > TpC\(^{19}\). While untreated glioblastoma has relatively low tumor mutation load, usually fewer than 100 mutations per tumor in the exonic region, compared to colorectal cancer, lung cancer and melanoma, if the patient suffers from compromised repair mechanisms such as MSH6 mutations, we will often observe hypermutations in the sGBM samples, where there is a drastic rise in number of mutations compared with the primary counterparts (typically over 1 000 coding region mutations per tumor)\(^{14,16}\). On average 17% of TMZ-treated patients will harbor hypermutation signatures, and it was reported to be more common in Caucasians than Asians, although the cause is still a mystery\(^{14}\).
3 Spatiotemporal studies and precision medicine in glioblastoma

There are many factors driving tumorigenesis and progression, but not every patient, or even every part of the tumor evolves at a uniform or identical pace. Studying longitudinal dynamics and spatial heterogeneity of cancer of large sample size would ultimately allow prediction of disease progression and outcome such that we can improve the precision and effectiveness of treatment as new targets arise (Figure 1).

GBM has been extensively studied using different cohorts and perspectives. Apart from basic bulk tumor sequencing, temporal analysis of paired samples allows comparisons of mutation landscapes to infer treatment consequences such as clonal switching and hypermutations. Spatial analysis using multi-focal sampling revealed intratumoral heterogeneity, while single-cell sequencing allows interpretations of cancer cell lineages and interactions that could support the above observations. Combining these approaches with the ever-expanding cohorts, we may confidently reconstruct the evolution trajectory of GBM, enabling more accurate prediction of disease progression and identification of impactful treatment targets in the future.

3.1 Intratumoral heterogeneity in GBM

In some sense, tumors can be described as new organs, with dynamic interactions among a wide range of tumor cells, surrounding epithelial and blood vessels, as well as infiltrating immune cells in the microenvironment. This complicates the composition of tumors and intratumoral heterogeneity that increases the difficulty of eradicating a tumor using a single therapeutic target. In addition to the branched evolution models based on clonal mutations, recent GBM and LGG studies deployed sample level strategies, ranging from multiple point sampling to single-cell sequencing of tumor samples in order to understand this phenomenon.

From the multisampling studies, intratumoral heterogeneity suggests a lineage of molecular aberrations as there are common, shared and private alterations in different locations of a tumor. Common alteration, that are found across all points of sampling, such as chromosome 7 amplification and chromosome
10 deletions in certain patients (sp49) [20], are interpreted as founding or truncal events[12], while shared alterations that occur among multiple, but not all, samples, and private alterations localized to an exclusive sample suggest localized or branched events. For instance, some critical genes, such as CIC and FUBP1, may see mutations at different loci in different low grade glioma samples that suggest independent but convergent evolution[15], while in another study of GBM, PTEN and EGFR mutations are identified as localized events[21]. In particular, EGFR, a critical driver gene, also exhibited "disjoint" mutations, featuring different point mutations in the different regions[21]. These observations support the branched model of tumor evolution[22]. Such heterogeneity is especially pronounced in patients with multiple lesions or when the secondary GBM is spatially distant from the primary[21].

In addition to point mutations, an early study in pGBM suggests heterogeneous changes in copy numbers of receptor tyrosine kinase (RTK) genes regarding the choice of RTK altered[12]. Clones within a tumor may have copy number alterations of different RTK such as PDGFRα and EGFR, leading to the observation of tumor mosaics. However, while different choices of alterations among multisampling studies provide evidence for the multiple drivers leading to GBM, the possibility of earlier "seeding" events that lead to multifocal tumors cannot be dismissed, especially in samples with few common events.

Profiling RNA expression also highlights intratumoral heterogeneity. As demonstrated by Sottoriva et al. [20], within a tumor the expression profiles can be dissimilar to the Verhaak molecular subtype classifiers[23]. This is also further supported by single-cell RNA sequencing studies, where we can clearly see clustered chromosome-wide amplification and deletion that arise from copy number changes, as well as mixtures of cells exhibiting different molecular subtypes[21,24]. When coupled with spatial information at sampling, single-cell sequencing can provide additional high-resolution inference on lineage patterns and suggest evolution trajectories.

### 3.2 Longitudinal evolution of brain cancers

Sampling and sequencing tumors at multiple time points portrays a valuable evolutionary landscape of tumors under therapy. A basic question arises in the comparison of genomic and transcriptomic landscapes before and after recurrence: which genetic or phenotypic alteration is conserved, newly emerging, or missing? As the recurring tumors commonly have highly heterogeneous profiles, such findings provide means to divide and conquer, to redefine tumors by founding and branched alterations, hence potentially improving the specificity of treatments.

#### 3.2.1 Low-grade glioma recurrence and progression

The recurrence of low-grade glioma either remains in low-grade, or progresses into high-grade glioblastoma. The aforementioned IDH1 R132H mutation is commonly shared in both initial and recurrent gliomas, indicating that it occurs at early stage and persists in progression[16]. Accordingly, targeting IDH1 becomes a promising therapeutic strategy. However, a recent study of longitudinal progression of glioma illustrated that while mutant IDH1 is playing a key role in cancer initialization, it does not seem critical for recurrent tumors, challenging the clinical efficacy of targeting such mutation[25]. In addition, the IDH1 mutation has strong propensity in co-occurring with TP53 and ATRX mutations. The co-occurrence of IDH1, TP53 and ATRX mutations implies strong dependencies among the three genes in gliomagenesis and progression. Unlike IDH1 mutational locus (R132H), TP53 and ATRX frequently harbor distinct mutational loci between initial tumors and recurrent tumors. This phenomenon, termed as clonal replacement/switching[26], suggests that mutations of TP53 and ATRX are relatively late events and they are indispensable functional drivers in recurrent gliomas.

Several studies have recently revealed events that drive the progression from LGG to sGBM. Transcriptomic sequencing of 272 gliomas identified PTPRZ1-MET (ZM) fusion in 3 out of 20 sGBM patients, suggesting the ZM translocation event as a key to drive glioma progression[27]. Wang et al. [26] discovered another genomic translocation activating MGMT in the progression of 1 out of 5 sGBM patients. Johnson et al. [16] performed whole-exome sequencing of primary-recurrence pairs from 23 initial low-grade glioma patients. This study highlights 6 out of 10 patients treated by TMZ (widely used in GBM but controversial in LGG) developed hypermutation, inducing alterations in RB and AKT-mTOR pathways. Bai et al. [28] investigated the malignant progression of 41 IDH1 mutant low-grade gliomas and demonstrated the activation of diverse oncogenic pathways such as
MYC and RTK-RAS-PI3K. Another integrated study analyzed sGBM data from 188 patients\(^\text{[15]}\). Through comparison of the mutational landscapes of LGG, pGBM and sGBM, this study highlights MET alterations including the skipping the exon14 in MET protein, MET amplification and ZM translocation are significantly enriched in sGBM. Furthermore, prolonged stability and hyper-activation of MET signaling pathway has been experimentally demonstrated to play substantial roles in the progression of LGG to sGBM.

### 3.2.2 Recurrence of primary GBM

As the somatic mutational landscape of LGG and primary GBM are different, it is expected that the evolution of recurrent GBM from primary GBM also has a distinct pattern from low-grade glioma and secondary glioblastoma. Despite the added temporal axis of variations, where transcriptome-based subtyping revealed that 63% of patients exhibited a subtype-switch from initial to recurrent tumors\(^\text{[20]}\), there are patterns in the evolution of recurrent GBM. Wang et al.\(^\text{[26]}\) portrayed a longitudinal genomic landscape of 114 glioblastoma patients, most of which have primary-recurrent-matched GBMs. While the driver mutations of IDH1, TP53 and ATRX, plus PI3K pathway alterations (PIK3CA, PIK3R1 and PTEN) are often carried over in recurrent GBM much like the low-grade glioma to sGBM progression, in recurrent GBMs significant enriched alterations of MSH6, NF1, RB1, PDGFRA and LTB4 were also uncovered\(^\text{[26]}\). In particular, this study experimentally demonstrated that inhibition of LTB4, encoding a protein binding to TGF-β, resulted in decreased glioma cell proliferation. In contrast, EGFR alterations including genomic amplification, point mutation and EGFRvIII (deletion of exons 2–7) that were common in pGBM, was less frequently observed in recurrent GBM\(^\text{[20]}\), suggesting that EGFR alterations might not be required for recurrent GBM. A more recent study using whole-genome sequencing uncovered the genomic landscape of IDH-wildtype GBM in the non-coding region, and highlighted that TERT promoter mutation appeared late as a prerequisite of rapid growth following chromosome 7 gain, 9p loss, or 10 loss at the early stage\(^\text{[29]}\).

### 4 Modelling GBM development

The data and the inferred knowledge allowed us to construct predictive models to forecast the risks of primary patients. A computational method named tumor evolutionary directed graph (TEDG)\(^\text{[30]}\) was developed to portray the underlying evolutionary trajectories of 93 GBM patients with their tumor genome sequenced. This directed graph uniquely recapitated major oncogenic events, i.e., point mutations and copy-number alterations, at the timeline of GBM progression history. To further unveil patient-specific evolutionary patterns, a statistical method was adopted to embed each patient-specific evolutionary tree on a sphere space, namely Moduli space. Mapping all the GBM evolutionary trees with primary-recurrent matched samples, 54 patients were found to follow a branched evolutionary mode, whereas 17 patients were supported to follow a linear evolutionary mode. The patients with branched-evolution tumor growths were in part confirmed by the discovery of clonal mutation replacement events, where the branching time points could be further modelled based on mutation load, suggesting the recurrent clone could appear as early as the initial tumor was diagnosed\(^\text{[30]}\).

With the prevalence of single-cell RNA sequencing, the tumor can be scrutinized with higher resolution. Using the expression profiles, cancer cells have been projected onto a lineage continuum from oligodendrocyte progenitors to astrocytes to stem-cells\(^\text{[31–32]}\). The continuity of expression and the potentially dynamic cell states could supplement the mutation-based evolutionary model in elucidating crucial roles of tumor-microenvironment interactions.

### 5 Treatment outlook

With breakthroughs in studying the spatiotemporal dynamics of glioblastoma, there is ongoing translation from findings into treatment. Several clinical trials have focused on agent targets based on the genomic landscape of gliomas. EGFR amplification, rearrangement, point mutations and other alterations are found in approximately half of glioblastomas\(^\text{[33]}\), in particular EGFRvIII deletion is found in nearly 20% of all GBM patients\(^\text{[34]}\). Some
studies have demonstrated that EGFRvIII-driven tumors are only sensitive to first generation EGFR tyrosine kinase inhibitors (TKI) erlotinib and gefitinib. Oncogenic FGFR-TACC fusion gene is found in nearly 3% of GBM, with promising actionability provided by some clinical trials. Another fusion gene, PTPRZ1-MET, has been found in 15% of secondary GBM patients, resulting in hyperactivation of MET signalling, and was associated with poor patient survival. A highly selective ATP-competitive small-molecule MET inhibitor PLB-1001, exhibited better blood-brain barrier penetrance and had an acceptable safety profile and achieved partial responses in a phase I clinical trial.

In addition, targeting tumor immune microenvironment provides a new direction for the treatment of primary tumors and delays tumor recurrence. However, in the glioma immune microenvironment there are intracranial primitive cells including microglias, astrocytes, neurons and oligodendrocytes, which differs from other tumors in the pathogenesis. This demands different therapeutic strategies against pro-tumor microenvironment. Woroniecka et al. found stereotyped T-cell transcriptional programs matching classical virus-induced exhaustion and that exhaustion signatures varied with tumor type as a severe event in glioblastoma. Van Den Bossche et al. demonstrated oncolytic virus promoted M2 macrophages shifted toward to M1 immunophenotype, inducing the inhibition of glioma initiation. The recent anti-PD-1 immunotherapy has not been helpful in GBM where less than 10% patients show long-term responses. Zhao et al. longitudinally profiled 66 patients under immune therapy and reported a novel treatment-resistant scenario characterized by the elimination of neoepitopes and the change of T cell diversity in GBM evolution.

Moreover, glioma stem cells (GSCs) are closely associated with tumorigenesis and recurrence. Shi Y et al. found that GSCs activated receptor-type tyrosine kinase BMX, which caused the destructive growth of tumor stem cells of glioma and is hardly expressed in normal neural stem cells, indicating the specificity of BMX in GSCs. To inhibit GSC activation, ibufibrate was used, in combination with conventional radiotherapy to effectively improve antitumor efficacy.

With ongoing development of novel therapeutic approaches to glioblastoma and better understanding of the underlying mechanisms, precision medicine for glioblastoma will hopefully emerge in the near future to rescue more lives from this deadly malady.

References

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脑胶质瘤治疗相关时空演化机制及其在精准治疗中的应用

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摘要 脑胶质母细胞瘤是成人中最恶性的颅内肿瘤，但其治疗方式在过去数十年未有突破。随着近年精准医学和下一代测序技术的发展，使研究胶质母细胞瘤背后的多维基因组学的复杂机制成为可能。其中继发胶质母细胞瘤及其配对的原发肿瘤是十分珍贵的数据，可用以分析低级别胶质瘤在时间和空间轴上的演化以及治疗对肿瘤的影响。本综述阐述胶质母细胞瘤的复杂性，包括各种驱动突变、空间上的异形性和不同的演化方式；此外，会讨论如何将这些基因学上的发现应用在肿瘤预后的预测以及精准治疗上。

关键词 胶质母细胞瘤，分子基因学，群落演化

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