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Current Advances in The Molecular and Histopathological Aspects of Glioblastoma Multiforme: A Review

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Abstract

Glioblastoma multiforme represents the most aggressive of all primary brain neoplasms in adults, characterized by extremely poor prognosis and limited therapeutic interventions. This paper reviews current knowledge regarding molecular and histopathological insights into GBM. Besides demonstrating necrosis and microvascular proliferation with high cellularity- which have made it a diagnosis of classification and clinical behavior- at the molecular level this tumor is unleashed by diverse genetic and epigenetic changes. This includes alterations in IDH mutation, PTEN loss, EGFR amplification as well as MGMT promoter methylation controlling tumor progression as well as treatment response to therapies. Further, therapeutic resistance arising due to the heterogeneity of cells within tumors plus associated recurrence has emphasized another dimension requiring focus in research studies regarding treatment options for cancer anywhere between baseline laboratory benches up through human patient applications. Additionally included are immune cell components among others such as extracellular matrix elements plus signaling pathways promoting invasion/survival processes inside mass development regions resulting finally to Spatial Transcriptomics-based single-cell sequencing mapping out spatial complexity yet providing directions towards potential biomarkers along with precise therapy choices. Progress does not dissuade the fact that GBM carries such a forlorn prognosis, hence the need for integrated research approaches that would incorporate histological, molecular, and microenvironmental information. It is, therefore, imperative to underscore comprehensive tumor profiling in guiding future therapeutic strategies toward clinical outcome improvement.

Keywords

glioblastoma multiforme, IDH mutation, PTEN loss, EGFR amplification, MGMT

Introduction

Brain tumors are among the most formidable challenges confronting clinical oncology because of their intricate biology, treatment resistance, and effects on essential neurological functions. Gliomas (CNS WHO 5 classification), arising from glial cells are 80% of malignant brain tumors. The World Health Organization (WHO) classifies gliomas by histopathological features and molecular markers- low-grade astrocytoma to high-grade glioblastoma. Glioblastoma multiforme is an aggressively/lethally primary brain tumor in adults which occupies not only predominantly but also significantly spaces

The integration of histopathology, molecular genetics, and neuroimaging has brought about significant advances in the diagnosis and management of brain tumors over recent decades. While histopathology continues to play a principal role in the classification of brain tumors, it is through the diagnostic schemas that incorporate molecular biomarkers that transformations have been affected regarding perceptions on tumor behavior and prognosis (Brat et al., 2015). Besides encouraging better precision in making diagnoses, these molecular breakthroughs provide insight into intra-tumoral diversity masked by present sub-classifications and are especially informative about diversity within GBM thus necessitating individualized treatment approaches.

GBM is a fast-growing tumor that does not respond well to treatment. It results in poor prognoses with about 14-16 months of life possible even under the best conditions involving surgery plus radiation as well as chemotherapy (Tan et al., 2020). There is no other more destructive pathological astrocytic version than GBM. The classic picture shows necrosis and pseudopalisading cell plus microvascular proliferation and marked degrees of atypia mitotic activity within the cells (Louis et al., 2016). These morphological hallmarks are appended by a sundry list of molecular changes that impact pathways involved in tumor growth, invasion, and response to therapy.

As of the recent 2016 and further updated 2021 WHO classifications of tumors of the CNS, molecular parameters including isocitrate dehydrogenase (IDH) mutations, 1p/19q codeletion, and O6-methylguanine-DNA methyltransferase (MGMT) promoter methylation have been incorporated into the classification of gliomas to reflect a trend toward more integrated diagnoses.<split>A GBM has IDH-wildtype and IDH-mutant forms; practically speaking, the former is much more common and carries a worse prognosis. This has improved precision in diagnosis and provided information about the origin and progression of tumors. The molecular landscape of GBM is heterogeneously defined by multiple genetic and epigenetic alterations. The major oncogenic drivers are typical amplifications of epidermal growth factor receptor (EGFR) mutations in the TP53 tumor suppressor gene loss of phosphatase and tensin homolog (PTEN), and PI3K/AKT/mTOR pathway activations. Other abnormalities include chromatin remodeling genes and DNA methylation- such as MGMT promoter methylation- that play a most significant role in therapeutic response pathways, especially to alkylating agents like temozolomide. MGMT methylation results in the silencing of a DNA repair enzyme that can make the cell more responsive to chemotherapy; therefore, it has already moved into being an important prognostic and predictive biomarker.

GBM is characterized by extreme cellular pleomorphism and mitotic activity, features typically seen in aggressive neoplasms. The most dramatic feature is pseudopalisading necrosis wherein viable tumor cells seemingly invade across an area of necrosis, a pattern classic for description of hypoxic microenvironments within tumors. Another feature of the pathology related to angiogenesis is microvascular proliferation reflecting active angiogenesis initiated by signals through pathologic vascular endothelial growth factor (VEGF) pathways. These histological features presently serve as diagnostic criteria and have therapy implications since pathways are a target of the available anti-angiogenic agents such as bevacizumab.

Recent findings underscore the crucial role of inter- and intra-tumoral heterogeneity in modulating disease progression and resistance. GBM comprises several genetically and phenotypically different cellular subpopulations within the same tumor, a fact indicated by failed monotherapy that actually calls for multi-targeted approaches (Patel et al., 2014). In addition, evidence has mounted in support of claims that GSCs also exist in normal therapy and standard radiotherapy as well as chemotherapy environments leading to disease relapse due to mere possession of self-renewing properties and resilience to cytotoxic treatments (Lathia et al., 2015). Besides genetic changes, DNA methylation,

histone modifications, and non-coding RNAs are increasingly recognized as important factors in the pathophysiology of GBM. For example, MGMT promoter methylation is the most critical epigenetic indicator in the prediction of therapeutic response (Hegi et al., 2005). Changes in the expression of microRNAs (miRNAs) have been associated with tumor growth, invasion, and angiogenesis. They offer possible prospects for biomarker discovery and therapeutic intervention (Kaur et al., 2020).

The super immunosuppressive environment found within GBM is just what is needed to crank up the aggressiveness of the tumor and encourage it to avoid immune destruction. This has huge clinical impacts because it makes un-leashing immune-mediated responses in the presence of dis-inhibitors like anti-PD1/PDL1 less effective in GBM than in other cancers where a wave of remarkable responses has been observed. However, there are plenty of studies going on at present aimed at developing methods by which the steady state microenvironment of GBM can be reprogrammed both for enhancing immune activation and sensitivity to therapeutic interventions. Great research efforts notwithstanding, the prognosis of GBM patients is dismal, and disease recurrence is almost ensured. This has pivoted large research efforts to better understand the molecular and histopathological mechanisms that not only underlie the initiation and progression of GBM but also its resistance to therapy. Cutting-edge technologies comprising single-cell RNA sequencing, multi-omics profiling, and spatial transcriptomic analyses are currently unraveling tumor biology with unprecedented resolution providing hope for new diagnostic markers and therapeutic targets (Neftel et al., 2019).

This review article aims at discussing the latest advances that have widened knowledge at the molecular and histopathological levels about glioblastoma multiforme. By drawing recent findings together, it attempts to bridge the gap between histological observations and molecular data with their bearing on diagnosis, prognosis, and future therapeutic strategies. These are best appreciated in more detail for developing precision medicine approaches that would bring enhanced clinical results to such a devastating disease.

Histopathological Features of GBM

Glioblastoma multiforme belongs to the most malignant primary brain tumors and is actually a grade IV astrocytoma by World Health Organization classification. Although modern achievements in molecular characterization have been made, peculiar histological features are still essential in establishing the diagnosis and understanding such aggressive clinical behavior of the tumor. Besides, histology has never lost its role in integrated practice for diagnostics of GBM (Louis et al., 2021). Microvascular proliferation is one of the most characteristic histopathological hallmarks; it is defined by the formation of multilayered or glomeruloid tufts of proliferating endothelial cells (figure 1). This neovascularization would certainly be able to support rapid growth and the metabolic needs of the tumor. The driving force behind microvascular proliferation is mainly overexpression of angiogenic factors (one such factor being vascular endothelial growth factor, VEGF) expressed under hypoxic conditions within the tumor microenvironment. These types of vascular structures are seen as a hallmark that differentiates GBM from lower-grade astrocytomas (Plate et al., 2012).

Another hallmark for diagnosis is pseudopalisading necrosis (figure 1). This means areas of necrosis surrounded by densely packed tumor cells aligned in a palisading pattern. The pseudopalisading arrangement can be understood as a reaction to hypoxia, such that viable tumor cells move away from the necrotic core and eventually find oxygen and nutrients. These are regions of necrosis caused by rapid growth of the tumor outpacing its supply of blood; thus, the aggressiveness of getting to GBM has important prognostic implications (Combs et al., 2014). Nuclear atypia and mitotic activity are also well-seen. The cells of the tumor show great pleomorphism with hyperchromatic, irregular nuclei and numerous mitotic figures-it's usually about five to six per high-power field-reflecting proliferative activity. Most often, the atypical mitotic figure is seen supporting genomic instability and, therefore, the disease process in high-grade gliomas. In some cases, giant cell morphology and epithelioid variant may sometimes be seen that further complicates the picture of this tumor (Wesseling & Capper, 2018).

GBM is highly heterogeneous at the cellular level. The composition of the tumor includes small cells, gemistocytic astrocytes, and multinucleated giant cells (figure 1). Some areas may show sarcomatous differentiation or take on mesenchymal-like aspects

particularly for GBM subtypes harboring mutations in TP53 or amplification of EGFR. It is this cell type diversity that makes histological grading a nightmare and matches up with intra-tumoral heterogeneity when taken to a molecular level (Brennan et al., 2013).

There is also considerable reactive change in the adjacent brain parenchyma. This may be represented by gliosis, perivascular lymphocytic infiltration, and neuronal satellitosis—tumor cells clustering around normal neurons. Infiltrative growth is another hallmark—cells extend into surrounding brain parenchyma, most of the times extensively beyond any margins that can be identified on imaging or even at gross pathology. It is this diffuse invasion that accounts for the high recurrence rate even after gross total resection (Giese et al., 2003).

Immunohistochemistry can be regarded as a useful adjunct to the evaluation of GBMs by traditional methods of staining with hematoxylin and eosin. Tumor cells are usually of astrocytic origin and are confirmed by positive staining for glial fibrillary acidic protein (GFAP). Expression may be focal or reduced in high-grade lesions. The proliferation marker, Ki-67, is well-recognized in immunohistochemistry for labeling indices generally over 15–20% labeling aggressive growth, and most essentially glioblastomas are high-grade tumors wherein labeling indices are above this level (Zhou et al., 2017). Other markers include p53, EGFR, and an antibody against the mutation-specific IDH1 R132H that assists more in tumor subtype classification and integrated diagnosis (Zhou et al., 2017).

One major histopathological distinction introduced by the 2016 and 2021 WHO classifications is the use of molecular features in conjunction with histology to diagnose GBM. For example, an IDH-wildtype astrocytic tumor with typical histological features of GBM is now classified as “glioblastoma, IDH-wildtype,” whereas tumors with an IDH1 or IDH2 mutation may be classified differently, even if they exhibit GBM-like morphology. This integration has redefined the histopathological evaluation, requiring molecular testing for definitive diagnosis in many cases (Louis et al., 2021). Special variants of GBM have also been recognized. Among them is gliosarcoma, showing a biphasic pattern of glial and mesenchymal components. Other forms include epithelioid glioblastoma, a rare variant that mostly occurs in younger patients who bear BRAF V600E mutations. These variants do not only present different histopathological appearances but also distinct molecular alterations and clinical behaviors (Mellai et al., 2018).

The histopathological assessment of GBM plays as much a critical role in prognosis and treatment plan. For example, it has correlated that extensive necrosis or high microvascular density presents with bad prognoses. Also, though the therapy response cannot be determined by histology, it guides the selection of further molecular tests (e.g. MGMT methylation) that are extremely useful in therapeutic decisions. Microvascular proliferation and necrosis, cellular pleomorphism, and infiltrative growth, though classic in the spectrum for diagnosis of GBM, remain highly relevant even in this era of molecular classification. The combination of classic microscopic features with modern immunohistochemical and molecular tools substantially firms up diagnostic accuracy and prognostic prediction in cases of glioblastoma. However, the morphological diversity and heterogeneity of GBM firmly underline challenges that advocate pathology to be assessed by an integrated approach involving multiple disciplines (Hegi et al., 2005).

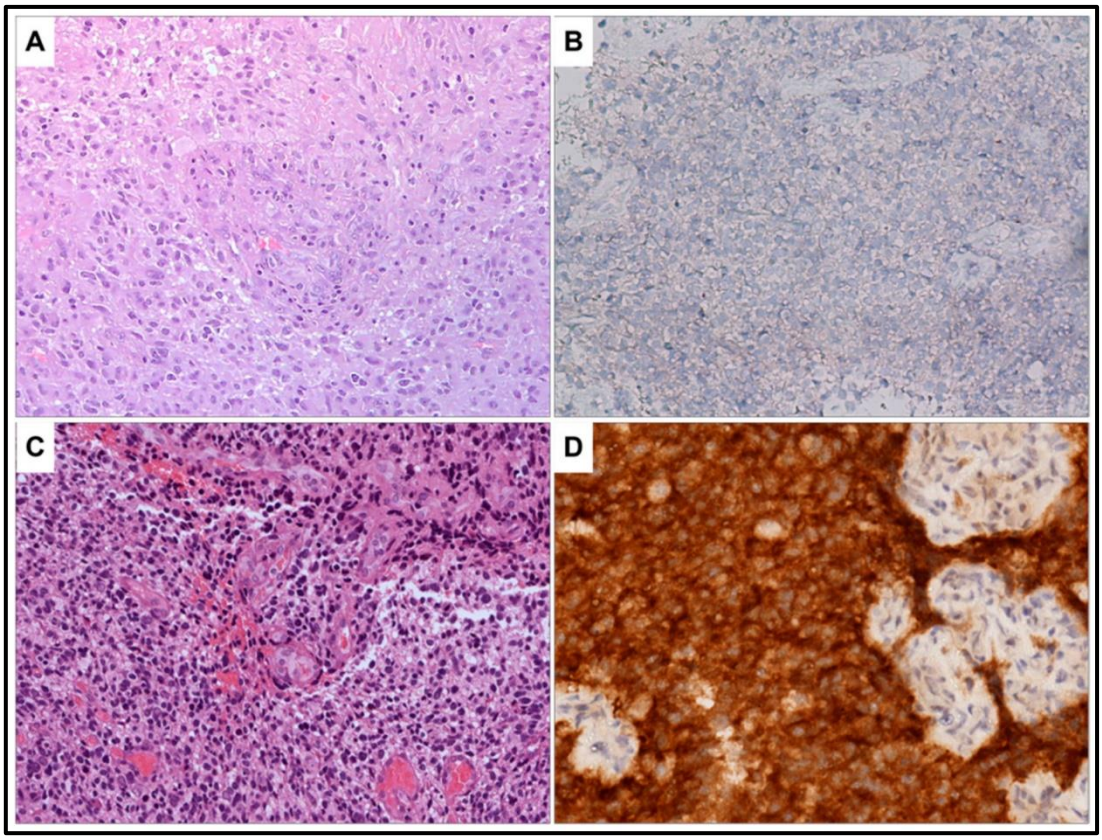

Figure 1. Histopathological and Immunohistochemical Features of Glioblastoma Multiforme (GBM). (A) Hematoxylin and eosin (H&E) staining showing pleomorphic tumor cells with hyperchromatic nuclei, prominent mitotic activity, and microvascular proliferation—hallmarks of high-grade gliomas. (B) Immunohistochemical staining for IDH1-R132H reveals weak or negative staining, consistent with IDH-wildtype GBM. (C) Another H&E-stained section depicting dense cellularity, vascular thrombosis, and pseudopalisading necrosis—a classic feature of GBM pathogenesis. (D) Immunohistochemical staining for glial fibrillary acidic protein (GFAP), showing strong cytoplasmic positivity, confirming the astrocytic origin of the tumor cells.

Molecular Alterations in GBM

A complex and heterogeneous molecular landscape drives the aggressive behavior, treatment resistance, and poor prognosis of Glioblastoma multiforme (GBM). The integration of molecular alterations into the diagnostic framework has significantly improved the understanding of GBM pathogenesis and has become essential in differentiating tumor subtypes, predicting prognosis, and guiding therapeutic decisions (Louis et al., 2021).

One of the most essential molecular differences in the classification of glioblastoma is revealed by the mutation status of isocitrate dehydrogenase (IDH). A certain group of GBMs contains IDH mutations and these are relatively better prognosis tumors that arise in relatively younger patients (Yan et al., 2009). The product of oncometabolite 2-hydroxyglutarate produced by mutant IDH1 or IDH2 alkylates epigenetic changes extensively through inhibition of α-ketoglutarate–dependent dioxygenases. Indeed, it has placed the mutation GBMs in a different category compared to classic, more than 90% cases and clinically more aggressive type, i.e., wildtype GBMs (Louis et al., 2021).

Methylation of the promoter of O6-methylguanine-DNA methyltransferase (MGMT) is the other features at the molecular level that relates to sensitivity towards the chemotherapeutic, alkylating agent temozolomide (TMZ). The MGMT protein constitutes a component of DNA repair mechanisms by reversing damage due to alkylation; promoter methylation removes its expression from the tumor so that the tumor cannot fix damage induced by TMZ and hence results in better treatment response with long survival. Thus, MGMT promoter methylation does serve a dual role in prognosis as well as prediction in GBM management (Hegi et al., 2005).

Epidermal growth factor receptor (EGFR) amplification and mutation represent among the most frequent molecular alterations in IDH-wildtype GBM,

approximately 50% of cases. The common EGFR variant, EGFRvIII results from an in-frame deletion of exons 2–7 rendering the receptor constitutively active; thereby signaling proliferation angiogenesis as well as resistance to apoptosis. Targeted therapies against EGFR have not yielded significant clinical benefits due to intratumoral heterogeneity and adaptive resistance mechanisms (Brennan et al., 2013). Another common alteration, particularly in IDH-mutant gliomas, involves mutations of the TP53 gene. This pathway responds to DNA damage by signaling for cell cycle arrest and apoptosis. Typically, cancers achieve p53 inactivation through mutation — classic loss of function leading to unregulated cellular proliferation and genomic instability. In contrast, TP53 mutations are relatively infrequent in IDH-wildtype GBM and other mechanisms - chiefly via MDM2 amplification - can also serve to inactivate p53 signaling (Cancer Genome Atlas Research Network, 2008)

Phosphatase and tensin homolog (PTEN) is commonly mutated in GBM, such that the PI3K/AKT/mTOR pathway becomes activated and signaling survival, metabolism, and growth of the cell. Normally, PTEN functions as a tumor suppressor by negatively regulating PI3K signaling. More explicitly, mutation or deletion of PTEN will uninhibit oncogenic signaling pathways and create resistance to therapy. Targeting the PI3K/AKT/mTOR axis is currently emphasized in research for therapeutics against GBM (Chakravarti et al., 2002). Changes to the retinoblastoma (RB) pathway are also very important in GBM. Mutations of CDKN2A/B result in the loss of function of tumor suppressor proteins p16^INK4a and p14^ARF, leading to cell cycle dysregulation and proliferation control not being imposed. These deletions frequently accompany amplification of either CDK4 or CDK6, thereby even more strongly promoting progression through the G1-S checkpoint of the cell cycle (Cancer Genome Atlas Research Network, 2008).

Mutations in the promoter of the gene coding for telomerase reverse transcriptase (TERT) are also among common molecular hallmarks in GBM. This pathway predominantly occurs in IDH-wildtype tumors. TERT mutations increase activity of telomerase, thereby enabling tumor cells to evade senescence and attain replicative immortality. These mutations carry a poor prognostic outcome and are extremely specific to primary GBM. Epigenetic changes are of great importance in the pathogenesis of GBM (Eckel-Passow et al., 2015). Methylation, and acetylation of histones, as well as the misregulation of non-coding RNAs- MicroRNAs and long non-coding RNAs- control gene expression and tumor characteristics. IDH mutations more frequently occur in gliomas that present the G-CIMP- a variation of the standard CpG island methylator phenotype that is associated with better prognosis and less aggressive tumor behavior (Noushmehr et al., 2010). In recent years, integrative genomic studies provide a basis for GBM molecular subtyping—classical, mesenchymal, and proneural add neural—subtypes by gene expression signatures (Verhaak et al., 2010). The classical type presents with EGFR amplification and TP53 mutations not present. The mesenchymal type expresses high mesenchymal markers and involves NF1 loss. Proneural is enriched for PDGFRA amplification plus IDH mutation and relates to better outcomes. While the neural subtype further complicates matters somewhat less well defined, these divisions served to highlight the molecular heterogeneity of GBM and underline personalized therapeutic avenues. Progress in single-cell RNA sequencing and space-based transcriptomics has verified that GBM is made up of changing cellular conditions rather than fixed subtypes. They found the presence of stem-like, growing, and developed cell types all in one tumor which leads to resistance against treatment as well as a return of the disease (Neftel et al., 2019).

The molecular changes that GBM presents are wide and varied, involving oncogenes, tumor suppressors, epigenetic regulators, and signaling pathways. It is thus able to be highly aggressive in its nature of growth; develop resistance to any form of administered therapy; and most often manifest a very complicated clinical course. As much detail as possible about such molecular characteristics is important in the process toward the development of biomarker-driven strategies, therapeutic targets discovery, and precision medicine for GBM (Tanaka et al., 2013).

GBM Microenvironment and Heterogeneity

Glioblastoma multiforme, because GBM is not defined solely by the malignant glial cells like in many other cancers but by a vast ensemble of stromal cells, immune infiltrates, extracellular matrix (ECM) components, and vascular structures interacting dynamically with tumor cells themselves, has probably one of the most elaborate and dynamic microenvironments among solid tumors. This fact adds significantly to its invasiveness,

therapeutic resistance, and recurrence. Besides, between- and within-tumor heterogeneity constitutes major obstacles to therapy effectiveness; it considerably changes patient outcomes as well as treatment strategies (Quail & Joyce, 2017).

Several non-neoplastic cell lineages compose the TME of GBM, or tumor-associated macrophages/microglia, astrocytes, endothelial cells and pericytes, neural stem cells, and some lymphoid and myeloid immune cells. Tumor-associated macrophages are very plentiful in GBM; these comprise up to 30-50% of the mass of a tumor. Most often they assume immunosuppressive pro-tumoral M2-like phenotypes supporting invasion as well as angiogenesis and immune evasion for a tumor (Poon et al., 2017). Such cytokines from TAMs as TGF-β, and IL-10 production together with expression of other immune checkpoint molecules such as PD-L1 ligand expressed on TAMs substantially inhibit all effector mechanisms involving T cells making tumors "cold" one in an immunological sense (Hambardzumyan et al., 2016).

Another hallmark of the GBM microenvironment is atypical angiogenesis. Much of this is supported by conditions of pathology-induced VEGF expression under hypoxic conditions. The architecture in GBM is highly vascular, but paradoxically in most cases vessels are dysfunctional - when qualified highly they are leaking, irregular, and there is poor perfusion. This then enables areas to form where there is inadequate oxygen supply and when supplied will fuel further angiogenesis via positive feedback loops involving HIFs. It is these hypoxic niches that maintain glioma stem-like cells (GSCs) which are very much central players in resistance and recurrence of the tumor (Hardee & Zagzag, 2012).

A subpopulation of tumor cells with stem-like properties includes self-renewal, multipotency, and increased therapy resistance, mainly based in the hypoxic and perivascular niches of the TME. They can repopulate the tumor after treatment. Such cells also change the microenvironment by secreting factors that recruit TAMs, remodel the ECM, and induce angiogenesis. The metabolic profile of GSCs is different from normal cell metabolism and has enhanced DNA repair capacity, therefore explaining their resistance to radiotherapy and chemotherapy (Lathia et al., 2015).

The ECM in GBM is another major component playing a role in the regulation of tumor behavior. Proteins comprising tenascin-C, laminin, and fibronectin belong to those proteins that enrich the ECM of GBM and support migration as well as invasion of cancer cells. Tumor and stromal cells secrete matrix metalloproteinases (MMPs) mainly MMP-2 and MMP-9 to break down barriers degrading the ECM then it facilitates the invasion into surrounding brain tissue. This type of invasiveness makes surgical resection completely impossible hence describes the high rate of recurrence which is the main characteristic feature in GBM (Xie et al., 2022).

Beyond its complex microenvironment, GBM displays extensive genetic, epigenetic, and cellular intratumoral heterogeneity. Initial findings-including those from the Cancer Genome Atlas revealed the presence of several molecular subtypes within one tumor thereby disputing any notion of a static homogeneous classification (Verhaak et al., 2010). Recent work applying single-cell RNA sequencing demonstrated dynamic transcriptional states among GBM cells corresponding to proneural, mesenchymal, classical, and neural phenotypes all coexisting within the same tumor. These are not immutable states; rather, tumor cells shift among them in response to environment and even therapeutic assault-consistently termed plasticity-of-state (Neftel et al., 2019).

Plasticity, thereby contributing to resistance in treatment. For example, radiation and chemotherapy may do well to kill the more differentiated tumor cells yet leave behind and even enrich for GSCs or cells in resistant states. In addition, single pathway targeted therapies (e.g., EGFR inhibitors) are frequently rendered useless since some subpopulations do not have that target or alternative signaling pathways to compensate for it. Hence, GBM treatment resistance is fueled by heterogeneity at both inter- and intra-tumoral levels (Sottoriva et al., 2013).

The immune landscape of GBM mirrors its heterogeneity. Although it resides in an organ that is generally considered immune privileged, there are highly immunosuppressive milieus induced by GBM. Other than TAMs, GBM also recruits T regulatory cells (Tregs), myeloid-derived suppressor cells (MDSCs), as well as tolerogenic dendritic cells providing service to the anti-tumor immune response. The expression of immune checkpoints upregulated on both tumor and immune cells further inhibits the activity of T-cells. Therefore, similar to other solid tumors such as melanoma or non-small-cell lung cancer, GBMs have not responded

significantly to immune checkpoint inhibitors (Jackson et al., 2019).

The major foci in current GBM research are the TME and heterogeneity. Major strategies under investigation include TAM reprogramming, GSC niche disruption, ECM component modification, and pathways to immune infiltration. Combination therapies that simultaneously target more than one cell type or signaling pathway within the TME are also being explored as a means of overcoming limitations imposed by monotherapy. Further, spatial transcriptomics coupled with advanced imaging techniques continue to now differentiate how spatial context and cell-cell interactions drive tumor evolution and therapy response (Quail & Joyce, 2017; Ravi et al., 2022).

Conclusions

Glioblastoma multiforme (GBM) has always been known and revealed as one of the most aggressive, deadly brain tumors with such specific histopathological features, complex molecular changes, and very dynamic tumor environments. Advancing knowledge of these particular fields has improved diagnostic accuracies and initiated new potential spaces for targeted treatments. These have not yet be fully overcome by addressing heterogeneity within a tumor and resistance mechanisms; hence, more detailed analyses of GBM biology are on the way to hopefully bring more effective and individualized strategies to therapy development based on clear insights at the molecular and cellular levels. Henceforth, future success in making better prognoses accessible alongside longer survival times in patients will require adding value through complementary histopathological expertise combined with cutting-edge macro-molecular insight.

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