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Origins of Glioblastoma: Initiation and Molecular Signatures of Extreme Aggressiveness

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Abstract

Compared with other malignancies, brain tumors rank among the most fatal types of cancer affecting humans, with the lowest survival rates. The overall 5-year survival rate for all primary brain tumors is approximately 5%, although this figure varies significantly on the basis of factors such as tumor type, location, size, patient age, and overall health. Glioblastoma, in particular, is among the most aggressive lethal cancers, making it one of the most devastating brain tumors. Its molecular pathogenesis is highly complex and involves genetic mutations in key regulatory genes, such as IDH12/, EGFR, PDGFRA, the hTERT promoter, and NF1, along with epigenetic alterations and contributions from the tumor microenvironment, all of which drive tumor progression and invasion. Addressing the extremely poor survival rate of glioblastoma requires a deeper understanding of its origins and the cells responsible for its initiation. Various cancer initiation theories—such as the two-hit hypothesis, random mutation model, and clonal selection hypothesis—have proven experimentally effective in explaining its development. However, the cancer stem cell hypothesis stands out, as it successfully accounts for glioblastoma's unmanageable aggressiveness and recurrence. Glioblastoma cancer stem cells (GSCs) can self-renew, differentiate into multiple tumor cell types, resist conventional therapies, and contribute to tumor heterogeneity, metastasis, and infiltration into surrounding brain tissue. These characteristics significantly impact patient prognosis and mortality. The exact cellular origin of glioblastoma remains a subject of ongoing research. However, studies suggest that glioblastoma may arise from neural stem cells (NSCs) or glial precursor cells, which undergo oncogenic mutations that drive uncontrolled proliferation. The molecular subtype of a tumor is often influenced by the lineage of its originating cells, with mesenchymal stromal cells emerging as potential glioblastoma-initiating cells, particularly in tumors with mesenchymal molecular features. This review explores the origin of glioblastoma and the biological factors that make it the most aggressive and lethal brain tumor.

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Introduction

The brain represents one of the most complicated organs in regard to cancer pathology, treatment, and prognosis. The abnormal, uncontrolled growth of cells within the brain or other cells close to it pours into the formation of brain tumors. These tumors can be classified as benign noncancerous, or malignant (cancerous). This classification is based on the origin and behavior of these tumors. Their slow growth is characteristic of benign noncancerous lesions. They remain captivated to their primary location of origin and do not intrude other tissues or spread to distant parts of the body. They also do not recur after surgical removal or radiation therapy. General examples include meningiomas and pituitary adenomas (1). Whereas malignant primary tumors are cancerous, tend to grow rapidly, and have the ability to invade surrounding brain tissue, they often recur even after surgical resection and chemoradiotherapy, making them difficult to remove completely. This aggressive nature causes these tumors to have a poor prognosis and are difficult to treat. Common types include glioblastomas, astrocytomas, and oligodendrogliomas (2). The tissue origins of these three types of brain malignancies vary accordingly. Glioblastomas originate from astrocytes, star-shaped brain cells, and spinal cord glial cells or from oligodendrocytes and their precursors. Astrocytomas also arise from astrocytes. They range from slow-growing-low grade to high-growing-high grade with more aggressive features. In some cases, glioblastomas are categorized as high-grade astrocytomas. Oligodendrogliomas develop from oligodendrocytes, which are myelin-producing glial cells (3). In a variety of cases, brain tumors may develop from other tumors that originate in remote organs. These tumors are classified as secondary brain tumors or metastatic brain tumors. They arise from cancer cells that have dispersed to the brain from other parts of the body, mainly the lungs, breasts, and skin (46-). Another classification for brain tumors was contributed by the World Health Organization (WHO). This classification system has evolved, with significant updates to incorporate new scientific and medical knowledge. The 5th edition was the most recent and was published in 2021. This edition emphasizes tumor molecular markers and traditional histological features (7, 8). In general, the incidence of brain cancer is relatively lower than that of many other types of cancer. For example, in comparison, breast cancer has an incident rate of approximately 142.77 per 100,000 persons, and the estimated number of new cases in 2024 is approximately 310,720. Moreover, the incidence rate of brain cancer is no more than 6.22 per 100,000 persons. According to U.S. statistics, an estimated 25,400 new cases of brain cancer were reported in 2024. In terms of incidence rates, brain cancer ranks far behind lung, prostate, and colorectal cancers. However, when mortality rates are considered, brain cancer is among the deadliest, surpassing many other tumor types. For example, the death rate for patients with breast cancer in 2024 was approximately 13%, whereas those with brain cancer had a staggering mortality rate of over 70%. These alarming statistics underscore the critical need for further research to deepen our understanding of brain tumors and improve treatment strategies (9). The majority of brain tumor-related deaths are attributed to glioblastoma, one of the most challenging cancers to treat. Understanding the high morbidity associated with glioblastoma requires a deeper investigation into its initiation mechanisms and identification of the specific cell types from which it originates. Resolving this fundamental question is crucial for developing effective treatment strategies and improving patient outcomes (9). This review explores the present knowledge about glioblastoma biology and theories that may contribute to explaining its origin and link it to the features that contribute to its stubbornness.

Glioblastoma: unmanageable features

Glioblastoma, also known as glioblastoma multiform (GBM), is the most aggressive and fatal primary brain tumor in adults, and it stands out for its quick growth, widespread infiltration into nearby brain tissue, resistance to therapy, and unavoidable recurrence. Its aggressive nature can be attributed to several key biological, molecular, and clinical features (10) (Fig. 1). The essential feature that makes this type of brain tumor difficult to manage and deadly is its extremely invasive characteristics. Malignant glioblastoma cells extensively penetrate the surrounding brain tissue. Therefore, removing all malignant cells surgically is nearly impossible. Microscopic tumor cells remain ingrained in normal brain tissue even when the noticeable tumor mass has been completely removed. As the tumor lacks a clear border, it spreads diffusely into vital brain regions, making surgical excision more difficult (11, 12). The aggressive proliferation of glioblastoma cells may be considered a second feature. Tumor growth and progression accelerate fundamental genetic mutations that disrupt cell cycle control, leading to the induction of unregulated cell proliferation. Mutations such as the deletion of tumor suppressor genes such as PTEN and TP53 or isocitrate dehydrogenase (IDH1/IDH2) lead to amplification of the EGFR (epidermal growth factor receptor) gene (1315-).

The accelerated proliferation feature induces extensive genomic instability resulting from random mutations, chromosomal abnormalities, and epigenetic changes such as MGMT promoter methylation (which affects the response to chemotherapy). All these molecular changes accelerate tumor evolution and adaptation to different stresses, increasing the difficulty of effective targeting (1619-). All these molecular changes add another important feature to glioblastoma tumors: the substantial heterogeneity of the tumor mass. Glioblastoma tumors may consist of several types of cell populations that harbor various genetic and molecular makeup. As a consequence of this heterogeneity, cancer cells become more resistant to known treatments. Some cells may resist the impact of treatment and grow back after therapy because heterologous tumor cells may react differently to the therapies used (2023-). In addition, glioblastoma cells can repair DNA damage caused by radiation therapy, allowing them to survive and continue growing (24). Another challenging feature of GBM that is an obstacle for chemotherapies is the blood–brain barrier, which may limit the delivery of chemotherapy drugs to the tumor site. Additionally, mechanisms such as enhanced drug efflux pumps (e.g., MDR1) and altered metabolic pathways allow tumor cells to resist drugs such as temozolomide (25, 26).

One of the most troubling features of this type of tumor is residual tumor cells that resist initial therapies. These

cells aggressively repopulate the tumor. Recurrent tumors often develop resistance to previous treatments that further complicate their management (2730-).

Rapidly growing glioblastoma tumor cells have emerging needs for oxygen and nutrients. This situation stimulates tumor cells to produce factors such as vascular endothelial growth factor (VEGF) that support the growth of new blood vessels to satisfy these needs. Therefore, angiogenesis results in aberrant and leaky blood vessels, which increase tumor invasiveness and treatment resistance (31- 34).

Another feature that increases the aggressiveness of glioblastoma tumors and complicates their management is the limitation of immune surveillance in the brain, which results from its organ nature in comparison to other parts of the body. This creates a "privileged" environment where tumors can grow without significant immune interference. Furthermore, glioblastoma cells can actively suppress any immune response by secreting immunosuppressive cytokines and recruiting regulatory T cells (T-regs) that inhibit antitumor immunity (3539-).

The location of glioblastoma tumors in the brain further complicates their management. Glioblastomas typically arise in the cerebral hemispheres but can occur anywhere in the brain. Their location in critical areas limits the extent of surgical resection to avoid damaging vital functions such as speech, movement, or cognition. Additionally, the delicate structure of the brain makes delivering high doses of radiation or chemotherapy without causing significant collateral damage challenging (4043-).

Overall, even with standard-of-care treatment (surgery, radiation, and chemotherapy with temozolomide), the median survival of glioblastoma patients is approximately 12–15 months. The features mentioned above contribute substantially to the poor prognosis of this cancer, whereas fewer than 5% of patients survive beyond five years, underscoring the aggressive and deadly nature of the tumor (4448-).

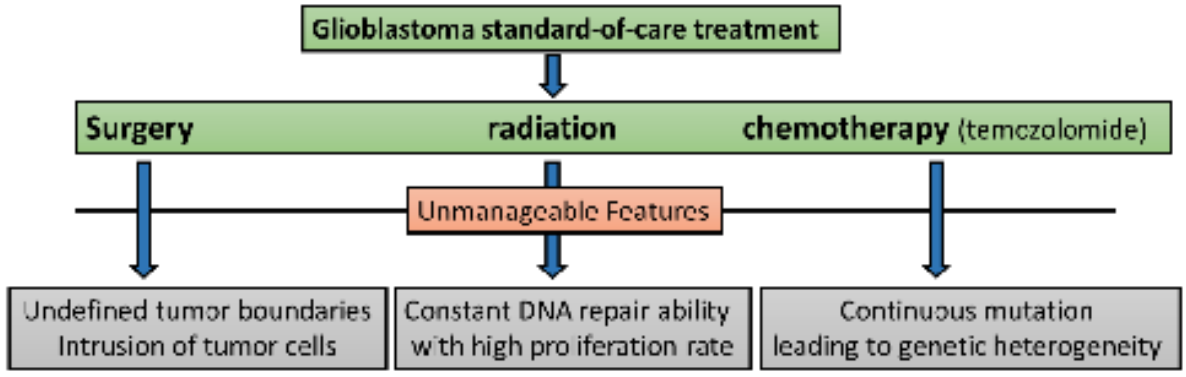

Figure 1: A representative diagram illustrating the key features of glioblastoma that contribute to its highly aggressive and difficult-to-manage nature.

Glioblastoma, initiation, and hallmarks

Like other malignancies, the origin and defining characteristics of glioblastoma (GBM) can be explained by the two-hit theory and the clonal selection hypothesis—two fundamental concepts in cancer biology that describe the process of carcinogenesis. These theories shed light on the evolutionary and genetic mechanisms driving cancer progression. In the case of glioblastoma, the accumulation of mutations in normal cells and the selective expansion of aggressive clones play significant roles in tumor development and progression.

The two-hit hypothesis and initiation of GBM

This hypothesis was the first to explain the initiation of retinoblastoma. When this theory is applied to the mutations that occur in both copies of a tumor suppressor gene, these claims can be extended to other cancers; these mutations can inactivate these essential genes, interfere with the cell cycle and prevent controlled cell proliferation. This theory proposes that the first mutation can arise in one allele and is impacted by an inherited germline mutation. Moreover, the second allele may be affected by a somatic mutation acquired during the lifetime (49, 50). Glioblastoma is usually not inherited because it is a sporadic cancer. Nonetheless, somatic mutations in essential tumor suppressor genes, including TP53, PTEN, and RB1, continue to be covered by the two-hit hypothesis. For example, chromosomal deletion (first hit) may cause a glioblastoma cell to lose one copy of the TP53 gene. A subsequent point mutation or epigenetic silencing inactivates the remaining functional copy of TP53 (second hit), leading to the loss of its tumor-suppressive function (Fig. 2). More than 90% of glioblastoma cases are classified as primary glioblastomas according to their genetic mutation changes, and they are found mainly in the elderly population. This type is genetically characterized by loss of heterozygosity 10q (70% of cases), EGFR amplification (36%), p16INK4a deletion (31%), and PTEN mutation (25%). As a result of mutation hits, a new type of glioblastoma, termed secondary glioblastomas, arises and develops in younger patients. In the manifestation of the two-hit theory, mutations in the TP53

pathway characterize the earliest detectable genetic alteration to secondary glioblastoma already present in 60% of precursor primary glioblastomas. The loss of heterozygosity during progression to glioblastoma, including 10q25-other (~70%), remains the most frequent genetic alteration in both primary and secondary glioblastomas. The mutation accumulation during the development of this tumor led to the separation of the genotypes into primary and secondary glioblastomas. These genetic mutation differences significantly impact the course of treatment and response to radiochemotherapy. Furthermore, chromosomal instability can lead to the loss of tumor suppressor genes (e.g., CDKN2A/B) and the activation of oncogenes at a time interval, supporting the two-hit model (5153-).

In a landmark study, Brennan and colleagues (2013) (54) cataloged genomic somatic alterations and mutations associated with glioblastoma tumor development through comprehensive genomic analysis. They describe significant novel mutated genes and complex rearrangements of signature receptors, including EGFR, PDGFRA, and telomerase reactivation, supporting tumor growth resulting from TERT promoter mutations. The role of MGMT DNA methylation is also significant (54). In many cases, mutations in tumor suppressor genes (TP53 and PTEN) contribute to glioblastoma initiation after the incidence of epigenetic silencing, which acts as a second hit in a match similar to what the two-hit hypothesis assumes (55). Panglioma analysis has expanded the knowledge of the somatic mutation landscape of glioma, revealing specific mutations (e.g., IDH1/IDH2) and revealing the relevance of DNA methylation profiles to the role of TERT pathway alterations in telomere maintenance. This knowledge was combined with the two-hit model for clinical classification of glioblastoma initiation and progression. These findings constitute a step forward in comprehending glioblastoma as a discrete disease subset and elucidating the mechanisms driving gliomagenesis (56, 57).

Genomic and epigenomic studies employing single-cell techniques have demonstrated that IDH1/IDH2 mutations can serve as early events in gliomagenesis. These findings align with the two-hit hypothesis, suggesting its relevance in glioblastoma development. Numerous studies collectively support the application of this hypothesis, offering valuable insights into the genetic alterations that drive glioblastoma tumorigenesis (58).

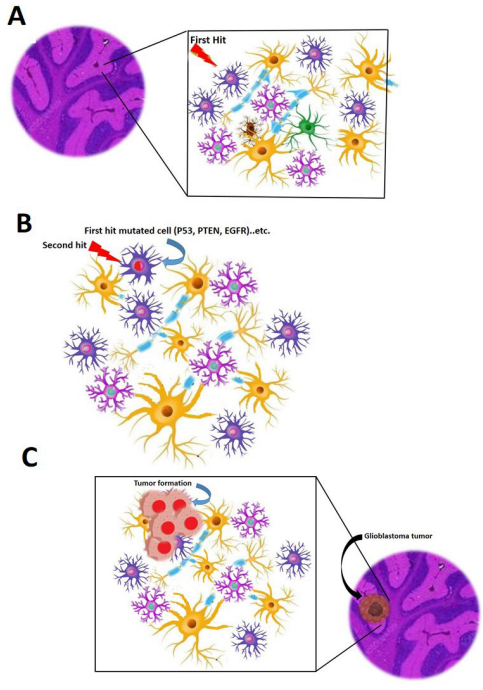

Figure 2: The two-hit hypothesis takes place when normal brain cells are hit by the first mutation event in one of its vital genes, such as a tumor suppressor gene or proto-oncogene (A). When a second mutation event occurs and may affect the same or other genes (B), tumors develop as a result of uncontrolled proliferation of the mutated cells, resulting in glioblastoma formation (C).

Clonal selection hypothesis in GBM

The clonal selection hypothesis is the other factor that potentiates the emergence of glioblastoma tumors. It represents the evolutionary process that drives tumor progression and recurrence. The clonal selection hypothesis has significant implications, particularly in understanding tumor heterogeneity and immune evasion.

The clonal selection hypothesis was initially introduced to clarify the diversity and specificity of the immune system. As research has progressed, shedding light on the mechanisms of cancer cell mutations and the environmental pressures that tumor cells face, scientists have proposed that tumors evolve in response to these challenges in a manner similar to immune system adaptation. This concept has since been integrated into cancer biology to explain the formation and progression of tumors into diverse and heterogeneous populations. (59).

The clonal selection hypothesis explains cancer initiation by suggesting that it originates from a single somatic cell that accumulates genetic mutations over its lifetime, particularly in genes that regulate the cell cycle (e.g., oncogenes and tumor suppressor genes) or through epigenetic modifications. These genetic alterations provide a selective advantage, such

as increased proliferation, resistance to apoptosis, or immune evasion. The mutated cell then expands through uncontrolled proliferation, gaining dominance over surrounding normal cells through natural selection. This process resembles the immune system›s clonal selection, where lymphocytes with specific receptors proliferate upon antigen exposure. In cancer, the «selected» clone proliferates due to its adaptability within the tumor microenvironment (60, 61).

Key mutations in genes such as TP53 and KRAS serve as «drivers» initiating clonal expansion. These mutations provide survival and growth advantages, enabling the mutated cells to outcompete others. As the tumor progresses, further genetic instability emerges, leading to clonal evolution and heterogeneity. Ongoing DNA replication errors, chromosomal abnormalities, and environmental stressors (e.g., hypoxia and immune pressure) accelerate mutation rates, resulting in the formation of subclonal populations (62). New subclones arise with additional mutations that increase their survival in specific tumor niches. Some develop chemotherapy resistance, allowing them to persist and dominate posttreatment tumors, whereas others acquire angiogenic properties, enabling them to thrive in nutrient-deprived areas and facilitating metastasis. The existence of multiple subclones within a tumor contributes to intratumor heterogeneity, resulting in distinct genetic mutations among subpopulations. This variability extends to differences in metabolism and invasiveness, leading to diverse clinical responses to chemotherapy and radiation therapy (63, 64). This heterogeneity complicates treatment, as therapies often target dominant clones, leaving resistant subclones to repopulate the tumor. More aggressive subclones may acquire mutations that promote invasion and dissemination, driving metastasis. Consequently, high tumor heterogeneity is frequently associated with poor clinical outcomes, as the tumor becomes more adaptable and resilient (65). While the clonal selection hypothesis in immunology describes the antigen-driven selection of lymphocytes, its fundamental principle—the selection of cells with advantageous traits—also applies to somatic evolution in cancer. Both processes involve the survival and expansion of the most adapted cells within their respective environments (66).

The clonal selection hypothesis provides a comprehensive framework for understanding GBM biology, including its initiation, progression, heterogeneity, and therapeutic resistance. Like many cancers, GBM can originate from a single neural progenitor or glial cell that accumulates driver mutations in critical cell cycle-regulating genes (e.g., TP53, IDH1, EGFR, and PTEN). These mutations give the cell a selective advantage, allowing it to evade growth control mechanisms and initiate clonal expansion. For example, EGFR amplification or TP53 loss disrupts cell cycle regulation, leading to uncontrolled proliferation. As a result, the mutated clone outcompetes normal cells in the brain microenvironment, forming a primary tumor mass (Fig. 3). GBM evolves through iterative mutation and selection, resulting in intratumor heterogeneity, a key factor in its aggressive nature. The evolutionary mechanisms driving this process include genetic instability due to mutations in DNA repair genes (e.g., MGMT, mismatch repair genes), which increases mutation rates and fosters subclonal diversification. (6769-). When tumor subclones acquire mutations that increase survival under microenvironmental stressors such as hypoxia, immune pressure, and nutrient scarcity, subclonal adaptation phenomena occur. The activation of genes such as HIF1α, which supports survival under hypoxic conditions, is activated. MET gene amplification promotes invasiveness. However, NF1 gene loss enhances resistance to apoptosis (70). Therefore, due to localized selection pressures, different tumor regions harbor distinct subclones that appear. The periumcrotic regions favor clones with enhanced angiogenic properties (e.g., VEGF overexpression). The invasive margins select the migratory phenotypes (e.g., CD44+ or OLIG2+ cells).

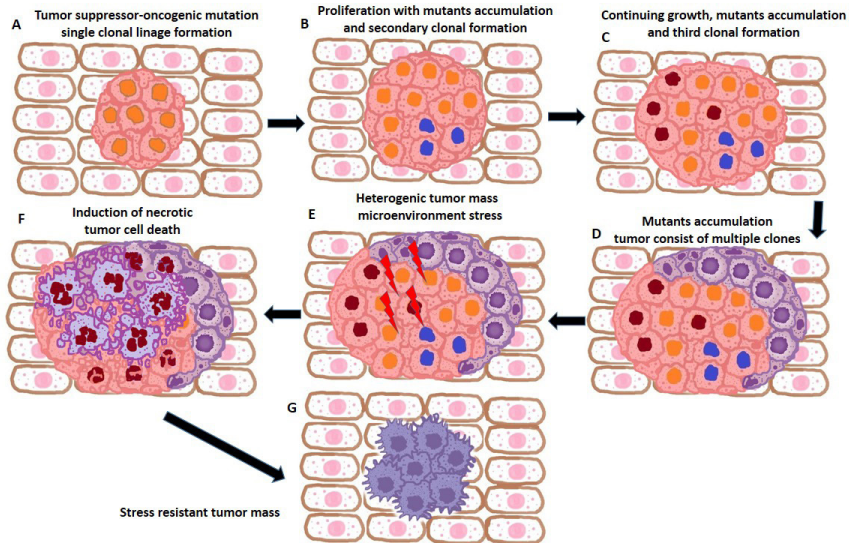

Figure 3: Illustration of the clonal selection hypothesis, which generally presumes that tumors consist of heterogeneous cells due to multiple mutation processes. After tumor initiation results from a mutation in a tumor suppressor gene or/and oncogene, a single clonal linage will exist (A), and continued tumor cell proliferation combined with the induction of a second mutation in other genes will induce mutation accumulation and secondary clonal formation (B). The same proliferation phenomenon will continue, and mutation accumulation will further increase to produce a tumor mass with multiple clonal formations (C and D). The larger tumor mass resulting from continued proliferation will suffer from microenvironmental stress, such as the accumulation of metabolic byproducts, hypoxia, a lack of nutrients, and inflammatory cytokines (E), which will lead to the induction of tumor cell death via necrosis (F), increasing the resistance of the tumor mass to microenvironmental stress as well as chemoradiotherapy (G).

Recent single-cell and 3D Omics sequencing studies have revealed distinct clonal hierarchies in GBM, with subclones coexisting in spatially segregated niches. The main source of the ability of GBM to resist therapies lies in the ability of GBM to achieve clonal selection. It results in the accumulation of therapy-resistant cells through clonal selection, which occurs by preexisting or therapy-induced subclonal expansion. The preexisting resistant clones contain mutations in drug targets (e.g., EGFRvIII) or DNA repair pathways (e.g., MGMT promoter methylation), enabling them to survive treatment and dominate posttherapy (71, 72). Adaptive resistance occurs when therapy-induced stress selects for clones with compensatory mutations, such as BRCA12/ loss, which evades homologous recombination repair. Another important feature is immune evasion, which is facilitated by subclones that downregulate MHC molecules or upregulate immune checkpoints (e.g., PD-L1), helping them escape T-cell surveillance. Hypoxic regions also favor clones with enhanced glycolytic metabolism (e.g., LDHA overexpression) or angiogenic capacity. Invasive subclones (e.g., MMP9+ cells) penetrate the blood–brain barrier (BBB), evading localized therapies and contributing to tumor spread (73, 74).

These adaptations allow glioblastoma subclones to thrive despite treatment pressures, making GBM highly resistant to conventional therapies. High heterogeneity in GBM is often associated with poor clinical outcomes because of the ability of tumors to evolve rapidly and withstand different therapeutic strategies (75).

A recent study linked the clonal architecture with spatially resolved transcriptional patterns and in vitro drug response to provide new insights into the clinical relevance of this cancer›s clonal composition, highlighting the importance of clonal architecture in understanding tumor behavior and developing targeted therapies. (76). Lerman et al. (2024) demonstrated the importance of the whole tumor sampling approach rather than one sample per patient method to reach a tangible understanding of glioblastoma tumor heterogeneity at diagnosis and the origins of tumor recurrence, with the impact of the clonal selectivity process on choosing therapies that are efficacious across the entire tumor. Three GBM tumors were sampled 43 times, and a single founding clone with multiple subclones was identified for each diagnosis–recurrence pair. Tumor-wide clonal alterations represent initial clonal expansions and a diverse set of large-scale copy number variations, driver mutations, and gene fusions. A second subset of alterations appeared tumor-wide at diagnosis but was not identified in paired recurrence samples. The cancer driver mutations were also subclonally distributed and included deletions, amplifications, and mutations. Evolutionary trees consisting of 5, 3, and 4 clone generations were discovered in the first, second, and third patients, respectively. Divergence of the recurrent tumors from their matched primary tumors occurred in the second and third generations of tumors. As a result, an average of 37% of potential driver mutations of oncogenesis and clonal expansion across the cohort appeared after divergence. Furthermore, each recurrent tumor contained at least one tumor-wide driver alteration subcloned or undetected at diagnosis (77, 78). Glioblastoma progression in its earliest possible stage was observed, and a high incidence of clonal extinction events and progressive divergence in clonal sizes, even after the acquisition of a malignant phenotype, was detected. Computational modeling suggested a dynamic result from clonal-based cell–cell competition. Through bulk and single-cell transcriptome analyses, coupled with lineage tracing, Myc transcriptional targets were found to have the strongest correlation with clonal size imbalances. The downregulation of Myc expression is sufficient to drive competitive dynamics in intracranially transplanted gliomas. Thus, the clonal selection hypothesis suggests that cell–cell competition drives clonal extinction and size imbalances in glioblastoma, influencing tumor evolution. Understanding these dynamics may inform therapeutic strategies targeting specific clonal populations to improve treatment outcomes in glioblastoma progression (79). The Myc oncogene seems to be a substantial participant in clonal competition and selection during gliomagenesis. In demonstrating the clonal selection hypothesis that maintains competition between clones, MYC expression dependency seems to be the driving force that plays a role in shaping glioblastoma evolution. This oncogene overexpression may contribute to the development of aggressive tumor clones distinguished for resistance to treatment. Ultimately, Myc expression may affect therapeutic strategies and provide insights into approaches to compete for tumor heterogeneity during gliomagenesis (80, 81). The devastating consequences of the clonal selection process become perceptible in recurrent tumors. The tumor formed after removing the primary tumor during the first surgery contains proteogenomic changes that cause the tumor to be drastically challenging to treat. The comprehensive proteogenomic analysis of matched samples of primary and recurrent GBMs allowed researchers to observe evolutionary changes after treatment. A significant shift in the tumor›s biological state from a proliferative phase at diagnosis to activated neuronal and synaptogenic pathways at recurrence was evident. These findings suggest that cancer cells adapt and change their characteristics in response to therapy. Recurrent GBM tumors activate posttranslational signaling pathways, such as the WNT/PCP and BRAF kinase pathways, while many oncogenic pathways, particularly the EGFR pathway, are downregulated and could be targeted for therapy. Targeting the BRAF kinase in recurrent tumors could disrupt neuronal transition and migration and halt tumor progression (82).

A novel fibroblast growth factor receptor (FGFR) fusion with fatty acid synthase (FASN) genes (FGFR3-FASN) alongside increased Ki67 expression resulting from clonal selection was linked to an aggressive GBM recurrent tumor just four months after initial treatment. Understanding such biological events could lead to new therapeutic strategies targeting this specific alteration in recurrent clones (83).

Complex multiomic analysis of 289 whole-genome sequencing (WGS) samples with various techniques, such as RNA sequencing, DNA methylation arrays, whole-genome bisulfite sequencing, and assays for transposase-accessible chromatin with sequencing (ATAC-seq), was carried out to explore the molecular heterogeneity of GBM. The analysis revealed substantial mutational events and numerous genetic driver alterations, emphasizing intertumoral heterogeneity.

Unavoidable differences in clonal architecture and clonal selection processes contribute to GBM pathogenesis. The differentiation status of tumor cells contributes to various molecular levels, including genetics, transcription, epigenetics, and chromatin modification characteristics. The distinct susceptibility to mutational and epigenetic modifications contributes to intratumoral heterogeneity, leading to the evolution of the clonal selection model in GBM. The clonal architecture determined by mutational signatures that differ across clonal and subclonal mutations contributes to the pathogenesis of GBM recurrence (84). Specific mutations in genes such as NF1 and EGFR can drive distinct transcriptional states and microenvironmental changes, influencing clonal selection and treatment outcomes through mechanisms such as immune infiltration and mesenchymal transitions. Another finding was that PDGFB promotes a neural progenitor/cell-like state. This persistent cellular heterogeneity contributes to transcriptional shifts closely linked to unique microenvironmental modifications, paving the way for targeted therapeutic strategies and precision medicine in recurrent GBM (85). The correlation between low levels of RAD18 expression and hypermutation in recurrent GBM patient samples after temozolomide treatment suggests that RAD18 may be a critical factor in the tumorigenic characteristics of clonal selection observed in GBM (86). The chromatin remodeling process can play a crucial role in GBM therapy resistance, as a possible part of clonal selection occurs in relapsed tumors. (87).

In conclusion, the clonal selection process in glioblastoma (GBM) is a multifaceted and evolving phenomenon that drives tumor growth, therapy resistance, and recurrence. Gaining insight into the genetic, molecular, and cellular mechanisms that fuel clonal evolution equips researchers and clinicians with tools to design more effective interventions against this aggressive disease. However, the inherent diversity and adaptability of GBM highlight the need for creative and comprehensive strategies to achieve lasting treatment outcomes and enhance patient survival.

Microenvironment in glioblastoma initiation

The role of the microenvironment in glioblastoma initiation started to gain significant attention in early studies. The earliest publications described the interaction between the surrounding cellular environment, which may influence tumor development and progression. The role of the tumor microenvironment (TME) in glioblastoma has been a research focus for many years. While pinpointing the first study is challenging, early research highlighted the importance of the TME in glioblastoma progression, particularly its influence on tumor heterogeneity, immune evasion, and therapy resistance. Furthermore, the glioblastoma tumor microenvironment promotes clonal evolution, multidrug resistance, and angiogenesis, complicating therapeutic interventions. Advances in understanding glioblastoma include the role of tumor-associated fibroblasts and immune cell differentiation pathways in regulating the tumor microenvironment and potential therapeutic agents. For example, studies have explored how glioblastoma cells interact with their microenvironment through signaling molecules, immune cells, and extracellular matrix components. (88, 89). Growth factors, inflammatory signals, and metabolic substrates within this microenvironment can transform normal cells into tumor cells. One such factor is the overexpression of platelet-derived growth factor (PDGF), which has been shown to contribute to the proliferation and survival of glioblastoma cells. In vitro studies have demonstrated that exposure to PDGF-AAs can induce genome instability in neural progenitor cells, leading to the acquisition of additional mutations and the development of tumorigenicity (90, 91).

Chronic inflammation is another key feature of the glioblastoma microenvironment. Activated microglia and astrocytes release proinflammatory cytokines and growth factors that can promote the proliferation and survival of glioblastoma cells. Additionally, neuronal injury and inflammation can trigger the activation of microglia and astrocytes, which release proinflammatory cytokines and growth factors. These factors can promote the proliferation and survival of mutated cells, facilitating tumor initiation (92).

One of the critical players in the glioblastoma microenvironment is astrocytes. These cells are glial cells (nonneuronal cells) found in the central nervous system (CNS), which includes the brain and spinal cord. They are crucial for maintaining homeostasis, supporting neuronal function, and regulating the environment around neurons. The key functions of astrocytes include providing structural and metabolic support to neurons, ensuring that they have the necessary nutrients and environment to function correctly, helping form and maintain the blood–brain barrier (which protects the brain by controlling what substances can enter the CNS), influencing how neurons communicate with each other by modulating synaptic transmission by releasing neurotransmitters and other signaling molecules, allowing astrocytes to react and form scar tissue after CNS injury or damage, helping limit further damage but also inhibiting regeneration, preventing excitotoxicity and maintaining proper neuronal signaling by helping to regulate the balance of ions (such as potassium) and neurotransmitters (such as glutamate) in the extracellular space (93). Moreover, astrocytes and their genetic mutations significantly influence the development and progression of glioblastoma (GBM), although the precise causal relationship is convoluted and involves multiple factors. As part of the tumor microenvironment, astrocytes contribute to the pathogenic effects of glioblastoma by facilitating tumor growth and survival through various mechanisms, such as transferring mitochondria and cholesterol and stimulating an immunosuppressive environment that supports tumor advancement (94). The deregulation of neuronal degeneration, particularly in white matter, and the inflammatory response associated with neuronal injury are key initiating events in GBM tumorigenesis (95). The role of astrocytes in glioblastoma progression does not involve genetic mutations only as a driving factor in glioblastoma. Dormant tumor cells resembling astrocytes have been identified as capable of transitioning to active phases, forming pseudolineages that play crucial roles in understanding tumor dynamics (96).

Nevertheless, the direct role of astrocyte mutations in triggering GBM remains unclear. Astrocyte-like neural stem cells that accumulate driver mutations may evolve into glioblastoma (GBM) through clonal expansion. These mutations in NSCs are key in GMB development, as the

mutagenesis of cancer-driving genes in NSCs leads to the migration of mutant cells and the formation of high-grade gliomas. Thus, astrocyte-like NSCs harboring mutations are causal in glioblastoma development (97).

Cancer stem cell hypothesis

Al-Hajj and colleagues achieved a landmark breakthrough at the University of Michigan in 2003. This team was able to isolate a distinct subpopulation of cells within breast tumors that demonstrated stem cell-like properties for the first time. They identified vital characteristics of these cells, such as the capacity for self-renewal and the ability to initiate tumor growth. These cells can be distinguished from other tumor cells by specific markers, particularly the $CD44^+/CD24^-/low$ phenotype. This groundbreaking discovery shifted the understanding of tumor initiation and heterogeneity, showing that not all cancer cells play an equal role in tumor growth and metastasis (98102-).

The concept of cancer stem cells (CSCs) in solid tumors, such as breast cancer, was motivated by earlier studies on hematopoietic stem cells and their function in leukemia (103105-). Investigators have noted that only a small fraction of leukemia cells can start and maintain the disease, suggesting that matching mechanisms might be involved in solid tumors (106). Al-Hajj and colleagues developed this idea by investigating whether a distinctive subset of cells within breast tumors retained stem cell-like features. Using advanced flow cytometry methods, they recognized a specific population of cells distinguished by the expression of high $CD44^+/CD24^-/low$ markers. This demonstrated self-renewal and the ability to initiate tumors in mice. They suggested that these cells might drive tumor initiation, progression, and recurrence (107110-).

The team selected to experiment with the $CD44^+/CD24^-/low$ marker combination because it can specify a unique subpopulation of cells exhibiting stem cell-like traits. (111, 112).

This choice was guided by earlier studies indicating that these markers are linked to tumorigenic potential. CD44 is commonly associated with cell adhesion and interactions with the extracellular matrix, while low or absent CD24 expression has been observed in cells displaying stem-like features. These marker combinations have become pivotal tools for identifying breast cancer stem cells and elucidating their role in tumor biology (98) (113, 114). To further validate these properties, serial transplantation experiments were performed. $CD44^+$ cells from the initial tumors were reisolated and injected into new mice. The ability of stem cells to consistently generate tumors across multiple generations provides compelling evidence of their self-renewal ability—a defining characteristic of stem cells. This methodology confirmed the existence of cancer stem cells and underscored their pivotal role in driving tumor growth and contributing to recurrence. (112)(115118-). This discovery prompted monumental works worldwide to identify the possibility of CSCs in different cancer types. Almost the same methodology and technique as those used by Al-Hajj et al. (2003) were used. However, investigators have employed other methods to recognize and identify cancer stem cells (CSCs) across various cancer types, with each experimental technique designed to target the distinct characteristics of CSCs. An overview of the methods employed and the rationale behind the use of specific markers for different cancers can be found in recent comprehensive reviews (119, 120). However, these experimental methods do not exceed one of the following methods established at the earliest time after 2003. Flow cytometry employs fluorescently labeled antibodies to identify specific cell surface markers, enabling researchers to isolate and analyze CSC populations on the basis of their marker expression (98). Aldehyde dehydrogenase (ALDH) is an enzyme that is often overexpressed in CSCs (121). This method helps identify CSCs in cancers such as breast and lung cancer. In xenotransplantation of isolated CSCs, these cells can be injected into immunodeficient mice to evaluate their ability to initiate tumors, demonstrating their tumorigenic potential (122). Organoid formation assays employ CSCs to form organoids in cultured 3D environments that mimic tumor growth. This approach assesses cancer stem cells› self-renewal capacity and differentiation capabilities (123). Another technique that can be employed to identify CSCs is single-cell RNA sequencing. This advanced technique analyzes gene expression at the single-cell level, identifying transcriptional profiles unique to CSCs (124). Traditional immunohistochemistry (IHC) methods use specific antibodies to detect CSC surface markers in tissue samples, providing spatial information about the localization of CSCs within the tumor via immunohistochemistry (IHC) (125). The type of marker that must be determined carefully after determining methods of CSC detection, which involves the stemness characteristics of the tested cells. Each cancer type has distinct biological characteristics. Consequently, these cancers express unique CSC markers that reflect their tissue of origin and functional properties. For example, for breast cancer, commonly used CSC markers include $CD44^+/CD24^-/low$ and ALDH1 (126). For colon cancer, two markers are used frequently: CD44 and EpCAM (127). In glioblastoma, CD133 is a key marker for identifying CSCs (128). In lung cancer, markers include ALDH1 and CD133 (129). Common liver cancer CSC markers include CD133, CD90, CD44, EpCAM, CD13, CD24, OV6, DLK1, α2δ1, ICAM-1, Lgr5, and keratin192 (130, 131. For kidney cancer, the CSC markers are CD133, CD24, CXCR4, and CD105 (132, 133). The prostate cancer CSC markers detected are CD133, CD44, ALDH1, and integrin α2β17 (134136-). Moreover, for sarcomas, the CSC markers are ALDH, CD133, CD44, and ABC transporters (137--139). All these markers are selected on the basis of their association with stem cell-like properties, such as self-renewal, differentiation, and tumor initiation. The variation in markers arises from differences in the molecular pathways and microenvironments that influence CSC behavior across different cancer types.

The CSC hypothesis has profoundly advanced our understanding of cancer biology and opened new avenues for research and treatment. It has become a cornerstone of modern oncology because it emphasizes tumor heterogeneity, drives targeted therapies, and inspires diagnostic innovations. The impact of the cancer stem cell (CSC) hypothesis on cancer research and treatment has significantly transformed the scientific understanding of cancer and revolutionized oncology research in several key ways. Notably, not all cancer cells contribute equally to tumor growth, metastasis,

or recurrence. This has led to a deeper appreciation of the hierarchical organization within tumors, where a small subset of CSCs drives tumor initiation and progression (140).

Therapeutic strategies could be improved on the basis of the knowledge of SCS features since conventional therapies, such as chemotherapy and radiation, primarily target rapidly dividing cells but often fail to eliminate CSCs, which are inherently resistant to these treatments. The CSC hypothesis may inspire the development of novel therapeutic strategies that specifically target CSCs to prevent tumor recurrence and metastasis (141). Identifying CSC-specific markers and signaling pathways has paved the way for targeted drug development and immunotherapies. For example, inhibitors targeting the Wnt, Notch, and Hedgehog pathways are actively explored as potential treatments to disrupt CSC survival and proliferation (142). For diagnostic purposes, CSC markers have been instrumental in developing diagnostic tools that predict patient prognosis and monitor treatment response (143--126). These tools enable personalized medicine approaches, tailoring therapies on the basis of the presence and activity of CSCs. (144146-).

Despite its widespread acceptance, the CSC hypothesis faces challenges, including difficulties in isolating and characterizing CSCs owing to their plasticity and dynamic nature. These challenges continue to drive innovation and debate, fostering advancements in methodologies and conceptual frameworks. However, ongoing challenges highlight the need for continued exploration and refinement of this paradigm (147).

Cancer stem cell hypothesis in glioblastoma

After confirming their presence in various solid tumors, researchers identified cancer stem cells (CSCs) in glioblastoma (GBM), following earlier discoveries of CSCs in leukemia and other solid tumors. Scientists have hypothesized that, like other cancers, GBM may contain a subset of cells with stem-like characteristics that contribute to tumor initiation, progression, and resistance to treatment.

A significant breakthrough occurred in 2004 when Singh et al. identified a specific subpopulation of glioblastoma cells characterized by the expression of a surface protein, the CD133 marker. These $CD133^{+}$ cells displayed essential stem cell properties, including self-renewal, multipotency (the ability to differentiate into various cell types), and the ability to initiate tumors when transplanted into immunodeficient mice. This landmark study strongly suggested that glioblastoma is driven by a small population of stem-like cells, thereby transforming the scientific understanding of this aggressive form of brain cancer. (148150-).

Events such as tumor initiation and progression—particularly in the context of malignant transformation, glioblastoma metastasis, and relapse following chemotherapy or radiotherapy—illustrate that specific glioblastoma cells can exhibit increasingly aggressive behavior by acquiring additional pro-oncogenic mutations. These mutations enable them to evade apoptosis and thrive under therapeutic stress, leading to treatment resistance. The cells that emerge as survivors from these intense selective pressures are recognized to exhibit phenotypic traits characteristic of cancer stem cells (CSCs). Understanding the mechanisms driving the emergence and maintenance of these small subsets of malignant cells is crucial, as they play a pivotal role in the overall aggressiveness of the tumor and the challenges faced in effective treatment strategies.

Hence, significant research efforts have been made to identify the genetic changes that occur in glioblastoma CSCs, which could be used to distinguish the ability of these cells to resist treatment and initiate tumor recurrence.

CSC resistance in chemotherapy models mirrored observations in recurrent GBM patients. These findings highlight the importance of targeting CSCs for effective GBM treatment (151). Recently, new types of treatments that interfere with several vital GBM cancer stem cell pathways have been discovered. Jhanwar-Uniyal et al. (2023)(152) investigated the role of the mTOR pathway in regulating glioblastoma CSCs via the use of inhibitors targeting the PI3K/AKT/mTOR and MAPK pathways. Many GBM tumors coexpressed the stem cell marker nestin and activated mTOR (pmTORSer2448), indicating a link between mTOR signaling and stemness. Treatment with the new compounds rapamycin and PP242 (mTORC1 inhibitors) or LY294002 (PI3K inhibitor) and the novel mTORC12/ inhibitors Torin1 and Torin2 effectively suppressed GBM CSC proliferation via the suppression of the stem cell marker NANOG and the arrest of self-renewal capacity. (152)

Caglar et al. (2023) (153) investigated the role of the cancer stem cell expression signature (CSC) in glioblastoma (GBM), and the researchers identified differentially upregulated genes primarily involved in transcriptional regulation. A protein–protein interaction network was constructed via the Search Tool for the Retrieval of Interacting Genes/Proteins (STRING). Hub genes, including DUSP6, FGFR3, EGFR, SOX2, NES, and PLP1, were identified via the MCC and MNC methods. The expression levels and prognostic values of these hub genes were validated in the TCGA GBM dataset via the Gene Expression Profiling Interactive Analysis 2 (GEPIA2) platform. The expression of four hub genes was elevated in GBM samples, with DUSP6 and SOX2 demonstrating significant prognostic value for patient survival. In conclusion, this study highlights DUSP6 as a promising therapeutic target in GBM (153, 154).

The origin of cancer stem cells (CSCs) in glioblastoma

One exciting theory proposed to explain the origin of glioblastoma is the cancer stem cell theory or what is now known as glioblastoma stem cells (GSCs). It has been postulated that glioblastoma stem cells (GSCs), such as normal neural stem cells, dedifferentiated glial cells, or oligodendroglial precursor cells, may originate from various sources in the brain. Understanding these potential origins is critical, as it influences the development of targeted treatments for glioblastoma.

The most significant focus of the cancer stem cell hypothesis suggests that tumors are organized hierarchically, with cancer stem cells at the top and progenitors with differentiated cells downstream. These cells share some crucial characteristics with normal stem cells, suggesting that glioblastomas may originate from neural stem cells, progenitor cells, or even differentiated cells, each with different probabilities of transformation into cancer, and this complexity can contribute to the heterogeneity observed in gliomas. The subventricular zone (SVZ) of the brain may play a vital role

in glioma development. This region (SVZ) is a brain region situated along the walls of the lateral ventricles and plays a vital role in neurogenesis and the generation of new neurons, even in adulthood. This area has distinctive characteristics that distinguish it from other brain regions. It serves as a neurogenesis hub, where neurons are continuously produced. The SVZ contains neural stem cells, progenitor cells, and neuroblasts, all of which collaborate to generate new neurons. These newly formed neurons migrate through the rostral migratory stream to the olfactory bulb, where they integrate into existing neural circuits. Researchers are investigating the mechanisms of SVZ neurogenesis to explore its potential as a source for regenerative therapies, particularly in the treatment of brain injuries (155). Since this species contains neural stem cells, it is thought to provide a favorable environment for tumor formation because of its high proliferative potential and the presence of growth factors. The complexity of the central nervous system and the various cellular compartments involved in tumorigenesis contribute to the challenges in understanding and treating these cancers. (156, 157)

Owing to the specificity of this area of the brain, many opinions have suggested that cancer stem cells (CSCs) in glioblastoma (GBM) may originate from the transformation of neural stem cells (NSCs) in the subventricular zone (SVZ) or subgranular zone (SGZ) of the hippocampus. Alternatively, CSCs could arise from dedifferentiated tumor cells reacquiring stemness properties. The two GBM subtypes, primary and secondary, are believed to originate from different pathways: primary GBM tumors originate directly from NSCs or progenitors, and secondary GBM tumors originate from transformed astrocytomas through a slow transformation process (158, 159).

Experimental evidence indicates that glioblastoma stem cells (GSCs) and normal neural stem cells (NSCs) in the subventricular zone (SVZ) exhibit notable characteristics. GSCs share the same expression patterns of tumor suppressor genes such as PTEN and p53 as do NSCs. Loss or mutation of both genes in GSCs enhances stem cell self-renewal and expansion. Codeletion of neurofibromatosis-1 (NF-1) with p53 accelerates glioma formation. Transcription factors such as the Sonic Hedgehog (Shh) pathway, which regulates the development and proliferation of NSCs, are dysregulated in glioblastomas. Growth factors such as EGF, PDGF, and TGF-beta play dual roles in normal SVZ function and glioma progression. The overexpression of these factors drives proliferation and tumorigenesis, with TGF-beta contributing to the immunosuppressive glioblastoma microenvironment. Cytoskeletal proteins such as nestin and doublecortin, which are markers of neural progenitors, are expressed in gliomas and linked to tumor progression. Nestin+ cells persist during gliomagenesis, whereas doublecortin overexpression protects glioma cells against hypoxia and glucose deprivation. GSCs reside in vascular niches similar to standard NSC niches and are supported by endothelial cells, growth factors, and extracellular matrix components. The vascular niche promotes GSC self-renewal and tumor growth, with nitric oxide production enhancing GSC tumorigenicity. This similarity suggests a potential origin of GSCs from these normal cell populations. (160, 161)

Genetic evidence has identified the subventricular zone (SVZ) as a potential cell-of-origin site for glioblastoma (GBM), specifically in IDH1 wild-type GBM, where driver mutations in the TP53, PTEN, EGFR, and TERT promoters are present. The reason behind finding these cells away from the SVZ was that neural stem cells (NSCs) harboring oncogenic mutations can migrate to remote brain areas and can form GBMs. (162, 163)

The microenvironment can specifically influence the functional status of GSCs. It regulates glioblastoma CSCs, particularly in perivascular and periumcrotic niches where stemness features can be lost or gained during differentiation or dedifferentiation via the microenvironmental signaling that governs the conversion between tumor cells and tumor stem cells through its intrinsic (Notch, OCT4, Wnt/βcatenin, BMI1, Nanog and c-Myc) and extrinsic (EGFR, PTEN, PI3K/AKT, Bmp) signals. The location of glioblastoma CSCs between the central necrosis zone and the proliferative zone seems to be influenced by hypoxia. The pericontinrotic niches arise from hypoxia-induced GSC generation, with other possible interpretations, such as astrocytes, immune cells, and pericytes, that can be identified by stemness antigens in perivascular niches. The cells near necrotic areas may contain precursors/stem cells that acquire stemness through dedifferentiation (164, 165).

Various sequencing techniques for samples matched in the tumor-free subventricular zone (SVZ), GBM tumor, and normal brain tissues have been used to detect the origins of cancer-driving mutations in glioblastoma CSCs. The results highlight the role of neural stem cells (NSCs) in the SVZ and their potential migratory behavior in GBM tumor development. Intrinsic DNA replication errors and exposure to mutagens are key factors in this process of mutation accumulation in NSCs located in the SVZ. Two dominant mutation signatures in both tumor-free SVZ and tumor tissue samples indicate a clock-like process of mutation accumulation over time.

TERT promoter mutations were present in all patients with IDH-wildtype GBM with driver mutations in tumor-free SVZ tissue. This mutation is significant because it prevents telomere shortening, allowing for extended cell proliferation without senescence.

It has been suggested that somatic mutations, particularly those caused by DNA replication errors, accumulate mainly in neural stem cells (NSCs) in the human brain and that progenitor cells originating from the SVZ have the potential to migrate, suggesting that driver mutations in stem cells can be found away from the primary tumor mass in GBM. Understanding these processes could help prevent the development and recurrence of GBM. (166) In that sense, current theories suggest that cancer stem cells in glioblastoma may originate from adult neural stem cells (NSCs) or oligodendrocyte precursor cells (OPCs). However, the subject remains debated and is influenced by species differences and tumor heterogeneity (167).

The genetic signature of GSCs and its contribution to GBM treatment resistance and recurrence

The genetic profiles of glioblastoma cancer stem cells (GSCs) are pivotal in driving treatment resistance and disease recurrence. These CSCs possess distinct molecular features that enhance their survival and adaptability within

the tumor microenvironment. Understanding the genetic signature of GSCs may help establish and develop targeted therapeutic approaches. A very recent review explained some of these genetic signatures if more information was needed (168). These cells are regulated by specific signaling pathways, namely, the Notch, Wnt, and Hedgehog (Hh) pathways. These pathways are essential for maintaining the properties of stemness, which in turn affects tumor behavior and the response to therapy. Targeting the Notch, Wnt, and Hh pathways with new potential therapeutic strategies is suggested to effectively suppress CSC proliferation and invasion, leading to significant delays in tumor recurrence. In one such study, the genetic signatures of twenty-four stem-like glioma cell lines (SLGCs) were studied with nonnegative matrix factorization expression metaprofiles. Five metaprofiles were identified and characterized by specific combinations of 7–12 factors. All SLGC lines expressed epidermal growth factor receptor (EGFR), its ligands, and other receptor tyrosine kinases. They also exhibited a neural signature and were predominantly IDH1 wild type, and there were variations in p53 and PTEN status among the lines. A positive correlation was found between the pluripotency factor Sox2 and the expression of several other factors, such as FABP7 and CD133. However, there was a weak or absent correlation with factors such as MGMT and Hif1α. Additionally, spherical growth was positively correlated with high levels of specific proteins, indicating complex relationships between stemness and growth behavior. Several other factors, including cathepsin-D, CD99, and EMMPRIN/CD147, are highly expressed across all SLGC lines, regardless of their stemness or growth characteristics (169). For that reason, precision medicine approaches should be investigated, and treatments tailored to the genetic characteristics of individual tumors are needed, which can provide more effective therapies for targeting CSCs in GBM patients (170). Furthermore, investigating how these cells promote therapy resistance is crucial for improving treatment strategies for recurrent GBM (rGBM), which is a critical factor in the growth and recurrence of GBM.

GSCs can evade immune surveillance by mimicking immune-suppressive functions; they solely recruit immune-suppressive cells into the tumor microenvironment. GSCs highly express specific markers (Oct4/Sox2high/FOXP3−) associated with regulatory T-cellcell (Treg) function within GBM tumors. The expression of genes such as TGFβ1, CD39, CD73, PD-L1, and galectin-1 by GBM CSCs is linked to immune suppression. Mechanistically, inhibiting TGFβ type II receptor (TGFBR2) or XBP1 signaling can reverse the immune-suppressive characteristics of recurrent GBM cells. The use of miRNA-based strategies, specifically miR-16-124/3p, shows promise in disrupting the immunosuppressive network. These inhibitions enhance the tumor-killing ability of CD4+ and CD8+ T cells and reduce the expression of anti-inflammatory markers. In this context, targeting the identified immunosuppressive mechanisms in GSCs could be pivotal for developing effective immunotherapies. These experiments provide the first evidence that GSCs mimic Treg cell functions, indicating a potential new avenue for therapeutic intervention in GBM (171).

As demonstrated by many investigations, the aggressiveness and heterogeneity of GBM make it a particularly difficult cancer to treat effectively, with high recurrence rates. Recently, the highly plastic states of the GBM among the three distinct cell states in IDH-wildtype proneural (PN), classical (CL), and mesenchymal (MES) states in response to various stimuli were discovered. The transition of GBM cells to the MES state is linked to increased invasiveness and resistance to treatment, contributing to tumor recurrence. However, the mechanisms driving this transition are not well understood (172, 174).

Pathways such as bromodomain-containing protein 2/phosphatase and tensin homolog/nuclear factor kappa B (PTEN/NF-kB/BRD2) were identified as crucial drivers of the MES transition. BRD2 is highlighted as an essential epigenetic modulator that influences the cell state transition (MES). PTEN was shown to regulate the chromatin binding of BRD2, BRD4, and p65/RelA, which are essential for the expression of MES genes. Specifically, the acetylation of RelA at lysine 310 is necessary for BRD2 to localize to the promoters of these genes. The loss of BRD2 function facilitates the transition of GBM cells from the mesenchymal state to the proneural state, increasing their sensitivity to ionizing radiation (IR). Furthermore, mutations in BRD2 bromodomain lead to a shift in cell state, and the use of BRD2-specific inhibitors can disrupt BRD2›s role in MES gene expression, increase sensitivity to IR, and reduce GBM cell invasion in animal models. Thus, targeting BRD2 with specific inhibitors could be a promising strategy for treating mesenchymal transition and potentially improving therapeutic outcomes (175).

Identifying distinct biomarkers that distinguish GSCs from differentiated tumor cells can provide insights into their functional roles and therapeutic susceptibilities. In addition to the oncogenes that have been recognized, such as SOX2, NANOG, CHRDL1, and OCT4, which regulate GSC self-renewal and tumorigenic potential, pathways such as the Wnt/β-catenin and STAT3-β-catenin pathways interact with FOXM1 to maintain GSC properties. Recently, a new classification of GSCs has been introduced on the basis of genetic signatures: proneural GSCs (PN GSCs) and mesenchymal GSCs (MES GSCs). A phenotypic categorization was also introduced on the basis of the ability to have high invasiveness, which contributes to therapy resistance. The proneural GSCs (PN GSCs) of a whole stem (GSf) express CD133, which is associated with active NOTCH1 and HER3 signaling pathways and contributes to invasion capacity. Moreover, the restricted stem (GSr) linked to active EGFR and PI3K/mTOR pathways lacks CD133 and grows adherently in vitro, which is associated with chemotherapy resistance (176).

Mesenchymal GSCs (MES GSCs) are distinguished by the downregulation of epithelial markers (e.g., E-cadherin) and the upregulation of mesenchymal markers (e.g., vimentin and N-cadherin). They also release exosomes with elevated levels of miR-1555-p, promoting mesenchymal transition in recipient glioma cells. Markers such as CD133, CD44, and Nestin are enriched in MES GSCs; therefore, they may be genetic signatures of glioma recurrence, highlighting their role in tumor relapse. Targeting these biomarkers and associated pathways—such as the Hedgehog, NOTCH, and

PI3K/AKT pathways—has shown promise in preclinical studies, as it inhibits tumor growth and prolongs survival. Additionally, targeting chemokine receptor 4 (CXCR4) effectively suppresses invasive growth and migration in GSCs. Moreover, longevity assurance homolog 2 (LASS2), a tumor suppressor gene that regulates ceramide synthesis, can reduce GSC migration and invasion by promoting apoptosis and inhibiting EMT (177).

The expression profile of recurrent glioblastomas often reflects GSC molecular signatures, underscoring their contribution to tumor relapse. Leveraging GSC-specific biomarkers in targeted therapies offers a path toward more precise and effective treatments tailored to individual patients, ultimately improving outcomes in glioma management (178). Identifying active genes in glioblastoma (GBM) is crucial for understanding its pathogenesis and improving patient prognosis. Various studies have identified numerous genes that are differentially expressed in GBM, contributing to its aggressive nature and poor prognosis. These genes are involved in various biological processes, including immune infiltration, angiogenesis, and oncogenic signaling pathways.

Conclusion

Glioblastoma multiforme (GBM) is among the most lethal forms of cancer known to humans. The standard treatment approach includes surgical resection, radiation therapy, and chemotherapy via temozolomide (TMZ). However, patients frequently experience tumor recurrence, resulting in a median survival of only 14.6 months. This high relapse rate is driven primarily by the biological and molecular complexity of the tumor. A thorough understanding of the cellular and molecular characteristics of the originating cell types is essential for addressing the root causes of GBM. In this review, we have examined key studies that shed light on the cells responsible for tumor initiation, as well as the factors that make GBM highly persistent and challenging to treat, ultimately contributing to its high mortality rate.

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ATT proposed the review topic, selected the subtitles, drafted the initial manuscript, and finalized the revised draft. EJS contributed to writing, refined the referencing style, and edited language corrections. BSA reviewed and finalized language corrections and ensured the accuracy of the final draft.

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