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Glioma stem cells: drivers of tumor progression and recurrence

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Abstract

Glioma, a common malignancy of the central nervous system, attracts significant clinical attention due to its poor prognosis. Glioma stem cells (GSCs), characterized by stem-like properties and substantial heterogeneity, play a crucial role in tumor initiation, progression, and potential recurrence. Moreover, through complex interaction mechanisms, they contribute to the challenges associated with treatment. This review seeks to explore the distinctive characteristics and underlying mechanisms of GSCs, aiming to provide novel theoretical insights and practical strategies for precision therapy in glioma.

Keywords Glioma stem cells, Glioma, Mechanism, Immunity, Metabolism

Introduction

When central nervous system (CNS) tumors are classified, gliomas account for 90% of all malignant CNS tumors [1]. Glioblastoma (GBM), the most aggressive subtype, constitutes 69% of glioma cases and is characterized by rapid progression and a median survival time of only 14 to 16 months [2]. Consequently, there is a critical need to develop more effective treatment strategies for GBM.

Glioma stem cells (GSCs) demonstrate characteristics such as self-renewal, the ability to differentiate into various lineages, unlimited proliferation, and significant invasiveness [3]. These attributes render GSCs pivotal in driving glioma initiation, progression, resistance to radiotherapy and chemotherapy, and malignant recurrence [4], Consequently, research on GSCs holds substantial theoretical significance and clinical application potential.

This review aims to underscore recent advancements in GSC research, examining their characteristics and underlying mechanisms to establish a theoretical foundation for the development of novel therapeutic strategies. By synthesizing existing research findings, this review further elucidates the critical role of GSCs in glioma development and progression, while also exploring potential future research directions.

Characteristics of GSCs

Definition

Within glioma tissues, GSCs constitute a minor cell population capable of self-renewal and differentiation into multiple lineages [5]. Self-renewal refers to the ability of GSCs to maintain population stability through asymmetric division while generating new GSCs [6], a process essential for the sustained growth and recurrence of tumors. Differentiation along multiple lineages demonstrates that GSCs possess the ability to transform into diverse cell types, thereby contributing to tumor heterogeneity (Fig. 1) and facilitating tumor recurrence and drug resistance [7]. The identification of GSCs has fundamentally altered the conventional understanding of glioma initiation and progression, offering novel insights for precision therapy in glioma.

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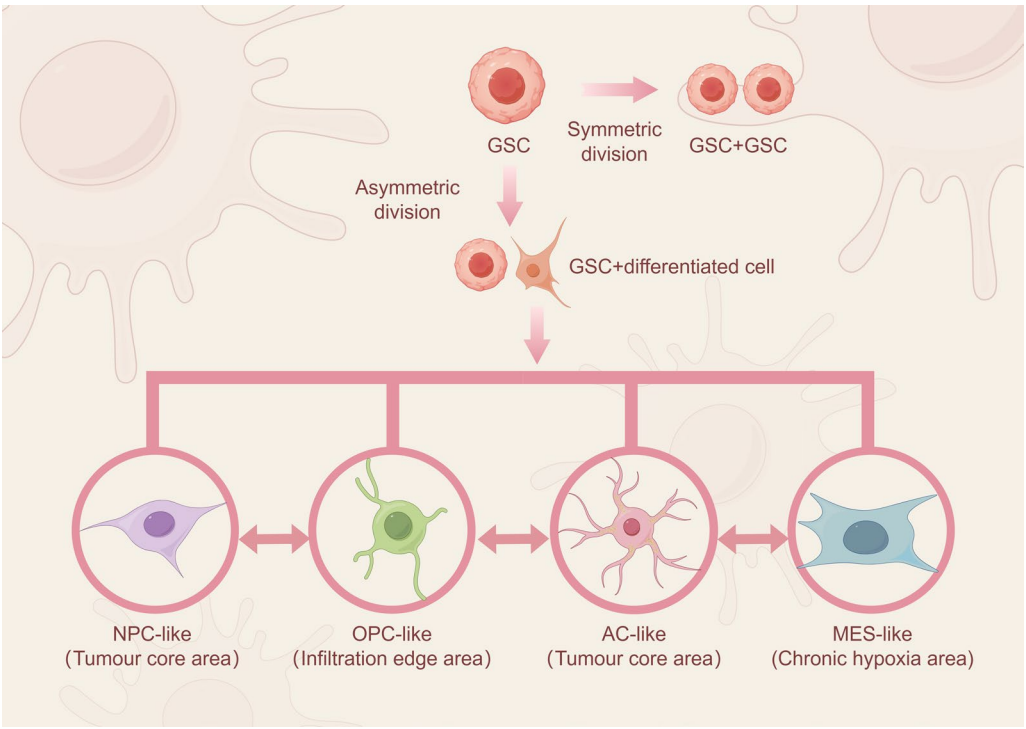

Fig. 1 GSC heterogeneity. GSC dynamically maintain population homeostasis through asymmetric division (yielding one stem cell and one differentiated progeny) and symmetric division (generating two stem cells). NPC-like and AC-like states predominantly localize to the tumor core. OPC-like cells are enriched at the infiltrative margin. MES-like subtypes preferentially reside within chronic hypoxic niches. Notably, undifferentiated GSC persist across all tumor regions, demonstrating microenvironmental plasticity. NPC-like: Neural progenitor-like; OPC-like: Oligodendrocyte progenitor-like; AC-like: astrocytic-like; MES-like: Mesenchymal-like (By Figdraw)

Table 1 GSCs stemness marker molecules and their roles

Maker	Description	Primary functions related to glioma	Citations
CD133/Prominin-1	Transmembrane glycoprotein	a. Facilitates tumor initiation and stemness maintenance b. Serves as a therapeutic target	[153–155]
Oct4/POU5F1	Transcription factor	a. Involves in the maintenance of glioma stemness b. Promotes tumor proliferation and migration c. Acts as a therapeutic target	[156–158]
Sox2	Transcription factor	a. Synergistically acts with Oct4 b. Maintains the undifferentiated state of stem cells c. Influences cancer cell cycle and proliferation efficiency	[5, 159, 160]
Nanog	Transcription factor	a. Positively correlates with tumor malignancy b. Participates in metabolic regulation of GSCs, facilitating their survival and proliferation in harsh environments c. Involved in GSC immune evasion	[156, 161]
CD44	Cell membrane glycoprotein	a. Characterized by high invasiveness and resistance to radiotherapy and chemotherapy b. Hypoxia can induce phenotypic changes in cells c. Predicts patient prognosis	[162, 163]
Nestin	Intermediate filament protein	a. Promotes the formation of tumor spheres	[164, 165]

Stemness marker molecules

GSCs are typically characterized by the expression of specific stem cell markers. Table 1 enumerates the principal stemness marker molecules and elucidates their mechanisms of action within GSCs. However, the application of CD133 as a classic stem cell marker in the study of GSCs has long been controversial. In GBM, the CD133-positive cell subpopulation exhibits significant stem cell characteristics, including enhanced sphere-forming ability in vitro, drug resistance, and tumorigenicity in vivo [8–10], making it an important marker for studying GSCs. Nevertheless, the limitations of CD133 are equally notable. Studies have shown that its expression is dynamically plastic: some GSCs can downregulate CD133 under hypoxic or metabolic stress conditions [11], while certain CD133-negative cells can regain stem cell characteristics through epigenetic reprogramming [12]. Single-cell sequencing further reveals the presence of CD133-independent stem cell subpopulations in GBM, which maintain their stemness by activating alternative signaling pathways, such as Notch3 [13, 14]. Despite the controversies surrounding CD133 in GSCs research, its functional relevance still renders it an important subject of study.

Moreover, CD109, a glycosylphosphatidylinositol-anchored glycoprotein, has been proposed as a marker for perivascular GSCs [15]. Studies have demonstrated a clear association between CD109 and the maintenance of GSC stemness as well as disease recurrence [16, 17]. GP130, a co-receptor for cytokines such as interleukin-6 (IL-6) and tumor necrosis factor (TNF), facilitates the activation of signaling pathways, thereby influencing the stemness characteristics of GSCs [18]. The interaction between CD109 and glycoprotein 130 has been identified as a mechanism that promotes the stemness and chemoresistance of GSCs by activating the IL-6/STAT3 signaling pathway. This interaction enhances the tumorigenic potential of GSCs and contributes to their resistance to conventional therapies [19]. Furthermore, the expression of integrin α2 (ITGA2) in GSCs is associated with STAT3 phosphorylation and the activation of epithelial-mesenchymal transition (EMT), underscoring the role of stemness markers in facilitating the invasive behavior of these cells [20]. Additionally, the cellular prion protein (PrPC) and its molecular chaperone Hsp70/90 organizing protein (HOP) are implicated in the regulation of GSC proliferation and self-renewal. The PrPC-HOP complex is crucial for maintaining the stemness of GSCs, and disruption of this complex results in diminished proliferation and impaired self-renewal [21]. In summary, the expression of stem cell markers in GSCs functions not only as an identifier for these cells but also significantly contributes to their proliferation, sustains their undifferentiated state, and augments their invasive potential.

Distribution of GSCs

With advancements in imaging technologies and the identification of molecular markers, the spatial distribution of GSCs within gliomas has become increasingly discernible, allowing for more precise identification of regions enriched with GSCs. Research employing CD133 + and Nestin + markers to locate GSCs has uncovered significant variations in GSC expression across different pathological grades of gliomas, with higher grades exhibiting an increased content of GSCs [22]. Furthermore, GSCs are typically distributed around the microvasculature, emulating the niche architecture of neural stem cells. This niche is modulated by adjacent cells and the cytokines they secrete, which regulate stem cell division and their departure from the niche. The microvasculature is integral to nutrient uptake and the overall functionality of stem cells, with the intricate vascular network ensuring the supply of vital nutrients and oxygen [23]. Additionally, the interaction between endothelial cells and mesenchymal stem cells (MSCs) within the perivascular niche is essential for sustaining stem cell functions and guiding their differentiation pathways. Endothelial cells release various signaling molecules, such as endothelin-1, which can affect MSC fate by promoting differentiation into specific lineages, such as osteogenic and chondrogenic pathways, through the activation of signaling pathways like AKT [24]. This interaction highlights the role of the microvasculature not only in nutrient provision but also in regulating stem cell behavior through paracrine signaling. The distribution of GSCs is influenced not only by their intrinsic properties but also by their close association with the microenvironment. The distribution of GSCs is influenced not only by their intrinsic properties but also by their close association with the tumor microenvironment (TME) in which they reside. The TME serves as the "soil" for GSC survival, encompassing the extracellular matrix, immune cells, vascular networks, and various signaling molecules. Hypoxic conditions significantly impact gene expression regulation, particularly genes involved in the hypoxia response, thereby affecting the growth and invasion of GSCs. Central to this process are hypoxia-inducible factors (HIFs), which regulate the expression of numerous genes that facilitate tumor progression and adaptation to low-oxygen environments. For example, HIF-1α is known to activate a range of downstream target genes that promote angiogenesis, cell survival, and metabolic adaptation, thus contributing to the aggressive nature of GSCs under hypoxic conditions [25, 26]. The hypoxic microenvironment in tumors, such as GBM, not only enhances GSC proliferation but also contributes to their resistance to conventional therapies. This resistance is partially attributed to the upregulation of stemness markers and other survival pathways mediated

by HIFs. For instance, the activation of the Wnt/β-catenin signaling pathway under hypoxic conditions has been demonstrated to enhance the invasive and metastatic potential of cancer cells, including GSCs. This pathway is frequently upregulated in response to HIF-1α activation, thereby reinforcing the invasive characteristics of these cells [27, 28]. Furthermore, hypoxia can induce the expression of genes involved in maintaining cancer stem cell properties, such as CD133 and vascular endothelial growth factor (VEGF), which are essential for the self-renewal and differentiation capabilities of GSCs. Consequently, the hypoxic environment not only facilitates the survival of these cells but also augments their ability to form neurospheres and migrate, thereby contributing to tumor progression and recurrence [29, 30]; Conversely, the inflammatory microenvironment enhances GSC migration and adhesion through the release of cytokines and chemokines, thus contributing to tumor heterogeneity and therapeutic resistance. The release of these factors is modulated by various elements, including the presence of other cell types such as oligodendrocyte progenitor cells and macrophages, which establish a supportive niche for GSCs at the tumor periphery [31]. The spatial distribution of GSCs within gliomas is characterized by a complex and dynamic process, shaped by the intrinsic properties of GSCs and the surrounding TME.

Regulatory mechanisms of GSCs

Regulatory mechanisms of GSCs in gliomas encompass insights from genetics, epigenetics, metabolic reprogramming the TME and the immune system.

Genetics and epigenetics

In 2008, the Cancer Genome Atlas (TCGA) database first identified the most prevalent mutated genes in gliomas, such as IDH1/2, TP53, and ATPX. Recent advancements in genomic research have significantly enhanced our comprehension of genetic mutations and structural variations in gliomas [32]. The evolution and diversity of clones during glioma progression are particularly intricate, involving epigenetic regulation that encompasses DNA methylation, histone activity, post-translational modifications (such as methylation and acetylation), and the microRNA modification profile [33]. Research has demonstrated that c-Myc, a crucial regulatory factor, is markedly expressed in GSCs and possesses the capability to activate transcriptional modules associated with stem cell characteristics, thereby inducing apoptosis [34]. GSCs exhibit heightened sensitivity to histone demethylase inhibition compared to their non-stem-like counterparts. This increased sensitivity is attributed to the distinctive chromatin features of GSCs, including the absence of the H3K9me3 mark and mutations in epigenetic regulatory genes (such as KDM4A, EZH2, and DNMT3A). KDM4A, as an H3K9me3-specific demethylase, is often overexpressed or functionally enhanced in GSCs. Its inhibition can directly restore H3K9me3 levels and trigger apoptosis [35, 36]. Mutations in EZH2 (which catalyzes H3K27me3) or DNMT3A (which regulates DNA methylation) may synergistically alter chromatin accessibility, further amplifying the vulnerability of GSCs to epigenetic interventions [37, 38]. In contrast, more differentiated glioma cells exhibit significant resistance to similar drugs due to the retention of more stable epigenetic marks, such as H3K9me3 and DNA methylation patterns [36].

Metabolic reprogramming

Metabolic reprogramming is increasingly acknowledged as a pivotal factor in tumor progression, reflecting a shift in the mechanisms by which cancer cells manage energy production and biosynthesis to support rapid growth and survival. This phenomenon is often characterized by the Warburg effect, wherein cancer cells preferentially utilize glycolysis over oxidative phosphorylation even in the presence of oxygen [39]. The hypoxic microenvironment induced by GBM not only affects the functionality of GSCs but also elevates the reliance on glycolysis [40], Although glycolysis is inefficient in adenosine triphosphate (ATP) synthesis, this metabolic shift serves as a beneficial foundation for the synthesis of novel molecules, such as nucleic acids, pyruvate, and NADPH [41]. Recent research has underscored the dual role of the pentose phosphate pathway (PPP) in cancer cells, including GSCs, under varying oxygenation conditions. Under hypoxic conditions, the PPP is frequently upregulated to satisfy the increased demand for nicotinamide adenine dinucleotide phosphate (NADPH), which is essential for maintaining redox homeostasis and supporting anabolic processes. This is particularly significant in the context of the Warburg effect, where the dependence on glycolysis can result in heightened oxidative stress. The role of the PPP in modulating oxidative stress and inflammation is well-documented, with transaldolase, a pivotal enzyme in the PPP, being implicated in the regulation of these processes and their contribution to carcinogenesis [42]. Metabolic pathways can alternate between glycolysis and the PPP, with glycolysis being activated under hypoxic conditions to facilitate cellular migration and invasion. Conversely, under normoxic conditions, the PPP is activated, promoting cell proliferation [43]. In scenarios of hypoxia combined with glucose deprivation, GSCs enhance the expression of high-affinity glucose transporter (GLUT) proteins to compete effectively with non-stem cells for glucose uptake [44]. Additionally, GSCs augment the synthesis of their metabolites by upregulating the expression

of fatty acid synthase and glutamine-metabolizing enzymes, which not only supply energy to GSCs but also modulate the immune microenvironment, thereby further supporting GSC survival and invasion. Compared to the upstream and downstream effects of genetic and epigenetic processes, the influence of the metabolome is more pervasive. Mutations in the IDH1 gene, particularly the R132H variant, have been extensively investigated in the context of GBM and other gliomas. These mutations confer a neomorphic enzymatic activity that disrupts the normal function of isocitrate dehydrogenase 1 (IDH1), resulting in the production of the oncometabolite 2-hydroxyglutarate (2-HG) instead of the typical product, alpha-ketoglutarate (α-KG) [45]. This aberrant enzymatic activity is implicated in the pathogenesis of gliomas by inducing a hypermethylated state of DNA and histones, which can activate oncogenes and inactivate tumor suppressor genes, thereby contributing to tumorigenesis [46]. Furthermore, the accumulation of 2-HG disrupts cellular metabolism and inhibits histone and DNA demethylases, leading to epigenetic modifications that impede cellular differentiation and promote tumor growth [47]. Additionally, mutations in the TERT promoter activate telomere maintenance mechanisms, providing a foundation for the immortalization of GSCs [48]; EGFRvIII reinforces proliferative signals through sustained activation of the PI3K/AKT pathway and synergizes with hypoxia-induced HIF-1α to upregulate glycolytic enzymes (such as PKM2 and LDHA), driving metabolic reprogramming [49, 50]. Loss of ATRX further leads to dysregulation of chromatin remodeling complexes, enhancing the adaptability of GSCs to hypoxic microenvironments, while TP53 mutations impair DNA repair and cell cycle regulation, exacerbating genomic instability [51–54].

Tumor microenvironment

The TME is a complex and dynamic entity composed of various non-cancerous components that play critical roles in either facilitating, supporting, or hindering tumor development [55]. Interactions between stem cells and the TME are well-documented. Hypoxia, a fundamental characteristic of GBM, enhances therapeutic resistance through various mechanisms, notably by inhibiting radicals, thereby reducing the efficacy of radiotherapy [56]. Within the TME of GBM, the interaction between endothelial cells and GSCs is crucial for tumor initiation and progression. This interaction is facilitated by the formation of a perivascular niche, where GSCs are frequently located in close proximity to endothelial cells. Endothelial cells secrete various soluble factors that promote the maintenance and proliferation of GSCs, thereby increasing the aggressiveness and therapy resistance of GBM [57, 58]. Furthermore, the cross-talk between GSCs and endothelial cells extends beyond the maintenance of stemness, involving modulation of the immune microenvironment. This interaction can result in the secretion of extracellular vesicles and exosomes, which carry bioactive molecules that influence the behavior of immune cells, potentially leading to immune evasion and further promoting tumor progression [59, 60]. Pericytes play a crucial role in supporting vascular architecture by characteristically expressing platelet-derived growth factor receptors. They can be derived from GSCs to sustain tumor growth and blood supply. During GBM angiogenesis, the number of pericytes increases in tandem with the disruption of the blood–brain barrier (BBB), serving as a marker for tumor neovascularization [55, 61]. GBM is referred to as a "cold tumor" due to its immunosuppressive microenvironment: hypoxia-induced metabolic suppression of immune cells [62], inhibition of effector T cell function by TGF-β/IL-10 secreted by endothelial cells [63], immune escape induced by GSCs delivering PD-L1 via exosomes [64, 65], immune escape induced by GSCs delivering PD-L1 via exosomes [66]. Transformation strategies require multi-target synergy: combining radiotherapy with STING agonists to activate antigen presentation [67], using CSF1R inhibitors to eliminate tumor-associated macrophages [68], employing IDH inhibitors to correct metabolic abnormalities and enhance immune recognition [69], and combining chimeric antigen receptor T (CAR-T) cells with immune checkpoint blockade to overcome immune tolerance [70, 71], ultimately achieving a therapeutic breakthrough in "cold-to-hot" tumor transformation.

Immune response

Natural killer (NK) cells function as the primary effector cells in GBM immunity, with their mechanisms involving interactions between NK cell receptors and GBM cell ligands, playing a critical role in the initial defense against infections and tumor development. The efficacy of NK cells is predominantly influenced by the complex equilibrium between activating and inhibitory signals transmitted through their surface receptors [72]. These receptors facilitate the ability of NK cells to differentiate between healthy cells and those that are infected or transformed, thereby ensuring appropriate immune responses. Activating receptors, such as NKG2D, identify stress-induced ligands on target cells, leading NK cells to exert cytotoxic effects and produce cytokines. In contrast, inhibitory receptors, including those from the killer cell immunoglobulin-like receptor (KIR) family, recognize self-molecules, such as MHC class I, and inhibit inappropriate NK cell activation. This balance is essential for preventing autoimmunity and ensuring that NK cells are activated only in the

presence of legitimate threats [73–75]. Additionally, the activity of NK cells is dynamically modulated by the surrounding microenvironment and the presence of cytokines. Cytokines such as interleukin-15 (IL-15) are pivotal in the priming and activation of NK cells, with proteins like cytokine-inducible SH2-containing protein acting as regulators to maintain homeostasis and prevent overactivation. This regulatory mechanism ensures that NK cells remain effective in their role in immune surveillance [76, 77]. GSCs can evade NK cell-mediated elimination through various mechanisms, including the downregulation of activating ligands, upregulation of ligands that engage inhibitory receptors, recruitment of other immunosuppressive cells [78], and alteration of the chemokine profile within the TME [79]. The remodeling of the chemokine profile within the TME is particularly critical. For instance, the CXCL12-CXCR4 axis mediates the homing of GSCs to perivascular niches and maintains their stemness [80]; CCL2 recruits tumor-associated macrophages (TAMs) and myeloid-derived suppressor cells to construct an immunosuppressive barrier [81–83]; CXCL1/CXCL8 activates neutrophils to release pro-tumor factors such as MMP9 and VEGF [84, 85]; CCL5 regulates the migration of effector T cells but can be hijacked by GSCs to induce the infiltration of regulatory T cells (Tregs) [86, 87]. These chemokines synergistically establish a chemokine gradient, guiding immune cells towards a pro-tumor phenotype and forming a protective microenvironment that hinders NK cell-mediated killing.

T cells are integral to tumor immunity, acting as pivotal effectors in the immune system's response to cancer. Among the diverse subsets of T cells, CD8 + cytotoxic T lymphocytes (CTLs) are particularly essential due to their capacity to directly eliminate tumor cells. These cells identify tumor antigens presented by major histocompatibility complex (MHC) class I molecules and execute their cytotoxic functions through the release of perforin and granzymes, which induce apoptosis in target cells. Nevertheless, the efficacy of CD8 + T cells can be undermined by various factors within the TME, such as the presence of immunosuppressive cells and molecules that inhibit their function. For example, Tregs can suppress CD8 + T cell activity, thereby promoting tumor immune evasion [88]. In addition to the role of CD8 + T cells, CD4 + T helper cells significantly contribute to antitumor immunity by facilitating the activation and maintenance of CD8 + T cell responses. These cells secrete cytokines that enhance the cytotoxic functions of CD8 + T cells (granzyme B expression increased 2.3-fold) and support the formation of memory T cells, thereby establishing long-term immune surveillance. Nevertheless, the role of CD4 + T cells is multifaceted, as they can also differentiate into Tregs, which promote tumor progression by suppressing effective antitumor immune responses [89]. GSCs can employ various mechanisms to evade T cell-mediated clearance, such as the high expression of PDL1 or CD86 to inhibit CTL activity, downregulation of MHC class I molecules to avoid immune recognition, expansion of immunosuppressive Tregs, and secretion of immunosuppressive factors. Furthermore, GSCs engage in metabolic competition by consuming glucose essential for T cell function and producing immunosuppressive metabolites like adenosine, which depletes tryptophan and induces T cell apoptosis.

Signaling pathways

In examining the complex biological characteristics of GSCs, it is essential to investigate their associated pathways and mechanisms of action. Figure 2 presents some of the common signaling pathways and their respective functions [90–100].

Furthermore, the interplay among various signaling pathways is crucial for the regulation of GSCs. Poly (A)-specific ribonuclease (PARN) has been identified as a pivotal regulator in the activation of the EGFR-STAT3 signaling pathway, thereby facilitating the self-renewal and proliferation of GSCs [101]; The interaction among the ERK, integrin α6, and N-cadherin signaling pathways enhances the invasive capabilities of GSCs [102]; Additionally, the crosstalk between the Wnt/β-catenin and TERT signaling pathways contributes to the maintenance of GSC stem cell characteristics and confers treatment resistance, with CD133 playing a significant role in this interaction [100]; The interaction between the NOTCH and p53 pathways has been demonstrated to influence GSC progression [103]; Circular RNAs, such as circKPNB1 and circZEB1, have been shown to promote the malignant phenotype of GSCs through the TNF-α/NF-κB pathway, establishing a positive feedback loop that enhances GSC proliferation and survival [96, 97]; Lastly, the FMR1/circCHAF1A/miR-211-5p/HOXC8 feedback loop promotes GSC proliferation and tumorigenesis via the MDM2-dependent p53 signaling pathway [104]. Comprehending the intricate interactions among these signaling pathways in GSCs is crucial for identifying novel therapeutic targets and advancing the development of more effective treatments for glioma.

Interventions targeting GSCs

Given the pivotal role of GSCs in the initiation and progression of GBM, as well as their intricate interactions with the TME, therapeutic strategies that focus on the targeted elimination of GSCs by modulating the microenvironment have garnered increasing research interest. However, the BBB, serving as a pivotal physiological

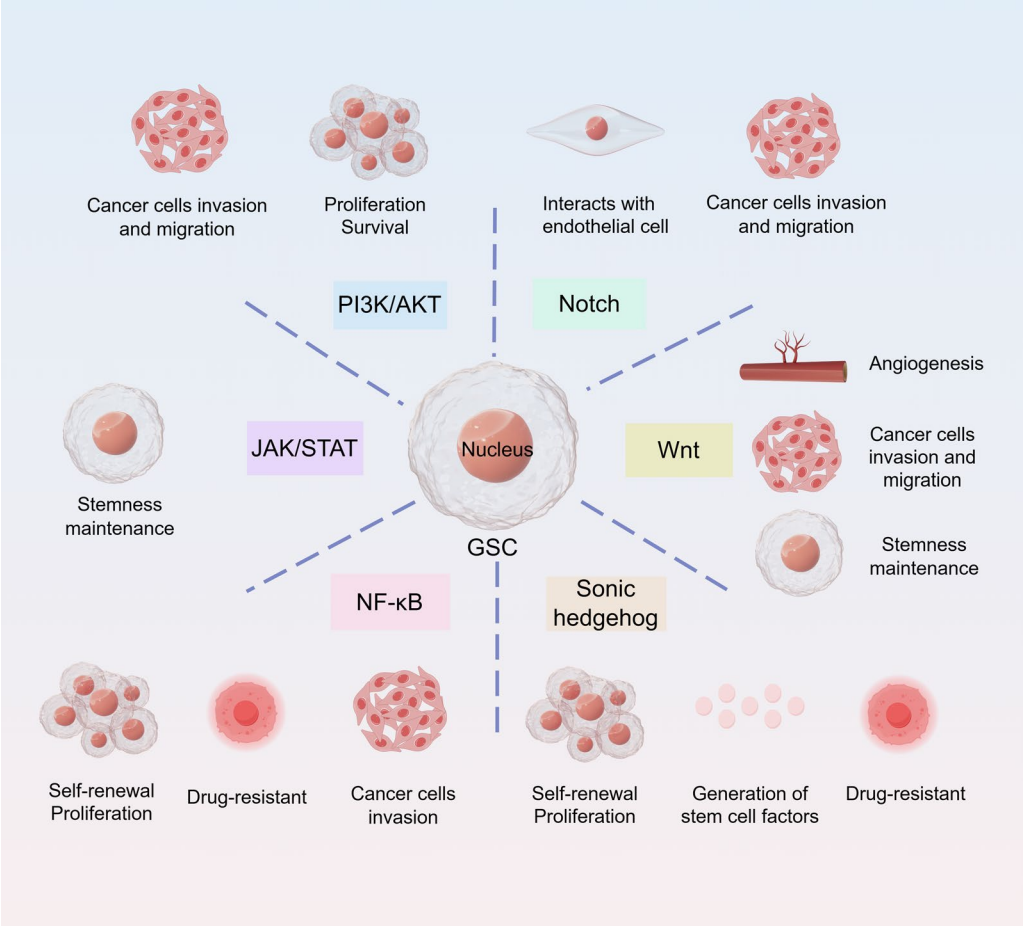

Fig. 2 Different signaling pathways and their related functions in GSC. The Notch signaling pathway mediates interactions between GSC and endothelial cells, thereby promoting cancer cell invasion/migration. The Wnt pathway regulates angiogenesis, cancer cell invasion/migration, and the maintenance of stemness in GSC. The Sonic hedgehog pathway drives GSC self-renewal, proliferation, and drug resistance, while also inducing the secretion of stem cell factors. The NF-κB pathway sustains GSC self-renewal and proliferation, enhances drug resistance, and increases invasive capacity. The JAK/STAT pathway plays a crucial role in the regulation of GSC stemness maintenance. The PI3K/AKT pathway facilitates cancer cell invasion/migration and supports stem cell proliferation and survival (By Figdraw)

barrier that restricts the delivery of drugs to the central nervous system, poses significant challenges to targeted therapy for GSCs due to its unique structure. Studies have demonstrated that more than 98% of small-molecule drugs and virtually all large-molecule therapeutic agents are unable to effectively penetrate the BBB [105]. In recent years, emerging technologies such as nanocarrier systems designed for BBB permeability, focused ultrasound-mediated modulation of barrier permeability, and immune cell-mediated targeted delivery systems have offered novel solutions to overcome this barrier. Consequently, this review emphasizes therapeutic approaches related to immunotherapy and metabolism while exploring nascent BBB penetration technologies. This integrated perspective provides a theoretical framework for developing multimodal treatment strategies.

Targeted BBB

The BBB, serving as the core protective mechanism of the central nervous system, comprises tightly connected endothelial cells, a basement membrane, and a pericyte complex. While effectively blocking pathogens, this complex also poses a significant limitation on the delivery efficiency of drugs into the brain. Targeted therapy against GSCs in GBM faces additional challenges due to the low permeability in hypoxic regions of the tumor core. Currently, innovative strategies to breach the BBB primarily focus on three major directions:

4.1.1. Physical intervention techniques temporarily open barriers in a controlled manner. Focused ultrasound combined with microbubbles (FUS-MB) technology leverages the ultrasonic cavitation effect to enhance the permeability of the BBB. Clinical trials have demonstrated that this approach can increase the

intracranial concentration of doxorubicin by 4.7 times [106, 107], and adjustments to ultrasound parameters and microbubble dosages can avoid brain tissue damage [108, 109]. Additionally, magnetically guided iron oxide nanoparticles can traverse the intact BBB through external magnetic field gradients, enabling effective drug delivery within the brain and significantly enhancing drug accumulation in the target region [110].

4.1.2. Biomimetic engineering strategies leverage natural cell penetration mechanisms to overcome barriers. PLGA nanoparticles coated with macrophage membranes exploit the homing properties of immune cells to enhance targeting efficiency by 12-fold [111]. When combined with genetic engineering modifications, these nanoparticles can extend their residence time in vivo and enhance their therapeutic efficacy [112]. Exosomes traverse BBB models through heparin sulfate proteoglycan-mediated transport mechanisms [113], while novel cationic lipid nanoparticles (LNPs), through optimized physicochemical properties, have successfully achieved effective delivery and gene silencing of siRNA in mouse glioblastoma, thereby activating T-cell-dependent antitumor immune responses [114].

4.1.3. Intelligent nanocarriers achieve precise drug release by responding to the tumor microenvironment. pH-sensitive polyethylene glycol-polylactic acid copolymers trigger drug release in acidic microenvironments [115], and by incorporating RGD peptides, they achieve dual targeting of both blood vessels and tumors [116]. Biomimetic liposomes mimic the structure of low-density lipoprotein (LDL) and enhance paclitaxel delivery efficiency through LDL receptor-mediated endocytosis [117].

Despite significant advancements, this field still faces challenges such as balancing delivery efficiency with safety, ensuring stability in large-scale production of carriers, and addressing receptor fluctuations due to tumor heterogeneity [118–120]. In response, cutting-edge research is shifting towards multimodal synergistic strategies, such as combining FUS-MB with engineered exosomes to simultaneously achieve BBB opening and active targeting [121], or utilizing artificial intelligence models to optimize drug selection and design by predicting drug permeability [122]. These interdisciplinary innovations are propelling neuro-oncological treatment towards an era of precise regulation.

Targeted immunotherapy

Immunotherapy, in particular, represents an innovative strategy in cancer treatment, attracting considerable attention due to its potential to induce durable responses in patients with various cancer types. Unlike conventional cancer therapies, which often rely on non-specific interventions such as surgery, radiation, and chemotherapy, immunotherapy leverages the specificity of the immune system to selectively target and eradicate cancer cells. Immunotherapy targeting GSCs primarily involves three principal strategies: tumor vaccines designed to elicit immune responses against antigens present on the surface of GSCs; CAR-T cell therapy, which entails the modification of T cells to specifically target and eradicate GSCs; and immune checkpoint inhibitors that obstruct tumor immune evasion mechanisms, thereby restoring T cell functionality [41]. NK cells have attracted considerable interest due to their potent cytotoxic capabilities and adaptability within the TME. Clinical trials have been conducted using autologous and IL-2-activated NK cells, while allogeneic NK cells or antibodies targeting NK cell inhibitory receptors serve to impede GBM cells from recognizing their own MHC class I molecules [123]. Notably, NK cells engineered with CARs and directed against HER2 have demonstrated the ability to eliminate glioma cells and neurospheres [124]. Additionally, research has shown that NK cells can be educated or primed to enhance their cytotoxic efficacy against glioma cells. For example, NK cell-derived exosomes, when applied to NK cells, have been found to enhance their antitumor activity, resulting in more effective targeting and destruction of tumor cells (removes 86% of tumours in 4 weeks) [125]. Concurrently, CAR-T cell therapy has emerged as a promising strategy for targeting GSCs. A primary approach in utilizing CAR-T cells against GSCs involves the identification and targeting of specific antigens that are overexpressed in GSCs but absent in normal cells. For example, the epidermal growth factor receptor variant III (EGFRvIII) serves as a tumor-specific antigen expressed in a subset of gliomas, including GSCs. CAR-T cells engineered to target EGFRvIII have demonstrated the capacity to recognize and eradicate GSCs, thereby reducing tumor growth and enhancing survival in preclinical models [126, 127]. In addition to EGFRvIII, other antigens such as IL-13 receptor alpha 2 (IL13Rα2) have been identified as viable targets for CAR-T cell therapy against GSCs. Research has shown that CAR-T cells targeting IL13Rα2 can effectively eliminate GSCs both in vitro and in vivo, resulting in significant tumor regression without impacting normal brain tissue [128, 129]. Furthermore, the TME plays a critical role in the efficacy of CAR-T cell therapy. The immunosuppressive characteristics of the TME in GBM can impede the activity of CAR-T cells. To address these challenges, strategies have been developed to enhance the functionality of CAR-T cells, such as incorporating additional features like a TGFβ-trap. This modification not only improves the anti-tumor

efficacy of CAR-T cells but also modulates the TME to foster a more pro-inflammatory and anti-tumorigenic milieu [130, 131]. Furthermore, HLA-G, a nonclassical HLA class I molecule, has been identified to form dimers that inhibit NK cell cytotoxicity through interactions with inhibitory receptors. This mechanism has been documented in various cancers, including gliomas, where the TME can promote the formation of HLA-G dimers, thereby shielding tumor cells from NK cell-mediated lysis [132]. Additionally, GSCs have been observed to express HLA-G, contributing to their resistance against NK cell-mediated cytotoxicity [133]. The interaction between killer cell KIRs on NK cells and HLA molecules on target cells is also crucial in modulating the immune response against GSCs. KIR/HLA interactions can inhibit NK cell activation, thus diminishing their capacity to mediate antibody-dependent cellular cytotoxicity (ADCC) against glioma cells. This inhibition exhibits selectivity and may vary contingent upon the specific therapeutic antibodies employed, as demonstrated in studies comparing the effects of rituximab and GA101 (obinutuzumab) [134]. Additionally, the expression of particular HLA alleles and their corresponding supertypes can modulate the immune response to GSCs. Certain HLA supertypes have been correlated with enhanced survival outcomes in patients receiving treatments such as hematopoietic cell transplantation [135], indicating that diversity in HLA presentation may augment immune responsiveness.

Targeted metabolic therapy

Considering the pivotal role of GSCs in metabolic regulation, strategies targeting metabolic pathways have emerged as promising approaches for the selective eradication of GSCs. One mechanism by which GSCs facilitate tumor progression involves the establishment of a positive feedback loop encompassing glycolysis, extracellular acidification, and immunosuppression. The glycolytic shift in GSCs results in lactate production, which is subsequently exported from the cells, leading to extracellular acidification. The acidic milieu within the TME exacerbates its immunosuppressive properties by inhibiting the function of CTLs and NK cells, while simultaneously facilitating the recruitment and polarization of immunosuppressive cell types, such as Tregs and TAMs [136, 137]. The interplay between GSCs and immune cells is pivotal in maintaining this feedback loop. GSCs are capable of secreting factors that modulate immune cell activity, thereby suppressing anti-tumor immune responses. For example, the secretion of transforming growth factor-beta (TGF-β) by GSCs can induce a metabolic shift in immune cells from oxidative phosphorylation to glycolysis, thereby augmenting the immunosuppressive environment [137]. Furthermore, TAMs, which are frequently polarized to the M2 phenotype within the TME, can release cytokines that promote glycolysis in GSCs, thereby perpetuating the feedback loop [138, 139]. Additionally, the acidic microenvironment resulting from increased glycolysis can activate acid-sensing ion channels on immune cells, thereby further facilitating tumor immune evasion. The intricate relationship between metabolic reprogramming and immune modulation underscores the complexity of the TME in GBM and highlights the challenges associated with developing effective therapies [140, 141]. Targeting this positive feedback loop offers a promising therapeutic strategy. By disrupting glycolysis or neutralizing the acidic environment, it may be feasible to restore immune cell function and enhance the efficacy of immunotherapies. For instance, inhibiting key glycolytic enzymes or employing buffering agents to counteract extracellular acidification could potentially disrupt the cycle of immunosuppression and improve patient outcomes [142, 143]. Additionally, regulating glucose metabolism in GSCs presents further opportunities for antitumor effects, as inhibiting GLUT1 can reduce glucose uptake by GSCs, thereby impairing their self-renewal capacity [144]. Hexokinase 2 (HK2), a critical enzyme that catalyzes the initial step of glycolysis, is frequently overexpressed in cancer cells, leading to increased glycolytic activity. This enzyme not only plays a crucial role in glucose metabolism but also facilitates cancer cell survival and proliferation by promoting the Warburg effect. Importantly, elevated HK2 expression was independently associated with poorer prognosis (Cox proportional hazards model, $P<0.006$) after adjusting for age [145–147]. Research has demonstrated that targeting HK2 can effectively suppress tumor growth. For example, the application of specific inhibitors such as Benitrobenrazide has been shown to impede cancer cell proliferation by directly targeting HK2, resulting in reduced glycolysis and increased apoptosis in cancer cells [148]. Similarly, xanthohumol, a natural compound, has exhibited anti-tumor effects on GBM by inhibiting glycolysis through the downregulation of HK2 expression. This effect was evidenced by a 50% reduction in tumor volume in murine models [148]. Comparable strategies can also be employed to disrupt fatty acid and glutamine metabolism in GSCs, further inhibiting their growth and survival. Nevertheless, given the heterogeneity of metabolic pathways and the adaptability of GSCs to metabolic stress, such therapies often necessitate combination with other treatment modalities to enhance efficacy in clinical applications. GSCs express high-affinity GLUT3, which confers a survival advantage in hypoglycemic microenvironments, thereby contributing to the maintenance of tumor hierarchy and

potentially bearing prognostic significance [149]. The promotion of pyrimidine synthesis not only sustains the nucleotide pool necessary for DNA and RNA synthesis but also fulfills the energy and metabolic demands of GSCs to adapt to diverse tumor microenvironmental conditions [150]. Consequently, targeting pyrimidine synthesis may disrupt the metabolic reliance of GSCs and enhance the therapeutic efficacy against GBM [44, 151, 152].

Conclusions and future perspectives

GSCs have emerged as pivotal drivers of glioma progression, orchestrating tumorigenesis through their self-renewal capabilities, invasive potential, and dynamic interactions with the TME. These cells maintain stemness via dysregulated signaling pathways, including IL-6/STAT3 and EMT, while establishing an immunosuppressive niche through exosomal PD-L1 secretion, Treg recruitment, and metabolic symbiosis. The "cold tumor" phenotype of GBM is reinforced by hypoxia-induced immunosuppression, endothelial-derived TGF-β/IL-10, and lactic acid-driven extracellular acidification, which collectively impair cytotoxic T/NK cell activity and promote immune evasion. GSCs further exploit glycolytic dominance to acidify the TME, activating acid-sensitive ion channels on immune cells and fostering a self-perpetuating cycle of immunosuppression. This cycle is amplified by TGF-β-mediated metabolic reprogramming of immune cells and reciprocal cytokine-enhanced glycolysis in GSCs, creating a feedforward loop that sustains tumor progression. Current therapeutic strategies aim to disrupt these interactions through dual targeting of metabolic and immune pathways. Inhibiting glycolysis (e.g., GLUT1/HK2 suppression), neutralizing acidic microenvironments, or combining IL-6/STAT3 blockade with immune checkpoint inhibitors demonstrates synergistic potential to restore antitumor immunity. Innovative approaches, such as bispecific antibodies co-targeting metabolic enzymes and immune checkpoints, oxidative phosphorylation inhibitors paired with PD-1/CTLA-4 blockade, and CAR-T therapies exploiting GSC glutamine addiction, highlight the promise of combinatorial regimens. Additionally, nanotechnology-driven delivery systems capable of bypassing the BBB and spatially controlled drug release are critical for enhancing therapeutic efficacy. Advances in spatial transcriptomics, CRISPR-based metabolic screens, and engineered immune cells (e.g., TGF-β-resistant CAR-T) will refine therapeutic precision. By integrating AI-driven predictive models to tailor therapies and address resistance, next-generation strategies may transform GBM into a manageable condition. Ultimately, dismantling the GSC-TME axis through metabolic-immune synergy represents a transformative frontier in glioma therapeutics, underscoring the need for translational innovation to bridge mechanistic insights with clinical realities.

Artificial intelligence

The authors declare that they have not use AI-generated work in this manuscript and all work are owned by the authors.

Abbreviations

GSCs	Glioma stem cells
CNS	Central nervous system
GBM	Glioblastoma
IL-6	Interleukin-6
TNF	Tumor necrosis factor
ITGA2	Integrin α2
EMT	Epithelial-mesenchymal transition
PrPC	Cellular prion protein
HOP	Hsp70/90 organizing protein
MSCs	Mesenchymal stem cells
TME	Tumor microenvironment
HIFs	Hypoxia-inducible factors
VEGF	Vascular endothelial growth factor
TCGA	The cancer genome atlas
H3K9me3	H3 lysine 9 trimethylation
ATP	Adenosine triphosphate
PPP	Pentose phosphate pathway
NADPH	Nicotinamide adenine dinucleotide phosphate
GLUT	Glucose transporter
2-HG	2-Hydroxyglutarate
α-KG	Alpha-ketoglutarate
BBB	Blood-brain barrier
CAR-T	Chimeric antigen receptor T
NK	Natural killer
KIR	Killer cell immunoglobulin-like receptor
TAMs	Tumor-associated macrophages
Tregs	Regulatory T cells
CTLs	Cytotoxic T lymphocytes
PARN	Poly (A)-specific ribonuclease
LNPs	Lipid nanoparticles
LDL	Low-density lipoprotein
CAR-T	Chimeric antigen receptor T
EGFRvIII	Epidermal growth factor receptor variant III
ADCC	Antibody-dependent cellular cytotoxicity
HK 2	Hexokinase 2

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JYH reviewed the literature and wrote the manuscript; XWY provided relevant literature and reviewed the manuscript; SSH proposed the framework of the article and reviewed the manuscript. All authors read and approved the final manuscript.

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