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Astrocyte transformation in glioblastoma: pathways supporting tumor aggressiveness

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Glioblastomas, the most common primary malignant brain tumors, are characterized by their aggressive nature and poor prognosis. One significant aspect of glioblastoma biology is the transformation of astrocytes into a reactive state that supports tumor progression. This transformation is mediated by various signaling factors secreted by tumor cells, including extracellular vesicles carrying non-coding RNAs and other bioactive molecules. This review explores the regulatory mechanisms and pathways linking glioblastoma and astrocytes and summarizes currently available *in vitro* models to study these interactions. Understanding the relationships between tumor cells and astrocytes is crucial for identifying novel therapeutic targets to combat this aggressive tumor.

Key words: glioblastoma, GBM, astrocytes, tumor microenvironment, astrogliosis, tumor-associated astrocytes

INTRODUCTION

Glioblastoma is the most prevalent and aggressive form of brain cancer. It accounts for 15% of all brain tumors and nearly half of all malignant brain tumors. The term "glioblastoma multiforme" is no longer used, but the abbreviation GBM remains widely accepted. Glioblastoma is classified as a grade IV astrocytoma under the WHO classification (Young et al., 2015; Ostrom et al., 2019). It can arise through two pathways: (I) the primary or *de novo* pathway, originating from astrocytes or precursor cells that acquire specific mutations; and (II) the secondary pathway, developing from lower-grade astrocytomas or oligodendrogliomas. According to the latest revised classification, GBMs are defined as "diffuse, astrocytic gliomas that are IDH-wildtype and H3-wildtype, exhibiting one or more of the following histological or genetic features: microvascular proliferation, necrosis, telomerase reverse transcriptase (*TERT*) promoter mutation, epidermal growth factor receptor (*EGFR*) gene amplification, or a concomitant gain of chromosome 7 and loss of chromosome 10 (+7/-10) (CNS WHO grade 4)" (Guo et al., 2023).

According to the latest WHO directives, GBM is now classified into histological subtypes (hist-GBM) and molecular subtypes (mol-GBM). Recent advancements in molecular methods have enabled further categorization of GBM into subtypes with isocitrate dehydrogenase (IDH) mutations and IDH-wild type subtypes. It has been observed that IDH-wild type diffuse gliomas are associated with a poorer survival prognosis, even if the histological features are less advanced. Therefore, even if a tumor exhibits histological characteristics that classify it as grade 2 or 3, the presence of specific molecular features can lead to its classification as a mol-GBM WHO grade 4 (Guo et al., 2023).

Currently, there is no effective cure for glioblastoma. The median survival time for patients after standard treatment is approximately 14 months, with a five-year survival rate of about 5% (Guo et al., 2023). Early nonspecific symptoms include headaches, dizziness, nausea, lethargy, seizures, hemiparesis, visual loss, stroke-like symptoms, memory problems, or personality changes (Young et al., 2015). Complete resection of glioma is impossible because of its infiltrative nature. Low-grade tumors also tend to transform into

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malignant glioma (Jaeckle et al., 2011). Achieving therapeutic drug concentrations in the brain is challenging due to the blood-brain barrier, and these concentrations can be toxic to healthy tissue. After tumor resection, chemotherapy and radiation therapy are typically started, with combined radiotherapy and temozolomide (TMZ) improving patient survival (Yalamarty et al., 2023). Bevacizumab (Avastin®) is also an FDA-approved treatment for recurrent glioblastoma (Diaz et al., 2017).

The interaction between GBM and its microenvironment is crucial for tumor development. GBM tumors show significant heterogeneity in the diversity of nerve cells and other associated cells, including microglia, astrocytes, pericytes, and endothelial cells (Schiffer et al., 2018). The presence of these cells in the tumor microenvironment (TME) and the tumor's ability to interact with the extracellular matrix (ECM) are essential for its survival (Ngo et al., 2022). Components of the ECM, such as hyaluronic acid, proteoglycans, and fibronectin, are overexpressed in the tumor microenvironment, enhancing the adhesion and migration capacities of tumor cells in various types of cancer (Kim & Kumar, 2014; Misra et al., 2015; Najjary et al., 2025). Integrative spatial analyses of human GBM samples revealed a region-specific organization of the ECM (De et al., 2025). Immunohistochemical studies further demonstrated that ECM components, including lumican, are enriched in perivascular niches, suggesting active ECM involvement in glioma progression and tumor-stroma interactions (Salinas et al., 2024). These studies also revealed ECM-mediated communication networks between tumor and stromal cells, particularly in aggressive niches such as microvascular proliferations, highlighting the ECM as a critical regulator of tumor structure and progression (De et al., 2025).

An important feature of GBM that plays a central role in shaping the tumor microenvironment is hypoxia. Hypoxic gradients contribute to the spatial organization of the tumor, promote aggressive mesenchymal-like hypoxic cell states, and are closely linked to necrosis and reduced T-cell infiltration (Greenwald et al., 2024). Hypoxia also drives angiogenesis, a process essential for tumor growth. Tumor cells secrete proangiogenic factors such as vascular endothelial growth factor-A (VEGF-A), which promote the formation of new blood vessels by stimulating endothelial cell proliferation, thereby ensuring the supply of oxygen and nutrients (Treps et al., 2017). Additionally, tumor cells and cells within the tumor microenvironment release mediators that sustain the tumor's self-renewing properties, such as transforming growth factor-β (TGF-β), fibroblast growth factor 2 (FGF-2) and epidermal growth factor (EGF) (Li et al., 2009; Fessler et al., 2015; Liu et al., 2021). Microglial cells also contribute to glioma invasion by secreting TGF-β and other glioma-promoting factors (Wesolowska et al., 2008; Hambardzumyan et al., 2016; Huang et al., 2024). These supportive roles of endothelial and microglial cells in tumor development are well established. Growing evidence also highlights the pivotal contribution of tumor-associated astrocytes to glioma growth and progression through diverse mechanisms of interaction with tumor cells.

Astrocyte activities in healthy and diseased brain

In the healthy brain, astrocytes, also known as astroglia, participate in synaptic plasticity, supply nutrients to neurons, and maintain ionic balance (Ortega et al., 2025). They are also a key component of the blood-brain barrier (BBB), which acts as a physical and metabolic barrier separating the interstitial fluid of the brain parenchyma from the blood flow (Manu et al., 2023). Astrocytic end-feet adhere tightly to each other and to the external surfaces of the vascular system, forming a compact structure that ensures BBB integrity. Astrocytes interact with pericytes and endothelial cells to facilitate the selective transport of substances across the barrier (Zhao et al., 2015; Heithoff et al., 2021).

Astrocytes respond to the central nervous system (CNS) injuries by a series of molecular and functional changes, transforming into an active form capable of repairing damaged tissue. This process is termed astrogliosis (Ortega et al., 2025). Overexpression of glial fibrillary acidic protein (GFAP) and other elements, such as nestin, transcription factors (e.g., STAT-3), growth factors (e.g., brain-derived neurotrophic factor [BDNF]), inflammatory cytokines (e.g., C-C motif chemokine ligand 2 [CCL2], interleukin 6 [IL-6]) and cell adhesion proteins (e.g., CD44), accompanies this process (Carrillo-de Sauvage et al., 2012; Chen et al., 2016; Teh et al., 2017; Priego et al., 2018; Xue et al., 2022; Al-Dalahmah et al., 2024; Manrique-Castano et al., 2024; Abou-El-Hassan et al., 2025).

Astrogliosis can have either positive or negative effects, depending on its intensity and context. Active astrocytes acquire the ability to proliferate, become hypertrophic, and accumulate around the site of injury (Wu et al., 2025). Studies in mouse models have shown that reactive astrocytes support axon regeneration in conditions including brain ischemia, primarily through the release of growth-promoting factors such as glial cell line-derived neurotrophic factor (GDNF) (Zhang et al., 2025). However, in certain pathological contexts - for instance, neuroinflammatory diseases - reactive astrocytes may adopt neurotoxic phenotypes and con-

tribute to axonal loss, highlighting their dual and context-dependent role in CNS injury and disease (Liddelow et al., 2017). The traditional A1/A2 framework classifies reactive astrocytes into two main categories: A1 astrocytes, which exhibit neurotoxic and pro-inflammatory properties, and A2 astrocytes, which are neuroprotective and anti-inflammatory. Increasing evidence shows, however, that this binary division oversimplifies the broad spectrum of astrocyte states observed across different injuries and diseases. Even so, the A1/A2 model remains a practical tool for describing and comparing astrocyte responses in diverse pathological contexts (Fan & Huo, 2021).

Advanced *in vitro* models to study GBM-astrocyte interaction

Astrocyte interactions with GBM can be studied *in vitro* using two-dimensional (2D) experimental setups, including co-culture systems or by treating cultured astrocytes with glioma-conditioned medium. However, to better understand how astrocytes respond to glioblastoma and interact with other cells, such as tumor-associated microglia, and the ECM, several more advanced three-dimensional (3D) models have been developed, including spheroids, organoids, organ-on-chip systems, and bioprinted constructs. These models more accurately replicate the physiological and pathological features of GBM compared with traditional 2D cultures, preserving cellular heterogeneity, chemical gradients, and ECM properties that are critical for tumor invasiveness and therapy resistance (Yadav & Purow, 2024).

Multicellular spheroids remain the most widely used 3D GBM models because they are relatively simple and reproducible (Bruns et al., 2023), while also recapitulating hypoxic gradients and dense cellular architecture (Del Rocío Aguilera-Marquez et al., 2025). From a translational perspective, spheroids are primarily used as cost-effective, scalable tools for initial drug cytotoxicity screening (Sabetta et al., 2023; Heinrich et al., 2024). However, their lack of fully organized ECM structure, vascular architecture, and complex biomechanical cues limits their predictive value for clinical outcomes compared with more advanced systems, such as hydrogel-based cultures or bioprinting models (Hill et al., 2021; Tung et al., 2024; Yadav & Purow, 2024).

Hydrogel systems offer greater control over ECM composition, stiffness, and biochemical signaling (Bruns et al., 2023). Biomaterials such as hyaluronic acid (HA) (Civita et al., 2019; Mier et al., 2025), polyethylene glycol (PEG) (Bruns et al., 2023), alginate (Del Rocío Aguilera-Marquez et al., 2025), and gelatin (Neufeld et al., 2021; Del Rocío Aguilera-Marquez et al., 2025) have been used to mimic the unique mechanical properties and composition of brain tissue. Another key advantage of hydrogel systems is the ability to tune matrix stiffness and HA molecular weight. In Andrade's study an *in vitro* blood–brain tumor barrier (BBTB) model based on a transwell system was used, with vascular endothelial cells seeded in the upper chamber, pericytes and astrocytes stabilizing the barrier on the lower side of the membrane, and a hydrogel containing glioblastoma spheroids placed beneath the insert. Low-molecular-weight HA promoted GBM invasion and increased BBTB permeability, while high-molecular-weight HA was associated with reduced invasiveness (Andrade et al., 2026). These observations highlight the critical role of ECM biomechanics in GBM progression. However, hydrogel systems are largely static models that lack perfusion, functional vascular networks, and dynamic mechanical forces such as shear stress or interstitial fluid flow. Despite these limitations, they still significantly improve physiological relevance compared to 2D cultures (Yadav & Purow, 2024).

Another notable example of available models is the Neuroimmune-Oncology Microphysiological Analysis Platform (NEO-MAP). NEO-MAP is a physiologically relevant organ-on-chip model that closely mimics the GBM microenvironment. This three-dimensional system reproduces the spatial organization of glial and tumor cells, with reactive astrocytes forming scar-like structures at the tumor boundary and microglia localized within the tumor core. Studies using NEO-MAP have shown that inhibition of the mTORC2 pathway in GBM induces A1-type astrocyte activation and promotes microglial polarization from a pro-tumoral to an anti-tumoral state, thereby enhancing tumor sensitivity to chemotherapy. Overall, NEO-MAP offers a platform for investigating tumor–glia crosstalk and evaluating potential therapeutic strategies under physiologically relevant conditions (Diep et al., 2025).

Among currently available technologies, 3D bioprinting is the most advanced strategy for reconstructing the spatial and cellular complexity of the GBM microenvironment. Bioprinting allows precise deposition of tumor cells, astrocytes, endothelial cells, pericytes, microglia, and ECM-mimicking biomaterials into architecturally defined structures with high reproducibility. These systems are especially useful for modeling tumor heterogeneity, vascular niches, and diffusion gradients that are difficult to reproduce in conventional cultures (Tung et al., 2024; Branco et al., 2025). Bioprinted GBM models can accurately recapitulate gradients of oxygen, nutrients, and metabolites, resulting in hypoxic and necrotic regions similar to those observed *in vivo* (Neufeld et al., 2021; Branco et al., 2025). Perfusable vascular channels created using bioinks enable the re-

construction of blood vessel-like networks lined with endothelial cells and pericytes, facilitating studies of angiogenesis, vascular permeability, and drug delivery across the BBTB (Neufeld et al., 2021). Bioprinting also provides unique opportunities to investigate the role of mechanical heterogeneity in GBM progression. Digital light processing (DLP)-based printing technologies can generate regions with distinct stiffness, replicating the heterogeneous ECM remodeling observed in patient tumors. A stiffer microenvironment promotes mesenchymal transition, invasiveness, and TMZ resistance in GBM cells, while a softer ECM favors more classical phenotypes (Tang et al., 2020; 2021). From a translational perspective, 3D bioprinting systems offer the highest clinical relevance among *in vitro* models, as they most closely recapitulate patient tumor transcriptomic signatures and therapeutic responses, and are compatible with patient-specific studies and CRISPR-based functional genomic screening (Tang et al., 2020). However, this technology involves high costs, significant technical complexity, the need for specialized equipment, and a lack of fully standardized protocols (Andreatta et al., 2020). Key properties of available 3D models for studying glioma are summarized in Table 1.

Transformation of Astrocytes in GBM

In the presence of GBM, astrocytes are transformed into tumor-associated astrocytes (TAAs, Fig. 1). The expression profile of TAAs differs significantly from that of normal astrocytes. TAAs predominantly express components of the antigen presentation pathway, such as MHC class II, which are important for T cell activation and the induction of local inflammation (Katz et al., 2012). However, this immune activation does not result in effective tumor elimination; instead, TAAs support tumor growth and confer resistance to therapy (Segura-Collar et al., 2023). Thus, TAAs are a valuable source of biomarkers associated with glioblastoma progression and potential therapeutic targets for future GBM treatments (Brandao et al., 2019; Zhang et al., 2020).

Tumor progression leads to the destruction of healthy tissue, necessitating astrogliosis for repair. However, the resultant activity of TAAs supports tumor growth. While the mechanisms behind astrocyte activation in GBM remain incompletely understood, several pathways have been proposed to play a role (Fig. 1). GBM secretes the receptor activator of NF-κB ligand (RANKL, also known as tumor necrosis factor ligand superfamily member 11, TNFSF11), which activates the NF-κB pathway through receptors on the astrocyte surface. This process leads to the formation of TAAs, capable of producing factors such as TGF-β, which promote tumor growth (Kim et al., 2014). Additionally, fibulin-3 released by glioblastoma cells promotes astrocyte activation by inducing NF-κB signaling, particularly at the tumor border (Nandhu et al., 2017).

GBM cells secrete extracellular vesicles (EVs), which are membranous nanoparticles containing proteins, lipids, and nucleic acids, that enable intercellular communication. GBM-derived EVs create an environment that stimulates astrocyte growth and induce their reprogramming into a tumor-supportive phenotype (Oushy et al., 2018; Wang et al., 2024). This communication is bidirectional and leads to extensive remodeling of the tumor microenvironment. Notably, EV composition is tumor- and cell line-specific, resulting in distinct microenvironmental effects. As shown by Wang et al. (2024), EVs from the F3-8 line induced antiviral responses in astrocytes, whereas those from G17-1 promoted ECM remodeling. GBM-EVs, particularly those derived from glioma stem cells (GSCs), drive astrocyte transformation by inducing morphological and functional changes, including podosome formation and enhanced proteolytic activity, which increase ECM remodeling capacity.

Table 1. Key properties of 3D models to study glioma-astrocyte interactions.

Model	Advantages	Limitations	TAA / astrocyte-targeting relevance
Spheroids	• Simple, low-cost, scalable • Suitable for high-throughput screening • Mimic basic tumor gradients and structure	• Limited ECM and vascular structure • Simplified microenvironment	• Screening of astrocyte-driven effects • Basic TME interaction studies
Hydrogel systems	• Controlled ECM composition and stiffness • Better mimic of brain-like environment • Improved cell-cell interaction modeling	• Static systems • No true perfusion or vasculature	• Studying astrocyte-ECM interactions • Testing TME-driven resistance mechanisms
3D bioprinting	• Highest structural complexity • Multicellular organization • Vascular-like and spatially defined models	• High cost and technical demand • Lack of full standardization	• Advanced astrocyte-targeted therapies in full TME context • Most clinically relevant platform

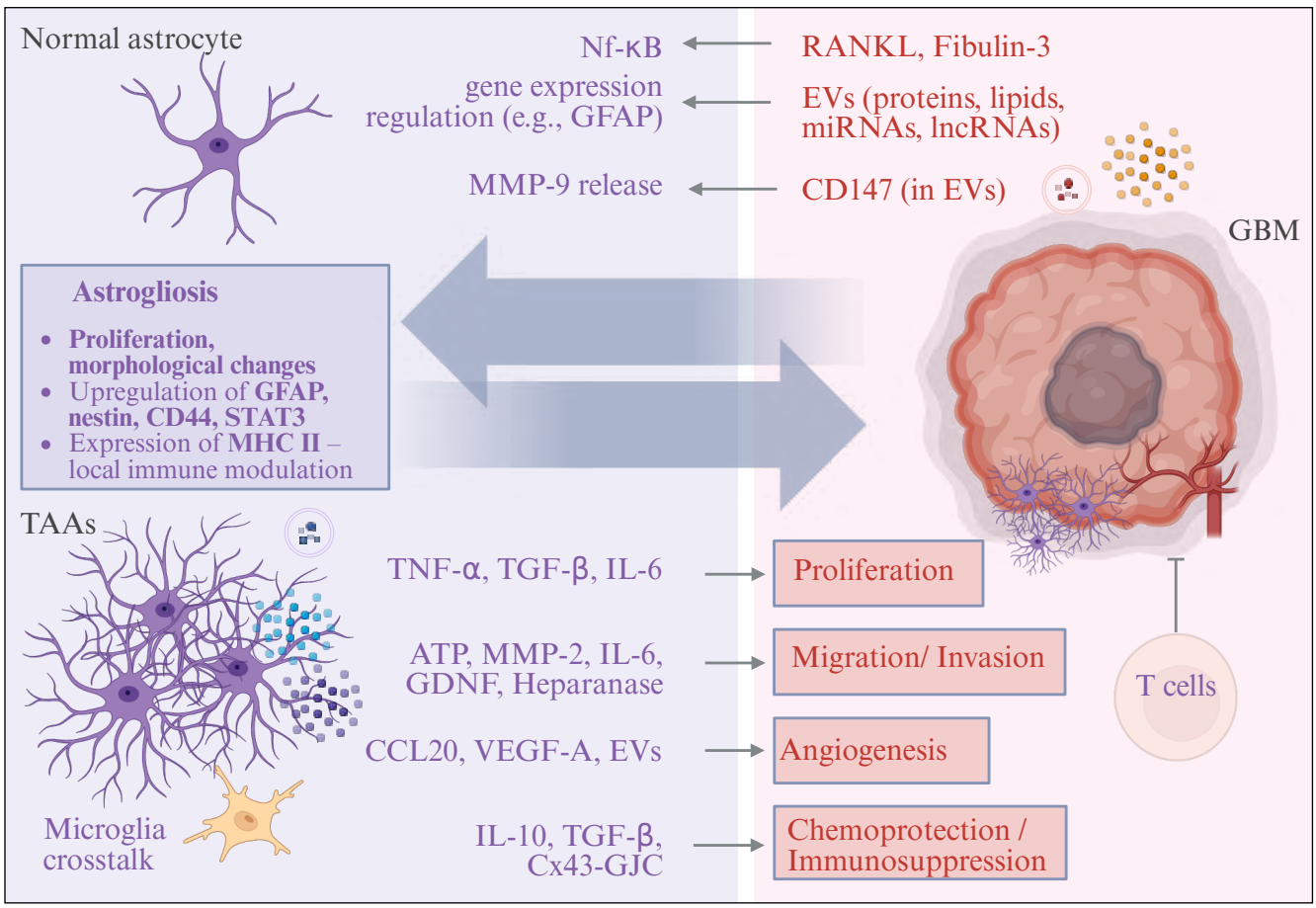

Fig. 1. Interaction between astrocytes and glioblastoma cells. Glioblastoma (GBM) cells secrete various factors – including RANKL, fibulin-3, and extracellular vesicles (EVs) containing CD147, miRNAs, and lncRNAs, which induce reactive changes in astrocytes through signaling pathways such as NF-κB. These changes result in astrogliosis, characterized by upregulation of GFAP, CD44, STAT3, and MHC-II expression. The resulting tumor-associated astrocytes (TAAs) secrete multiple pro-tumorigenic factors (e.g., TGF-β, IL-6, VEGF-A), promoting glioma cell proliferation, invasion, survival, and immune evasion. This interaction creates a supportive tumor microenvironment and facilitates glioblastoma progression.

They also modulate key signaling pathways, including suppression of TP53 and activation of MYC, promoting a pro-tumorigenic state (Hallal et al., 2019).

It was also shown that CD147 contained in glioma-derived EVs enhances the release of matrix metalloproteinase-9 (MMP-9) from astrocytes by potentiating intracellular signaling pathways, primarily through JNK activation, leading to ECM degradation and increased tumor invasiveness. This effect was further amplified by ionizing radiation, which increased CD147 in EVs, suggesting a potential therapy-induced promotion of tumor progression (Colangelo & Azzam, 2020).

Moreover, GBM-EVs transport microRNAs (miRNAs) and long non-coding RNAs (lncRNAs), that reprogram astrocyte gene expression and function. For example, lncRNA-ATB present in GBM-derived EVs enhances GFAP expression in astrocytes by sequestering inhibitory miR-204-3p. This induces an astrocyte phenotype associated with increased motility and glioma invasion (Bian et al., 2018). Similarly, GBM influences astrocytes through lncRNAs such as HOXDeRNA (LINC01116), which acts as a transforming factor by epigenetically reprogramming transcriptional networks and driving their conversion (Deforzh et al., 2024). In addition, GSC-derived EVs deliver miR-3065-5p, which targets DLG2 and contributes to astrocyte reprogramming characterized by increased proliferation (Li et al., 2024). Long-term exposure to GBM-EVs further induces tumor-like features in astrocytes, including anchorage-independent growth in soft agar (Oushy et al., 2018).

Reactive astrocytes are consistently associated with gliomas and are found in both the peritumoral region and the tumor core. Outside the tumor, they display a regular distribution pattern, while within the tumor, they often represent a continuation of peritumoral gliosis and become trapped within the infiltrating mass. These astrocytes are frequently localized around blood vessels or form dense fibrous networks. They may also

accumulate in regions of high proliferative activity and surround areas of focal necrosis. In infiltrative zones, their processes are often displaced from blood vessels by invading tumor cells (Schiffer et al., 2018).

Different subpopulations of astrocytes in the GBM microenvironment

Astrocytes from different brain regions display diverse molecular, morphological, and functional properties (Farmer & Murai, 2017; John Lin et al., 2017). Similarly, TAAs also show remarkable heterogeneity, in both morphology and gene expression profiles, which is strongly influenced by their location within the GBM microenvironment (Fig. 2).

Katz et al. (2012) demonstrated that CD44 and tenascin-C are overexpressed in tumor-associated astrocytes. This overexpression was primarily confined to astrocytes in the tumor's perivascular niche, indicating distinct TAA populations within the GBM microenvironment. A recent study by Ghosh et al. (2025) demonstrated that astrocytes in the GBM microenvironment form several distinct subpopulations, closely dependent on their location relative to the tumor. Using spatial transcriptomics and a murine model of GBM, the authors found that astrocytes at the tumor border exhibit increased expression of *Gfap*, *Aldh1l1*, *Aqp4*, *Pdpn*, *Lcn2,* and *S1pr*. In this region, they also display a characteristic elongated, bipolar morphology and are densely packed, forming a glial scar-like structure that serves as a physical and functional barrier separating the tumor from healthy brain tissue. Tumor border-associated astrocytes uniquely express transglutaminase 1 (*Tgm1*), which is involved in scar stabilization. Importantly, these astrocytes retain expression of the glutamate transporter GLT-1 (*Slc1a2*), indicating partial preservation of homeostatic functions and glutamate buffering near still-viable neurons (Ghosh et al., 2025).

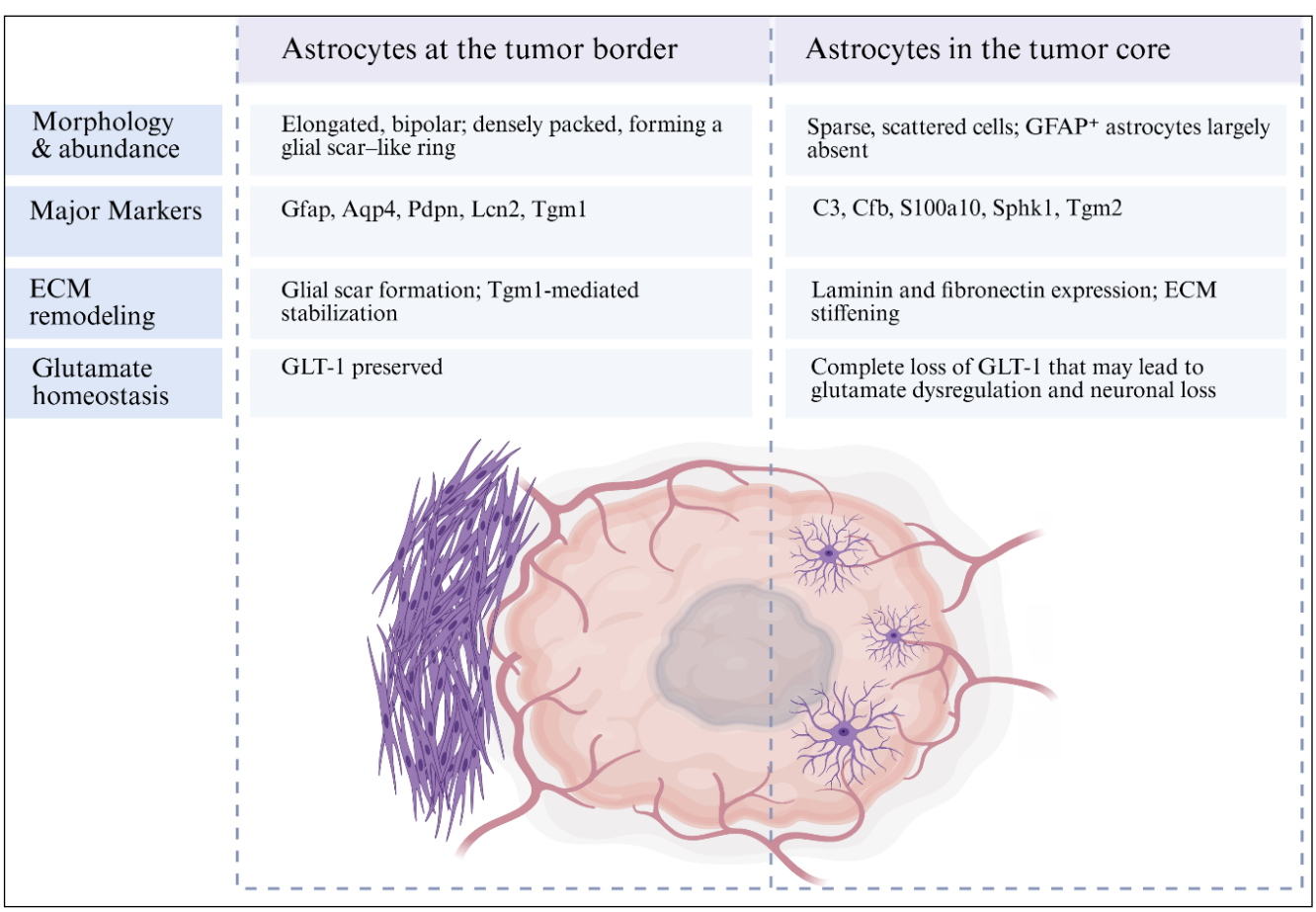

Fig. 2. Heterogeneity of tumor-associated astrocytes at the glioblastoma border and core. Astrocytes in glioblastoma show spatially defined heterogeneity. At the tumor border, astrocytes are elongated, densely packed, and form a glial scar–like structure, dominated by subtypes expressing *Gfap*, *Aldh1l1*, *Aqp4*, *Pdpn*, *Lcn2,* and *S1pr*, while retaining GLT-1–mediated glutamate homeostasis. In contrast, astrocytes within the tumor core are sparse, largely GFAP$^-$, express *C3*, *Cfb*, *S100a10*, *Sphk1*, *Tgm2*, and ECM proteins (laminin, fibronectin), and lose GLT-1 (Ghosh et al., 2025).

In contrast, astrocytes within the tumor core are less numerous. This compartment is enriched in neurotoxic and immunomodulatory astrocyte states, while classical GFAP$^+$ astrocytes are largely absent. Tumor core-associated astrocytes express inflammatory and neurotoxic markers such as *C3* and *Cfb*, as well as *S100a10* and *Sphk1*, and completely lose GLT-1, which likely leads to glutamate dysregulation and may contribute to neuronal loss (Sattler et al., 2013). Additionally, astrocytes in the tumor core exhibit high expression of transglutaminase 2 (*Tgm2*), along with ECM components such as laminin and fibronectin, which may contribute to ECM stiffening and promote tumor progression (Berg et al., 2021; Ghosh et al., 2025).

This study confirmed that TAAs do not form a homogeneous population but exhibit complex spatial, molecular, and functional heterogeneity closely linked to the tumor microenvironment. These findings also challenge the traditional A1/A2 astrocyte classification by revealing multiple context-dependent astrocyte states specifically shaped by GBM-associated cues and the spatial niche within the tumor.

Impact of TAAs on GBM cells

Given the significant differences in the morphology and expression patterns of astrocytes in the tumor core and tumor border, it is plausible that they also represent different activities and functional consequences for tumor growth.

In response to brain tissue damage, reactive astrocytes express factors such as tumor necrosis factor-α (TNF-α) (Abou-El-Hassan et al., 2025) and TGF-β (Henrik Heiland et al., 2019). Although many studies indicate that astrocytes support glioblastoma progression through the secretion of growth factors and cytokines, there is also evidence suggesting that astrocyte-derived signals can have opposite effects on glioma cells (Yao et al., 2014; Ribeiro et al., 2024). For example, Ribeiro et al. (2024) demonstrated in an *in vitro* model of GBM spheroids that factors released by reactive astrocytes inhibit spheroid growth. In contrast, increased GBM cell proliferation was observed after treating tumor cells with medium derived from astrocytes exposed to GBM-EVs, indicating that prolonged tumor–astrocyte interactions can reprogram astrocytes toward a tumor-supportive phenotype (Oushy et al., 2018).

Recent evidence indicates that TAAs support GBM growth and invasiveness by supplying tumor cells with high-energy metabolites that facilitate their survival within the infiltrative niche. As demonstrated using orthotopic glioblastoma models combined with spatial transcriptomics, astrocytes at the tumor leading edge exhibit increased ATP release. This ATP is subsequently captured and used by GBM cells through the activity of brain-type creatine kinase (CKB) (Yang et al., 2025). This mechanism enables GBM cells to replenish their metabolic reserves within the invasive zone, thereby promoting their migration and proliferation in regions characterized by limited nutrient availability.

TAAs influence not only tumor proliferation but also its ability to migrate and invade, which is primarily achieved by modulation and/or production of ECM-degrading enzymes. GBM invasion is typically observed near blood vessels and along the tumor edge (Schiffer et al., 2018). Astrocytes near the tumor mediate matrix metalloproteinase-2 (MMP-2) activation *via* the uPAR-plasmin cascade, allowing remodeling of the ECM and tumor expansion (Le et al., 2003). Reactive astrocytes also secrete IL-6, which has been shown to support GBM cell migration through induction of MMP expression (Li, 2010; Chen et al., 2016). Reactive astrocytes express heparanase in response to ischemic brain injury (Li et al., 2012), indicating their capacity to produce this enzyme under pathological conditions. Given that heparanase promotes ECM remodeling through degradation of heparan sulfate proteoglycans and is a key driver of glioblastoma growth and invasion (Kundu et al., 2016), the potential contribution of astrocyte-derived heparanase to GBM progression warrants further investigation. It was also shown that GDNF secreted by astrocytes promotes glioma cell migration *in vitro*, as well as tumor progression *in vivo*. The authors proposed that astrocyte-derived GDNF may act as a chemoattractant, enhancing GBM migration (Shabtay-Orbach et al., 2015).

TAAs also support tumor cell survival. In particular, TAAs facilitate GBM adaptation to hypoxic conditions. Astrocytes respond to both moderate and severe oxygen deprivation by undergoing reactive astrogliosis. This response is largely driven by HIF-2α, which in astrocytes shows stronger and more sustained stabilization than HIF-1α (Pantazopoulou et al., 2021). Under hypoxia, astrocytes increase secretion of chemokine C–C motif ligand 20 (CCL20), which binds to C–C chemokine receptor type 6 (CCR6) on GBM cells and activates HIF-1α signaling, supporting neoangiogenesis through increased VEGF production (Jin et al., 2018). Other hypoxia-associated factors released by astrocytes include VEGF-A, TGF-β1, and IL-1α, which contribute to angiogenesis and ECM remodeling (Loeffler et al., 2005; Pantazopoulou et al., 2021; Wu et al., 2025). In the long-term glioblastoma cerebral organoid (ltGLICO) model described by Nicholson et al. (2024), the lack of functional vascularization led to chronic hypoxia and oxidative stress, reshaping the tumor microenvironment. In this context, astrocytes act as key

partners of GSCs and, under hypoxic pressure, secrete protumorigenic ligands such as FGF-1. This factor directly promotes GSC expansion and survival, providing a mechanistic link between hypoxia, microenvironmental remodeling, and increased aggressiveness of GBM (Nicholson et al., 2024).

Another important mechanism supporting the invasion of tumor cells is the formation of functional connexin 43-mediated gap junctions (Cx43-GJs) between astrocytes and glioblastoma cells (McCutcheon & Spray, 2022; Mier et al., 2025). The invasiveness of GBM was reduced when tumor cells were placed in a Cx43-deficient astrocyte environment (Sin et al., 2016).

TAAs in chemoprotection and immunoprotection

Astrocytes may protect GBM from immune responses by secreting anti-inflammatory factors such as interleukin 10 (IL-10). TAAs actively shape the immunosuppressive tumor microenvironment, primarily through activation of the JAK/STAT3 pathway in astrocytes and crosstalk with microglia. This interaction between the two cell types in the presence of glioma promotes the release of cytokines such as TGF-β, IL-10, and G-CSF (Henrik Heiland et al., 2019). Consequently, pharmacological inhibition of the JAK/STAT pathway - for example, with ruxolitinib - has been proposed as a strategy to reprogram the immune landscape of GBM and enhance immunotherapy efficacy by converting "cold" tumors into "hot" ones (Henrik Heiland et al., 2019). Furthermore, TAAs contribute to the suppression of T cell responses (Faust Akl et al., 2025). Specifically, Faust Akl et al. (2025) showed that glioblastoma-derived IL-11 induces TRAIL expression in astrocytes *via* STAT3 signaling. TRAIL-positive astrocytes trigger apoptosis of tumor-specific T cells. Their presence correlated with shorter survival and faster recurrence, while TRAIL blockade with an oncolytic HSV-1 engineered to express a single-chain TRAIL-blocking antibody restored T cell activity and improved outcomes in preclinical models.

The above-mentioned Cx43-GJs between astrocytes and glioma cells are also important for protecting tumor cells against chemotherapy. Astrocytes in direct contact with glioma cells significantly reduce chemotherapy-induced apoptosis, particularly in response to agents such as temozolomide and vincristine, and this protective effect depends on functional Cx43-GJs. Specifically, astrocytes protect tumor cells from chemotherapy by sequestering intracellular calcium through gap junction channels, which helps prevent calcium overload and reduces apoptosis (Chen et al., 2015). Along these lines, other studies have shown that astrocytes establish tunneling nanotubes (TNTs) with GBM cells, enabling mitochondrial transfer that rescues tumor cells from chemotherapy-induced metabolic stress and apoptosis (Civita et al., 2019). Furthermore, in PEGDA (poly(ethylene glycol) dimethyl acrylate)-based systems, astrocytes promoted resistance to temozolomide and NF-κB inhibitors, likely through the formation of glial scar-like protective barriers (Pustchi et al., 2020).

Another astrocytic protein investigated in the context of GBM because of its association with inflammation and prognosis is leucine-rich α-2 glycoprotein 1 (LRG1). Astrocytic LRG1 expression correlates with increased peritumoral inflammation, $CD8^+$ T cell infiltration, and a higher incidence of pseudoprogression - MRI changes that resemble tumor growth but result from treatment-induced inflammatory responses. Consequently, LRG1 may represent a potential histopathological biomarker to help distinguish pseudoprogression from true tumor progression, although its elevated expression is also associated with shorter progression-free survival (Furuta et al., 2025).

TAAs can therefore support the proliferation, apoptosis resistance, and invasion of GBM cells (see Fig. 1 for a summary of these interactions). A better understanding of these mechanisms may provide new markers and therapeutic strategies for GBM.

CONCLUSIONS

Glioblastoma remains the most aggressive primary tumor of the central nervous system. The recurrent nature of the disease results from the highly infiltrative phenotype of tumor cells, their ability to penetrate functionally essential brain structures, a dampened immune response, and the limited permeability of the BBB. In this context, the tumor microenvironment is a critical driver of disease progression.

An increasing number of studies highlight the fundamental role of TAAs, which arise through the reprogramming of normal astrocytes by GBM-derived secreted factors such as RANKL, fibulin-3, and EVs carrying miRNA and lncRNA. TAAs form a functionally heterogeneous population that supports glioma cell survival and proliferation by releasing cytokines and growth factors (including TGF-β and IL-6), modulating the activity of proteolytic enzymes such as MMP-2, and supplying high-energy metabolites like ATP, which are particularly important for sustaining tumor cells within invasive niches.

Another key aspect is the contribution of TAAs to the establishment of an immunosuppressive microenvironment. The underlying mechanisms include secretion of IL-10 and TGF-β, modulation of T-cell

activity, and induction of T-cell apoptosis through TRAIL. TAAs may also promote chemoprotection of GBM cells, for example, by forming gap junctions with tumor cells.

Understanding the complex, bidirectional interactions between astrocytes and glioma cells is essential for identifying new therapeutic entry points within the tumor microenvironment. Strategies targeting these interactions are still lacking. Emerging experimental approaches that go beyond standard-of-care therapies include advanced cellular immunotherapies (e.g., multi-antigen CAR-T cells), oncolytic virus engineering (e.g., modified HSV-1 delivering pro-apoptotic molecules), and innovative drug-delivery systems such as convection-enhanced delivery (CED) or the use of progenitor cells as therapeutic carriers. In light of current knowledge, future therapeutic strategies for GBM should also focus on the precise modulation or elimination of functional TAAs subpopulations, as well as on disrupting their key signaling pathways (such as STAT3 signaling). Targeting the astrocytic microenvironment may substantially weaken the metabolic, immunological, and pro-invasive support that enables GBM cells to survive and progress, thereby paving the way for the development of more effective therapeutic interventions.

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