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Role of the Microenvironment in Glioma Pathogenesis

Maya Anjali Jayaram[1] and Joanna J. Phillips[1,2]

[1]Department of Neurological Surgery, Brain Tumor Center, University of California, San Francisco, California, USA; email: Joanna.phillips@ucsf.edu

[2]Division of Neuropathology, Department of Pathology, University of California, San Francisco, California, USA

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Abstract

Gliomas are a diverse group of primary central nervous system tumors that affect both children and adults. Recent studies have revealed a dynamic cross talk that occurs between glioma cells and components of their microenvironment, including neurons, astrocytes, immune cells, and the extracellular matrix. This cross talk regulates fundamental aspects of glioma development and growth. In this review, we discuss recent discoveries about the impact of these interactions on gliomas and highlight how tumor cells actively remodel their microenvironment to promote disease. These studies provide a better understanding of the interactions in the microenvironment that are important in gliomas, offer insight into the cross talk that occurs, and identify potential therapeutic vulnerabilities that can be utilized to improve clinical outcomes.

INTRODUCTION

Gliomas are a diverse group of primary central nervous system (CNS) tumors. Circumscribed, low-grade tumors such as pilocytic astrocytoma are the most common type of primary brain tumor in children and adolescents. In contrast, diffuse gliomas such as glioblastoma, isocitrate dehydrogenase–wild-type (IDH-wt), referred to as GBM, are the most common type of primary malignant brain tumor in adults. Recent updates to the World Health Organization CNS tumor classification now more prominently incorporate molecular and clinical features. Gliomas are considered to be circumscribed or diffuse, and diffuse gliomas that occur primarily in adults (adult-type) are distinguished from those that occur primarily in children (pediatric-type) (1). Tumors are then graded on the basis of molecular and histologic features. An important distinction is that while the pediatric-type, diffuse low-grade gliomas are typically associated with a more favorable outcome, the adult-type, diffuse gliomas frequently progress over time. As their name suggests, diffuse gliomas are characterized by infiltration within the adjacent brain parenchyma. In adult-type, diffuse gliomas and pediatric-type, high-grade gliomas this infiltration can be extensive (**Figure 1**). Characteristic patterns of infiltration in diffuse gliomas include perineuronal, perivascular, and along white matter tracts (2), suggesting important interactions with components of their microenvironment.

Diffuse gliomas in adults consist of three tumor types: glioblastoma, IDH-wt (GBM); astrocytoma, IDH-mutant; and oligodendroglioma, IDH-mutant and 1p/19q-codeleted. While GBM presents as a high-grade, malignant tumor, both astrocytoma, IDH-mutant, and oligodendroglioma, IDH-mutant and 1p/19q-codeleted, can present as lower-grade tumors but frequently evolve to more aggressive, high-grade tumors. In pediatric and young adult patients, diffuse high-grade gliomas include diffuse midline glioma, H3K27-altered; diffuse hemispheric glioma, H3G34-mutant; diffuse high-grade glioma, H3-wild-type and IDH-wt; and infant-type hemispheric glioma.

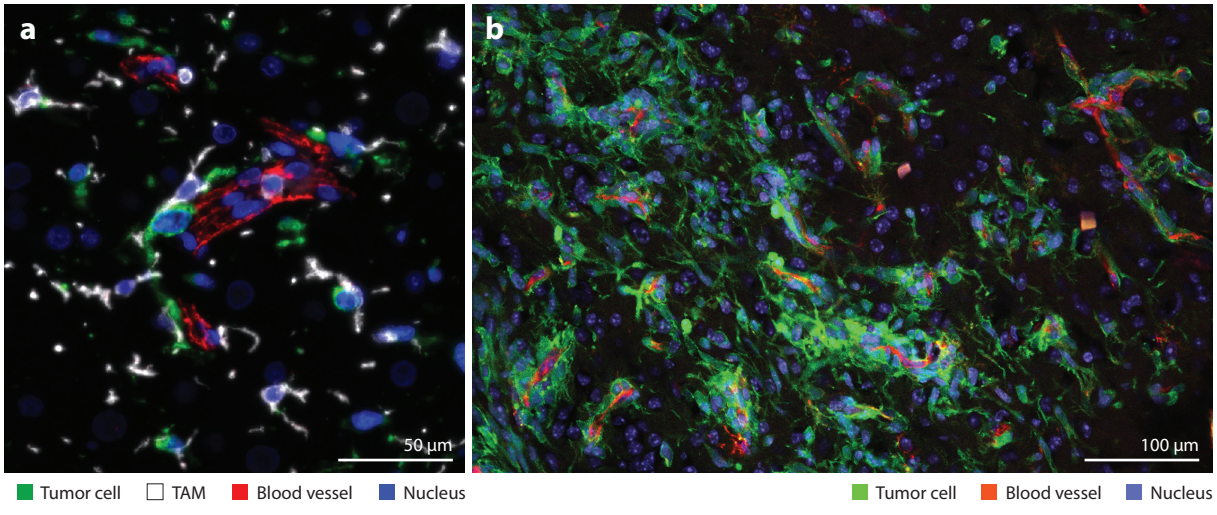

Figure 1

Diffuse glioma infiltration within the brain. (*a*) Representative image of an IDH-mutant astrocytoma demonstrating tumor cells (IDH1 R132H-mutant, *green*), TAMs (Iba1, *white*), blood vessels (CD31, *red*), and nuclei (*blue*). (*b*) Representative image from an immunocompetent murine model for GBM, IDH-wild-type, demonstrating infiltrating tumor cells (EGFR, *green*), lectin-stained blood vessels (*red*), and nuclei (*blue*). Abbreviations: EGFR, epidermal growth factor receptor; GBM, glioblastoma; IDH, isocitrate dehydrogenase; TAM, tumor-associated macrophage/microglia.

Thus, gliomas represent a heterogeneous group of tumors with distinct molecular features, biology, age of presentation, location, and outcome. Despite these differences, there are common themes (3). For several glioma types, transformation of an oligodendroglial precursor cell (OPC) or earlier neural precursor cell is the likely cell of origin (4–7). For example, in pediatric-type diffuse high-grade gliomas, precursor cells arrested within specific developmental windows, due to dysregulated development, are thought to be more permissive to transformation (5–7). In adult-type diffuse gliomas, a hierarchy of cellular states is thought to contribute to tumor heterogeneity and therapy resistance (4). While different mechanisms are at play, the recapitulation of developmental programs in gliomas may have implications for how glioma cells interact with components of their microenvironment. For example, in the healthy brain, bidirectional communication between neurons and glia regulates synapse formation and glial proliferation (8, 9).

At the intersection of the temporal, spatial, and molecular heterogeneity observed in gliomas is the tumor microenvironment (TME). Both cellular and acellular components of the microenvironment contribute to gliomagenesis, and alterations in the TME have been shown to promote glioma development, progression, invasion, and therapy resistance. The application of several new technologies, including single-cell sequencing and incorporation of mass cytometry, to the study of gliomas and their microenvironment has had a profound impact on our appreciation for the role of the TME in disease. Over the last few decades, substantial progress has been made in our understanding of glioma biology, intratumoral heterogeneity, tumor evolution, and therapeutic resistance mechanisms (3, 10). Yet, the median survival for a patient with GBM remains less than 2 years, and patients with lower-grade tumors can have significant morbidity (11–13). Understanding the mechanisms by which the TME contributes to gliomas is critical, as this knowledge can be used to identify tumor vulnerabilities and develop novel therapeutic targets.

In this review, we focus on three types of interactions that occur in the TME: glioma-neural, glioma-immune, and glioma-ECM (extracellular matrix). Using recent examples from the literature, we illustrate how cross talk between tumor cells and components of the TME impacts disease. While many of these examples come from the study of adult-type diffuse gliomas, we also include several important studies investigating pediatric-type H3K27-altered diffuse midline glioma and neurofibromatosis 1 (NF1)-associated low-grade glioma.

NEURAL-GLIOMA CROSS TALK

Dynamic cross talk between neurons and glia, including oligodendrocytes, oligodendrocyte precursor cells, and astrocytes, is critical during normal development and throughout the life span for normal brain function. At the synapse, astrocytes regulate synapse formation and function. In the absence of astrocytes, neurons in culture form very few synapses with decreased function (14). Using retinal ganglion cell cultures, several astrocyte-secreted factors and their role in glutamate-mediated synapses have been identified. For example, astrocyte-derived thrombospondins promote the formation of structural synapses (15, 16). Astrocyte-derived glypicans (GPCs), a family of glycosylphosphatidylinositol (GPI)-linked heparan sulfate proteoglycans (HSPGs) that can be released from the cell membrane, promote the formation of functional CNS synapses (8). Neuronal-glial communication is also critical for glial proliferation, differentiation, and function. Indeed, both paracrine signaling from electrically active neurons and direct electrochemical coupling via excitatory, glutamatergic synapses play a role (9, 17–19). In the cortex, activity-dependent synapses elicit a mitogenic response in neural progenitor cells and OPCs, promoting oligodendrogenesis and increased myelination (20). Glutamate receptors are

critical for excitatory neurotransmission and include the α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid receptor (AMPAR), important in fast neurotransmission, and the N-methyl-D-aspartate (NMDA) receptor, important in slow neurotransmission. In addition to their expression on the postsynaptic neuron at the synapse, AMPARs are expressed on many types of glia, including astrocytes, oligodendrocytes, and microglia (see 21). Given the interconnected nature of neurons and glia, it is perhaps not surprising that glioma cells can co-opt this communication. Recent studies, however, have elegantly tested the importance of these interactions on gliomagenesis. In the following section, we highlight several studies that demonstrate how bidirectional communication between non-neoplastic neural cells and gliomas promotes tumor development and growth (**Figure 2**). This is a rapidly developing field with many potential therapeutic implications.

Seizures can occur when there is an imbalance between excitatory and inhibitory signaling. Patients with diffuse gliomas are commonly afflicted by recurrent unprovoked seizures, and multiple factors likely contribute. Astrocytes play an important role in homeostasis, regulating extracellular ion concentrations and the levels of neurotransmitters such as glutamate (22). Peritumoral reactive astrocytes may have decreased homeostatic function with decreased potential for glutamate and potassium uptake (23). Tumor-derived factors can also contribute. In IDH-mutant diffuse glioma, the oncometabolite D-2-hydroxyglutarate (2-HG) released by tumor cells may mimic glutamate promoting excitatory signaling in adjacent neurons (24). In GBM, tumor cells release glutamate

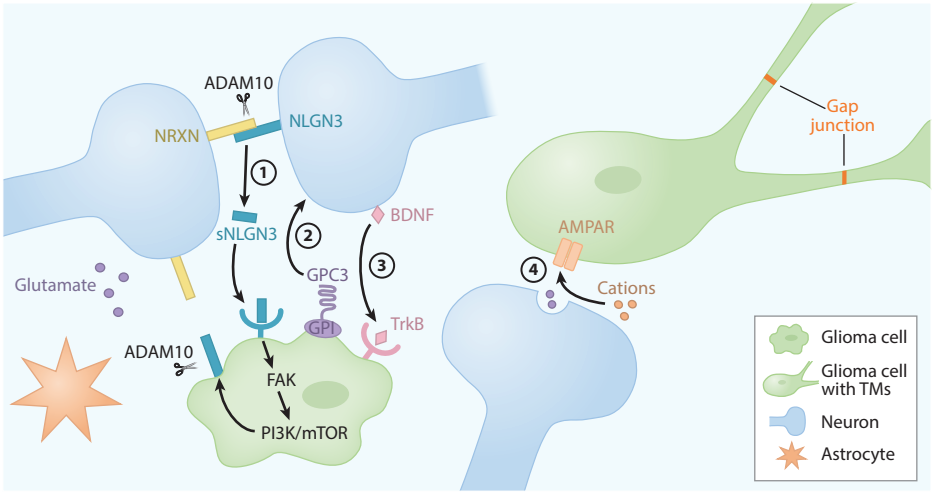

Figure 2

Cross talk in the tumor microenvironment between glioma cells, neurons, and non-neoplastic astrocytes contributes to glioma growth and invasion. (①) Neuronal activity-dependent release of NLGN3 promotes FAK and PI3K/mTOR signaling in gliomas, upregulating several synapse-related genes including *NLGN3*. (②) Release of GPI-anchored GPC3 from glioma cells contributes to neuronal hyperexcitability. (③) Neuronal activity-dependent release of factors such as BDNF that can promote glioma proliferation, survival, or migration. (④) Glutamate binding activates AMPAR, allowing an influx of cations driving membrane depolarization. Glioma membrane depolarization is associated with increased glioma proliferation and migration and increased formation of TM networks via gap junctions between tumor cells. Abbreviations: ADAM10, a disintegrin and metalloproteinase domain-containing protein 10; AMPAR, α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid receptor; BDNF, brain-derived neurotrophic factor; FAK, focal adhesion kinase; GPC3, glypican-3; GPI, glycosylphosphatidylinositol; mTOR, mammalian target of rapamycin; NLGN3, neuroligin-3; NRXN, neurexin; PI3K, phosphatidylinositol-3-kinase; TM, tumor microtube; TrkB, tyrosine receptor kinase B. Figure adapted from Reference 31 with permission from Elsevier.

into the extracellular environment (25), where GBM glutamate levels up to 100-fold higher than in normal brain have been reported (26). In 2001, Takano et al. (27) demonstrated that glutamate released from tumor cells could increase glioma growth in vivo in an NMDA-dependent manner, suggesting the possible involvement of neurons. One factor influencing glutamate release is the activity of the cystine/glutamate transporter (SLC7A11/xCT), which mediates uptake of extracellular cystine in exchange for glutamate. In a murine model for GBM, Buckingham et al. (28) demonstrated that pharmacologic inhibition of SLC7A11 function inhibited tumor cell release of glutamate and delayed tumor-associated epileptic activity. In human GBM, expression of the catalytic subunit of SLC7A11 is upregulated and associated with reduced overall survival (29). These studies helped establish the importance of extracellular glutamate levels in the TME. Today there is overwhelming evidence that bidirectional neuronal-glioma signaling can promote glioma growth via both paracrine factors and direct electrochemical communication. This cross talk may be an important and previously underappreciated factor promoting the human disease.

Paracrine Factors

Using optogenetics to drive neuronal activity in vivo, Venkatesh and colleagues (30, 31) demonstrated activity-dependent growth and proliferation of high-grade glioma orthotopic xenografts. Several secreted neuronal activity-dependent mitogens were identified, including brain-derived neurotrophic factor (BDNF) and neuroligin-3 (NLGN3), both of which could promote glioma proliferation (31). NLGN3, a synaptic protein and member of the neuroligin family of cell adhesion proteins, is expressed on both neurons and oligodendrocyte precursor cells (32). With neuronal activity, the sheddase ADAM10 (a disintegrin and metalloproteinase domain-containing protein 10) is released, cleaving NLGN3 from the cell surface and releasing it into the glioma microenvironment (30). In glioma cells, NLGN3 promotes focal adhesion kinase (FAK) phosphorylation and activation of downstream PI3K/mTOR (phosphatidylinositol-3-kinase/mammalian target of rapamycin) signaling driving cell proliferation (30, 31). The importance of activity-dependent release of Nlgn3 was demonstrated using an orthotopic xenograft model, as knockout of *Nlgn3* blocked glioma growth (30). Interestingly, activity-dependent release was decreased after conditional deletion of *Nlgn3* in neurons or OPCs, suggesting that both neuronal- and OPC-derived NLGN3 may contribute (30). Glioma cells exposed to Nlgn3 also upregulate several synapse-related genes including *NLGN3*. NLGN3, expressed on postsynaptic neurons, interacts with neurexin (NRXN), expressed on presynaptic neurons, to organize synaptogenesis. Thus, neuronal activity promotes a potential glioma-driven increase in neuronal activity in a feed-forward manner (30).

The potential importance of neuronal activity-dependent paracrine signaling on early glioma development is highlighted by two important studies that examined glioma growth in the context of normal sensory stimulation. NF1-associated optic nerve gliomas are low-grade gliomas that can cause significant morbidity. In a murine model for NF1-associated optic nerve glioma, neuronal activity induced by visual stimulation was sufficient to promote glioma growth. Strikingly, during a critical developmental window, both the initiation and the maintenance of tumors could be blocked by either decreasing visual stimulation or inhibiting Nlgn3 function (33). The potential importance of neuronal activity-dependent NLGN3 in the growth of a broad range of pediatric and adult glioma subtypes is supported by in vitro data demonstrating NLGN3-mediated growth in cultured cells from pediatric diffuse gliomas (H3K27-altered diffuse midline glioma and diffuse high-grade glioma, H3-wild-type and IDH-wt), adult diffuse gliomas (IDH-wt GBM, IDH-mutant oligodendroglioma), and Nf1 optic glioma (30, 33). Using an autochthonous mouse genetic model with conditional knockout of *Tp53* and *Nf1* in OPC, Chen et al. (34) modulated glioma development by altering olfactory neuron stimulation. In this case, olfaction-mediated

gliomagenesis was dependent on neuronal activity-dependent IGF-1 (insulin-like growth factor 1) signaling. Together, these data raise the intriguing possibility that inhibition of specific activity-dependent mitogens, potentially during critical developmental windows, may represent a novel therapeutic strategy for gliomas.

Direct Electrochemical Communication

It had been known that glutamate and glutamate activation of Ca^{2+}-permeable AMPARs on tumor cells facilitate glioma cell proliferation and migration (27, 35, 36). Yet, it was not clear whether direct electrochemical communication existed between neurons and gliomas. In 2019, two groups demonstrated bona fide, functional neuron-glioma synapses mediated by the AMPAR (37, 38). Using in vivo xenograft models for high-grade gliomas, neuronal activity-dependent tumor cell depolarization conferred increased glioma cell proliferation and promoted neurite-like protrusion formation and invasion (37, 38). Blocking neuron-glioma synaptic communication using an AMPAR inhibitor or genetically expressing a dominant-negative form of GluA2, a subunit of AMPAR, reduced tumor growth and prolonged mouse survival (37, 38). Neuronal activity can also induce nonsynaptic potassium-evoked currents in a subgroup of glioma cells (37–39). By establishing gap junctions through neurite-like protrusions termed tumor microtubes (TMs), glioma cells can form interconnected, functional networks (40, 41). Both direct neuron-glioma synapses and neuronal activity-dependent nonsynaptic potassium-evoked currents contribute to the generation of intercellular calcium waves identified in gap junction connected cells of the network (37–39). Disruption of these networks in murine xenograft glioma models suggests that they can promote glioma progression, invasion, and therapy resistance (40, 42) (for a review, see 41).

GBMs are highly heterogeneous tumors, and only subsets of glioma cells are electrochemically coupled with neurons or interconnected via gap junctions. Understanding the factors that drive this interconnected network and how it contributes to glioma growth and therapy resistance are major areas of research. Interestingly, glioma cells with an OPC-like transcriptional program are enriched for the expression of synapse-related genes, perhaps reflecting neuronal activity-dependent signaling in normal OPC development (37). In addition, certain molecular alterations in a tumor may promote neuronal activity. Alterations in PI3K/AKT/mTOR signaling are very common in IDH-wt GBM and IDH-mutant diffuse glioma (43, 44). In a functional screen of PIK3CA variants identified in human GBM, investigators identified subsets of driver variants that generated tumors with dysregulated synapse-associated gene expression (45). These tumors had increased hyperexcitability during early tumor formation, and tumor-bearing mice had increased seizure activity. In one PIK3CA variant, GPC3 (glypican-3), a member of the glypican family of HSPGs previously associated with synapse-organizing protein complexes, was upregulated. Deletion of GPC3 decreased early tumor hyperexcitability and prolonged overall survival (45). Spatial location within the tumor may also influence connectivity. Using in vivo imaging and single-cell transcriptional profiling, invasive tumor cells that lacked connectivity were enriched for neuronal, neural-progenitor cell, or nonmesenchymal expression signature as compared with TM-connected and less motile glioma cells (46). Consistent with this concept, spatial analyses suggest that glioma expression of AMPARs is highest at the invasive edge (46). While tumor cell populations likely utilize diverse strategies to invade and proliferate, bidirectional signaling with neurons to promote activity-regulated glioma growth is emerging as an important factor.

Astrocyte-Glioma Interactions

Non-neoplastic astrocytes in the TME can also communicate with glioma cells via gap junctions and other paracrine factors. In a murine glioma model, Cx43-mediated gap junctions between

non-neoplastic astrocytes and glioma cells promote tumor invasion (47, 48). As mentioned above, tumor-associated reactive astrocytes can contribute to extracellular glutamate levels due to reduced uptake (23). Reactive astrocytes can also promote an immunosuppressive microenvironment via the release of anti-inflammatory cytokines such as transforming growth factor beta (TGF-β), interleukin 10 (IL-10), and granulocyte colony-stimulating factor (G-CSF) (49). Astrocyte secretion of chemokines may also directly modify tumor cells. For example, CCL20 released from astrocytes can promote glioma adaptation to hypoxic stress by promoting hypoxia-inducible factor 1 alpha (HIF-1α) expression (50).

The examples cited above highlight the dynamic interactions that occur in the TME between glioma cells, neurons, and glia. In many of these examples, tumors recapitulate neurodevelopmental processes and interactions. Defining the impact of these interactions on patient outcomes and identifying potential therapeutic vulnerabilities are critical next steps. Advances in both intraoperative and noninvasive techniques to measure neuronal activity in patients, including electrocorticography and magnetoencephalography, will be crucial to understand how neuronal activity may impact patient outcomes (51). In this regard, intraoperative electrocorticography in patients with IDH-wt GBM demonstrated increased cortical excitability in the glioma-infiltrated brain (37). Moreover, IDH-wt GBM exhibiting increased functional connectivity within the infiltrated brain was associated with worse overall survival (52).

THE IMMUNE MICROENVIRONMENT

The composition of the immune infiltrate in diffuse gliomas is diverse and can include macrophages, microglia, myeloid-derived suppressor cells, lymphocytes (CD8 cytotoxic T cells, CD4 regulatory T cells, and B cells), natural killer (NK) cells, and neutrophils (**Figure 3**). The dominant population, however, is composed of resident microglia and bone marrow–derived macrophages, collectively referred to as tumor-associated macrophages/microglia (TAMs). Indeed, up to 30–50% of cells in gliomas may comprise TAMs (53). The immune composition and TAM population, however, are not uniform and vary across glioma molecular subtypes, temporally within the same tumor subtype, and with patient age (54–60). Elucidating the factors that regulate this diverse population and the mechanisms by which these non-neoplastic cells shape both glioma behavior and the overall immune microenvironment are critical areas of investigation. In the following section, we review some of the recent investigations in this area with a focus on the dynamic cross talk that occurs and its role in gliomagenesis.

TAM-Glioma Cross Talk

Heterotypic signaling between glioma cells and TAMs has emerged as an important factor in promoting an immunosuppressive TME and driving tumor progression. The importance of TAMs in gliomas is highlighted by studies in which orthotopic tumor development or invasion is abrogated or delayed by altering chemokine function, such as granulocyte-macrophage colony-stimulating factor (GM-CSF) and chemokine (C-C motif) ligand 5 (CCL5), or chemokine receptors, such as colony-stimulating factor-1 receptor (CSF-1R), CX3CR1, and CCR2, that direct myeloid cell migration or function (61–65).

In some contexts, glioma-microglia cross talk may be critical to promote very early tumor development. Patients with NF1 have inactivation of one copy of NF1 and are at risk of developing NF1-associated tumors, including optic nerve glioma, with biallelic inactivation of NF1. In a murine model for NF1-associated optic nerve glioma, glia cells including microglia are $Nf1^{+/-}$ (66). Compared with $Nf1^{+/+}$ microglia, $Nf1^{+/-}$ microglia exhibited a unique activation phenotype with high levels of activated c-Jun-NH_2-kinase (JNK) and increased proliferation and migration

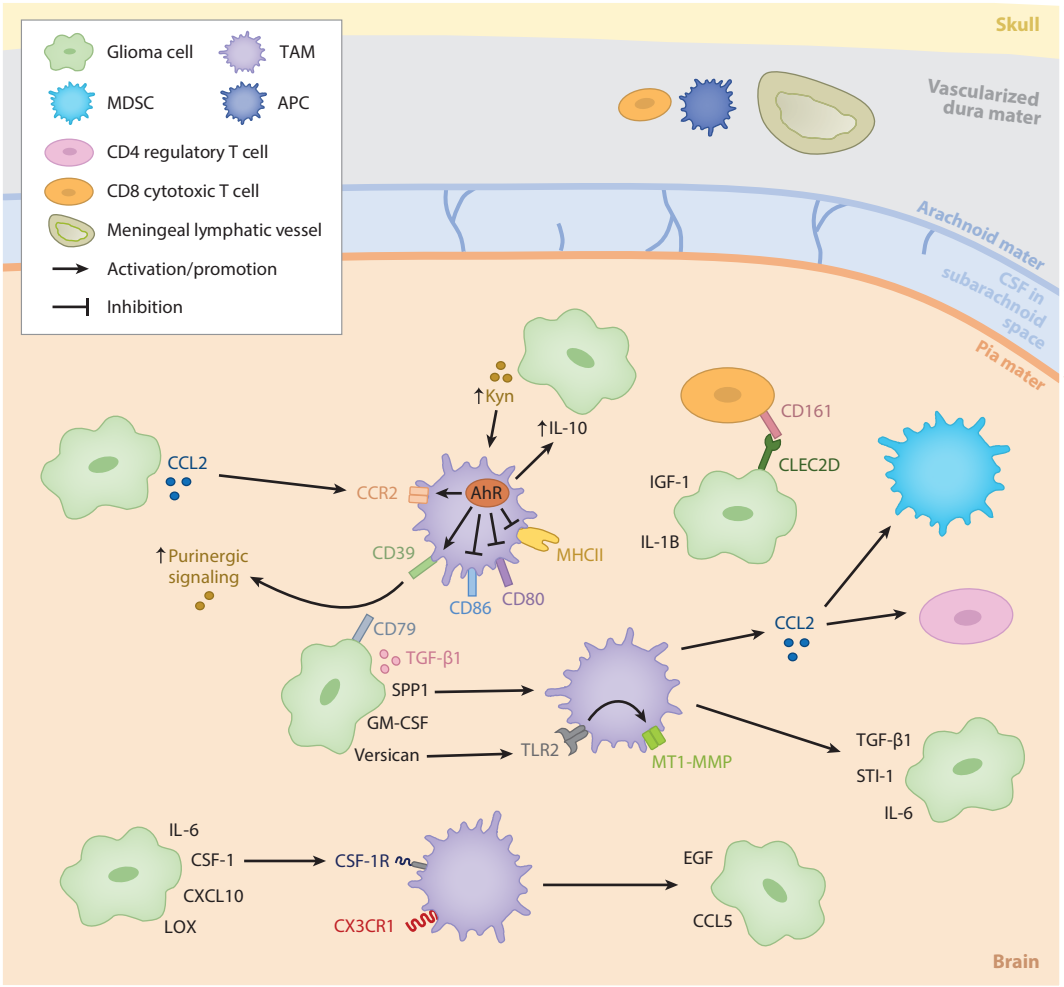

Figure 3

Cross talk within the glioma microenvironment between glioma cells and immune cells. Glioma cells actively regulate their immune microenvironment including TAMs, MDSCs, CD4 T regulatory cells, and CD8 T cells. The brain meninges consist of the pia mater (*orange*), the arachnoid mater (*blue*), and the vascularized dura mater (*gray*), which is adherent to the skull (*tan*). The lymphatic vessel network (*olive green*) within the dura acts as a neuroimmune interface where CNS antigen presentation occurs and T cell trafficking is regulated. Abbreviations: AhR, aryl hydrocarbon receptor; APC, antigen-presenting cell; CCL, chemokine (C-C motif) ligand; CCR, chemokine (C-C motif) receptor; CNS, central nervous system; CSF, colony-stimulating factor; CX3CR1, CX3C motif chemokine receptor 1; CXCL10, chemokine (C-X-C-motif) ligand 10; EGF, epidermal growth factor; GM-CSF, granulocyte-macrophage colony-stimulating factor; IGF, insulin-like growth factor; IL, interleukin; Kyn, kynurenine; LOX, protein-lysine 6-oxidase; MDSC, myeloid-derived suppressor cell; MHCII, major histocompatibility complex class II; MT1-MMP, membrane type 1 metalloprotease; SPP1, osteopontin; STI, stress-inducible protein; TAM, tumor-associated macrophage/microglia; TGF-β1, transforming growth factor beta 1; TLR2, Toll-like receptor 2. Figure adapted from Reference 88 with permission from Elsevier.

(66). Inhibition of this activated phenotype, or inhibition of microglial function, decreased glioma proliferation in vivo (62, 67). In complementary studies, genetic reduction of the microglia chemotaxis receptor CX3CR1 decreased microglial recruitment and delayed tumor formation (62, 67). Similarly, in a murine model for BRAF-fusion-driven glioma, CCR2 expression in TAMs was required for tumor development (68).

In the TME, tumor-educated TAMs release paracrine factors that promote glioma growth, survival, and invasion. Paracrine factors released by TAMs that can promote tumor growth include IL-1β, TGF-β1, stress-inducible protein 1 (STI-1), epidermal growth factor (EGF), and CCL5 (61, 69–72). For example, neutralizing antibodies against CCL5 resulted in decreased glioma proliferation in vivo (61). TAMs can also induce the expression of growth factor receptors on tumor cells such as platelet-derived growth factor receptor beta (PDGFR-β) (73).

Glioma cells actively shape their immune microenvironment by releasing factors that recruit TAMs to the TME or alter their function once present. For example, 2-HG produced by IDH-mutant tumor cells alters tryptophan metabolism in TAMs, resulting in increased production of kynurenine, a ligand of the aryl hydrocarbon receptor (AhR) (70). Upon ligand activation, AhR translocates to the nucleus, where it regulates the expression of genes important for the innate and adaptive immune response, including several factors that may promote an immunosuppressive microenvironment. In IDH-mutant glioma TAMs, activation of AhR increased the production of IL-10 and decreased expression of major histocompatibility complex class II (MHCII) and costimulatory molecules CD86 and CD80 (70). In IDH-wt GBM, kynurenine produced in tumor cells can activate AhR in TAMs, upregulating expression of CD39, important in purinergic signaling, and CCR2, the receptor for the chemokine CCL2 (74). CSF-1R signaling is important for microglia and macrophage differentiation and survival. Inhibition of CSF-1R in a murine model for glioma decreased tumor growth, prolonged survival, and, in late-stage tumors, decreased proliferation and increased apoptosis, leading to tumor regression (65). Rather than decreased TAM numbers, however, inhibition of CSF-1R altered TAM functional phenotype and increased phagocytic function (65). The inhibition of CSF-1R also highlighted a TAM-glioma feedback loop where glioma cells recruit microglia and macrophages to the tumor via secretion of chemotactic factors, including GM-CSF, interferon-γ, and chemokine (C-X-C-motif) ligand 10 (CXCL10) (65). To date, several tumor-secreted factors have been implicated in shaping the TAM response to promote tumor growth, including CCL2, CSF-1, TGF-β1, IL-6, protein-lysine 6-oxidase (LOX), and osteopontin (SPP1) (69, 71, 75, 76).

Programming the Immunosuppressive Microenvironment in Diffuse Glioma

Several factors likely conspire to produce the relatively lymphocyte-depleted, immunosuppressive microenvironment of diffuse glioma (77). Tumor-educated macrophages and microglia play a critical role, as they are responsible for antigen presentation and regulating the lymphocytic response. As mentioned previously, TAMs release paracrine factors, such as IL-10, which acts to suppress CD8 T cell recruitment, and CCL2, which promotes the recruitment of regulatory T cells and myeloid-derived suppressor cells (78, 79). TAMs can also directly impact antigen presentation and T cell activation. As illustrated above, activation of AhR can promote an immunosuppressive microenvironment via alterations in both paracrine and cell surface factors (70). TAMs may also help to create a metabolic microenvironment that promotes immunosuppression. Spatially-resolved single-cell analyses identified TAM expression of CD39 (ENTPD1), an integral plasma membrane ectonucleotidase that hydrolyzes ATP and ADP to AMP, in close proximity to tumor cells expressing the ecto-5′-nucleotidase CD73 (NT5E) (80). As CD73 catabolizes AMP to adenosine, this proximity would be predicted to contribute to elevated adenosine levels and increased purinergic signaling promoting immunosuppression. A broad range of CNS tumors have elevated CD73 expression, including both adult IDH-wt GBM and pediatric H3K27-altered diffuse midline glioma (80). In a murine model for GBM, response to immune checkpoint therapy was improved in the absence of CD73 expression in the TME (81). Additional factors contributing to the lymphocyte-depleted phenotype of diffuse glioma are T cell sequestration in the bone marrow and lymphopenia due to treatment with dexamethasone and chemoradiation (82–84). Glioma cells

can also directly regulate their immune microenvironment. In IDH-mutant diffuse glioma, 2-HG in the microenvironment can alter T cell maturation and effector function and reduce CXCL10 expression (85). In GBM, a population of polyclonal CD8 and CD4 T cells was identified that express NK cell receptors, including KLRB1 (CD161 protein) (86). Engagement of CD161 with its ligand CLEC2D (LLT1) inhibited key aspects of T cell function, including cytotoxicity and cytokine secretion. Using single-cell RNA-sequencing data and humanized GBM models, the investigators demonstrated that CLEC2D, expressed on tumor cells, engages T cell CD161 and inhibits T cell killing of glioma cells. In this context, knockout of CD161 on T cells conferred prolonged survival (86).

Until recently, it was unknown how brain antigens in the cerebrospinal fluid (CSF) made their way to the draining cervical lymph nodes. The brain meninges consist of three layers: the pia mater tightly attached to the surface of the brain; the arachnoid mater overlying the subarachnoid space; and the vascularized dura mater, which is fused to the cranial bones. Recent studies have highlighted the role of the meninges in fluid transport and CNS antigen presentation. The glial-lymphatic (glymphatic) system permits fluid exchange between the CSF and interstitium, while the lymphatic vessel network within the dura acts as a neuroimmune interface where CNS antigen presentation occurs and T cell trafficking is regulated (87, 88). While local factors in the glioma immune microenvironment, such as TGF-β1 and CCL2, discussed above, can promote an immunosuppressive microenvironment, factors that regulate APC (antigen-presenting cell) and T cell trafficking within the dura or parenchymal tertiary lymphoid structures in glioma may represent promising new therapeutic targets to promote an antitumor T cell response. In this regard, blood vessel remodeling may also be important. High endothelial venules (HEVs) are specialized postcapillary venules that facilitate the transmigration of lymphocytes in secondary lymphoid organs. In murine models for gliomas, therapeutically induced HEVs have been associated with increased T cell recruitment and improved response to immune-modulating therapies (89–91).

Therapeutic Opportunities and Challenges

Major advances have been made in recent years to improve immunotherapy for patients with cancer, including tumor vaccination strategies and adoptive cell therapies such as the engineering and use of chimeric antigen receptor (CAR) T cells. Another breakthrough has been the use of immunomodulators, including immune checkpoint blockade, to promote an antitumor immune response (see 92, 93). To date, however, the success of immunotherapeutic approaches in diffuse glioma has been limited. For example, in the TME, the immune checkpoint molecule programmed cell death ligand 1 (PD-L1), expressed on TAMs and glioma cells, interacts with programmed cell death protein 1 (PD-1) on T cells, inducing T cell–mediated immune tolerance. In recurrent GBM, a phase 3 clinical trial of anti-PD-1 therapy failed to demonstrate benefit; however, neoadjuvant administration of PD-1 inhibitor, which may antagonize negative T cell regulators during a critical therapeutic window, may improve efficacy (94–96). In the periphery, immunotherapy has been most successful in tumor types that contain high numbers of infiltrating T cells, such as lung cancer and subtypes of melanoma (97). As TAMs are thought to play a major role in shaping the immunosuppressive microenvironment of glioma, they are a potentially promising therapeutic target. Yet, our understanding of their regulation and function is limited. While some clinical studies have suggested a correlation between TAM accumulation and poor patient prognosis (98) several studies suggest that TAM phenotype and function may be most relevant for the outcome (99). In addition, molecular subtypes of gliomas exhibit different patterns of immune infiltrate. For example, GBMs with alterations in *PTEN* secrete the enzyme LOX, which promotes cross-links in the ECM and TAM recruitment (75). MAPK-driven gliomas appear to harbor a unique microenvironment with increased CD8 T cell infiltrates in some molecular subsets and different sensitivities

to immunomodulating therapies in GBM (54, 100, 101). Even different transcriptional subtypes of GBM exhibit different immune-related signatures (60, 102). Recent advances in single-cell and spatial technologies are enabling unprecedented analysis and profiling of myeloid populations in the resting and diseased brain, including in diffuse glioma (59, 78, 98, 103). These studies highlight the heterogeneity of immune cell populations in the CNS and emphasize the importance of cellular cross talk in the TME. Immunotherapeutic strategies targeting glioma will need to consider this heterogeneity and will require a more complete understanding of how the glioma-associated immune response is regulated and how it evolves with disease progression. It is also unclear how the tumor-associated immune microenvironment influences neuronal activity and seizures. Given the potential importance of neuronal activity on glioma growth and invasion, discussed above, this is an important avenue of investigation, particularly since several inflammatory mediators present in glioma, such as IL-1β and TGF-β, are known to alter neuronal and glial functions (for a review, see 104). Taken together, these studies highlight the importance of glioma-immune cross talk in the TME and suggest that therapeutic targeting of this communication may be beneficial.

THE GLIOMA MATRISOME: THREE-DIMENSIONAL TUMOR MICROENVIRONMENT

Glioma development and progression are dependent on cross talk within the TME, including interactions with neurons, non-neoplastic glia, and immune cells. Integral to this communication are the matrisome, or ECM, and the matrisome-affiliated proteins, including cell surface molecules and soluble factors, that make up the three-dimensional microenvironment of a cell (105). This network provides essential biochemical and biomechanical cues that shape cell–cell interactions (106) and cell behaviors (for a review, see 107). The glycosylation of matrisome components, including posttranslational modifications consisting of the addition of single monosaccharides or elaborate oligo/polysaccharides, contributes to its tremendous diversity of structures and functions (108) (for a review, see 109). In the CNS, genetic and pharmacologic modulation of the matrisome, including its glycosylation, illustrate the essential role that it plays in regulating signal transduction, cell adhesion, proliferation, and differentiation. For example, glycosylation of matrisome components is necessary for neurite outgrowth, axon guidance, synaptogenesis, and innate immunity (for a review, see 110). Within the embryonic ventricular zone, disruption of the interaction between ECM laminins and integrin β1 expressed by neural stem cells results in dysregulated proliferation of progenitor cell populations (111, 112). As discussed, glioma-propagating cells or cancer stem cells share features with glial progenitor/neural stem cells and are thought to drive gliomagenesis and therapy resistance (113, 114). Thus, there is a great need to understand the factors that provide a supportive microenvironment, or niche. The perivascular niche is thought to be particularly important. For example, integrin alpha 6, which binds extracellular laminin, is enriched on glioma stem/progenitor cells cultured as tumorspheres in vitro and is expressed on tumor cells within the perivascular regions of human GBM (115). Knockdown of integrin alpha 6 in tumor cells inhibited self-renewal, proliferation, and tumor formation, suggesting integrin signaling in the perivascular niche helps to maintain glioma progenitors (115).

The dominant components of the ECM in many peripheral organs are fibrous proteins, such as collagens, elastins, fibronectins, and integrins, that impart them with a relatively stiff ECM influencing cell signaling and tissue function. In contrast, the ECM of the nondiseased brain is relatively soft (for a review, see 106). While fibrous proteins are present, glycosaminoglycan (GAG)-containing molecules predominate, including hyaluronan/hyaluronic acid (HA) (116), proteoglycans (PGs), and tenascins (**Figure 4**). GAGs are a family of polysaccharides that interact with diverse partners, including soluble factors, membrane proteins, and components of the ECM.

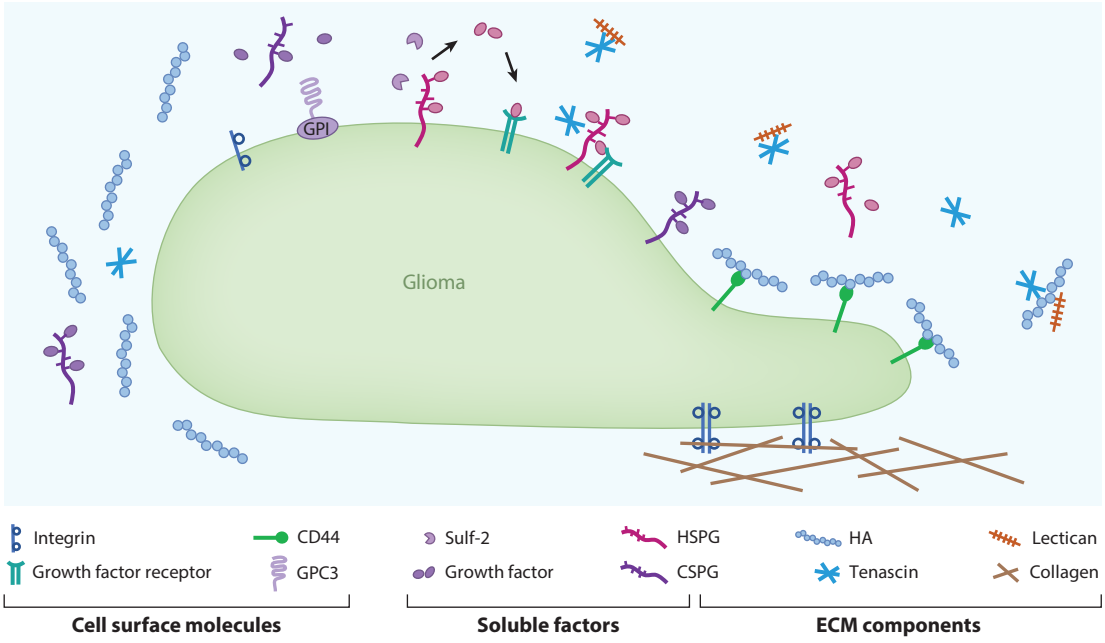

Figure 4

Interactions between glioma cells and the three-dimensional microenvironment of a cell. Representative components of the matrisome and matrisome-affiliated proteins include the ECM, cell surface molecules, and soluble factors. Note there are several types of HSPGs and CSPGs and they can exist in multiple forms, including GPC3 and lecticans, respectively. Abbreviations: CSPG, chondroitin sulfate proteoglycan; ECM, extracellular matrix; GPC3, glypican-3; GPI, glycosylphosphatidylinositol; HA, hyaluronic acid/hyaluronan; HSPG, heparan sulfate proteoglycan; Sulf-2, extracellular sulfatase Sulf-2.

This network of interactions shapes a wide range of cellular processes. Disruption of GAG biosynthesis, catabolism, and distribution, due to experimental manipulations in the laboratory or as a result of human genetic conditions, demonstrates their profound role in normal development and homeostasis (117). Defining the networks of the ECM, soluble factors, and cell surface molecules that act to promote glioma growth and invasion is critical, as they represent potential therapeutic vulnerabilities. In the following section, we highlight the importance of this network in diffuse glioma and its impact on the cross talk between glioma cells and neural or immune components.

Glioma Invasion

HA is composed entirely of a GAG that is released extracellularly by hyaluronan synthase (HAS) enzymes located in the plasma membrane and enzymatically remodeled or degraded by hyaluronidase. Hypoxia, a hallmark of GBM, promotes HA secretion (118), and in vitro specific molecular weights of HA can promote glioma invasion (119). While HA interacts with several matrisome components, CD44 was the first HA receptor identified. In GBM, expression of cell surface receptor CD44 is upregulated particularly within the mesenchymal transcriptional subtype (120, 121). CD44 is also enriched in long, thin microtubule-based protrusions that form when glioma cells are plated on the HA matrix (122). By knocking down CD44, Wolf et al. (122) demonstrated that CD44-HA interactions are required to support microtubule protrusions, glioma adhesion, and glioma migration in HA-rich microenvironments. Furthermore, disruption of HA interactions, via HA agonists or soluble HA-binding proteins, attenuated HA signaling and inhibited tumor cell invasion and anchorage-independent growth in vitro (123).

While the human brain ECM is relatively soft, ECM stiffness is markedly increased in human GBM, and stiffness is correlated with a transcriptional signature of aggressive disease (124). In contrast, lower-grade IDH-mutant gliomas are much less stiff, but, upon progression, they become stiffer (124). Tenascin C (TNC), an ECM protein that can complex with lecticans and HA, is upregulated in both GBM and progressed high-grade IDH-mutant gliomas. Using an orthotopic GBM xenograft model, knockout of TNC prolonged murine survival and reduced ECM stiffness and mechanosignaling in tumors, indicated by reduced pFAK (phosphorylated FAK) and pMLC (phosphorylated myosin light chain) (124). To directly test the impact of increased mechanosignaling on glioma, the authors expressed an autoclustering β1 integrin mutant (V737N) in IDH-mutant glioma cells. The derived tumors had increased mechanosignaling, and mouse survival was decreased (124). The importance of mechanical cues and their impact on glioma cell proliferation and invasion is well established. When glioma cells are plated on highly rigid ECMs, tumor cells form prominent stress fibers, develop mature focal adhesions, migrate rapidly, and have increased proliferation (125). Pharmacologic inhibition demonstrated that mechanosensing requires a competent actin cytoskeleton, Rho GTPase-based signaling, and nonmuscle myosin II-based contractility (125). The sensitivity of proliferation to the matrix was dependent, in part, on alterations in growth factor signaling pathways, including increased epidermal growth factor receptor (EGFR) clustering with condensing of phosphorylated EGFR into vinculin-positive focal adhesions (126).

Glioma Growth, Proliferation, and Survival

PGs comprise a protein core and GAG side chains. While the protein core determines cellular localization, including that of integral membrane, GPI-linked, or secreted proteins, the GAG structure regulates interactions with diverse extracellular components. The two most common types of PGs in the brain are HSPGs and chondroitin sulfate proteoglycans (CSPGs). Lecticans are a subset of CSPGs that bind HA and include versican (VCAN, CSPG2), neurocan, brevican, and aggrecan. Using a murine model for metastatic lung carcinoma, Kim et al. (127) identified versican as a factor in tumor-conditioned media that activates myeloid Toll-like receptor 2 (TLR2) signaling. Using *Tlr2*$^{-/-}$ mice, the authors demonstrated that increased metastatic disease was dependent on TLR2-mediated myeloid cell activation and TNF-α secretion. Furthermore, the knockdown of versican, specifically the RNA splice variant V1, reduced metastatic foci and prolonged survival (127). In GBM, versican is upregulated at the transcriptional level and has significantly altered glycosylation compared with the non-neoplastic brain (108, 128). In a murine model for GBM, the versican isoforms V0/V1 were identified in a screen for soluble factors that trigger TLR2 signaling and membrane type 1 metalloprotease (MT1-MMP; MMP14) expression on TAMs (129) (**Figures 3** and **4**). Previously, TAM expression of MT1-MMP was shown to activate glioma-derived pro-MMP-2 and promote glioma growth (130, 131). Consistent with this finding, silencing of versican in tumor cells was sufficient to confer reduced tumor growth and prolong overall survival in a murine model (129).

CSPG4/NG2 is an integral membrane PG found on the surface of several immature progenitor cells, including oligodendrocyte progenitor cells and pericytes (for a review, see 132). On OPCs, the largest population of dividing cells in the adult brain, CSPG4/NG2 modulates receptor tyrosine kinase (RTK) signaling to promote cell proliferation and cell migration (133–135). CSPG4/NG2 modulation of RTK signaling may be related to both its ability to act as a reservoir or coreceptor for ligands, such as with PDGF-AA and fibroblast growth factor 2 (FGF-2) (133), and its ability to directly interact with receptors, such as for FGFR1 and FGFR3 (136). In 2011, Sugiarto et al. (137) demonstrated that CSPG4/NG2 regulates EGF-dependent proliferation and self-renewal of OPCs. CSPG4/NG2 was also required to establish OPC polarity, in part,

by achieving asymmetric segregation of active EGFR. Thus, CSPG4/NG2 may play a role in the loss of asymmetric division in a subset of glioma precursor cells (137).

HSPGs also regulate ligand-mediated signaling through their interactions with ligands, receptors, or both via the formation of ternary complexes that enhance ligand-receptor stability and signaling (117). In GBM, hepatocyte growth factor (HGF) is an important mitogen that promotes tumor cell proliferation via activation of the Met receptor. Using a truncated HGF isoform, containing both the HS binding site and the primary Met binding site, Cecchi et al. (138) generated a mutant ligand that lacked HS binding. Overexpression of the mutant HGF inhibited functional Met signaling in tumor cells and inhibited tumor growth despite maintaining Met binding (138). GPCs are GPI-linked HSPGs that are tethered to the cell membrane. GPC4 and GPC6 are expressed by astrocytes and can promote functional synapse formation via their ability to increase GluR1 AMPAR surface levels and clustering on neurons (8). As discussed above, alterations in PIK3CA in GBM are associated with upregulated tumor cell expression of GPC3, a GPI-linked HSPG, which is released into the extracellular environment and can promote neuronal hyperexcitability and gliomagenesis (45) (**Figure 2**). HSPG GAG side chains undergo extensive posttranslational modifications, including sulfation on the 6-*O* position of glucosamine (117). While several intracellular enzymes regulate HSPG biosynthesis and sulfation, the extracellular sulfatases, SULF1 and SULF2, regulate HSPG-dependent signaling in the extracellular environment. In a murine model for GBM, the knockdown of SULF2 decreased multiple RTK signaling pathways including PDGFR-α (139), known to be involved in glioma growth and invasion (140, 141). Decreased RTK activity was associated with decreased tumor cell proliferation and prolonged survival.

The above studies highlight how factors present in the ECM, on the cell membrane, and secreted into the microenvironment can promote glioma growth and invasion. Importantly, this network of factors also has a profound impact on the immune response, neuronal signaling, and angiogenesis. Therapeutic targeting of the ECM has the potential to block this dynamic cross talk that is important in glioma development and progression.

CONCLUSION

The glioma TME is a dynamic entity that is in direct communication with the tumor. With a focus on the interactions between glioma cells and neural, immune, and ECM components, we highlight how paracrine signaling, electrochemical synapses, and direct cell contact with the TME shape the biochemical and biomechanical input to a tumor. We also highlight how the tumor actively promotes this cross talk to alter fundamental aspects of its microenvironment, such as altering immune cell function, neuronal activity, and ECM composition. It is now possible to interrogate alterations in genetics, epigenetics, and proteomics at single-cell resolution within human gliomas. These analyses reveal a dynamic TME in which the tumor and the TME coevolve. Strategies to disrupt these interactions and exploit tumor vulnerabilities will hopefully lead to the design of novel therapeutic agents that improve patient outcomes.

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