Context-Dependent Glioblastoma-Macrophage/Microglia Symbiosis and Associated Mechanisms

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Glioblastoma (GBM) is a lethal form of primary brain tumor in human adults. The impact of tumor-intrinsic alterations is not exclusively confined to cancer cells but can also be extended to the tumor microenvironment (TME). Glioblastoma-associated macrophages/microglia (GAMs) are a prominent type of immune cells that account for up to 50% of total cells in GBM. Emerging evidence suggests that context-dependent GBM–GAM symbiotic interactions are pivotal for tumor growth and progression. Here, we discuss how specific genetic alterations in GBM cells affect GAM biology and, reciprocally, how GAMs support GBM progression. We hypothesize that understanding context-dependent GBM–GAM symbiosis may reveal the molecular basis of GBM tumorigenesis and lead to novel candidate treatment approaches aiming to improve GBM patient outcomes.

GBM Genetic Alterations and Associations with the TME

Cancer has been recognized as a genetic disease for more than a century as it can be driven by either the activation of oncoproteins or the inactivation of tumor suppressor genes (TSGs), triggering tumor formation and progression [1]. In-depth studies on cancer genomics have yielded comprehensive atlases of oncogene and TSG alterations for specific types of cancer, which can reveal specific cancer vulnerabilities (Box 1). GBM is the most aggressive and highly lethal form of primary brain tumor in human adults [2,3], and the current standard of care offers only modest survival benefit to GBM patients [4–6]. Genomic profiling of patient tumors has led to GBM subclassification based on gene alterations affecting core signaling pathways, specifically those associated with P53/ARF/MDM2, receptor tyrosine kinase (RTK)/RAS/PI3K/PTEN, and RB/CDKN2A alterations [4,7–9]. These subclassifications have motivated clinical trials for testing targeted therapies, such as those against RTK signaling [4]. Regrettably, attempts to inhibit aberrant signaling that results from these gene alterations have not been successful in improving GBM patient outcomes. The underlying basis for these failures relates to the inherent cellular heterogeneity (see Glossary) of GBM that ensures the survival of cancer cells irrespective of treatment [4,10]. Glioma stem cells (GSCs) are subpopulations of cells endowed with stem cell properties (e.g., self-renewing capacity and tumor-propagating potential), which have been shown to promote GBM intratumoral heterogeneity [11,12].

The impact of aberrant cancer-associated signaling is not limited to cancer cells and extends to normal cells of the tumor microenvironment (TME) [13]. The function and composition of the GBM TME are influenced by cancer cell-intrinsic signaling pathways and secreted factors [13–16]. Reciprocally, the TME can promote GBM progression by modulating multiple cancer biological properties, including cell proliferation, survival, migration, and immune surveillance [5,17]. Within the TME, glioblastoma-associated macrophages/microglia (GAMs) constitute the most abundant cell population and account for up to 50% of the total number of live cells in human adults [4]. Genomic profiling of human GBM has led to GBM subclassification based on gene alterations affecting core signaling pathways, specifically those associated with P53/ARF/MDM2, receptor tyrosine kinase (RTK)/RAS/PI3K/PTEN, and RB/CDKN2A alterations [4,7–9]. These subclassifications have motivated clinical trials for testing targeted therapies, such as those against RTK signaling [4]. Regrettably, attempts to inhibit aberrant signaling that results from these gene alterations have not been successful in improving GBM patient outcomes. The underlying basis for these failures relates to the inherent cellular heterogeneity (see Glossary) of GBM that ensures the survival of cancer cells irrespective of treatment [4,10]. Glioma stem cells (GSCs) are subpopulations of cells endowed with stem cell properties (e.g., self-renewing capacity and tumor-propagating potential), which have been shown to promote GBM intratumoral heterogeneity [11,12].

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**Highlights**

Symbiotic glioblastoma-macrophage/microglia (GBM–GAM) interactions reveal synthetic lethality in GBM harboring a deficiency in a specific tumor suppressor gene (e.g., PTEN, NF1, or TP53).

Cancer cell-intrinsic activation of oncoproteins (e.g., EGFR and CLOCK) can shape a protumor immune response by modulating GAM biology.

GBM–GAM symbiosis can contribute to GBM progression by promoting glioma stem cell (GSC) stemness, GBM cell proliferation, survival, and migration as well as by suppressing T cell-mediated immune responses in mouse and patient-derived xenograft (PDX) models.

Characterizing GBM–GAM symbiosis might reveal personalized therapeutic targets. For example, LOX and CLOCK inhibition can impair tumor progression and GAM infiltration, specifically in PTEN-deficient and CLOCK-high GBM in mouse and PDX models, respectively.

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cells in the GBM tumor mass [17,18]. GAMs are composed of multiple subpopulations, including bone marrow-derived macrophages (BMDMs, hereafter referred to as macrophages) and brain-resident microglia (hereafter referred to as microglia) (Box 2) [18,19]. GAMs can also be classified as polarized toward proinflammatory and ‘alternatively activated’ phenotypes (Box 3), which exhibit antitumor/immune-stimulatory and protumor/immunosuppressive effects, respectively [20]. However, it is highly likely that GAMs are composed of heterogeneous subpopulations that are not polarized to either state with variable immune functional capabilities, such as antigen presentation, phagocytosis, and tumor-supportive functions [21]. A study of a large cohort of GBM patients has shown that tumors with mutations in PTEN, epidermal growth factor receptor (EGFR), and neurofibromin 1 (NF1) are significantly enriched with GAMs [22]. With respect to the transcriptomic classification of GBM, the most biologically aggressive mesenchymal subtype is enriched for alterations in PTEN, TP53, NF1, and RB1 [23] and is highly enriched with immunosuppressive macrophages [24]. Transcriptional regulatory network analyses have also revealed that the master regulators and their target genes for GAMs in mesenchymal GBM are associated with genetic aberrations in NF1 and PTEN/PK3/mTOR/AKT pathways [25]. Together, these findings support a relationship in which GBM cells and GAMs can influence each other under specific genetic backgrounds.

Increasing evidence underscores the myriad of interactions and intertwined roles of cancer cells and GAMs in GBM biology. In this opinion article, we highlight and discuss how the alterations of specific TSGs and oncogenes in GBM cells affect GAM biology (including migration, adhesion, and polarization) and, reciprocally, how GAMs support GBM growth and progression. We propose to discuss context-dependent GBM–GAM symbiotic interactions, therapeutic potential via targeting GBM–GAM symbiosis, and future perspectives. We hypothesize that these emerging insights not only expand our understanding of GBM tumorigenesis but also might

Box 1. Personalized Medicine for Cancer Patients
Discoveries of synthetic lethality and oncogene addiction have revealed specific cancer vulnerabilities that can be exploited for treatment with vulnerability-targeted agents [88,74–76]. For example, treatment with inhibitors targeting epidermal growth factor receptor (EGFR) or poly ADP-ribose polymerase (PARP) provides significant benefits to cancer patients with an EGFR mutation [77,78] or a BRCA mutation [79–81], respectively. Unfortunately, many gene alterations (including alterations of oncogenes and TSGs) have yet to reveal druggable targets. Recent discoveries with new conceptual approaches (e.g., synthetic essentiality and collateral lethality) suggest that targeting lethal gene–gene interactions within cancer cells may provide a means to identify novel cancer therapeutic targets [75,81,82]. Now emerging is the concept of lethal gene–gene interactions between cancer cells and the TME [83]. Together, such lethal gene–gene interactions suggest opportunities for novel treatment approaches.

Box 2. Identity of Macrophages and Microglia in GBM
GAMs are composed of two subpopulations, including bone marrow-derived macrophages (BMDMs, hereafter referred to as macrophages) and microglia [17,18]. Distinguishing these two populations is complicated due to the lack of specific macrophage or microglial markers [18,84]. Combining Cx3cr1GFP/wt; Cd22EGFP/Flk knock-in mice with a genetically engineered mouse model (Fgfbp-driven glioma) or GL261 model has allowed for the ability to distinguish macrophages from microglia in tumors, in which macrophages account for approximately 85% of GAMs in these glioma models [18]. However, a growing body of evidence suggests that the ratio between macrophages and microglia in GBM is dependent on the methods used to quantify them as well as on tumor genetic backgrounds and subtypes. For example, macrophage abundance in GL261 tumors is significantly enhanced in the irradiation bone marrow transplantation (IR-BMT) lineage tracing model compared with the Flt3-Oec; Rosa26:mTmG lineage tracing model [84]. Based on genetic lineage tracing in murine models, transcriptional profiling followed by functional studies has revealed that CD49d is specifically absent in microglia and can distinguish microglia from macrophages in mouse and human GBM tumors [84]. Mutations in isocitrate dehydrogenase 1 and 2 (IDHmut) are generally observed in low-grade glioma, and GBM patients with IDHmut have a significantly better prognosis than those with wild-type (WT) IDH [85]. Analysis of glioma patient samples has demonstrated that IDHmut tumors harbor more microglia and fewer macrophages than IDH WT tumors [85,86]. With regard to GBM tumor subtypes, macrophage and microglia signatures have been reported to be highly enriched in GBM patients with mesenchymal and neural subtypes, respectively [84].

Glossary

- Alternately activated macrophages: skewed towards an immunosuppressive and protumor phenotype in cancer.
- Bone marrow-derived macrophages (BMDMs): here, infiltrating macrophages in GBM, originate from the bone marrow.
- Brain-resident microglia: specialized macrophages in the brain that originate from progenitors seeding the embryonic yolk.
- Cell heterogeneity: fundamental property of biological systems that covers different aspects of cells, ranging from genetic diversity to cell-to-cell variability; driven by stochastic molecular interactions involved in all cellular processes.
- Circadian rhythm: an organism’s internal clock that regulates the sleep–wake cycle and repeats on each rotation every 24 h (e.g., in humans).
- Extracellular vesicles (EVs): lipid bilayer-delimited particles that are naturally secreted by cancer cells and/or stromal cells in the tumor.
- GBM–GAM symbiosis: symbiotic interaction where GBM cells and GAMs benefit from each other via secretion of soluble factors and vesicles or through a cell-to-cell contact mechanism.
- Glioblastoma-associated macrophages/microglia (GAMs): macrophages and microglia that infiltrate into GBMs, promote tumor progression, and exhibit immunosuppressive functions.
- Hypoxia-inducible factor 1-alpha: the master transcriptional regulator of cellular responses to hypoxia.
- Lysyl oxidase (LOX): a copper-dependent amine oxidase that plays a key role in the biogenesis of connective tissue matrices (by crosslinking collagen and elastin) and in regulating macrophage migration.
- Oncogene addiction: dependency of tumor cells on a single oncogene to maintain their protumor activity.
- Organoids: (tumor organoids) 3D multicellular in vitro tissue construct that can mimic its corresponding in vivo TME; it can be used to study aspects of tumor biology in tissue culture dish.
- Patient-derived xenograft (PDX): cancer patient-derived xenograft model that can reflect properties of the original patient tumors.
- Phagocytosis: process where phagocytes, such as macrophages, engulf particles, debris, or other cells.
Box 3. Proinflammatory and Alternatively Activated Macrophages

Macrophages are highly plastic cells consisting of distinct phenotypes, including a ‘classically activated’ proinflammatory phenotype and an ‘alternatively activated’ anti-inflammatory phenotype, which can be activated by specific factors or cytokines [97,98]. For example, macrophages are polarized toward a proinflammatory phenotype upon stimulation with lipopolysaccharide, IFN-γ, or TNFα, which in turn produce proinflammatory cytokines, such as interleukin 1 beta (IL-1β), IL-12, and IL-23, to kill cancer cells and microorganisms. Instead, macrophage alternative polarization can be triggered by exposure to IL-4, IL-10, or IL-13, which in turn produces anti-inflammatory factors, such as IL-10, transforming growth factor beta 1 (TGF-β), C-C motif ligand 17 (CCL17), and CCL22, to promote tumor progression, angiogenesis, and tissue repair [87–89]. These two phenotypes have been widely used to define macrophage subpopulations in cancer and immunology; however, this dichotomy is facing challenges due to the fact that (i) macrophages can respond to the dynamic TME in vivo, (ii) both phenotypes often coexist in tumors, and (iii) phenotype-specific macrophage markers are still missing [90,91]. However, accumulating evidence demonstrates that GAMS are usually biased toward an alternatively activated phenotype, promoting tumor progression and creating an immunosuppressive microenvironment [19]. Consequently, reprogramming GAMS toward a proinflammatory phenotype has been reported as an effective anti-tumor strategy in certain models. For example, treatment with colony stimulating factor 1 receptor (CSF-1R) inhibitor BLZ945, angiotensin 2/vascular endothelial growth factor (Ang-2/VEGF) bispecific antibodies, and oncolytic viruses (G47DG expressing murine IL-12) can skew GAMS toward a proinflammatory phenotype and extend survival in GBM mouse and PDX models [92–94]. Moreover, this strategy can synergize with immunotherapies (e.g., anti-PD1 antibody (Ab) or anti-CTLA4 Ab therapy) to extend survival in CT2A and 005 GSC GBM mouse models [94]. In addition, clinical evidence further suggests that anti-PD1 Ab therapy resistance is often observed in GBM patients with high numbers of alternatively activated GAMS and PTEN deletion/mutation [95].

provide a roadmap for the development of novel putative therapeutic strategies that disrupt this context-dependent circuit in GBM.

The Influence of TSG Alterations on GAM Biology

Factors secreted by GBM cells in the TME are known to recruit and polarize GAMS [20]. Increasing evidence shows that GBM cell-derived factors are regulated by specific TSG alterations (Figure 1). PTEN is a TSG that acts in opposition to PI3K signaling in GBM, and PTEN is mutated and/or deleted in about 30–40% of GBMs [8,23]. The Cancer Genome Atlas (TCGA) analyses and immunohistochemical staining in GBM patient tissue microarrays (TMAs) have revealed that PTEN mutations are significantly associated with increased intratumoral macrophages but have no effect on microglia [24]. These findings strongly suggest that PTEN deficiency in GBM cells might specifically trigger macrophage infiltration. This hypothesis is further supported by in vitro and in vivo studies (including mouse and human GBM models) showing that depletion of PTEN in PTEN-deficient WT GBM increases macrophage recruitment, whereas overexpression of PTEN in PTEN-deficient GBM has the opposite effect [24]. Mechanistically, PTEN deficiency in GBM cells activates SRC and AKT pathways, which, in turn, activates yes-associated protein 1 (YAP1) signaling [24]. YAP1 is a transcriptional coactivator that binds to the promoter of lysyl oxidase (LOX) and upregulates LOX expression in GBM cells [24]. LOX is a secreted enzyme that can be internalized by macrophages through the β1 integrin receptor, which, in turn, causes reactive oxygen species (e.g., hydrogen peroxide) to promote macrophage migration via activation of PYK2 signaling [24]. Moreover, in PTEN-null, but not PTEN-WT, GBM models (e.g., human and mouse orthotopic GBM models established in severe combined immunodeficient (SCID) and C57BL/6 mice), LOX inhibition suppresses tumor macrophage infiltration and increases the survival of tumor-bearing mice [24]. In addition, activation of PI3K/AKT signaling in human GBM cells and GSCs has been shown to promote GBM progression via activation of other signaling pathways, such as the WNT/β-catenin pathway (e.g., increasing GBM cell proliferation and GSC self-renewal, inhibiting GBM cell apoptosis, and inducing GSC differentiation into endothelial-like cells relative to controls) [26,27]. For example, GSCs in GBM can secrete WNT-induced signaling protein 1 (WISP1) to promote GAM survival and GSC stemness through activation of the c68β1 integrin/AKT pathway in vitro and in GBM patient-derived xenograft (PDX) models [28]. Inhibition of the WNT/β-catenin/ WISP1 pathway by short hairpin RNA (shRNA) knockdown (KD) or usage of the pharmacological inhibitor camosic acid in human GSCs suppresses GBM growth and extends survival through a
mechanism that involves reduced numbers of intratumoral GAMs and disrupted GSC maintenance in NOD SCID gamma (NSG) mouse models [28]. Collectively, these findings suggest that PTEN/PI3K/AKT signaling is essential for producing soluble factors (e.g., LOX and WISP1) in human or mouse GBM cells that recruit and maintain GAMs via activation of macrophage β1 integrin signaling.

These data not only reveal some of the molecular bases underlying an interplay between PTEN-deficient GBM cells and GAMs but also highlight a synthetic lethal interaction between mutation/deletion of PTEN and suppression of macrophage chemokines. This, in turn, might offer synthetic lethal approaches to target GBM–GAM symbiosis in PTEN-null GBM.
NF1 is a TSG that is mutated or deregulated in approximately 40% of the mesenchymal subtypes of GBM and in 16% of all GBM patients [8,29]. In addition to cancer cell-intrinsic effects, tumor development in the context of NF1 loss has been reported to be regulated by steroid hormones in the CMV-CreERT2; NF1<sup>fl/fl</sup>; ROSA26 neurofibroma mouse model, thereby suggesting that tumor NF1 status can influence the TME [29]. Indeed, NF1-deficient GSCs isolated from tumors of the NF1 genetically engineered mouse model (NF1<sup>fl/fl</sup>; GFAP-Cre mice) on a C57BL/6 background produce the chemokines C-X-C motif ligand 1 (CX3CL1) and C-C motif ligand 5 (CCL5), thus recruiting microglia into the TME, and this effect is further amplified by loss of Pten in GSCs isolated from NF1<sup>fl/fl</sup>; Pten<sup>fl/fl</sup>; GFAP-Cre mice [30]. TCGA data show that GBM patients with NF1 abnormalities are associated with higher expression of intratumoral GAM-specific genes, such as ITGAM (integrin subunit alpha m; also known as CD11B) and AIF1 (allograft inflammatory factor 1; also known as IBA1), than patients with WT NF1 [31]. Additional support characterizing GBM cell NF1 status as a possible determinant of intratumoral GAM was offered by a study in which conditioned medium from NF1 shRNA KD human GBM cells increased the recruitment of macrophages and microglia relative to conditioned medium from shRNA control cells in a transwell migration assay [31]. Together, these findings suggest that loss of NF1 in GBM cells might promote intratumoral GAM infiltration. Evidently, further studies are needed, and these might also reveal some of the molecular mechanisms underlying GAM infiltration in human GBM. Accordingly, in this regard, GBM PDX models might lead to the development of novel synthetic lethal approaches to target GBM–GAM symbiosis in NF1-deficient GBM.

Another TSG alteration that affects GBM cellular composition is that of TP53. TP53 mutation is frequent in human GBM, and, in addition to its effects on cell cycling and apoptosis [8,32], tumor expression of mutant p53 increases the expression of CCL2 and tumor necrosis factor alpha (TNFα) via activation of nuclear factor kappa B (NF-κB) signaling in human GBM cells (e.g., U87 and 19NS) [32]. Increased CCL2 and TNFα, in turn, can recruit macrophages and microglia into the GBM TME [32]. The GL261 glioma mouse model in C57BL/6 mice harbors Trp53 mutant tumor cells [33]; these can communicate with GAMs via the release of extracellular vesicles (EVs) and soluble factors [34,35]. For example, GL261 cell-derived EVs contain microRNA miR-21 that can increase microglia proliferation [34]. GL261 cells additionally produce secreted phosphoprotein 1 (SPP1) as a chemokine to trigger Iba1<sup>+</sup> macrophage tumor infiltration via integrin αvβ5 signaling in tumors [35]. Moreover, results from several genetically engineered GBM mouse models (e.g., Ntv-a; Pdgfb; shTrp53; Pten<sup>−/−</sup>; Trp53<sup>−/−</sup> and Pten<sup>−/−</sup>; Trp53<sup>−/−</sup>; Idh1<sup>R132H</sup>; Pdgfb) demonstrate that GSCs with Trp53 deficiency can activate mTOR signaling in microglia, thus skewing microglia into an alternatively activated phenotype via activation of signal transducer and activator of transcription 3 (STAT3) and NF-κB signaling [36]; this in turn, suggests a potential therapeutic strategy involving inhibition of the mTOR/STAT3/NF-κB pathway in Trp53-deficient GBM. In addition to modulating GAM migration and polarization, Trp53 deficiency might also affect the phagocytic activity of GAMs. For instance, Trp53 and Sparc co-deficiency in mouse models promotes phagocytosis of glioma cells by GAMs and induces decreased animal survival [37]. Together, these findings highlight the key role of p53 mutations in influencing GAM tumor infiltration, polarization, and phagocytic activity in GBM human and mouse models.

Consequently, we posit that there are significant symbiotic interactions between GAMs and GBM cells harboring deficiencies of PTEN, NF1, and TP53 and suggest that targeting this context-dependent GBM–GAM symbiosis might reveal synthetic lethality. However, further studies are needed to design synthetic lethal approaches to target GBM–GAM symbiosis for specific TSG-deficient GBM (e.g., PTEN, NF1, or TP53). In addition, it will be relevant to determine whether this GBM–GAM symbiosis exists in GBMs that harbor a deficiency in other TSGs, such as CDKN2A and CDKN2B, as this might offer additional candidate personalized therapeutic strategies.
Role of Oncogene Alterations on GAM Biology

In addition to TSGs, the activation and amplification of oncogenes can also play an important role in modulating GAM infiltration and polarization in GBM (Figure 2). Among these is EGFR amplification and activation, which is a hallmark of GBM and presents in about 60% of human GBM cases [8,38–40]. TCGA analyses and immunohistochemical staining using patient TMAs have revealed that EGFR activation correlates positively with intratumoral macrophages in GBM [40,41]. Mechanistically, EGFR activation in GBM cells can modulate the expression and activity of a variety of factors that are important for macrophage adhesion, infiltration, and polarization. For example, carbonic anhydrase IX (CAIX) mRNA and protein expression amounts are highly

![Diagram of Oncogene Alterations](https://biorender.com/)
upregulated in human GBM cells (e.g., U87 and U251) under hypoxic conditions and are associated with poor patient prognosis [42]. Moreover, EGFR activation plays a role in CAIX upregulation via stabilization of hypoxia-inducible factor 1-alpha in human GBM cells that, in turn, promotes macrophage adhesion and polarization toward an alternatively activated phenotype [42]. EGFR activation can additionally increase TNFα-induced vascular cell adhesion molecule-1 (VCAM-1) expression in human (U251) and mouse (ALTS1C1) GBM cells via a P38/STAT3 pathway-dependent mechanism, and increased VCAM-1 promotes macrophage adhesion [43]. These macrophages conversely stimulate GBM cell proliferation, invasion, and further production of TNFα, thus forming a positive feedback loop between GBM cells and macrophages [43]. In addition to TNFα, stimulation of EGFR with EGF in human GBM cells (e.g., U251, D54, and A172) can activate protein kinase C epsilon type (PKCε) and NF-κB pathways, in turn upregulating VCAM-1 to promote macrophage adhesion and thus increase GBM cell invasiveness in vitro [40]. Notably, half of EGFR-amplified human GBM tumors harbor an EGFR truncating mutation (EGFRvIII) [38]. Coexpression of EGFR and EGFRvIII in human GBM cells (e.g., U87 and A172) activates KRAS, and activated KRAS increases CCL2 expression, which, as noted previously, can recruit CD68+ macrophages and TMEM119+ microglia into the GBM TME of GBM mouse models established in BALB/C nu/nu mice [44]. The findings described previously provide novel insights into EGFR-dependent GAM regulation and offer potential therapeutic targets for GBM patients that specifically harbor EGFR amplifications or mutations.

From another angle, circadian rhythm is an important regulatory system that plays a pivotal role in regulating cancer cell proliferation, metabolism, and DNA repair [45-47]. Circadian locomotor output cycles kaput (CLOCK) is a key circadian regulator, which can function as an oncogene or a TSG based on TME factors [48]. In GBM, CLOCK is amplified in ~5% of human GBM cases and functions as an oncogene [48]. Inhibition of CLOCK by shRNA KD or pharmacological inhibition via SR9009 treatment in human GBM PDX lines (e.g., T3565, T387, GSC272, and GSC20) reduces GSC self-renewal due to its effects on cell metabolism [48,49]. Unbiased profiling studies have shown that high cancer cell stemness correlates with increased immunosuppressive activities in human GBM, including enhancing the GAM component [50], suggesting a potential role of stemness regulation in triggering GAM infiltration in GBM. Examination of TCGA GBM datasets revealed that a microglia signature (but not a macrophage signature) was enriched in CLOCK-high relative to CLOCK-low GBM patients [48]. Furthermore, high expression of CLOCK in human (e.g., GSC272 and GSC20) and mouse (e.g., QPP7) GSCs has been shown to recruit CX3CR1+ microglia into GBM tumors [48]. Mechanistically, this is presumed to occur with CLOCK upregulating the expression of olfactomedin-like protein 3 (OLFML3) in GSCs, which serves as a chemokine for microglia [48]. Inhibition of CLOCK by shRNA KD or pharmacological inhibition via SR9009 treatment in human PDX lines (e.g., GSC272, GSC20, T387, and T3565 in SCID and NSG mice) and mouse (e.g., CT2A in C57BL/6 mice) orthotopic models can increase survival by reducing GSC stemness and microglia tumor infiltration relative to controls [48,49]. Similarly, tumor-bearing mouse survival was also enhanced following shRNA KD of OLFML3 in the GSC272 mouse model [48]. These data suggest that blockade of the GSC-microglia interplay via inhibition of OLFML3 might be a potentially promising therapeutic strategy in CLOCK-high GBM. However, the mechanism by which OLFML3 induces microglia tumor infiltration remains unknown. Future studies might identify druggable mediators of the effect of OLFML3 on microglia, which might thereby expand the number of putative therapeutic targets in CLOCK-high GBM.

Taken together, these findings highlight the notion that oncogene alterations (e.g., EGFR and CLOCK amplifications and/or mutations) in GBM cells can change the properties of GAMs (e.g., migration and polarization), which might in turn contribute to promoting GBM progression, at least from what has been assessed from mouse models. Although further studies are needed,
these findings provide a rationale for developing personalized therapeutic strategies that target specific oncogene alteration-mediated GBM-GAM symbiosis (e.g., EGFR and CLOCK). Moreover, these findings provide a framework for the discovery of druggable targets in GBM that harbor specific alterations of other important oncogenes, such as PI3K, cyclin-dependent kinase 4 (CDK4), and platelet-derived growth factor receptor alpha (PDGFRα).

**Impact of GAMs on GBM Progression**

Once macrophages and microglia have infiltrated into the TME, they are educated by GBM cells [20]. The inverse is also true (i.e., recruited GAMs can reciprocally promote GBM progression [20]) (Figure 3). A growing body of evidence indicates that GAMs promote GSC self-renewal, and stemness is known to be important for sustained tumor growth and resistance to therapy in GBM mouse models and patient samples [5,25]. Mechanistically, GAMs can regulate GSC self-renewal by secreting stemness-supporting factors, including heparin-binding EGF-like growth factor, IL-12, IL-1β, and CCL8 [51–53] as well as lipocalin 2, hepatocyte growth factor, vascular endothelial growth factor, and IL-6 [54] in mouse and human GBM models. Furthermore, recent studies have demonstrated that pleiotrophin (PTN) is expressed and secreted by GAMs, and PTN helps sustain GSC stemness through its receptor PTPRZ1, which activates AKT signaling in GBM PDX lines [55]. shRNA KD of PTN in alternatively activated macrophages impairs their ability to promote GSC growth, and inhibition of GSC-associated PTPRZ1 dramatically impairs GSC maintenance and tumorigenic potential in human PDX GBM models, such as T4121 and T0912 models in SCID mice [55]. GAMs that are skewed toward an alternatively activated

![Impact of Glioblastoma-Associated Macrophages/Microglia (GAMs) on Glioblastoma (GBM) Progression](https://biorender.com/)

**Figure 3.** Impact of Glioblastoma-Associated Macrophages/Microglia (GAMs) on Glioblastoma (GBM) Progression. Once infiltrated into the GBM tumor, GAMs contribute to GBM progression by promoting glioma stem cell (GSC) stemness, GBM cell proliferation, survival, altered metabolism, and migration as well as by suppressing CD4+ and CD8+ T cell activity, stimulating angiogenesis, and recruiting additional macrophages in mouse and human GBM models. This figure was created using BioRender (https://biorender.com).
phenotype appear to be essential for maintaining GSC populations in GBM mouse models and patients [17,56]. Reprogramming of GAMs toward a proinflammatory phenotype using vitamin B3 or amphotericin B not only impairs the stemness and tumorigenicity of human and mouse GSCs in vitro and in vivo but also can sensitize brain tumors to chemotherapy in PDX models (e.g., BT048 and BT53M) [57,58]. In addition to macrophages, microglia can also contribute to GSC maintenance in GBM. For instance, when microglia are stimulated by CD8+ T cell-derived CCL4, they secrete CCL5 that can increase GSC stemness by activating CD44/AKT/GSK3β/CREB signaling in GSCs isolated from Nf1lox/lox; GFAP-Cre mice [59]. Together, these findings suggest that GAMs are a component of the GSC niche, which can sustain GSC properties via secretion of a variety of stemness-supporting factors.

In addition to maintaining GSC populations, GAMs can influence multiple GBM biological properties, including proliferation, survival, and migration [20,60]. The influence may occur through a variety of mechanisms that include the secretion of soluble factors, the release of exosomes, as well as cell-to-cell contact. For example, GAMs secrete IL-6 and IL-1β that can enhance 3-phosphoinositide-dependent protein kinase 1 (PDK1)-mediated activation of phosphoglycerate kinase 1 (PGK1) [61] as well as PI3K/PKCδ-mediated activation of glycerol-3-phosphate dehydrogenase (GPD2) [62], respectively, in GBM cells (such as U87 and U251). These activations, in turn, can promote GBM cell glycolysis and proliferation [61,62]. Inhibition of the PDK1/PGK1 (shRNA KD of PGK1 or PDK1 inhibitor OSU-03012 treatment) or PI3K/PKCδ/GPD2 (shRNA KD of PDKCD or GPD2) axes in GBM cells or neutralization of macrophage-derived IL-6 or IL-1β can attenuate GAM-associated effects on tumor cell glycolysis, proliferation, and tumorigenesis in human (e.g., U87 and U251) and mouse (e.g., GL261) models [61,62]. In addition to cytokines, GAMs also secrete SPP1 [24], lysophosphatidic acid [63], and transforming growth factor (TGF)-β1 [64] and produce exosomes containing arginase-1 [65] to promote GBM biological activities (e.g., proliferation, survival, and migration). These protumor effects can also be achieved through a GAM-to-GBM contact mechanism that involves GAM-induced upregulation of PDGFRB in GBM cells [66]. Lastly, GAMs can promote GBM growth and progression via indirect mechanisms, which may include the recruitment of additional macrophages [35], the suppression of CD4+ and CD8+ T cell infiltration, the effector activity of these cells [35,36,67], as well as an increase in angiogenesis [24]. In summary, these studies suggest that GAMs may contribute to GBM progression by regulating several cancer hallmarks, which include increasing GBM cell survival, metabolism, migration and proliferation, promoting macrophage infiltration and angiogenesis, sustaining GSC stemness, and suppressing antitumor T cell activity (Figure 3). In addition, these findings highlight the therapeutic potential of targeting GAMs during the symbiotic interactions between GAMs and GBM cells. However, further studies are needed to characterize whether these GAM effects are context dependent (including species dependent). Ideally, these forward-looking investigations might lead to the development of novel candidate personalized therapies to treat GBM.

**Concluding Remarks**

While therapeutic strategies involving the identification of synthetic lethality or inhibiting crucial oncogene addiction have achieved success in treating some types of cancer [68], such approaches have yet to impact GBM patient outcomes. This could also be said for immunotherapeutic approaches that have been tested in treating GBM but have not succeeded. This opinion article has specifically highlighted the molecular circuitry between GBM cells with specific genetic backgrounds and the GAM component of the TME. Our current understanding suggests a context-dependent GBM–GAM symbiosis that appears to be essential for sustained tumor growth and progression but may also inform the development of personalized therapy in GBM (Figure 4, Key Figure).

**Outstanding Questions**

Among the different genetic alterations, which ones are the drivers regulating GAM recruitment and polarization? Answering this question might enable key elements of GBM–GAM symbiosis and associated therapeutic strategies.

What are the specific differences and similarities of GBM–GAM symbiosis in GBM in terms of different genetic alterations or in terms of the same genetic backgrounds at different tumor stages? Can we achieve successful clinical outcomes in GBM patients by blocking GBM–GAM symbiosis under specific genetic backgrounds? The definition of these issues might help to develop personalized therapies for GBM patients.

What are the specific regulatory and functional differences between macrophages and microglia in GBM? scRNA-seq studies might help to address this question. This knowledge might allow for the identification of specific macrophage- or microglia-targeted therapies for GBM.

What signaling pathways are essential for regulating GBM–GAM symbiosis? Such knowledge might help to identify novel therapeutic targets.

Can scRNA-seq identify new subpopulations and/or states of GBM cells, macrophages, and microglia as well as new GBM–GAM interactions? Answering this question might inform novel elements of GBM–GAM symbiosis.

Can organoid cultures mimic the in vivo TME during GBM–GAM symbiosis? This knowledge might allow for the design of translational preclinical studies targeting GBM–GAM symbiosis.

Does GBM–GAM symbiosis affect T cell-mediated immune responses and immunotherapies? If so, how? This can have key implications for developing novel strategies to improve antitumor efficiency from immunotherapies in GBM patients.

Is there a context-dependent GBM myeloid-derived suppressor cell (MDSC) symbiosis in GBM? Similar to macrophages, MDSCs originate from the bone marrow, have heterogeneous subpopulations, and can play an
Although our knowledge of the role of GBM–GAM crosstalk in tumorigenesis has increased in the last few years, multiple open questions remain regarding the molecular mechanisms underlying this symbiosis and how we might target this crosstalk, especially when taking into consideration the unique genetic features of individual GBM types (see Outstanding Questions). Absent from the previous text is a discussion of one of the hallmark features of GBM, intratumoral heterogeneity [69–72]. The heterogeneity and dynamic plasticity of GBM cells as well as the heterogeneity of GAMs (e.g., macrophage versus microglia and proinflammatory versus alternatively activated or their overlapping phenotypes) highlight the challenges in identifying patient-specific GBM–GAM circuits that might be therapeutically accessible.

**Key Figure**

**Context-Dependent Glioblastoma (GBM)–Macrophage/Microglia Symbiosis**

![Image of the figure showing oncogene activation, TSG inactivation, adhesion, migration, polarization, survival, metabolism, migration, proliferation, stemness, angiogenesis, and T cell activity.](https://biorender.com/)

**Figure 4.** Inactivation or activation of specific tumor suppressor genes (TSGs) or oncogenes in GBM cells can regulate the adhesion, migration, and polarization of macrophages and microglia via the secretion of soluble factors and exosomes or through a cell-to-cell contact mechanism in mouse and human GBM models. Reciprocally, such glioblastoma-associated macrophages/microglia (GAMs) can promote GBM cell survival, proliferation, metabolism, migration, and self-renewal. GAMs can also promote GBM progression via indirect mechanisms (e.g., stimulating angiogenesis and suppressing CD8+ and CD4+ T cell activity). This figure was created using BioRender (https://biorender.com/).

Single-cell RNA sequencing (scRNA-seq) may provide insights regarding patient-specific targets. With respect to preclinical research, an emerging alternative is to grow GBM tumors as ex vivo organoids, which may better recapitulate certain tumor features [72,73]. GBM organoid and macrophage/microglia cocultures could serve as a powerful model system to study the molecular circuits underlying GBM–GAM symbiosis under specific genetic backgrounds and to test therapeutic agents targeting their crosstalk.
Altogether, our current knowledge of the molecular GBM–GAM symbiosis and its functional impact on GBM progression is still at an early stage. Recent studies have provided valuable information showing that GBM–GAM interactions are of fundamental importance to the biologically aggressive characteristics of GBM. We anticipate that future studies may lead to a detailed understanding of context-dependent GBM–GAM symbiotic interactions and may offer a roadmap for the development of novel putative anticancer therapeutic strategies that disrupt this dynamic circuitry.

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Declaration of Interests
No interests are declared.

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