Mechanism and therapeutic potential of tumor-immune symbiosis in glioblastoma

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Glioblastoma (GBM) is the most aggressive and lethal form of brain tumor in human adults. Myeloid-lineage cells, including macrophages, microglia, myeloid-derived suppressor cells (MDSCs), and neutrophils, are the most frequent types of cell in the GBM tumor microenvironment (TME) that contribute to tumor progression. Emerging experimental evidence indicates that symbiotic interactions between cancer cells and myeloid cells are critical for tumor growth and immunotherapy resistance in GBM. In this review, we discuss the molecular mechanisms whereby cancer cells shape a myeloid cell-mediated immunosuppressive TME and, reciprocally, how such myeloid cells affect tumor progression and immunotherapy efficiency in GBM. Moreover, we highlight tumor-T cell symbiosis and summarize immunotherapeutic strategies intercepting this co-dependency in GBM.

Glioblastoma (GBM) is the most common and lethal form of brain tumor in human adults, with a median survival of ~14–16 months following initial diagnosis [1,2]. The current standard of care for patients with GBM includes maximal surgical resection followed by radiotherapy and/or chemotherapy, which offers minimal clinical benefits [1,2]. Moreover, clinical trials for targeted therapies (e.g., therapies targeting receptor tyrosine kinase signaling) have also failed to improve patient outcomes, largely due to inter/intratumoral genetic heterogeneity and instability, and insufficient target engagement within the brain [3].

GBM can be highly infiltrated by immune cells in the TME (see Glossary) [4–6]. The most frequent immune population within the GBM TME are myeloid-lineage cells, which include tumor-associated macrophages and microglia (TAMs), MDSCs, and neutrophils [7–10]. Increasing evidence underscores that these myeloid cells not only promote GBM tumor growth, but provoke an immunosuppressive TME to induce resistance of immunotherapies, including immune checkpoint inhibitor (ICI) therapies [7,9,11–15]. In-depth studies focusing on the immune landscape have revealed that immune cells in GBM are multifaceted [4,16,17]. For example, the proportion and functional status of immune cells vary based on the genetic background, molecular state (e.g., mesenchymal, classical, and proneural), and disease stage of GBM [18–20]. Upon infiltration into the TME, immune cells are educated by cancer cells to promote tumor progression, inhibit antitumor immunity, and induce immunotherapy resistance [15,21,22]. Together, these findings vastly expand our knowledge of the context-dependent symbiotic interaction between cancer cells and immune cells in GBM. In this review, we discuss the molecular mechanisms by which cancer cells shape an immunosuppressive TME by regulating the biology of myeloid cells and lymphocytes, and, reciprocally, the mechanisms by which immune cells affect GBM progression and immunotherapy efficiency. Moreover, we discuss the therapeutic potential of targeting tumor-myeloid cell symbiosis in combination with immunotherapies (e.g., ICI therapies) for treatment of GBM.

Highlights

- Heterogeneity is a hallmark of glioblastoma (GBM), which includes cancer cell heterogeneity (e.g., distinct genetic and epigenetic alterations) and tumor microenvironment (TME) heterogeneity (e.g., distinct stromal cell types with different phenotypes). Heterogeneity generates context-dependent GBM-TME crosstalk that is critical for tumor growth and treatment resistance.
- Among the TME, myeloid cells (e.g., macrophages, microglia, myeloid-derived suppressor cells, and neutrophils) are the most prominent and dominant cells that contribute to tumor progression and immunosuppression via symbiotically interacting with cancer cells and lymphocytes.
- Blockade of the tumor-immune symbiosis inhibits tumor progression and improves the effectiveness of immunotherapies (e.g., immune checkpoint inhibitor therapies) in GBM.
GBM-TAM crosstalk
TAMs are the most abundant type of cells in the GBM TME (accounting for up to 50% of total live cells of the whole tumor mass) and comprise two major subpopulations: bone marrow-derived macrophages (hereafter referred to as macrophages) and microglia (Box 1) [23]. Emerging evidence demonstrates that the context-dependent symbiotic interaction between cancer cells and TAMs is critical for GBM tumor growth by regulating distinct cytokines, chemokines, metabolites, and other factors [21]. Here, we discuss different molecular mechanisms underlying tumor-TAM crosstalk in GBM (Figure 1 and Table 1).

Genetic alterations
Genetic alterations in cancer cells can regulate macrophage and microglia biology [21]. As such, isocitrate dehydrogenase (IDH) mutations are associated with higher infiltration of microglia, while IDH wild-type GBMs harbor relatively higher levels of intratumoral macrophages [20]. By contrast, PTEN mutations in cancer cells induce an increased infiltration of macrophages into the GBM TME, but do not affect microglia [3]. However, GBM tumorigenesis is not triggered by a single genetic alteration, but by combined alterations in a series of core signaling pathways [24,25]. For example, mesenchymal GBM is enriched for mutational alterations in PTEN, TP53, NF1, and RB1 [26]. Loss of PTEN, TP53, and NF1 in cancer cells upregulates lysyl oxidase (LOX), CCL2, tumor necrosis factor (TNF)α, CCL5, and CX3CL1, which, in turn, triggers the infiltration of macrophages and/or microglia into the GBM TME [21]. Further evidence demonstrates that activation of PI3K/AKT/mTOR signaling [27] or loss of NF1 [28] in cancer cells increase the expression and secretion of chitinase-3-like protein 1 (CHI3L1), which, in turn, increases macrophage infiltration and immunosuppressive polarization. These findings highlight the role of cancer cell genetic alterations in affecting TAM biology in mesenchymal GBM, which is supported by the observation that mesenchymal GBM tumors from patients harbor significantly more TAMs compared with other tumor subtypes (e.g., classical and proneural) [29]. Within the GBM tumor, more macrophages surround mesenchymal-like cancer cells than do other subtypes of cancer cell (e.g., oligodendrocyte progenitor-like cancer cells) [17,30]. Together, we posit that inactivation of PTEN, TP53, and NF1 in mesenchymal cancer cells might regulate TAM biology by secreting distinct chemokines and factors (Table 1). However, further studies are needed to investigate whether the loss of RB1, which is highly mutated in mesenchymal GBM, affects TAM biology. By contrast, some chemokines and factors [e.g., colony stimulating factor (CSF)2, SLIT2, P-selectin, and SPP1] are highly expressed in mesenchymal GBM and can trigger TAM infiltration.

Box 1. Bone marrow-derived macrophages and brain-resident microglia in GBM
Lineage-tracing experiments in mice have demonstrated that monocytes can migrate to the brain and differentiate into macrophages during GBM progression. By contrast, microglia originate from yolk sac progenitors during embryonic development [21,121]. The morphological differences between macrophages and microglia have been observed using high-resolution open-skull 2-photon microscopy [122]. scRNA-seq and cellular indexing of transcriptomes and epitopes through space (CITE-seq) have shown that microglia usually occur in newly diagnosed GBM tumors. By contrast, macrophages are more prevalent in recurrent GBM tumors and in hypoxic regions of tumors. In addition, TAMs are highly plastic cells and can be polarized toward both immunosuppressive and immunostimulatory phenotypes, thus exhibiting distinct effects [21]. Moreover, TAMs show a phenotype-specific spatial distribution in which immunosuppressive and immunostimulatory TAMs are enriched in the tumor periphery and core, respectively [19,123].

The characterization of heterogeneous populations of microglia and macrophages in GBM tumors is still arduous due to the lack of specific markers. Integrin alpha 4 (ITGA4, also known as CD49D) has been identified as a specific macrophage marker to distinguish them from microglia in GBM [124]. Moreover, scRNA-seq and multicolor fluorescence-activated cell sorting (FACS) analyses revealed that P2RY12, TMEM19, CX3CR1, and Hexb are highly expressed in tumor-associated microglia, whereas FCGR2B, CLEC10A, CD1C, CD1B, CD207, and CD209 are highly expressed in macrophages [20,117,125,126]. By taking advantage of advanced technologies (e.g., CyTOF, spatial tissue characterization, and scRNA-seq), more compelling evidence supports the observation that microglia and macrophages have distinct transcriptomic profiles and expression signatures in GBM [4,16,121,127].

Glossary
Bone marrow-derived macrophage: type of macrophage that originates from bone marrow-derived hematopoietic stem cells.
Chimeric antigen receptor (CAR) T cell therapy: highly personalized form of adoptive T cell therapy that takes advantage of patient’s own T cells and is engineered to express a CAR, which comprises the antigen-recognition site of an antibody fused with the cytoplasmic domains of the T cell receptor chain and co-stimulatory receptors.
Cytometry by time-of-flight (CyTOF): technology that enables single-cell analysis of protein expression by using rare heavy metal isotope-conjugated antibodies.
Extracellular vesicles (EVs): membrane-bound submicron vesicles released by cells into the TME; includes exosomes (50–200 nm), microvesicles (100–1 μm), and large oncosomes (>1 μm).
Glioma stem cells (GSCs): population of highly malignant and self-renewing glioma cells, which have a crucial role in tumor maintenance and therapeutic resistance in GBM.
Immune checkpoint inhibitor (ICI): inhibitors that block immune checkpoint molecules (e.g., PD1 and CTLA4) to activate antitumor immune responses.
Indoleamine-2,3-dioxygenase 1 (IDO1): essential enzyme that catalyzes tryptophan to kynurenicine, leading to immunosuppression by regulating Tregs and effector T cells.
Isocitrate dehydrogenase (IDH): energy metabolic enzyme involved in the Krebs cycle.
Microglia: brain-resident monocytes differentiated from yolk sac progenitors during embryonic development.
Myeloid-derived suppressor cell (MDSC): immature myeloid cells from bone marrow that can induce immunosuppression and promote tumor progression.
Neutrophils: subset of granulocytes that act as the first line of immune defense in the body.
Regulatory B cells (Bregs): small population of B cells that can induce immunosuppression.
Regulatory T cells (Tregs): specific subpopulation of CD4+ T cells with strong immunosuppressive activity.
Single-cell RNA sequencing (scRNA-seq): approach for detection and quantitative analysis of RNA.
and immunosuppressive polarization. However, it is unclear whether their expression is regulated by the core genetic alterations observed in mesenchymal GBM [31–34]. Together, these findings suggest that genetic alterations in cancer cells enhance the expression and secretion of different chemokines and factors, which, in turn, regulate TAM biology in the GBM TME. Mirroring cancer cell actions, TAMs promote GBM progression by reprogramming cancer cells into a more aggressive state (Figure 1) [3,18,35]. For example, TAM-secreted cytokines, such as oncostatin M (OSM) and interleukin 11 (IL11), shift cancer cells toward a mesenchymal and stem cell-like state by activating the STAT3 pathway [17,18,35]. Additionally, TAM-derived extracellular vesicles (EVs) exhibit a similar protumor effect. Experimental evidence demonstrates that EV-containing miRNAs (e.g., miR-27a-3p, miR-22-3p, and miR-221-3p) trigger a proneural-to-mesenchymal transition by regulating the CHD7-ReiB/STAT3 pathway [36]. Therefore, blocking the genetic alteration-mediated tumor-TAM crosstalk is a promising therapeutic strategy for GBM.

Epigenetic alternations

Although genetic alterations in mesenchymal GBM have an important role in regulating TAM biology, tumor subtype features are fluid and dynamic. Multiple subtypes may coexist in the same tumor [5] and can shift during tumor progression and upon therapeutic intervention [37]. The cellular and molecular heterogeneity of the GBM TME suggests that, in addition to genetic alterations, alternative mechanisms contribute to the symbiotic interaction between cancer cells and immunosuppressive polarization. Reciprocally, GBM-educated TAMs convert cancer cells toward a more aggressive state (e.g., inducing MES transition and increasing stemness) via secretion of distinct factors (e.g., OSM and IL-11) and EVs, thus promoting tumor progression. Abbreviations: ALKBH5, alkB homolog 5; CCL2/5, C-C motif chemokine ligand 2/5; CHI3L1, chitinase-3-like 1; CLOCK, circadian locomotor output cycles protein kaput; CSF1, macrophage-colony stimulating factor; CX3CL1, C-X-C motif chemokine ligand 1; CXCL8, C-X-C motif chemokine ligand 8; EVs, extracellular vesicles; GBM, glioblastoma; HGF, hepatocyte growth factor; IL, interleukin; Kyn, kynurenine; LOX, lysyl oxidase; MES, mesenchymal; OLFML3, olfactomedin-like 3; OSM, oncostatin M; PGE2, prostaglandin E2; SETDB1, SET domain bifurcated histone lysine methyltransferase 1; TAM, tumor-associated macrophage and microglia; Trp, tryptophan; VEGFA, vascular endothelial growth factor A.

Figure 1. Molecular mechanisms underlying the cancer cell-TAM crosstalk in GBM. Genetic alteration (e.g., mutation/deletion of PTEN, TP53, NF1, and amplification/mutation of EGFR), epigenetic regulation (e.g., regulation of epigenetic factors SETDB1, ALKBH5, and CLOCK) and metabolic regulation (e.g., regulation of lipid and Trp metabolism) in cancer cells would trigger the expression and secretion of various cytokines and other factors (as indicated) that promote TAM infiltration and immunosuppressive polarization. Reciprocally, GBM-educated TAMs convert cancer cells toward a more aggressive state (e.g., inducing MES transition and increasing stemness) via secretion of distinct factors (e.g., OSM and IL-11) and EVs, thus promoting tumor progression. Abbreviations: ALKBH5, alkB homolog 5; CCL2/5, C-C motif chemokine ligand 2/5; CHI3L1, chitinase-3-like 1; CLOCK, circadian locomotor output cycles protein kaput; CSF1, macrophage-colony stimulating factor; CX3CL1, C-X-C motif chemokine ligand 1; CXCL8, C-X-C motif chemokine ligand 8; EVs, extracellular vesicles; GBM, glioblastoma; HGF, hepatocyte growth factor; IL, interleukin; Kyn, kynurenine; LOX, lysyl oxidase; MES, mesenchymal; OLFML3, olfactomedin-like 3; OSM, oncostatin M; PGE2, prostaglandin E2; SETDB1, SET domain bifurcated histone lysine methyltransferase 1; TAM, tumor-associated macrophage and microglia; Trp, tryptophan; VEGFA, vascular endothelial growth factor A.
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and TAMs. Glioma stem cells (GSCs) are intrinsically immune suppressive of both adaptive and innate immunity [38]. They can escape immune surveillance by recruiting immunosuppressive programmed death-ligand 1 (PD-L1) + macrophages. Independent of genetic selection, the infiltration of these immunosuppressive macrophages is triggered by epigenetic changes in GSCs following serial transplantation through immunocompetent hosts [39], suggesting a crucial role of cancer cell epigenetic regulation in influencing TAM biology. This hypothesis is reinforced further by a growing body of evidence highlighting the essential role of several epigenetic factors in regulating the GBM-TAM crosstalk (Figure 1 and Table 1). First, a gain-of-function screen of epigenetic regulators identified circadian regulator CLOCK as a key hit in GSCs that promotes microglia infiltration into the TME by transcriptionally upregulating olfactomedin-like 3 (OLFML3) and legumain (LGMN) [40]. In addition, CLOCK-regulated LGMN polarizes microglia toward an immunosuppressive phenotype, which, in turn, promotes tumor progression and suppresses antitumor immunity [41]. Second, SET domain bifurcated 1 (SETDB1) is a member of the methyltransferase family that can activate the AKT/mTOR pathway in cancer cells to upregulate the expression and secretion of CSF1, which, in turn, induces macrophage infiltration and immunosuppressive polarization [42]. As a result, these macrophages mediate the oncogenic effect of SETDB1 in GBM mouse models. Conversely, depletion of macrophages using liposomal clodronate impairs SETDB1 overexpression-induced tumor growth [42]. Finally, N6-methyladenosine (m6A) is one of the most abundant RNA modifications during GBM tumorigenesis [43]. This process can be erased by alkB homolog 5 (ALKBH5) demethylase [44]. Functionally, depletion or inactivation of ALKBH5 in cancer cells suppresses the expression and secretion of CXCL8, thus impairing hypoxia-induced macrophage recruitment and immunosuppression, as well as GBM tumor growth [45]. Together, these findings suggest that epigenetic regulation is involved in regulating tumor-TAM symbiosis in GBM, and highlight a therapeutic potential for targeting such epigenetic regulators.

**Metabolism**

From another angle, metabolic dysregulation is a hallmark of cancer [46]. Cancer cell metabolism not only ensures sufficient energy for maintaining tumor potential, but also regulates TAM biology [47,48], thus inducing a metabolism-dependent GBM-TAM symbiotic interaction (Figure 1 and Table 1). Lipid metabolism is one of the mechanisms that regulate this symbiosis. For example, the loading of lipids in cancer cells upregulates the expression and secretion of protumor factors [e.g., vascular endothelial growth factor A (VEGFA) and hepatocyte growth factor (HGF)] under hypoxic conditions, which, in turn, triggers the infiltration of macrophages both in vitro and in GBM mouse models [49]. Moreover, peroxidation of the polyunsaturated fatty acid arachidonic acid (AA) by cyclooxygenase-2 (COX2) in cancer cells leads to the production of prostaglandin E2 (PGE2) [50,51], which can promote TAM immunosuppressive polarization in GBM [51]. Unfortunately, the clinical application of COX2 inhibitors in patients with GBM is limited [50]. Recent studies demonstrated that PGE2 production can also be triggered by activation of arsenite-resistance protein 2 (ARS2)-induced monoaoylglycerol lipase (MAGL) signaling in GSCs,
and inhibition of the ARS2-MAGL axis impairs GBM progression and TAM-immunosuppressive polarization [51]. Thus, these findings suggest that deciphering the mechanism of fatty acid metabolism during GBM-TAM symbiosis would reveal new therapeutic targets for GBM.

Tryptophan (Trp) metabolism is an important mechanism contributing to therapy resistance across many cancer types, including GBM [52]. Trp-catabolic enzymes, such as indoleamine-2,3-dioxygenase 1 (IDO1), IDO2, and tryptophan-2,3-dioxygenase (TDO2), can mediate the first step of the kynurenine (Kyn) pathway and are upregulated in glioma cells [53]. The metabolites (e.g., Kyn and kynurenic acid) of this pathway can activate the aryl hydrocarbon receptor (AHR) on immune cells, including TAMs, thus affecting their function in the GBM TME [54]. Specifically, AHR is essential for TAM recruitment and Trp-induced TAM activation in GBM. Suppressing AHR genetically and pharmacologically inhibits GBM progression by impairing TAM infiltration and immunosuppressive polarization [20,54].

In line with the metabolic changes in cancer cells, aberrant amino acid metabolism in TAMs also contributes to tumor growth. GBM is characterized by a highly acidic TME due to severe hypoxia. To survive in such a low pH environment, myeloid cells (e.g., TAMs and MDSCs) catabolize arginine to polyamines, thus maintaining an immunosuppressive TME to promote GBM tumor growth [55]. Depletion of arginine or administration of a polyamine inhibitor (e.g., difluoromethylornithine) synergizes with radiation to improve the survival of GBM-bearing mice [55,56]. Together, these findings highlight that cancer cell and/or TAM metabolism (e.g., fatty acid, Trp, and arginine metabolism) can promote tumor growth by regulating GBM-TAM symbiosis.

**GBM-MDSC crosstalk**

MDSCs are a highly heterogeneous population of myeloid cells contributing to tumor immunosuppression (Box 2) [57]. Emerging evidence demonstrates a clear GBM-MDSC symbiosis, in which cancer cells contribute to MDSC infiltration and activation (Figure 2 and Table 1). During tumor progression, cancer cells secrete CCL20, IL8, CXCL1, CXCL2, and macrophage migration inhibitory factor (MIF) to recruit MDSCs from the bone marrow [58,59]. In line with cancer cell-derived chemokines, TAM-derived CCL2 also attracts CCR2^+^Ly6C^+^ monocytic MDSCs (M-MDSCs) into the TME in response to cancer cell-secreted soluble factors (e.g., osteoprotegerin and CCL20), thus resulting in local immunosuppression [55,60,61]. However, the number of infiltrating Ly6G^+^
polymorphonuclear (PMN)-MDSCs in CCL2- and CCR2-deficient mice is unchanged [61,110], suggesting that CCL2-CCR2 signaling does not contribute to the infiltration of PMN-MDSCs in GBM. Further evidence demonstrates that M-MDSCs express high levels of the MIF receptor...
CD74. Targeting M-MDSCs with the MIF-CD74 interaction inhibitor ibudilast significantly abrogated MDSC-induced immunosuppression [59]. Another MIF receptor, CXCR4, also participates in the recruitment of M-MDSCs into the GBM TME via SDF1α-CXCR4 signaling [64]. Together, these findings highlight the molecular mechanism underlying GBM-induced M-MDSC recruitment. Although the infiltration of PMN-MDSCs in GBM has remained poorly understood, a recent study revealed that CXCL1 and CXCL2 simultaneously enhance the infiltration of M-MDSCs and PMN-MDSCs into the TME in vivo [65].

Once MDSCs infiltrate the TME, they are further activated by different cytokines [e.g., macrophage (M)-CSF, granulocyte-macrophage (GM)-CSF, IL6, IL10, transforming growth factor (TGF)β, B7-H1, and IFNγ] [58] and EVs to promote tumor growth both directly and indirectly (Figure 2). First, GBM-derived exosomes promote MDSC expansion by transporting different miRNAs (e.g., miR-1246, miR-29a, and miR-92a) under hypoxic conditions [66,67]. Mechanistically, hypoxia-induced heterogeneous nuclear ribonucleoprotein A1 (hnRNPA1) facilitates miRNA packaging into exosomes. The secreted exosomes are then taken up by local MDSCs, which, in turn, promotes MDSC activation via the dual-specificity phosphatase 3 (DUSP3)/ERK pathway [66]. Upon activation, MDSCs express PD-L1 in a hypoxia inducible factor (HIF)1α-dependent manner, thus resulting in T cell exhaustion and inhibition of antitumor immunity [68]. Second, GBM-associated MDSCs produce exosomes containing PD-L1, thus inducing a rapid increase in PD-L1 in B cells via caveolae-mediated endocytosis. As a result, these B cells convert to regulatory B cells (Bregs) to inhibit antitumor immunity and promote GBM tumor growth [69].

Third, MDSCs can function as an intermediate between cancer cells and T cells, whereby GBM-derived EVs promote the expansion of M-MDSCs, which in turn, suppress T cell-mediated antitumor immunity in GBM [70]. Finally, metabolism regulates the protumor function of MDSCs in GBM. MDSCs can generate polyamines and fatty acids to maintain their protumor and immunosuppressive function in GBM [55,71]. As a result, inhibition of the arginine-ornithine-polyamine axis reduces the survival of myeloid cells, including MDSCs, activates antitumor immunity, and impairs GBM tumor growth [55]. Together, these findings highlight a vital role of MDSCs in regulating antitumor immunity and tumor growth in GBM mouse models.

**GBM-neutrophil crosstalk**

Neutrophils are the most abundant circulating leukocytes and are essential for innate and adaptive immune responses [8]. Since classical neutrophils and PMN-MDSCs share the same set of phenotypic cell surface markers (e.g., CD11b+CD14−CD15+/CD66b+ and CD11b+Ly6G+Ly6Clo for human and mouse, respectively), functional analysis is required to distinguish between them [58,72]. PMN-MDSCs display strong immunosuppressive activity by suppressing T cells, whereas neutrophils do not [58]. Due to a lack of effective identification and isolation methods, researchers tend to investigate the function of total neutrophils (CD66b+ and Ly6G+ for human and mouse, respectively) in GBM, which may contain both classical neutrophils and PMN-MDSCs [73,74]. Here, we discuss recent findings highlighting the role of cancer cell-neutrophil crosstalk in GBM progression (Figure 2).

Recent studies revealed that neutrophil infiltration in GBM is associated with tumor genetic backgrounds and molecular states. For example, IDH mutation in GBM tumors suppresses neutrophil infiltration [4], whereas telomerase reverse transcriptase (TERT) mutation promotes such immune cell infiltration [62]. Neutrophils infiltrate the early stage of mesenchymal GBM tumors, in which chemokines (e.g., CXCL1, CCL3, CXCL2, G-CSF, IL1β, and ICAM1) are secreted by cancer cells [63]. In addition, several other cancer cell-derived factors (e.g., IL8, CXCL3, CXCL5, and osteopontin) have been shown to function as potent neutrophil chemokines [75–77]. However, it is unclear whether the expression of these chemokines is associated with genetic mutations
Neutrophils also manifest substantial plasticity in the GBM TME, although their roles in tumor progression remain controversial. On the one hand, neutrophils may exhibit antitumor properties by releasing reactive oxygen species (ROS) [78]. On the other hand, neutrophils promote GBM progression through distinct mechanisms. First, neutrophils promote ferroptosis-mediated tumor necrosis during GBM progression. Interestingly, neutrophils are spatially and temporally correlated with necrosis in the TME, in which neutrophils transfer myeloperoxidase-containing granules into cancer cells to induce ferroptosis, thus inducing necrosis via accumulation of lipid peroxides [73]. Depleting neutrophils or inhibiting ferroptosis diminishes neutrophil-induced cancer cell necrosis and tumor aggressiveness [73]. Second, neutrophils induce therapy resistance by upregulating GSC self-renewal and mesenchymal transition. For example, in an irradiated GBM model, Ly6G+ neutrophils and MDSCs promote the conversion of cancer cells into GSCs by regulating the nitric oxide synthase 2 (NOS2)-nitric oxide (NO)-ID4 signaling axis [79]. Moreover, antiangiogenic therapy increases neutrophil infiltration in GBM, which, in turn, upregulates GSC proliferation, migration, and mesenchymal transition via activation of S100A4 signaling [80]. Third, neutrophils promote GBM progression by releasing DNA into the extracellular space to form neutrophil extracellular traps (NETs) [81]. Consequently, NETs interact with the receptor for advanced glycation end-products (RAGE) on cancer cells to promote CXCL8 expression and secretion by upregulating the ERK/NF-κB signaling pathway (Table 1). The secreted CXCL8 further binds to CXCR2 on neutrophils to form additional NETs, thus inducing a positive feedback loop to promote GBM progression [77].

The opposite effects of neutrophils in GBM may relate to tumor stages. Neutrophils can infiltrate the early stage of tumors in preclinical models, and depletion of neutrophils at this initial stage promotes tumor growth and reduces survival of GBM-bearing mice. By contrast, depletion of neutrophils at the late stage of tumor growth has no such effect [63]. This phenomenon is also supported by functional studies showing that neutrophils from healthy mice suppressed the tumorigenicity of GSCs in a syngeneic mouse model, whereas neutrophils from tumor-bearing mice promoted tumor progression and immunosuppression [63]. Mechanistically, the antitumor effects of neutrophils during the early tumor stage primarily rely on their cytotoxicity, ferroptosis, and immune-stimulating activities [74]. However, tumor-educated neutrophils change their phenotypes to exhibit a protumor function [63]. Further studies revealing the molecular mechanisms underlying the context-dependent GBM-neutrophil symbiosis will help to develop therapeutic strategies.

**GBM-T cell crosstalk**

T cells are lymphocytes with a vital role in antitumor responses. Naïve T cells can be recruited and trained in the thymus for differentiation into different subpopulations, including innate T cells (e.g., natural killer T cells) and adaptive T cells (e.g., cytotoxic CD8+, helper CD4+, and memory T cells) [82]. Emerging evidence demonstrates that different subsets of T cells can affect cancer cell biology via distinct mechanisms. For example, CD8+ cytotoxic T cells can induce cancer cell apoptosis via cell–cell interactions or secretion of effector molecules. CD8+ T cells tend to directly kill cancer cells by recognizing MHC-I and activating cytotoxic signals. However, cancer cells may adaptively express low MHC-I to escape recognition and attack by CD8+ T-cells [10]. By contrast, CD4+ T cells recognize MHC-II molecules on antigen-presenting cells (e.g., dendritic cells and macrophages) [83]. Nevertheless, a recent single-cell RNA sequencing (scRNA-seq) study proposed that glioma-infiltrating CD4+ T cells also express cytotoxicity genes [84], enabling them to affect cancer cells directly by secreting TNFα and IFNγ [10]. In addition, CD4+ T cells...
may help CD8+ T cells to mediate antitumor immunity in GBM [10]. For instance, CD4+ T cell-derived IL2 activates CD8+ T cells by promoting the expression of IL2 receptor α subunit (CD25), which, in turn, exhibits an antitumor activity [85]. Finally, regulatory T cells (Tregs) have been identified as a protumor subpopulation of CD4+ T cells in GBM tumor tissues and the circulatory system [86]. Tregs comprise two subpopulations: induced Tregs (iTregs) and natural Tregs (nTregs). In GBM, thymus-derived nTregs are the predominant Treg population and are closely associated with tumor progression and immunotherapy resistance [87]. Although this emerging evidence supports the role of T cell-mediated antitumor immunity in the TME, GBM is recognized as a ‘cold’ tumor due to bone marrow sequestration of T cells [88–90].

T cell receptor (TCR) sequencing on a multiregion of tumors has revealed that TCR repertoires within the TME are highly heterogeneous and spatially restricted [91]. These findings suggest a potential context-dependent regulation of cancer cells on T cell biology (Table 1). Compared with other GBM subtypes, mesenchymal cancer cells and GSCs express more numerous inhibitory immune checkpoint molecules (ICMs), such as PD-L1 [92,93], which is triggered by the Wnt ligand and activated epidermal growth factor receptor (EGFR) through promoting the binding of β-catenin/T cell-specific factor (TCF)/lymphoid enhancer-binding factor (LEF) to the CD274 promoter [94]. Consequently, cancer cell-expressed or EV-delivered ICMs (e.g., PD-L1) suppress T cell function and proliferation by ligating their corresponding receptors on T cells [95,96]. Similarly, the Wnt/β-catenin signaling pathway helps GSCs to escape T cell-mediated killing by suppressing MHC-I [93]. Alternatively, cancer cells and GSCs express various factors (e.g., ICOSLG, IDO1, and CCL1) to enhance Treg expansion in the TME [61,88,97,98]. As a result, T cells tend to be skewed toward Tregs rather than toward cytotoxic CD8+ T cells in GBM [99]. Disrupting the GBM-T cell crosstalk by inhibition of cancer cell-derived IDO1 improved the survival of GBM-bearing mice by suppressing Tregs [88,100]. Similarly, elimination of Tregs in GBM using anti-GITR (a GITR agonistic antibody that promotes Treg differentiation into CD4+ effector T cells) inhibited Treg-mediated immunosuppression and activated CD4+ T cell-mediated antitumor immunity, thus killing cancer cells [99]. These findings highlight a direct regulatory mechanism of cancer cells on T cells in GBM.

Additionally, myeloid cells may serve as a central hub to pass immunosuppressive signals from cancer cells to T cells. For example, cancer cell-derived IL6 and Kyn can upregulate PD-L1 expression on myeloid cells by activating the STAT3 pathway and TAMs via AHR, respectively, which, in turn, suppresses T cell function in the TME [54,101]. Together, these findings reveal the molecular basis for how cancer cells symbiotically interact with T cells and how this symbiosis affects tumor progression in GBM.

**Impact of GBM-immune symbiosis on the effectiveness of immunotherapies**

Tumor-immune symbiosis is critical for regulating antitumor immunity in GBM. Therefore, inhibition of this symbiosis may affect the effectiveness of immunotherapies, including ICI therapies [21,38,102]. Indeed, recent evidence showed that myeloid cell infiltration in GBM correlates with increased immunotherapy resistance [7,14,103]. Here, we summarize recent findings highlighting the role of targeting the symbiosis between cancer cells and immune cells (e.g., T cells, TAMs, MDSCs, and neutrophils) to improve the effectiveness of immunotherapies, especially ICI therapies, in GBM (Figure 3, Key figure).

Given the remarkable T cell dysfunction in GBM, multiple therapeutic approaches (e.g., ICIs) have been developed to target the GBM-T cell interaction. A randomized clinical trial suggested that patients newly diagnosed with GBM or with relapsed GBM would benefit from anti-PD1 therapy [12,104]. However, a Phase III randomized study did not conclusively demonstrate that patients
with recurrent GBM benefited from this anti-PD1 therapy [102]. We propose that these controversial results may relate to context-dependent GBM-T cell symbiosis. Indeed, recent studies demonstrated that nonresponders to anti-PD1 therapy harbor more PTEN mutations, whereas responders contain more mitogen-activated protein kinase (MAPK) pathway alterations (e.g., PTPN11 and BRAF mutations) and higher phospho-ERK1/2 expression in cancer cells [103,105]. Given the critical role of PTEN mutations in triggering macrophage infiltration in GBM [3] and the evidence showing that tumors that do not respond to ICI harbor a higher infiltration of myeloid cells (including TAMs and monocytes) but lower infiltration of T cells compared with responding tumors from GBM mouse models and patients [7,105], it is plausible that anti-PD1 therapy resistance in PTEN-deficient tumors may relate to TAMs. This hypothesis is supported by emerging evidence demonstrating that blockade of TAM immunosuppressive function through distinct strategies can improve the effectiveness of ICIs in GBM. First, macrophages from nonresponding tumors show higher expression of immunosuppression-related genes (e.g., PD-L1). Functionally, inhibition of macrophage PD-L1 and its alternative binding partner CD80 restores the antitumor effect of ICI therapies (e.g., combined anti-PD1 and anti-CTLA4 therapy) [7].

Second, the occurrence of TAM heterogeneity in GBM has encouraged researchers to develop new strategies to target specific TAM subpopulations. Studies using scRNA-seq and cytometry by time-of-flight (CyTOF) technologies identified novel macrophage populations that can contribute to ICI therapy resistance in GBM. For example, a unique population of CD73+ macrophages has been identified that exhibit an immunosuppressive function in GBM. Depleting CD73
in mice decreased the immunosuppressive CD206^Arg1^VISTA^PD1^CD115^ macrophage cluster, but increased the immunostimulatory inducible (i)NOS^+ myeloid cell clusters. As a result, the antitumor effect of combined anti-PD1 and anti-CTLA4 therapy was significantly improved by CD73 depletion [14].

Third, targeting the signaling axis (e.g., the ligand–receptor axis) responsible for GBM-TAM crosstalk is an additional promising strategy for enhancing the effectiveness of ICI therapies in GBM. For example, inhibition of the IL6 (cancer cell)-IL6R (TAM) axis [101], the PROS1 (TAM)-AXL (GSC) axis [106], the MAGL-PGE2 (GSC)-KLF4 (TAM) axis [51], the SLIT2 (cancer cell)-ROBO1/2 (TAM) axis [33], the CSF1 (cancer cell)-CSF1R (TAM) axis [107], and the CD47 (cancer cell)-SIRPα (TAM) axis [108,109] genetically and/or pharmacologically showed a robust synergy with ICI therapies in GBM mouse models. Finally, since immunostimulatory macrophages can be triggered by distinct cytokines (e.g., IL12 and IL23), cytokine-targeted therapies may help to reconstruct the TME to improve ICI efficiency. For example, addition of oncolytic herpes simplex virus (oHSV) G47D expressing IL12 significantly improved the efficacy of anti-CTLA4 and anti-PD1 therapy in GBM-bearing mice by increasing intratumoral immunostimulatory macrophages and enhancing T effector to Treg ratios [13].

Similar to blockade of GBM-TAM crosstalk, targeting the GBM-MDSC/neutrophil symbiosis might also improve the effectiveness of ICI therapies in GBM [38]. For example, CCR2 is highly expressed in M-MDSCs, and inhibition of CCR2 using CCR2-deficient mice or its antagonist CCX872 synergized with anti-PD1 therapy in GBM-bearing mice [110]. Similarly, depletion of neutrophils using anti-Ly6G antibodies enhanced the therapeutic efficiency of anti-PD1 therapy in a GBM mouse model [111]. These findings highlight that disrupting GBM-myeoid cell symbiosis is a promising therapeutic strategy for improving the antitumor response to ICI therapies in GBM.

In addition to ICIs, adoptive T cell therapy [e.g., chimeric antigen receptor (CAR) T cell therapy] is an alternative strategy to eliminate cancer cells by engineered T cells [112]. One such trial in patients with recurrent GBM used EGFRVIII-CAR T cells. However, the antitumor efficiency of this treatment was dampened by therapy-induced adaptive changes in the local TME and antigen loss in cancer cells [113]. To overcome these challenges, one strategy is to engineer EGFRVIII-CAR T cells to co-express a bispecific T cell engager (BiTE) against EGFR wild-type cancer cells [114]. The second strategy is to design a new CAR using a toxin as the targeting entity. This approach is based on the cancer cell-binding potential of chlorotoxin (CLTX). As a result, CLTX-CAR T cells efficiently limited tumor growth in the absence of off-target effects in GBM mouse models [115]. Together, these findings demonstrate that targeting GBM-T cell symbiosis is a promising strategy for improving the effectiveness of adoptive T cell therapy.

**Concluding remarks and future perspectives**

The remarkable developments in the field bring light to our understanding and ability to target the highly complicated TME and immune landscape of GBM [4,16,24,29,63,116,117]. Various inspiring findings, as discussed earlier, suggest that crosstalk between cancer cells and immune cells is essential for regulating tumor progression and immunotherapy resistance in GBM. During tumor progression, cancer cells recruit and educate immune cells (e.g., myeloid cells and T cells). Reciprocally, such infiltrating immune cells increase cancer cell aggressiveness and GSC stemness, and induce immunotherapy resistance. These findings suggest that targeting the tumor-immune symbiosis is a promising therapeutic strategy for GBM. Moreover, cancer cells, GSCs, and immune cells in the GBM TME are highly dynamic and plastic with respect to different disease stages, molecular states, mutational status, and treatments [4,21,38], which results in
context-dependent tumor-immune symbiosis and informs the development of personalized therapies for GBM.

Although ICI therapies exhibit a robust antitumor effect in various solid tumors, their applications in GBM have not yet been attained, likely due, at least in part, to the infiltration of immunosuppressive myeloid cells [7,14,102,103]. Based on its critical role in suppressing antitumor immunity, different approaches have been developed to enhance the response of GBM to ICI therapies in mouse models by regulating GBM-myeloid cell crosstalk [21,38,118]. Hence, in-depth mechanism studies underlying the GBM-immune symbiosis are critical for identifying effective therapeutic targets. Current strategies of targeting the tumor-immune symbiosis aim to block the ligand–receptor interaction, which not only inhibit GBM progression, but also reshape T cell biology, thus improving the antitumor efficiency of ICI therapies. Nevertheless, current knowledge of the tumor-immune symbiosis in GBM is still at an early stage (see Outstanding questions).

In addition to cancer cells, immune cells exhibit a remarkable heterogeneity in the TME. For example, recent unbiased scRNA-seq and CyTOF studies revealed unique TAM subpopulations, including CD73\(^{\text{high}}\) macrophages and a specific high-grade glioma-associated microglia (HGG-AM), that have an important role in GBM development [14,119,120]. Genetic studies confirmed that depletion of CD73 in GBM-bearing mice exhibited a robust antitumor effect and synergized with ICI therapy [14]. Similarly, HGG-AM has protumor effects by secreting IL1\(\beta\) to promote GSC proliferation via apolipoprotein E (ApoE)-mediated NLRP1 inflammasome formation [120]. Supporting by this initial success, further comprehensive studies using scRNA-seq, CyTOF, and additional advanced technologies (e.g., advanced imaging, whole-exome sequencing, CRISPR knockout screening, high-throughput screening, brain tumor organoids, nanotechnology, tumor-on-a-chip systems, and exosome delivery systems) followed by detailed molecular studies are needed to identify context-dependent tumor-immune symbiosis and to reveal the molecular basis underlying its role in promoting tumor growth and immunotherapy resistance. As a result, these studies will lead to the identification of novel and effective therapeutic strategies intercepting the context-dependent tumor-immune symbiosis in GBM.

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Declaration of interests

No potential conflicts of interest were disclosed by the authors.

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