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# Signal Pathways Involved in the Interaction Between Tumor-Associated Macrophages/TAMs and Glioblastoma Cells

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It is commonly recognized, that glioblastoma is a large complex composed of neoplastic and non-neoplastic cells. Tumor-associated macrophages account for the majority of tumor bulk and play pivotal roles in tumor proliferation, migration, invasion, and survival. There are sophisticated interactions between malignant cells and tumor associated-macrophages. Tumor cells release a variety of chemokines, cytokines, and growth factors that subsequently lead to the recruitment of TAMs, which in return released a plethora of factors to construct an immunosuppressive and tumor-supportive microenvironment. In this article, we have reviewed the biological characteristics of glioblastoma-associated macrophages and microglia, highlighting the emerging molecular targets and related signal pathways involved in the interaction between TAMs and glioblastoma cells, as well as the potential TAMs-associated therapeutic targets for glioblastoma.

**Keywords:** glioblastoma, macrophage, microglia, signal pathway, interaction, therapeutic targets

## INTRODUCTION

Glioblastoma multiform (GBM) is the most common primary malignant tumor of the central nervous system with an annual incidence rate of 3-5/100,000 and a dismal prognosis of 14.6 months, accounting for about 50% of all gliomas (1, 2). Both intrinsic characteristics of cancer cells and extrinsic interaction with the sophisticated tumor microenvironment (TME) lead to treatment

resistance and tumor aggression (3). TME comprises complex non-cell constituents, such as extracellular matrix, interstitial fluid, growth factors, cytokines, chemokines, and angiogenic molecules, and multicellular components including both immune and non-immune cells that form a tumor-supportive milieu in which tumor cells grow and infiltrate (4). The miscellaneous non-neoplastic cells closely interact with each other and neoplastic cells in the TME, contributing to strong interdependence that drives tumor aggression (5).

It has been largely demonstrated that glioma cells strongly interplay with the most abundant non-neoplastic immune infiltrates in the TME called tumor-associated macrophages (TAMs)/microglia (6, 7). Tumor-associated macrophages (TAMs) display remarkable diversity and plasticity in TME and can change their characteristics accordingly in response to environmental cues (8). Traditionally, TAMs are classified as two extreme polarizations with M1 polarization (classically activated macrophages) on one end and M2 polarization (alternatively activated macrophages) on another end, which is oversimplified in the context of GBM. A more informative macrophages classification leads to a spectrum of macrophage populations based on their function (9). Investigations revealed that macrophages with different phenotypes coexist within the same mouse and human TAM population. Generally, TAMs presented as a common theme of regulatory and immunosuppressive phenotype with high diversity (10).

Furthermore, TAMs account for 30-50% of GBM tumor bulk, so targeting TAMs may be a reasonable and promising adjunctive therapy for these difficult-to-control cancers (11). To fully understand the complex interaction between TAMs and glioblastoma cells, this paper reviews the biological characteristics of glioblastoma-associated macrophages and microglia, with emphasis on molecular targets and related signal pathways arising from the interaction between TAMs and glioblastoma cells, as well as the related potential therapeutic strategies for glioblastoma treatment.

## THE BIOLOGY OF GLIOBLASTOMA-ASSOCIATED MACROPHAGES AND MICROGLIA

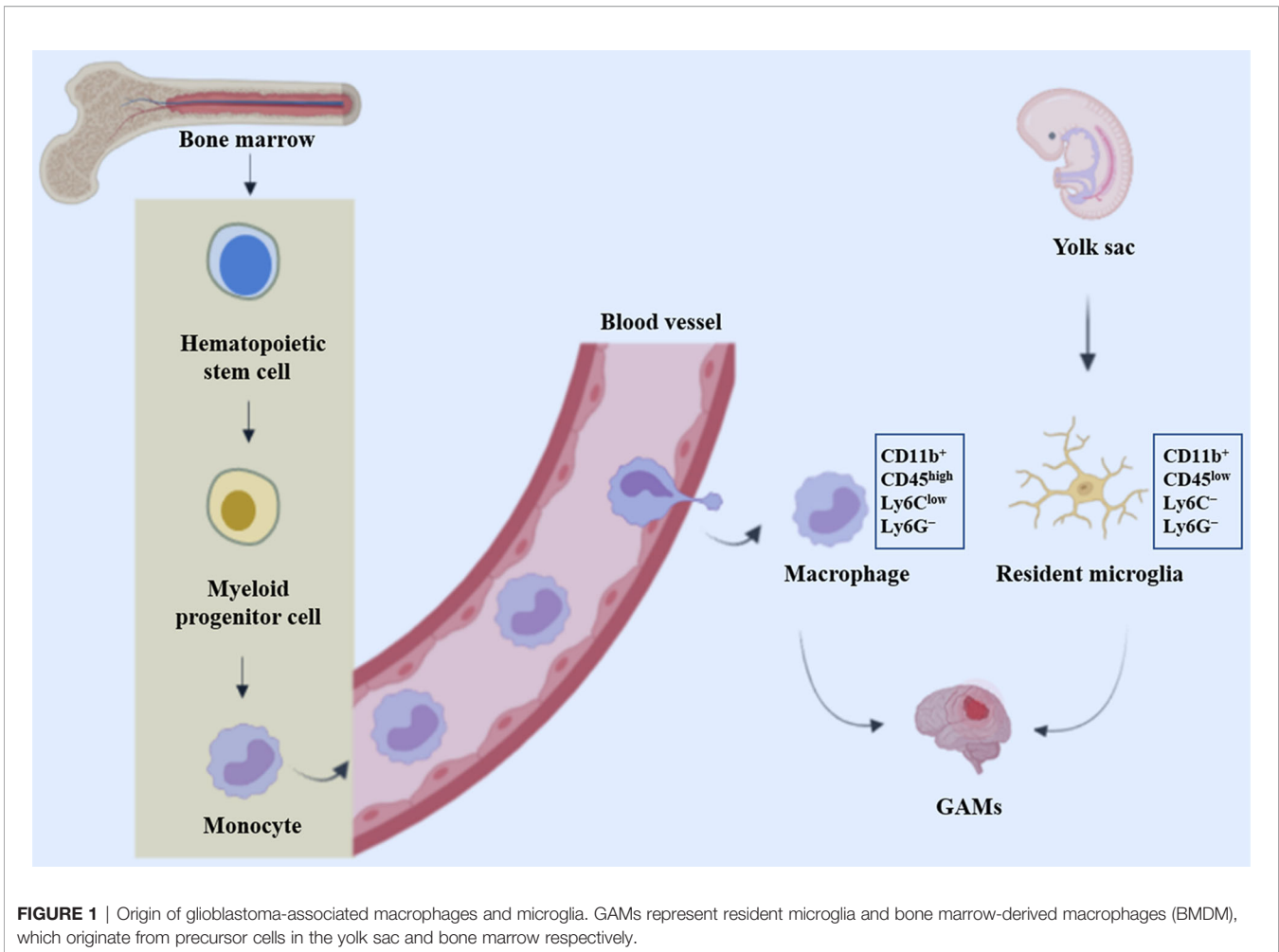
TAMs are widely believed to represent two types of non-neoplastic immune cells that are similar in morphology and function but differ in the ontology: resident microglia and bone marrow-derived macrophages (BMDM) (12). Microglia are originated from myeloid precursors inhabited in the primitive yolk sac and are distributed throughout the brain during embryogenesis (13). These resident mononuclear cells function as key immune effector cells, playing pivotal roles in health and disease conditions of the central nervous system (CNS). In addition, other ontogenesis of brain microglia may reflect different waves of yolk sac hematopoiesis (14). Unlike resident microglia, macrophages typically penetrate through the blood-brain barrier into the CNS in the context of neuropathology,

either through peripheral circulation or through direct channels connecting the skull bone and brain (15) (**Figure 1**).

Microglia are first discovered and described by Pio Del Rio Hortega about a century ago. Microglia ontogenesis and its homeostasis regulating mechanisms in health and disease conditions have been a hotspot for many decades (16). The main reasons for the confusion were the use of particular experimental systems, including chimera mice generated by bone marrow (BM) transplantation of lethally irradiated recipients, and monocyte classification schemes dependent on the expression of specific cell surface molecules (17). Through bone-marrow transplantation, researchers found that under homeostatic circumstances, a considerable proportion of microglia was superseded by donor-derived monocytes (18). Other similar studies have also indicated that both the endogenous microglia self-renewal and the dynamic recruitment of BM-derived microglial progenitors from the blood circulation contribute to augment in microglia density in reaction to CNS damage (19–23). Circulating progenitor cells contribute little to the brain microglia pool, suggesting that microglial proliferation during microgliosis (microglial activation) is mainly attributed to the local expansion of pre-existing resident microglia (24). These seemingly contradictory findings are eventually resolved with the use of chimeric animals produced by parabiosis, which does not necessitate either irradiation or transplantation. No microglia recruitment from the bloodstream was observed using two acute and chronic microglial activation (axotomy and neurodegeneration) (24). Additionally, Ajami et al. observed that acute peripheral monocytes recruitment in an experimental mouse model of autoimmune encephalitis (EAE) notwithstanding, these infiltrating cells vanished on remission and did not contribute to the endogenous microglia pool (25). Furthermore, recent fate-mapping studies have identified immature yolk sac progenitors as the predominant source of CNS microglia.

Taken together, these studies disclose that mouse myeloid progenitors from the blood circulation are not substantially participating in the pool of adult microglia after birth, thus determining that the pool of adult microglia mainly stems from yolk sac derived progenitors and maintain themselves by virtue of longevity and limited self-renewal (13, 24, 26). Single-cell RNA sequencing (scRNA-seq) of CD11b+ myeloid cells in naïve and GL261 glioma-bearing mice demonstrated considerable cellular and functional heterogeneity of myeloid cells in TME and is indicative of sex-specific discrepancies in responses of myeloid cells to gliomas (27). The ontogenesis of miscellaneous myeloid cells in the CNS is discussed in greater detail in a previous review (11). Furthermore, additional studies showed microglia located in different compartmentalization of mouse brain possess different transcriptomic information, suggesting that there are different microglia sub-phenotypes in both the human and mouse depending on their topological distribution and protein expression levels (28)

There is sufficient evidence that all tissue macrophages originate from a hematopoietic stem cell (HSC) pool during embryogenesis in the fetal liver (29–31). On day 12.5 of the



embryogenesis (E12.5), HSCs develop into fetal monocytes characterized as two subsets including  $CCR2^+Ly6C^+CX3CR1^{int}$  and  $CCR2^+Ly6C^-CX3CR1^{high}$  (30, 32). Lineage tracing experiments showed that the  $Ly6C^+$  subset was an imperative precursor of the  $Ly6C^-$  subset with a restricted lifespan (29). In addition, the  $Ly6C^+$  monocytic population emigrates from the fetal liver into the blood, leading to the downregulation of  $Ly6C$  and the initial expression of  $CX3CR1$ , which culminates in the tissue infiltrating macrophages (33). With few exceptions, splenocytes (34) and skin or gut macrophages remain in the tissue postnatally with longevity and limited self-renewal (31, 35, 36). After birth and during adulthood, hematopoiesis occurs mainly in the bone marrow, but also in the spleen, where  $Ly6C^+$  monocytes are produced and extravasated from the bone marrow into the bloodstream by monocyte chemoattractant proteins (MCPs). Under healthy circumstances, monocytes have extremely short circulation half-lives with a period of 19 h for  $Ly6C^+$  and  $\sim 2.2$  days for  $Ly6C^-$  (29). However, in the presence of pathological lesions such as brain tumors or inflammation, the blood-brain barrier (BBB) is disrupted, monocytes infiltrate and fill in the inflamed brain tissue, where

they differentiate into BM-derived macrophages (BMDMs) (31, 37, 38).

In the monitoring mode, microglia are morphologically highly ramified and, when activated, they rapidly transform into an amoeboid shape (39). However, BMDM is morphologically similar to activated microglia and is indistinguishable on histological sections. When lineage tracing is not available, they can be discriminated employing differential expression of the  $CD11b/CD45$  markers with  $CD45$  low in microglia and high in macrophages, together with the  $Ly6C$  and  $Ly6G$  markers ( $CD11b^+CD45^{low}Ly6C^-Ly6G^-$  for microglia and  $CD11b^+CD45^{high}Ly6C^{low}Ly6G^-$  for macrophages) (40). In addition, emerging evidence support the view that microglia and macrophages are located in different regions of malignant gliomas, with macrophages appearing to be recruited early in tumorigenesis and to inhabit perivascular region (40). However, data on the dominant monocyte population in these tumors, with some studies demonstrating a microglia predominance (41), while others report infiltrating bone marrow-derived macrophages representing the majority of the glioma-associated macrophage (GAM) population (40, 42). These

discrepancies could be attributed to specific experimental mouse model systems including the RCAS model and GL261 or T387 cell lines utilized in each of these studies, indicating that variations in GAM populations may be distinguishingly determined by the molecular characteristics of the glioma. In the future, the distinctive roles of microglia and blood monocytes in disease pathogenesis should be investigated thoroughly, to clarify the fate and origins of blood monocytes.

## EFFECTS OF GLIOBLASTOMA ON TAMs

### Recruitment of TAMs

Glioblastoma cells recruit microglia and monocyte to evolve tumor niche through the establishment of chemokine gradients, resulting in the accumulation of TAMs in and around glioma tissue with an amoeboid morphology. Many factors mediate the recruitment of TAMs, such as chemokines, ligands of complement receptors, neurotransmitters, and ATP (43). It remains to be determined whether there exist distinct factors that attract intrinsic resident microglia or peripheral monocyte-derived macrophages to the tumor.

### Classical Chemokine Signals

Monocyte chemoattractant protein-2 (CCL2) is the first chemoattractant factor to be discovered and CCL2/CCR2 signaling is significant in chemo-attraction during neuro-inflammatory processes (44). In some experimental glioblastoma models, tumor cells released CCL2 to attract macrophages (45), and CCL2/CCR2 blockade prolonged mouse survival (40, 41). Similarly, CCL2-expressing glioma cells produced a 10-fold increase in Ox42-positive cell density in rat models, while tumors overexpressing CCL2 increased more than three-fold, resulting in reduced rat survival (45). Moreover, Felsenstein et al. found that TAMs in human GBM specimens and syngeneic glioma model expressed CCR2 to various extents. Inoculating a CCR2-deficient strain for glioma model revealed a 30% reduction of TAMs intratumorally (46). Jung et al. revealed that Necrotic cells induced the expression of CCL2/MCP-1 and CCL20/MIP-3 $\alpha$  in glioblastoma cells through activation of NF- $\kappa$ B and AP-1 and facilitated the recruitment of microglia into tumor tissues (47). However, Okada et al. observed a stronger correlation between MCP-3, rather than MCP-1 expression and the density of infiltrating microglia and macrophages, challenging to some extent the importance of MCP-1 to human glioma biology (48).

CX3CR1 is a receptor for the cytokine CX3CL1 (fractalkine), which is mainly expressed in microglia and is a reliable marker for microglia imaging *in vivo*. The CX3CL1 and CX3CR1 signaling cascade play pivotal roles in neuron-microglia communication, and downregulation of CX3CR1 compromises synapse plasticity during development (49). Nevertheless, there are inconsistent data concerning the importance of CX3CL1 in tumor-induced TAM infiltration (50–53). CSF-1/CSF-1R is another signal pathway involved in microglial recruitment. CSF-1 released from glioma cells functions as a chemo-

attractant, and CSF-1R antagonist reduced the infiltration of TAMs and ameliorated glioblastoma invasion *in vivo* (54, 55). In addition, glioma cells also secrete hepatocyte growth factor (HGF) and scatter factor (SF) as chemo-attractants for microglia, but this has only been validated in a microglial cell line (56). CXCL12 is another potent chemokine for microglia and macrophage, especially recruiting TAMs toward hypoxic areas (57). The growth factor glial cell-derived neurotrophic factor (GDNF) was initially discovered as a secreting factor from the glial cell line B49, promoting the survival and differentiation of dopaminergic neurons. Mouse and human gliomas also secrete GDNF, which function as a strong chemoattractant for microglia (58).

### Emerging Chemokines and Molecules Involved

Recently, a growing number of emerging chemokines have been validated to be implicated in recruiting TAMs. For instance, Zhou et al. demonstrated that Glioblastoma stem cells (GSCs)-secreted periostin (POSTN) to recruit TAMs through the integrin  $\alpha$  $\beta$ <sub>3</sub>, as blocking this signaling by an RGD peptide inhibited TAM recruitment. Silencing POSTN in GSCs markedly reduced TAMs infiltration, inhibited tumor growth, and prolonged survival of mice bearing GSC-derived xenografts (59). Osteopontin (OPN) is an effective chemokine for macrophages, which blocks the ability of glioma cells to recruit macrophages significantly. Integrin  $\alpha$   $\beta$ <sub>5</sub> (ITG $\alpha$ v $\beta$ 5) is highly expressed on TAMs and constitutes a major OPN receptor. OPN deficiency in glioma cells led to a marked reduction in pro-tumor macrophages infiltrating the glioma (60). Profiling and functional studies in GBM models established that PTEN deficiency activates YAP1, which directly upregulates the expression of lysyl oxidase (LOX) expression. Mechanistically, secreted LOX induced TAMs recruitment *via* activation of the  $\beta$ 1 integrin-PYK2 pathway in macrophages. LOX inhibition dramatically attenuated macrophage infiltration (61). Differentiated Glioblastoma Cells (DGCs) exhibited a significant augment in YAP/TAZ/TEAD activity compared with GSCs. The transcriptional target CCN1 of YAP/TAZ was released abundantly from DGCs, but not in GSCs, which promoted macrophage migration *in vitro* and macrophage infiltration into tumor niche *in vivo* (62). CLOCK and its heterodimeric partner BMAL1 prompted GSC self-renewal and triggered tumor-supportive immune response through transcriptional upregulation of OLFML3, a novel chemoattractant recruiting immune-suppressive TAMs into the TME. In GBM models, *CLOCK* or *OLFML3* depletion decreased intra-tumoral microglia density and extended overall survival (63).

Some emerging molecules recruit TAMs directly as chemokines, while others indirectly modify TAMs infiltration by modulating classical chemokine signals. Takenaka et al. reported that glioblastoma cells produced kynurenine to activate aryl hydrocarbon receptor (AHR) in TAMs, which promoted CCR2 expression, subsequently driving TAMs recruitment in response to CCL2 (64). An et al. demonstrated that EGFR and EGFRvIII cooperated to induce TAMs infiltration through KRAS-mediated upregulation of the chemokine CCL2 (65). By analyzing proteomic and transcriptional data available for GBM tumors from The Cancer Genome Atlas (TCGA), Lailler et al. manifested

that GBM with high expression of phosphorylated ERK1/2 increased density of TAMs with a tumor-supportive M2 polarization. Using three human GBM cell lines in culture, they confirmed the existence of ERK1/2-dependent regulation of the production of CCL2 (66). Han et al. demonstrated that SETDB1 promoted AKT/mTOR-dependent CSF-1 induction and secretion, leading to macrophage recruitment in the tumor, and subsequently contributing to tumor growth (67). Additionally, De Boeck et al. found that IL-33 expression was positively correlated with the density of TAMs in a large subset of human glioma specimens and murine models, nuclear and secreted functions of IL-33 regulated chemokines that collectively recruited and activated circulating and resident innate immune cells. Conversely, loss of nuclear IL-33 crippled TAMs recruitment remarkably, inhibited glioma growth, and prolonged survival (68).

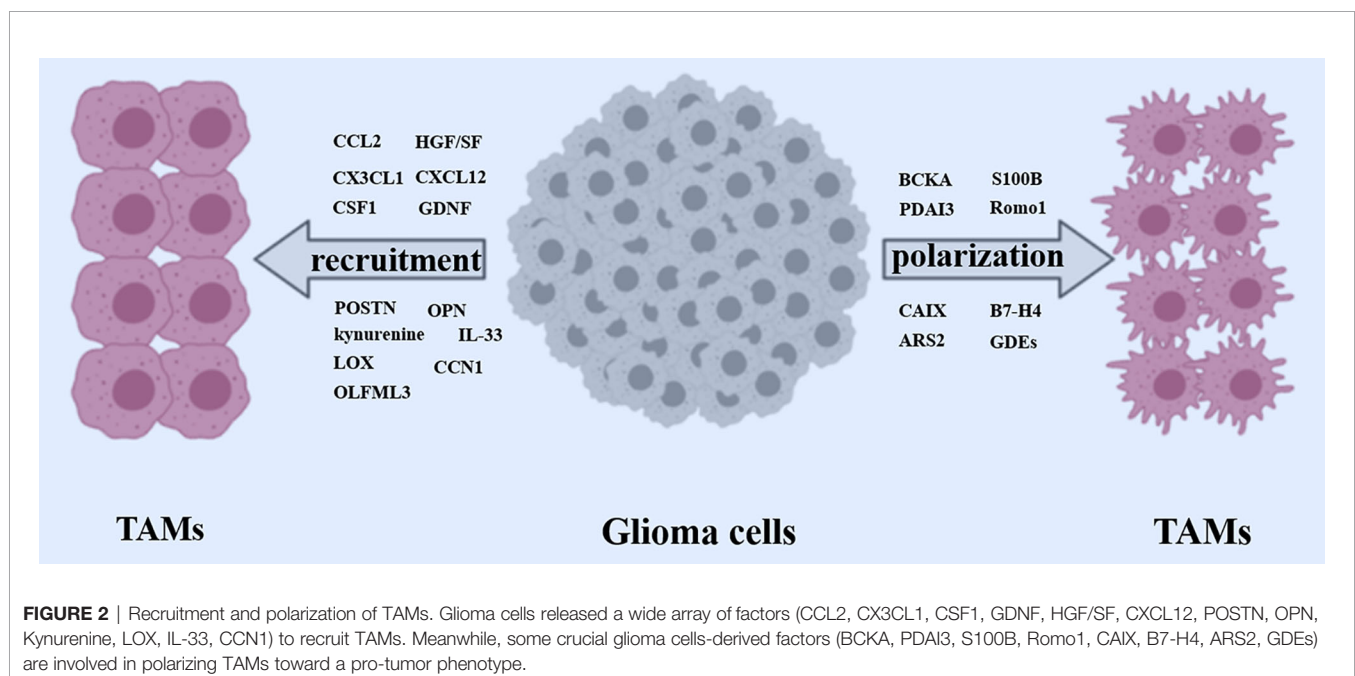
Transcriptome analysis indicated that most RSK1hi GBMs present as the mesenchymal subtype, and RSK1 expression was significantly associated with gene expression signature of immune infiltrates, especially in activated natural killer cells and M2 macrophages. In an independent cohort, Glaucia et al. confirmed that RSK1hi GBMs excluded long survivors, and RSK1 expression was positively associated with the protein level of the mesenchymal subtype marker lysosomal protein transmembrane 5, as well as with the TAM-associated CD68 (69). Tao et al. demonstrated that the Wnt-induced signaling protein 1 (WISP1) secreted by GSCs signals through Integrin  $\alpha6\beta1$ -Akt to sustain M2 TAMs through a paracrine mechanism. Silencing WISP1 markedly disrupted GSC maintenance, reduced TAMs infiltration, and potently suppressed GBM growth (70). In conclusion, there are a variety of glioma-derived factors involved in TAMs infiltration toward the glioma (**Figure 2**). Digging novel key

factors and the involved mechanism is still an attractive orientation moving forward in the future.

## Polarization of TAMs

TAMs are a heterogeneous population, not only because of their ontogenetic origin and distribution within the tumor but also to their functions. Historically, upon activation, TAMs were classified into two distinctive subsets, including M1 and M2 phenotype/polarization (71). Specifically, M1 is characterized by the classical activation of inflammatory receptors TLR2/4 and the secretion of pro-inflammatory cytokines such as TNF and IL-1 $\beta$ , polarized by lipopolysaccharide (LPS) either alone or in combination with Th1 cytokines such as IFN- $\gamma$  and GM-CSF, with a pro-inflammatory phenotype. On the contrary, M2 is defined as the anti-inflammatory phenotype with the production of ARG1, IL-10, and IL-4, polarized by Th2 cytokines such as IL-4 and IL-13 (71, 72). TAMs are considered to resemble an M2 polarization in the context of GBM (73). Nevertheless, transcriptional analyses have shown that this dichotomous classification is an oversimplification of the otherwise sophisticated biology of these cells (74). Microglia and macrophages possess both M1 and M2 phenotypes in the setting of murine brain tumors (75). For instance, both IL-1 $\beta$  and ARG1 were found to be enriched in TAMs (40). In human GBM, TAMs more closely resemble the expression profile of non-polarized M0 macrophages (76).

There are various GBM-derived factors involved in the polarization of TAMs toward a pro-tumor M2-like phenotype. S100B, a member of the multigene family of Ca<sup>2+</sup>-binding proteins, is overexpressed by glioblastoma. Gao et al. demonstrated that low concentrations of S100B attenuated microglial activation through the induction of the STAT3



**FIGURE 2** | Recruitment and polarization of TAMs. Glioma cells released a wide array of factors (CCL2, CX3CL1, CSF1, GDNF, HGF/SF, CXCL12, POSTN, OPN, Kynurenine, LOX, IL-33, CCN1) to recruit TAMs. Meanwhile, some crucial glioma cells-derived factors (BCKA, PDAI3, S100B, Romo1, CAIX, B7-H4, ARS2, GDEs) are involved in polarizing TAMs toward a pro-tumor phenotype.

signal pathway (77). Glioblastoma-associated macrophages (GAMs) have a higher expression of ERp57/PDIA3 than in the microglia present in the surrounding parenchyma. Chiavari et al. demonstrated that reduced PDIA3 expression/activity in glioblastoma cells markedly limited the microglia pro-tumor polarization toward the M2 phenotype and the secretion of pro-inflammatory factors (78). Yin et al. demonstrated that arsenite-resistance protein (ARS2), a zinc finger protein directly activated the novel transcriptional target MGLL, encoding monoacylglycerol lipase (MAGL), which stimulate M2-like TAM polarization through the production of prostaglandin E2 (PGE2) (79). Under hypoxic conditions, the expression of CAIX (carbonic anhydrase IX) regulated through EGFR/STAT3/HIF-1 $\alpha$  axis significantly increased in GBM, contributing to the polarization of tumor-associated monocytes/macrophages (TAM) toward a more tumor-supportive phenotype (80). Reactive oxygen species (ROS) modulator 1 (Romo1) is highly expressed in macrophages and is associated with the poor prognosis of glioblastoma patients. using the glioblastoma murine model, Sun et al. found that the overexpression of Romo1 led to the M2 polarization of bone marrow-derived macrophages (BMDMs) through the mTORC1 signaling pathway (81).

TAMs acquire an immunosuppressive phenotype in the GBM microenvironment. Silva et al. showed that glioblastoma cells excreted large amounts of branched-chain ketoacids (BCKAs), metabolites of branched-chain amino acid (BCAA) catabolism. Tumor-excreted BCKAs can be taken up and re-aminated to BCAAs by TAMs. BCKAs exposure attenuated the phagocytic activity of macrophages (82). In both *in vitro* and *in vivo* GBM mouse models, GBM-initiating cells induced mTOR signaling in the microglia but not bone marrow-derived macrophages. mTOR-mediated regulation of STAT3 and NF- $\kappa$ B activity promoted an immunosuppressive microglial phenotype, which hindered effector T-cell infiltration, proliferation, and immune reactivity, thereby contributing to tumor immune evasion and tumor aggression (83). Yao et al. identified that B7-H4+ glioma infiltrated macrophages/microglia showed immunosuppressive phenotype which could be regulated by IL-6 excretion. IL-6-activated STAT3 bound to the promoter of the B7-H4 gene and enhanced B7-H4 expression on TAMs, resulting in an immunosuppressive phenotype of TAMs, which contributed to GBM progression (84).

The components of tumor-derived exosomes such as microRNAs and proteins induce macrophages to M2-like polarization to support tumor growth (85, 86). Xu et al. found that compared with normoxic glioma-derived exosomes (N-GDEs), hypoxic glioma-derived exosomes (H-GDEs) drastically facilitated autophagy and M2-like macrophage polarization, which subsequently promoted glioma proliferation and migration *in vitro* and *in vivo*. The interleukin 6 (IL-6) and miR-155-3p were highly expressed in H-GDEs. Further experiments showed that IL-6 and miR-155-3p induced M2-like macrophage polarization through the IL-6-pSTAT3-miR-155-3p-autophagy-pSTAT3 positive feedback loop, contributing to glioma progression (87). The Glioblastoma-derived exosomes (GDEs) traversed the monocyte cytoplasm, resulting in a reorganization of the

actin cytoskeleton, and skewed monocytes toward the immune suppressive M2 phenotype, including programmed death-ligand 1 (PD-L1) expression. Mass spectrometry analysis demonstrated that the GDEs contain a variety of contents, including members involved in the signal transducer and activator of transcription 3 (STAT3) pathway that functionally mediate this pro-tumor immune-suppressive switch (88).

In addition to genetic regulation, a scenario of distinct histone modifications was identified to underlie the polarization of microglia by glioma, which demonstrates the contribution of epigenetic mechanisms to glioma-induced “transcriptional memory” in TAMs resulting in the tumor-supportive phenotype (89). Altogether, GAMs are genetically and epigenetically educated by a variety of factors from within glioblastoma cells or GDEs, leading to a pro-tumor immunosuppressive polarization, which results in GBM progression (Figure 2).

## Chemoradiotherapy and TAMs

The impacts of conventional therapies on TME have been largely investigated, indicating that chemoradiotherapies not only exert a direct cytotoxic effect on tumor cells but also modulate the immune infiltrates either in an anti-tumor or pro-tumor direction, depending on tumor types and chemotherapeutic agents (90–92). Chemoradiotherapy has a huge impact on TAMs recruitment and polarization. A clinical microdialysis study demonstrated that radiotherapy induced an immediate inflammatory reaction leading to TAMs recruitment, which was correlated with a short survival time in malignant glioma (93). Irradiation leads to the alteration of multiple pathways in the context of GBM. Particularly, it modifies the macrophage polarization, rendering them more supportive of tumor growth (91). Although controversies exist, mainstreams reported that chemotherapy induced TAMs recruitment and programmed them toward an immunosuppressive tumor-supportive polarization, contributing to tumor angiogenesis, T cell immunity suppression, and activating anti-apoptotic programs in cancer cells to induce chemoresistance (90, 94). Therefore, incorporating TAMs-targeting therapy into chemoradiotherapy may provide a promising choice for GBM treatment.

## Metabolic Reprogramming of TAMs

TAMs are characterized by remarkable plasticity and dynamic metabolic trait (95). In reaction to the altered metabolic profile of TME, TAMs evolve toward a cellular state which prioritizes utilizing glycolysis, fatty acid synthesis (FAS), and glutamine-glutamate metabolism (96, 97), influencing TAMs recruitment and polarization. Reciprocally, these functionally reprogrammed TAMs secrete a wide range of altered cytokines and angiogenic factors contributing to tumor growth and survival (98–100). Won et al. elucidated in review (101) that loco-regional metabolic signals released from tumor environments (glucose, glutamine, cysteine, lactate, IDO, adenosine, itaconic acid, acidic pH) have a huge impact on the polarization fate and immunosuppressive functions of TAMs, thus possibly leading to immune tolerance and treatment resistance in GBM. Therefore, regulation of the promoters and enhancers of tolerized genes involved in metabolism and lipid

biosynthesis may reverse the immune tolerance, transcriptionally rewiring the intracellular signaling of innate immune cells to make macrophages more competent in response to stimulation (10). Similarly, Carroll et al. found that inhibition of fatty acid synthase, which catalyzed the synthesis of long-chain fatty acids, prevented the proinflammatory response in macrophages (102). Intriguingly, metabolic profiling showed that exposure to  $\beta$ -amyloid stimulated acute reactive microglial inflammation accompanied by metabolic reprogramming from oxidative phosphorylation to glycolysis. Moreover, metabolic strengthening with recombinant interferon- $\gamma$  treatment counteracted the defective glycolytic metabolism and inflammatory functions of microglia (103). Such microglial metabolic switch may also exert huge influences on GBM development.

## TAMS REMODELING GBM PROLIFERATION AND INVASION

The fact that a great number of TAMs accumulated in and around glioma bulk has intrigued the investigators to explore their roles in tumor proliferation, migration, and invasion. As expected, accumulating evidence indicated that TAMs promote glioma growth and invasion *in vitro* and *in vivo*. One study has noted long before that the motility of the murine glioma cells was increased threefold at the presence of microglial cells *in vitro*. By contrast, endothelial cells and oligodendrocytes only slightly promoted glioma motility (104). *In situ*, organotypic brain slices can be used to monitor glioma growth. These slices showed reduced invasion and growth of gliomas when microglia cells were removed with liposomes filled with the toxin clodronate (105). In addition, an alternative *in vivo* approach made the use of transgenic mice expressing the herpes simplex virus thymidine kinase gene under the control of the CD11b promoter, which was specifically expressed by microglia in the central nervous system. When ganciclovir was infused into the brain, there was a prominent reduction in microglia number, concomitantly resulting in attenuated glioma growth *in vivo* (106).

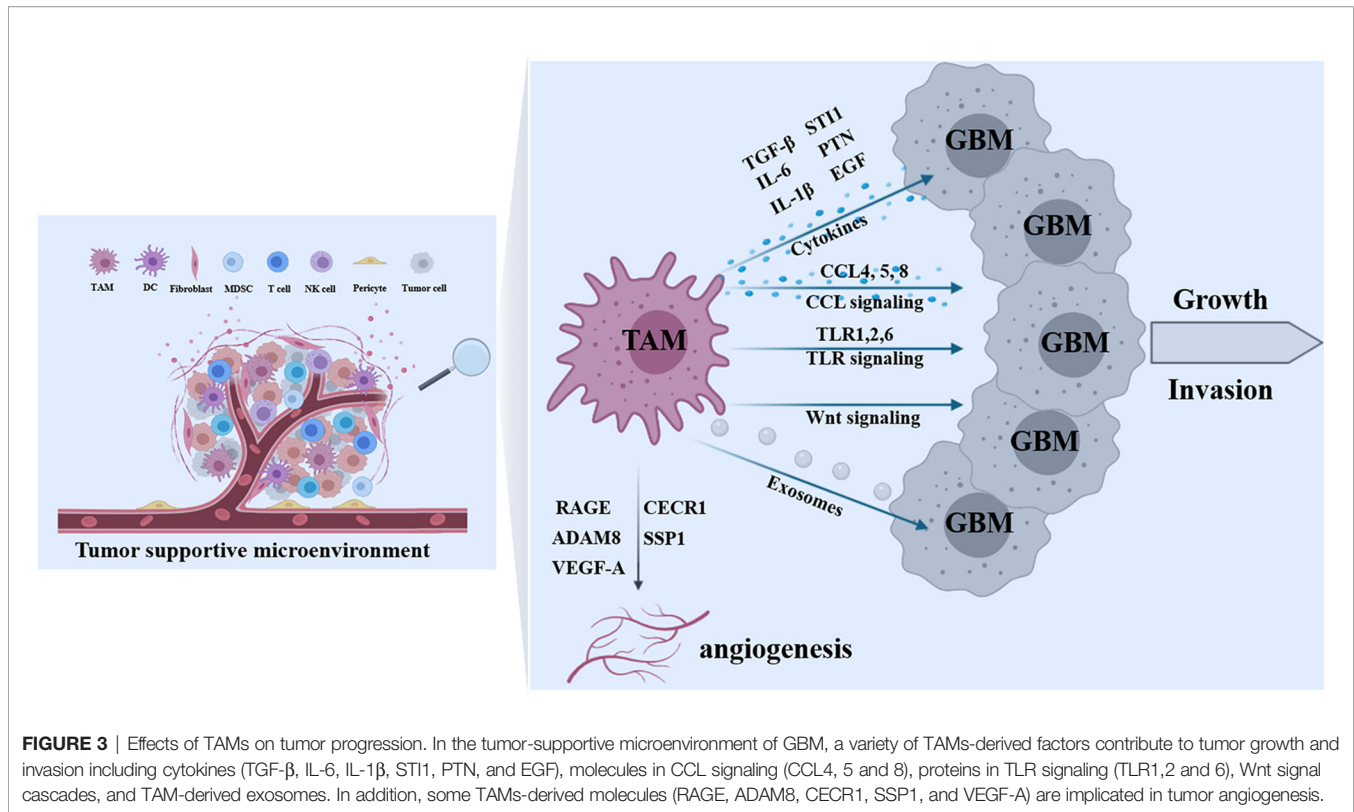
## Cytokine Signaling

As mentioned above, there are a variety of factors from glioblastoma cells to induce TAMs recruitment and polarization. Meanwhile, various factors from TAMs have been reported to promote glioma proliferation, migration, and invasion (Figure 3). CCL2 released from glioma is a critical chemokine for TAMs and simultaneously triggers IL-6 release from microglia, thereby promoting the invasiveness of glioma cells (107). IL-6 secreted by *in situ* macrophages regulated the direction of a PKG1-catalyzed reaction by increasing PDK1-dependent PKG1 phosphorylation in glioblastoma cells, promoting glycolysis and proliferation of tumor cells (108). Lu et al. demonstrated that interleukin 1 $\beta$  (IL-1 $\beta$ ) produced by M2 macrophages activated phosphorylation of the glycolytic enzyme glycerol-3-phosphate dehydrogenase (GPD2) at threonine 10 (GPD2 pT10) through phosphatidylinositol-3-kinase-mediated activation of protein kinase- $\delta$  (PKC $\delta$ ) in glioma cells. Blocking IL-1 $\beta$  generated by macrophages or

Inhibition of PKC $\delta$  or GPD2 pT10 in glioma cells attenuated the glycolytic rate and proliferation of glioma cells (109). In addition, microglia synthesized and released stress-inducible protein 1 (STI1), a cellular prion protein-ligand that increased the proliferation and migration of glioblastomas *in vitro* and *in vivo* (110). Microglia release epidermal growth factor (EGF), which also stimulates glioblastoma cell invasion (54). Transforming growth factor- $\beta$  (TGF- $\beta$ ) is predominantly produced by microglia when studied in co-culture systems, and blocking the TGF- $\beta$  function impairs glioma growth (111). In addition, TGF- $\beta$ 2 induced the expression of matrix metalloproteinase-2 (MMP2) and suppressed the expression of tissue inhibitors of metalloproteinases (TIMP)-2, which degraded the extracellular matrix to promote glioma invasion (112). Targeting TGF- $\beta$  signaling was initially considered as a potential anti-tumor therapy. However, systemic inhibition of TGF- $\beta$  signaling led to acute inflammation and disturbance of immune system homeostasis (111). CECR1 is a potent regulator of TAM polarization and is consistently highly expressed by M2-type TAMs, particularly in high-grade glioma. CECR1 mediated paracrine effects in M2-like TAMs stimulated MAPK signaling and activated the proliferation and migration of glioma cells (113). Shi et al. found that TAMs secreted abundant pleiotrophin (PTN) to stimulate glioma stem cells (GSCs), thus promoting GBM malignant growth through PTN-PTPRZ1 paracrine signaling. Co-implantation of M2-like macrophages (MLCs) promoted GSC-driven tumor growth, but depletion of PTN expression in MLCs mitigated their pro-tumorigenic activity. Disrupting PTPRZ1 abrogated GSC maintenance and tumorigenic potential. Moreover, interference of PTN-PTPRZ1 signaling by shRNA or anti-PTPRZ1 antibody potently suppressed GBM tumor growth and prolonged animal survival (114).

## CCL/CCR Axis

Furthermore, the chemokine (C-C motif) ligand is an important cluster of molecules involved in the process of TAMs-mediated glioma progression. Wang et al. found that both hypoxia and macrophage supernatant promoted GBM cells invasion and matrix metalloproteinase (MMP)-9 expression, and hypoxia modulated the invasive activity of GBM cells by upregulating CCR5 expression. The supernatant of hypoxic macrophages also showed a greater pro-invasion effect than that of normoxic macrophages by increasing CCL4 secretion. Moreover, they found that interferon regulatory factor-8 (IRF-8) was possibly involved in hypoxia-modulated CCL4 expression of macrophages. Taken together, the study found that the CCL4-CCR5 axis played significant roles in TAM-mediated glioblastoma invasion, and hypoxia enhanced the interaction between these two types of cells by upregulating both CCL4 and CCR5 expression, respectively (115). Chemokine (C-C motif) ligand 5 (CCL5) was reported to modulate the migratory and invasive activities of human glioma cells in association with MMP2 expression. In response to CCL5, glioma cells synchronously upregulated intracellular calcium levels and p-CaMKII and p-Akt expression levels. Inhibition of p-CaMKII suppressed CCL5-mediated glioma invasion and upregulation of MMP2. Glioma cells tended to migrate toward GAM-



conditioned media activated by the granulocyte-macrophage colony-stimulating factor (GM-CSF) in which CCL5 was abundant. This homing effect was related to MMP2 upregulation and could be ameliorated either by controlling intracellular and extracellular calcium levels or by CCL5 antagonism (116). In addition, CCL8 was highly expressed by TAMs and contributed to pseudopodia formation by GBM cells. CCL8 dramatically activated ERK1/2 phosphorylation in GBM cells and promoted invasion and stemlike traits of GBM cells through CCR1 and CCR5. Blocking TAM-secreted CCL8 by neutralized antibody markedly attenuated invasion of glioma cells (117).

### TLR Signal Pathways

Toll-like receptors are prominent detectors of DNA fragments or bacterial cell wall components and are crucial for mediating immunologic responses to pathogens (118). TLRs signaling pathways play an important role in the interaction between microglia and glioma, among which TLR2 is considered to be the main TLR that triggers MT1-MMP upregulation in microglia. Therefore, the implantation of mouse GL261 glioma cells into TLR2 knockout mice resulted in markedly smaller tumor volume and better survival rates compared with wild-type control mice. TLR2 forms heterodimers with TLR1 and TLR6, which was critical for modulating MT1-MMP expression, while silencing of both TLR1 and TLR6 resulted in reduced MT1-MMP expression. In addition, treatment with TLR2-neutralizing antibodies reduced glioma-induced

microglial MT1-MMP expression and attenuated glioma growth (119). In a screen for endogenous ligands secreted from glioma cells, versican was identified as a candidate molecule for triggering TLR2 signaling cascade (120). Versican exists as different splice variants such as V0, V1, and V2. The V0 and V1 isoforms are highly expressed in mouse and human gliomas and decreased glioma versican expression is correlated with reduced microglial MT1-MMP expression *in vitro* and *in vivo*. Furthermore, implantation of versican silenced glioma cells resulted in smaller tumors and longer survival rates relative to controls. Remarkably, the effect of versican signaling on glioma growth was reliant on the presence of microglia. Versican-mediated TLR2 expression polarized microglia into a pro-tumorigenic phenotype featured by the upregulation of MT1-MMP and MMP9 expression. This feed-forward loop presented us with a great example of the interdependent microglia-glioma interactions that contributed to glioma growth and invasion (121). Additionally, the MMP2 enzyme is released in a pro-form that needs to be cleaved to become active. The prominent enzyme for pro-MMP2 cleavage is the membrane-bound metalloprotease MT1-MMP. In this regard, slices obtained from MT1-MMP-deficient mice showed substantially smaller tumors. In addition, glioma growth was further reduced after microglia were removed from organotypic sections without MT1-MMP, suggesting that MT1-MMP is not the only glioma promoter expressed by microglia. In human glioma samples, MT1-MMP expression was positively correlated with the increasing malignancy of glioma (106).



## Wnt Signal Cascades

The Wingless-type MMTV integration site family (Wnt) proteins such as Wnt3a, Wnt5a, Wnt7a, Wnt5b, and Wnt2 participated in many biological processes (122). The Wnt signaling pathways consist of the  $\beta$ -catenin-independent pathway and the  $\beta$ -catenin-dependent pathway. The  $\beta$ -catenin-independent Wnt signaling pathway can be further divided into the Wnt/Planar Cell Polarity (PCP), Wnt/Calcium (Ca<sup>2+</sup>), and Wnt-dependent stabilization of proteins (STOP) signaling pathways (123). In the Wnt/ $\beta$ -catenin signaling pathway, Wnt proteins interact with the transmembrane receptor Frizzled and their co-receptor low density lipoprotein receptor-related protein 5/6 (LRP5/6), contributing to the stabilization of  $\beta$  catenin, its translocation to the nucleus, and consequent transcription of target genes essential to stem cell self-renewal, cell differentiation, polarization, and invasion (122,123). Increasing evidence indicated that Wnt signaling pathways play significant roles in the maintenance and progression of gliomas (124–126). Wnt signaling-induced proteins released from GSC mediate TAMs recruitment and M2-like polarization (70). In turn, Wnt proteins secreted from TAMs may further contribute to GBM stemness, mostly through the  $\beta$ -catenin dependent Wnt signaling, and may even increase its invasiveness and aggressiveness, mostly through  $\beta$ -catenin-independent Wnt signaling (127).

## Exosomes Signaling

Glioblastoma-derived exosomes (GDEs) can reprogram macrophages, converting M1 into TAMs and augmenting tumor-supportive functions of M2 macrophages. In turn, these GDEs-reprogrammed TAMs, release exosomes decorated by immunosuppressive and tumor-growth promoting proteins. TAM-derived exosomes disseminate these proteins in the TME contributing to tumor cell proliferation and migration. One study demonstrated that mechanisms underlying the promotion of glioblastoma growth involved Arginase-1+ exosomes produced by the reprogrammed TAMs. A selective Arginase-1 inhibitor, nor-NOHA reversed growth-promoting effects of arginase-1 carried by TAM-derived exosomes, suggesting that GBEx-reprogrammed Arginase-1+ TAMs emerge as a major source of exosomes promoting tumor growth and as a potential therapeutic target in glioblastoma (128).

## TAMS FACILITATE ANGIOGENESIS OF GBM

TAMs not only directly act on glioma cells, but also affect angiogenesis to indirectly impact tumor growth. In PTEN-null GBM models, TAMs secreted SSP1 (secreted phosphoprotein 1), which sustained glioma cell survival and stimulates angiogenesis (61). Signaling through the receptor for the advanced glycation end product (RAGE) was important for the process. RAGE ablation abrogated angiogenesis, which could be reconstituted with wild-type microglia or macrophages. Moreover, this TAMs activity correlated with the expression of VEGF, which is a critical pro-angiogenic factor (129). ADAM8, a metalloprotease-disintegrin strongly expressed in tumor cells and associated

immune cells of GBMs is related to angiogenesis and is correlated with poor clinical prognosis. Furthermore, the angiogenic potential of ADAM8 in primary macrophages was mediated by the regulation of osteopontin (OPN), a crucial inducer of tumor angiogenesis. By *in vitro* cell signaling analyses, the study found that ADAM8 regulated OPN expression *via* JAK/STAT3 pathway in primary macrophages (130). M2-like immunosuppressive macrophages promote angiogenesis, whereas M1-like pro-inflammatory macrophages suppress angiogenesis. Zhu et al. showed that extracellular adenosine deaminase protein Cat Eye Syndrome Critical Region Protein 1 (CECR1) was highly expressed by M2-like macrophages in GBM where it defines macrophage M2 polarization and contributed to tumor expansion. Immunohistochemical evaluation of GBM tissue samples showed that the expression of CECR1 was correlated with microvascular density in the tumors. In a three-dimensional co-culture system consisting of human pericytes, human umbilical vein endothelial cells, and THP1-derived macrophages, CECR1 knockdown by siRNA and CECR1 stimulation of macrophages inhibited and promoted new vessel formation, respectively. Further investigation manifested that CECR1 function in (M2-like) macrophages mediated cross-talk between macrophages and pericytes in GBM *via* paracrine PDGFB–PDGFR $\beta$  signaling, promoting pericyte recruitment and migration, and tumor angiogenesis (131). In addition, Cui et al. observed that soluble macrophages-derived immunosuppressive cytokines, predominantly TGF- $\beta$ 1, and surface integrin ( $\alpha_v\beta_3$ )-mediated endothelial macrophage interactions were required for inflammation-driven angiogenesis (132). The study demonstrated tuning cell-adhesion receptors using an integrin ( $\alpha_v\beta_3$ )-specific collagen hydrogel regulated inflammation-driven angiogenesis through Src-PI3K-YAP signaling, highlighting the importance of altered cell-ECM interactions in inflammation. Dual integrin ( $\alpha_v\beta_3$ ) and cytokine receptor (TGF $\beta$ -R1) blockade suppressed GBM tumor neovascularization by simultaneously targeting macrophage-associated immunosuppression, endothelial-macrophage interactions, and altered ECM (132). Wang et al. validated that myeloid cell-restricted VEGF-A deficiency led to a growth delay of intracranial tumors and prolonged survival. Endothelial tube formation was significantly decreased by conditioned media from mutant macrophages (133). Recently, due to varied granulocyte influx, Blank et al. subdivided GBM samples into groups with low (GBM-IPMNL) and high numbers of granulocytes (GBM-hPMNL), which were related to activation of the microglia/macrophage population (134). Moreover, microglia/macrophages of the GBM-hPMNL specimens were highly associated with tumor blood vessels, accompanied by remodeling of the vascular structure. While microglia/macrophages represented the main source of alternative proangiogenic factors, additionally granulocytes participated through the production of IL8 and CD13, suggesting that tumor-infiltrating myeloid cells might play a crucial role for the limited efficacy of anti-angiogenic therapy bypassing VEGF mediated pathways through the expression of alternative proangiogenic factors (134).

**TABLE 1** | Interactions between Glioblastoma and GAMs.

Effects	Key factors	Mechanisms	References	
<b>Glioblastoma on GAMs</b>				
<b>Recruitment</b>	CCL2	Chemokine	(40, 41, 45–47)	
	CX3CL1	Chemokine	(49–53)	
	CSF-1	Chemokine	(54, 55)	
	HGF/SF	Chemokine	(56)	
	CXCL12	Chemokine	(57)	
	GDNF	Chemokine	(58)	
	POSTN	GSCs secreted POSTN to recruit TAMs through the integrin $\alpha\beta_3$	(59)	
	OPN	OPN signals through the receptor Integrin $\alpha\beta_5$ on TAMs	(60)	
	LOX	LOX induced TAMs recruitment via activation of the $\beta_1$ integrin-PYK2 pathway in macrophages	(61)	
	CCN1	CCN1, a transcriptional target of YAP/TAZ, functions as a chemokine to recruit TAMs	(62)	
	OLFML3	OLFML3 functions as a novel chemoattractant	(63)	
	Kynurenine	Kynurenine activated aryl hydrocarbon receptor in TAMs, which promoted CCR2 expression, subsequently driving TAMs recruitment in response to CCL2	(64)	
	EGFR/EGFRIII	EGFR and EGFRIII cooperate to induce TAMs infiltration through KRAS-mediated upregulation of the chemokine CCL2	(65)	
	ERK1/2	ERK1/2 mediate TAMs recruitment through regulation of the production of CCL2	(66)	
	SETDB1	SETDB1 promoted AKT/mTOR-dependent CSF-1 induction and secretion, leading to macrophage recruitment in the tumor	(67)	
	IL-33	IL-33 recruits TAMs through the regulation of chemokines	(68)	
	RSK1	N/A	(69)	
	WISP1	WISP1 signals through Integrin $\alpha\beta_1$ -Akt to recruit TAMs	(70)	
	<b>Pro-tumor Polarization</b>	S100B	S100B induced microglia activation through the induction of the STAT3 signal pathway	(77)
		PDAI3	PDAI3 induced microglia pro-tumor polarization toward the M2 phenotype and the secretion of pro-inflammatory factors	(78)
ARS2		ARS2 activated its novel transcriptional target MGLL, encoding monoacylglycerol lipase (MAGL), stimulated M2-like TAM polarization through the production of PGE2	(79)	
CAIX		CAIX regulated through EGFR/STAT3/HIF-1 $\alpha$ axis induced pro-tumor polarization of TAMs	(80)	
Romo1		Romo1 led to the M2 polarization of bone marrow-derived macrophages through the mTORC1 signaling pathway	(81)	
BCKAs		Exposure to BCKAs attenuated the phagocytic activity of macrophages	(82)	
mTOR		mTOR-mediated regulation of STAT3 and NF- $\kappa$ B activity promoted an immunosuppressive microglial phenotype	(83)	
IL-6		IL-6-activated STAT3 enhanced B7-H4 expression on TAMs, resulting in an immunosuppressive phenotype of TAMs	(84)	
GDEs		The components of GDEs such as IL-6 and miR-155-3p induced M2-like macrophage polarization through the IL-6-pSTAT3-miR-155-3p-autophagy-pSTAT3 positive feedback loop	(85–88)	
Versican		Versican-mediated TLR2 expression polarized microglia into a pro-tumorigenic phenotype featured by the upregulation of MT1-MMP and MMP9 expression	(120)	
<b>GAMs on Glioblastoma</b>				
<b>Proliferation and invasion</b>	IL-6	IL-6 increased PDPK1-dependent PGK1 phosphorylation in glioblastoma cells, promoting tumor cell glycolysis and tumorigenesis	(107, 108)	
	IL-1 $\beta$	IL-1 $\beta$ activated phosphorylation of the glycolytic enzyme glycerol-3-phosphate dehydrogenase (GPD2) at threonine 10 (GPD2 pT10) through PI3K/PKC $\delta$ signal pathways to promote tumor growth	(109)	
	STI1	N/A	(110)	
	TGF- $\beta$ 2	TGF- $\beta$ 2 induced the expression of MMP2 and suppressed the expression of (TIMP)-2 to promote glioma invasion	(111, 112)	
	CECR1	CECR1 stimulated MAPK signaling and activated the proliferation and migration of glioma cells	(113)	
	PTN	PTN promoted GBM malignant growth through PTN-PTPRZ1 paracrine signaling	(114)	
	CCL4	CCL4-CCR5 axis participated in TAMs-mediated glioblastoma invasion	(115)	
	CCL5	CCL5 upregulated mmp2 through the CaMKII and p-Akt signals	(116)	
	CCL8	CCL8 dramatically activated ERK1/2 phosphorylation in GBM cells and promoted invasion and stemlike traits of GBM cells through CCR1 and CCR5	(117)	
	TLR2	TLR2 forms heterodimers with TLR1 and TLR6 modulating MT1-MMP expression to promote tumor invasion and growth	(119)	
	Wnt	TAMs secreted Wnt proteins, contributing to GBM invasiveness and aggressiveness mostly through $\beta$ -catenin-independent Wnt signaling	(124–126)	
	<b>Angiogenesis</b>	Exosomes	GBex-reprogrammed Arginase-1+ TAMs emerge as a major source of exosomes promoting tumor growth	(128)
		SSP1	N/A	(61)
		RAGE	N/A	(129)
		ADAM8	ADAM8 induced angiogenesis via JAK/STAT3 pathway mediated OPN expression	(130)
CECR1		CECR1 promoted pericyte recruitment and migration, and tumor angiogenesis via paracrine PDGFB-PDGFR $\beta$ signaling,	(131)	
TGF- $\beta$ 1		TGF- $\beta$ 1/integrin ( $\alpha_v\beta_3$ ) interaction between macrophages and endothelial promoted GBM angiogenesis	(132)	
VEGF-A		N/A	(133)	

## POTENTIAL THERAPEUTIC TARGETS

For the past decades, investigators have largely focused on the intrinsic genetic mutations that occur in the tumor cells and the molecular mechanisms contributing to tumor progression. With the deepening understanding of TME in recent years, it is now acceptable that numerous signals emanated from the TME play pivotal roles in tumor growth. Concerning TME-mediated tumor aggression, it is important to recognize that glioblastoma is a sophisticated microcosm in which the interaction between neoplastic and non-neoplastic cells will not only affect gliomagenesis (135) but may also modify glioma responses to standard therapy. TAMs play critical roles in GBM growth and invasion, which provided a rationale for TAM-targeted therapies as feasible alternatives for GBM treatment. Generally, there are two strategies in terms of TAMs targeting therapies in GBM, including altering their pro-tumor function, often referred to as re-education, and blocking their recruitment.

### Re-Education of TAMs

BLZ945, a small-molecule CSF1R inhibitor, has been shown to ameliorate glioma progression by educating TAMs into an anti-tumor phenotype in a PN mouse model of GBM (136). However, further preclinical trials examining the long-term effect of BLZ945 reported rapid tumor rebound after a resting phase of 4 weeks (137). In detail, this resistance was mediated by TAMs *via* the excretion of insulin growth factor 1 (IGF-1) after the secretion of IL-4, probably produced by T cells in response to the drug. IGF-1 interacts with its cognate receptor IGF1R on the surface of tumor cells to activate the phosphatidylinositol 3-kinase (PI3K) signaling pathway, subsequently resulting in tumor resistance and proliferation (137). In a clinical trial with unselected adult recurrent GBM patients, unfortunately, CSF1R inhibitor as a single agent reported no effectiveness (138). A recent non-randomized, open-label, phase I/IIa, dose-escalation study targeting TAMs is at recruiting status, involving a single injection of Temferon, an investigational gene therapy-based approach consisting of autologous CD34+ enriched hematopoietic stem and progenitor cells exposed to transduction with a lentiviral vector driving myeloid-specific IFN- $\alpha 2$  expression (NCT03866109) (139). This strategy may provide a promising opportunity for GBM patients, as it showed prominent effectiveness in a mouse model of breast cancer.

### Blocking TAMs Recruitment

The CCL2/CCR2 signal pathway plays an essential role in monocyte recruitment toward the tumor niche. Downregulation of CCL2 levels prolonged the survival of GBM-bearing mice (40). Several clinical trials are currently underway to block CCL2 and CCR2 in solid tumors (140). Another promising target to block TAM recruitment is the SDF-1 receptor CXCR4. Some CXCR4

antagonists, such as peptide R or LY2510924, have demonstrated successful results in GBM mouse models (141, 142); However, they have not been validated in clinical trials. Another CXCR4 inhibitor, Plerixafor, has been tested for toxicity and efficacy in a completed Phase I/II clinical study in GBM patients after RT and temozolomide (NCT01977677). This study demonstrated that Plerixafor was well tolerated as adjunctive therapy for radiotherapy and chemotherapy in patients with newly-diagnosed GBM and improved the local control of tumor recurrence (143). Periostin has been reported as an interesting target for attenuating the tumor-supportive TAMs by interrupting integrin  $\alpha v \beta 3$  signaling (59). CD47 is another target to block TAM recruitment. Currently, there are two ongoing Phase I trials testing the efficacy of two monoclonal antibodies, IBI 188 (NCT03763149) and SRF-231 (NCT03512340), which are being conducted as monotherapies in patients with advanced malignant tumors and lymphomas.

## CONCLUSION

It is undeniable that there are complex and interdependent interactions exist between tumor cells and non-tumor cells within glioblastoma that promote the progression of GBM. As the main component of GBM, TAMs play an important role in the formation and growth of GBM. Although many emerging factors involved in TAMs and glioma cells interactions have been identified and tested in several pre-clinical studies over the past few years (**Table 1**), which factors are key to regulating this interesting interaction remains to be determined. Still, it is not clear how microglia and BMDMs interact in the tumor and whether they acquire different properties and perform different functions. It is not known whether histologically or molecularly different glioma types exhibit different functional phenotypes of TAMs. Nevertheless, targeting TAMs has emerged as a promising approach for GBM treatment. Further dissecting the mechanisms and interactions between TAMs and tumor cells or other immune cells will shed light on new GBM treatments. In addition, it is still needed to re-evaluate the efficacy of drugs that have been already used and investigated in the light of TAMs reprogramming. Integrating TAMs targeted therapies into available standard therapies or immunotherapies would be a promising field worthy of investigation.

## AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

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## GLOSSARY

ADAM8	a disintegrin and metalloprotease
AHR	aryl hydrocarbon receptor
ARS2	arsenite-resistance protein 2
BCKAs	branched-chain ketoacids
BMDM	bone marrow-derived macrophages
CAIX	carbonic anhydrase IX
CCL	chemokine (C-C motif) ligand
CCN1	cellular communication network factor 1
CECR1	Cat Eye Syndrome Critical Region Protein 1
CSF-1	colony stimulating factor-1
DGCs	differentiated Glioblastoma Cells
EGFR	epidermal growth factor receptor
FAS	fatty acid synthesis
GAMs	glioblastoma-associated macrophages
GBM	glioblastoma multiform
GDEs	glioblastoma-derived exosomes
GDNF	growth factor glial cell-derived neurotrophic factor
GM-CSF	granulocyte-macrophage colony-stimulating factor
GPD2	glycolytic enzyme glycerol-3-phosphate dehydrogenase (GPD2) at
pT10	threonine 10
GPD2	glycerol-3-phosphate dehydrogenase 2
GSCs	glioblastoma stem cells
H-GDEs	hypoxic glioma-derived exosomes
HGF	hepatocyte growth factor
IGF-1	insulin growth factor 1
IL-1 $\beta$	interleukin 1 $\beta$
IL-6	interleukin 6

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IRF-8	interferon regulatory factor-8
ITG $\alpha$ $\beta$ 5	Integrin $\alpha$ $\beta$ 5
LOX	lysyl oxidase
LPS	lipopolysaccharide
LRP	lipoprotein receptor-related protein
MAGL	monoacylglycerol lipase
MCP-1	monocyte chemoattractant protein-1
MMP2	matrix metalloprotease-2
N-GDEs	normoxic glioma-derived exosomes
OPN	osteonectin
PD-L1	programmed death-ligand 1
PGE2	prostaglandin E2
PI3K	phosphatidylinositol 3-kinase
PKC $\delta$	protein kinase-delta
POSTN	periostin
PTN	pleiotrophin
PCP	Planar Cell Polarity
RAGE	advanced glycation end product
Romo1	reactive oxygen species modulator 1
RSK1	ribosomal S6 kinase 1
SETDB1	SET domain bifurcated 1
SF	scatter factor
SSP1	secreted phosphoprotein 1
STAT3	signal transducer and activator of transcription 3
STI1	stress-inducible protein 1
TAMs	tumor-associated macrophages
TGF- $\beta$	Transforming growth factor- $\beta$
TIMP	tissue inhibitors of metalloproteinases
TME	tumor microenvironment
WISP1	Wnt-induced signaling protein 1
Wnt	The Wingless-type MMTV integration site family