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REVIEW

Inflammatory Mediators in Glioma Microenvironment Play a Dual Role in Gliomagenesis and Mesenchymal Stem Cell Homing: Implication for Cellular Therapy

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Abstract

Glioblastoma is the most aggressive malignant primary brain tumor, with a dismal prognosis and a devastating overall survival. Despite aggressive surgical resection and adjuvant treatment, average survival remains approximately 14.6 months. The brain tumor microenvironment is heterogeneous, comprising multiple populations of tumor, stromal, and immune cells. Tumor cells evade the immune system by suppressing several immune functions to enable survival. Gliomas release immunosuppressive and tumor-supportive soluble factors into the microenvironment, leading to accelerated cancer proliferation, invasion, and immune escape. Mesenchymal stem cells (MSCs) isolated from bone marrow, adipose tissue, or umbilical cord are a promising tool for cell-based therapies. One crucial mechanism mediating the therapeutic outcomes often seen in MSC application is their tropism to sites of injury. Furthermore, MSCs interact with host immune cells to regulate the inflammatory response, and data points to the possibility of using MSCs to achieve immunomodulation in solid tumors. Interleukin 1β, interleukin 6, tumor necrosis factor α, transforming growth factor β, and stromal cell–derived factor 1 are notably up-regulated in glioblastoma and dually promote immune and MSC trafficking. Mesenchymal stem cells have widely been regarded as hypoimmunogenic, enabling this cell-based administration across major histocompatibility barriers. In this review, we will highlight (1) the bidirectional communication of glioma cells and tumor-associated immune cells, (2) the inflammatory mediators enabling leukocytes and transplantable MSC migration, and (3) review preclinical and human clinical trials using MSCs as delivery vehicles. Mesenchymal stem cells possess innate abilities to migrate great distances, cross the blood-brain barrier, and communicate with surrounding cells, all of which make them desirable "Trojan horses" for brain cancer therapy.

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Glioblastoma (grade IV) is the most malignant form of primary brain cancer and makes up 15% of all primary brain tumors. Despite the most aggressive treatment strategies (radiation, chemotherapy, and surgery), patients have a median survival of approximately 15 months.[1] Tumors arising from glial cells account for 75% of malignant brain tumors and are classified by histopathologic and genetic findings. Grade IV gliomas are classified by *IDH* (for expansion of gene symbols, use search tool at www.genenames.org) alterations, p53, *ATRX* loss, and 1p/19q codeletions and stratified into 4 subtypes: classic, neural, mesenchymal, and proneural.[1] Genetic alterations and immunosuppression drive gliomagenesis, promoting tumor cell growth, proliferation, cellular invasion, and therapeutic resistance.[2] Malignant tumors have been described as chronic injuries[2] wherein inflammation plays a large role in advancing the proliferation, progression, and aggressiveness of tumor growth.[3] One major problem encountered with the treatment of gliomas is the blood-brain barrier (BBB). This structural and

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ARTICLE HIGHLIGHTS

- Glioblastomas avoid elimination by the immune system through the secretion of immunomodulating factors.
- Mechanisms for gliomagenesis involved in immunosuppression and tumor progression also mediate the recruitment of therapeutic and transplantable mesenchymal stem cells (MSCs) for cellular therapy.
- Mesenchymal stem cells as cellular therapy may offer 2 advantages for glioma: site-specific targeting and immunomodulation via cargo delivery.
- Mesenchymal stem cells (adipose tissue–derived MSCs, bone marrow MSCs, umbilical cord MSCs) display favorable results as therapeutic delivery vehicles and could be considered as a viable alternatives to neural stem cells because of their availability, ease of extraction, simple expansion protocols, and low immunogenicity permitting their transplant across major histocompatibility barriers.
- Mesenchymal stem cells display promising potential as immunotherapy for glioblastoma via cargo delivery of immunostimulants.

biological barrier impedes accumulation of effective therapeutic concentrations into the tumor bulk. Administration of pharmacological agents are conservatively regimented due to the vulnerability of healthy cells and the risks of off-target effects ultimatley impeding effective pharmacological concentrations for therapeutic efficacy. This stringent balance of systemic toxicity vs tumor ablation has hindered the translation of therapies with strong tumoricidal effects that have otherwise shown robust efficacy, preclinically. Moreover, histopathologic and tumor composition studies have revealed considerable heterogeneity in the tumor bulk, rendering directed and targeted therapy even more complex.

The tumor niche consists of stromal cells (endothelial, fibroblasts, pericytes), reactive astrocytes, tumor cells with varying lineage heterogeneity, and invading immune cells (microglia, macrophages, granulocytes, B cells, and T cells). However, the inability to stimulate an antitumor immune response is due to multiple soluble factors released by tumor cells that mediate immune reprogramming and allow the recruitment of immunosuppressive cells. Clinical data suggest extensive infiltration of peripheral monocytes that have assumed an immunosuppressive state; this infiltration and accumulation in the tumor bulk is directly correlated with glioma grade, with glioblastoma (grade IV) being the most infiltrated.[4] Mesenchymal stem cells (MSCs) from bone marrow (BMSCs), adipose tissue (AMSCs), or umbilical cord (UC-MSCs) have been preclinically investigated for the treatment of brain cancer by delivering various antiglioma cargo to modulate the tumor niche. An effective treatment strategy for glioma would preferentially target the tumor and enable the release of a therapeutic payload to transformed cells while sparing healthy cells in proximity. Mesenchymal stem cells have emerged as one potential cellular vehicle for the delivery of therapeutic cargo and may be an effective candidate as immune cargo delivery vehicles to brain cancer. The influence of inflammatory cytokines originating from the tumor niche enable MSCs to selectively migrate to tumor areas.[5,6] There is scarcity in the literature regarding the role of the immune system in glioma initiation, but strong evidence suggests that immune cells inhabiting the tumor niche are able to support gliomagenesis.[7] Such mechanisms include immunomodulation initiated by secretion of soluble factors,[8] induction of T-cell anergy,[9] polarization of microglia and macrophages toward an immunosuppressive state,[10] extracellular matrix reconstruction to allow for tumor cell migration and invasion, and activation of the tumor stromal compartments for support and maintenance of cancer cell niche for survival. These aforementioned factors work together in synchrony to create a tumor microenvironment that favors tumor cells harboring a selective mutational advantage to evade immunosurveillance.

Mesenchymal stem cells have widely been regarded as hypoimmunogenic, enabling MSC administration across major histocompatibility complex (MHC) barriers. While MSCs are not immunoprivileged, they are regarded as immunoevasive and largely go undetected by the immune system. Mesenchymal stem cells possess innate abilities to migrate great distances, cross the BBB, and communicate with surrounding immune cells, all of which make them desirable "Trojan horses" for brain

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cancer therapy. In this review, we will highlight (1) the bidirectional communication of glioma cells and tumor-associated immune cells, (2) the inflammatory mediators released by glioma that could be exploited for the recruitment and migration of therapeutic transplantable MSCs, and (3) future implications for utilizing MSCs as cargo delivery vehicles for glioblastoma (GBM) immunomodulation (Figure 1).

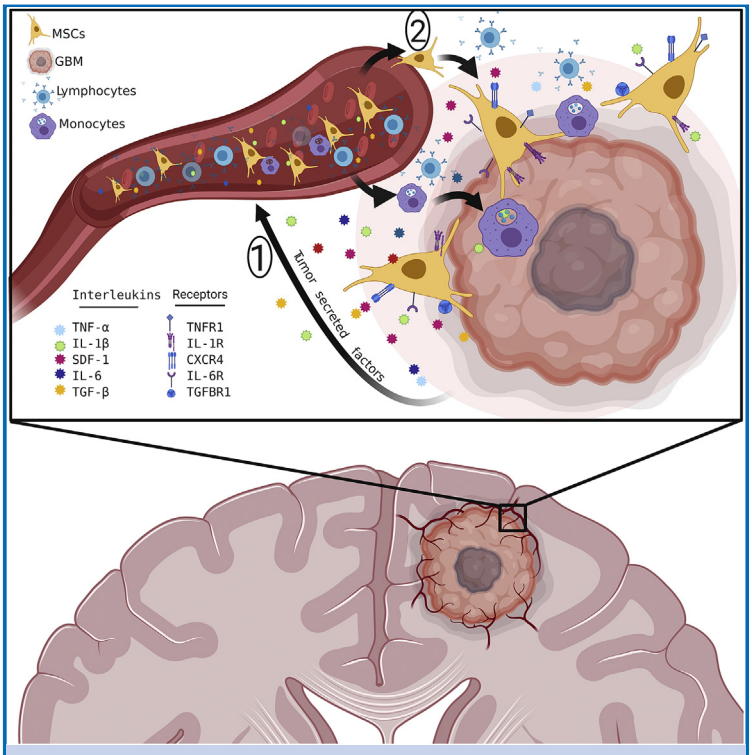

FIGURE 1. Mode of mesenchymal stem cell (MSC) and immune cell migration and trafficking to glioblastoma (GBM). (1) Inflammatory cytokines released by GBM (2) promote extravasation of MSCs, lymphocytes, and monocytes and direct them toward tumor location. IL = interleukin; TGF = transforming growth factor; TNF = tumor necrosis factor.

NOXIOUS LOOP BETWEEN TUMOR-ASSOCIATED IMMUNE CELLS AND GLIOMA

Despite aggressive therapy, the progression of glioma in patients suggests a gross failure in host immune mechanisms. Various strategies in the clinic have employed immunostimulants to reactivate immune surveillence, such as interleukin (IL) 12 (ClinicalTrials.gov Identifier: NCT02079324), GM-CSF, tumor necrosis factor (TNF) α, and interferon (IFN) β (ClinicalTrials.gov Identifier: NCT02530047). Notwithstanding ongoing research and numerous strategies combating glioma, methods to reverse immunosuppression in patients have produced little success. This problem is generally due to cells gaining a mutational advantage over the course of the evolutionary process of gliomagenesis, rendering tumor cells virtually undetected by surveillance mechanisms. During tumor initiation and evolution, competent immune cells, such as microglia, natural killer cells, macrophages, and dendritic cells (DCs), attempt to destroy the tumor via the release of cytotoxic and proinflammatory factors such as TNF-α and IL-6, leading to the recruitment of helper $CD4^+$ and cytotoxic $CD8^+$ T cells from the periphery to the tumor bed.[11] This movment is aided by the release of the proinflammatory cytokine IL-1β, that contributes to the loss of BBB integrity via the expression of genes favoring vessel plasticity. Loss of BBB integrity permit infiltration of myeloid-derived suppressor cells (MDSCs) in the tumor bulk. Recruited macrophages up-regulate inducible nitric oxide synthase and secrete IFN-β, IL-12, and MCP1 in the growing tumor.[12]

Tumor-infiltrating leukocytes, measured by the presence of $CD45^+$ from human biopsy samples following resection, constitute 40.3% of grade IV gliomas; 85.6% of which are $CD33^+$ MDSCs.[4] Composition of the tumor-associated macrophage (TAM) and polymorphonuclear cell subsets in MDSCs, defined by investigators as $CD33^+$/HLA-DR^+ antigen and $CD33^+$/HLA-DR^- antigen constitute 64.7% and 15.8%, respectively, of the immune concentration found in tumor bed. TAMs and brain-resident microglia comprise the dominant nonneoplastic cell type in the tumor and largely contribute to the initial breakdown of immunosurveillance and eventual immunosuppression via the reciprocal release of multiple soluble factors: GM-CSF, SDF1, HGF/SF, CX3CL1, GDNF, ATP, MCP1, MCP3, (Figure 2).[11] In an effort to evaluate the prognostic potential of peripheral MDSCs on survival, investigators evaluated peripheral immune composition and concentration of MDSCs in 259 blood samples from patients with newly diagnosed and recurrent GBM. The study found that reduced MDSC concentrations in blood resulted in better

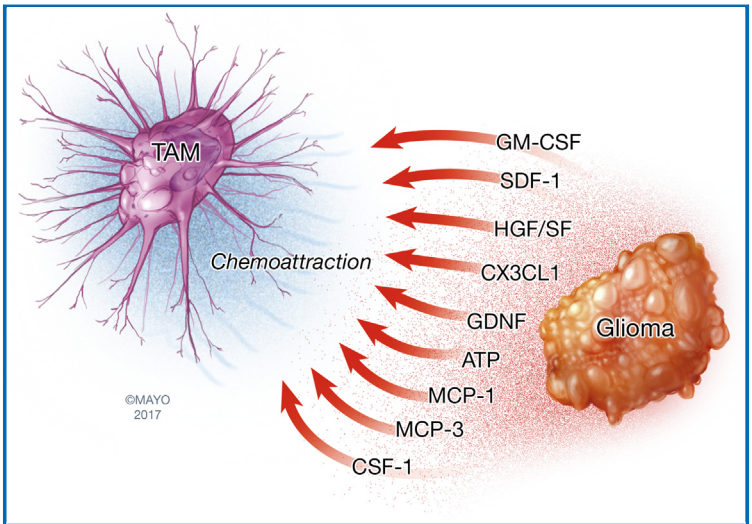

FIGURE 2. Immunosuppressive soluble factors. ATP = adenosine triphosphate; CSF-1 = colongy stimulating factor 1; CX3CL1 = chemokine ligand 1; GDNF = glial cell line-derived neurotrophic factor; GM-CSF = granulocyte-macrophage colony stimulating factor; HGF/SF = hepatocyte growth factor/scatter factor; MCP-1 = monocyte chemoattractant protein 1; MCP-3 = monocyte chemotactic protein; SDF-1 = stromal derived factor 1; TAM = tumor-associated macrophage.

outcomes[13]; strategies to target MDSCs may offer a new avenue for GBM immunotherapy that would slow tumor growth at onset of disease.

TAMs and MDSCs are associated with poor survival and contribute to innate immunosuppression via cross-talk with surrounding tumor cells.[14] Results from one study suggest that the recruitment of MDSCs to the tumor bed is, in part, initiated by the secretion of macrophage migration inhibitory factor by tumor cells.[15] Upon arrival, infiltrated microglia and macrophages transition from the proinflammatory "M1" state to an anti-inflammatory "M2" phenotype. This transition is facilitated by the uptake of tumor-derived factors such as MCP1,[16] CSF1,[17] and MCP3.[18] Over the course of tumor progression and through various tumor mechanisms, recruited macrophages lose their phagocytic ability, cytotoxic T-cell proliferation is inhibited, and there is an increase in infiltration of regulatory T cells, resulting in a chronic immunosuppressive state and tumor tolerance. With M2 or TAMs being the primary phenotype found in the tumor microenvironment, a more robust shift occurs, and an up-regulation of anti-inflammatory factors ensues.[19] These factors dampen tumor clearance and allow for a more permissive environment where cancer cells thrive.[20] A toxic loop is now established within the tumor niche as more tumor-supportive immune cells, such as regulatory T cells and MDSCs, arrive.

Soluble factors involved in the recruitment of MDSCs and leukocytes to the tumor have been implicated in the tropism and favored movement of neural stem cells (NSCs) and MSCs to glioma. These cells have been extensively inestigated as delivery vehicles for anti-glioma cargo.

STEM CELL APPLICATIONS FOR GLIOMA

Stem cell applications have been studied extensively in the field of regenerative medicine and are currently making headway in solid cancers. The 2 most studied stem cell applications for brain cancer therapy are MSCs and NSCs. Their application relies on the tropic and homing capacity toward brain tumors and the therapeutic delivery of anti-glioma cargo. Precise and targeted applications for brain tumors via tumor antigens and neoantigens are currently being explored to avoid off-target toxicity. However, MSCs and NSCs have been found to be effective vehicles that colocalize to tumor cells when administered locally and systemically. While preclinical applications are being explored for MSCs in GBM, NSCs are currently undergoing phase 1 and 2 clinical trials in human patients. The first human safety and feasibility phase 1 study with stem cell therapy employed genetically modified NSCs expressing cytosine deaminase, an enzyme that converts the prodrug 5-fluorocytosine (5-FC) to the chemotherapeutic 5-fluorouracil. Engineered NSCs were administered intracerebrally during resection and patients were given a 7-day oral dose of 5-FC (NCT02015819/NCT01172964).[21] Follow-up results documented safety, and autopsy results revealed NSC homing to distant microsatellite tumors in the brain. The concluded pilot study documented proof of concept regarding NSCs' tropism to glioma. Furthermore, the study confirmed the diffusion of 5-FC out of NSCs into adjacent and highly proliferative brain tumor cells mediating cell

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killing by proxy.[22] Current work is focused on dose escalation regimens to identify an optimal therapeutic dose for phase 2. Similarly, NSCs were engineered to express carboxylesterase enzyme that mediates the conversion of the prodrug CPT-11 (irinotecan) to SN-38, a topoisomerase 1 inhibitor potent at killing cancer cells. Application is currently under way in phase 1 clinical trials for the intracerebral injection of patients with recurrent glioblastoma (NCT02192359). Although the administration of a prodrug with NSC-enzyme therapy has been proven safe for the intracereberal injection in recurrent GBM, NSCs have also been engineered to deliver oncolytic adenoviral therapy with concomitant administration of chemotherapy and radiation in patients with primary GBM following a phase 1 clinical trial (NCT03072134). Preclinical data on human NSCs documented efficacy in patient-derived xenograft mouse models of human commercial U87 and U251 GBM lines.[23,24]

Currently, NSC-mediated cargo delivery to GBM owes its therapeutic success to the "bystander effect." Cancer cell death is mediated via the diffusion of drug or virus out of the exogenously delivered NSCs through cell junctions and into adjacent cancer cells. They have limited effects as naive NSCs compared with MSCs. Although NSCs are known to be hypoimmunogenic, most NSC-mediated delivery utilizes an immortalized commercial cell line (HB1.F3.CD21)[22,25] that is well characterized and found to be stable over sequential passaging and culture propagation. However, oncogenic transformation and serial karyotyping during propagation of immortalized cell lines must be closely monitored. Autologous MSCs applications, such as those isolated from adipose tissue offer minimally invasive protocols for the procurement of stem cells. Nonimmortalized primary NSCs can be isolated from the periventricular zone of adult brains during surgery or from fetal brains; the amount required for therapeutic application (or multiple dosing) necessitates high procurement of NSCs to meet sufficient dose requirements for human trials. Consequently, consecutive culture passaging of primary NSCs increases the risk of potential lineage commitment and differentiation that could lead to loss of migratory ability toward gliomas. Current clinical applications of NSCs to brain tumors use allogeneic lines, with the assumption that NSCs are hypoimmunogenic and express low levels of MHC class I and II. A study investigated the immunogenicity of human NSCs with HLA-incompatible donors in a one-way mixed lymphocyte reaction found that NSCs stimulated lymphocyte in all nonmatched donors, suggesting that this sensitivity may be sufficient to enable immunorecognition of exogenously delivered and allogenically grafted NSCs. The investigators reported that although immunogenicity is low, it is not negligible.[26] Long-term risk would ultimately lead to activation of peripheral lymphocytes and eventual clearance of the therapeutic vehicle (NSCs) more rapidly.

Although NSCs display a relatively good safety index for the treatment of gliomas, MSCs are becoming seriously considered for the purpose of glioma therapy. Mesenchymal stem cells are immunoevasive and hypoimmunogenic, isolation is minimally invasive, large expansion protocols for primary human clinical use can be achieved, and risks for cell rejection can be eliminated with the use of autologous MSCs, given ease of procurement. Thus, simplicity of extraction, little to no notable cell manipulation (ie, immortalization), intrinsic immunomodulatory ability, and their tropic capacity to areas of insult make MSCs optimal cellular therapeutics.

MSC APPLICATIONS FOR GLIOMA

Mesenchymal stem cells are multipotent adult stem cells residing in various tissues and organs in the adult body. These cells have been widely used in the field of regenerative medicine for tissue regeneration, wound healing, vehicles for cargo delivery, and immunomodulation. It was originally believed that MSCs integrate into the site of injury and differentiate into surrounding stroma to enable tissue regeneration. This theory has since been challenged (due to poor MSC persistence and retention after implantation); it is believed that MSCs act in a paracrine fashion through site-specific modulation and can endogenously regulate tissue at the site of implantation, primarily through soluble factor secretion. In this fashion, MSCs act dynamically in response to an insult and regulate the inflammatory

TABLE 1. Human Clinical Trial Applications of MSCs in Solid Tumors

Source of MSC	Diagnosis	Trial phase	Route of administration	Parameters to evaluate	Clinical trial ID	Cytokine, factors, or drug involved
Bone marrow	Prostate cancer	I	Intravenous	Ratio of MSC genomic to prostate DNA in bodily fluids (blood, seminal vesicles) in resected prostate	NCT01983709	Toxins (not specified)
Bone marrow	Ovarian carcinoma	I	Intraperitoneal	Maximum tolerated dose	NCT02530047	INF-β
Adipose tissue	Pancreatic cancer	I	Intravenous	Clinical response and adverse effects	NCT04087889	Not specified
Adipose tissue	Recurrent ovarian cancer	I	Intraperitoneal	Safety and tolerability Response rate, progression-free survival, and overall survival	NCT02068794	Oncolytic measles virus encoding thyroidal sodium iodide symporter
Not specified	Head and neck cancer	I	Intratumoral	To assess safety and tolerability	NCT02079324	GX-051 (IL-12)
Bone marrow	Refractory solid tumors	I	Intravenous	To assess safety and tolerability	NCT01983709	ICOVIR5
Bone marrow	GBM, gliosarcoma, anaplastic astrocytoma	I	Intra-arterial	To evaluate safety, toxicity, and immuno-mediated cytokine responses To evaluate progression-free survival	NCT03896568	Oncolytic adenovirus (DNX-2401)

GBM = glioblastoma; ID = identification number; IL = interleukin; INF = interferon; MSC = mesenchymal stem cell.

response in a context-dependent manner. Mesenchymal stem cells have been widely used in clinical trials as intrinsic modulators of chronic inflammation or autoimmune disorders.[27] Their multipotency, immune privilege, and immunomodulatory capabilities make them a viable source for autologous or allogenic applications. Allogenic MSCs have a wide portfolio of diverse applications for human clinical trials in solid tumors. Numerous preclinical and clinical trials are assessing their potential in several disease models such as Crohn disease, tissue repair, graft-vs-host, cancer, and neurodegenerative diseases.[28-30]

Although MSCs are widely used in regenerative medicine, their applications in solid tumors are currently being explored in early-phase human clinical trials using ClinicalTrails.gov (search words: cancer, MSCs, GBM) (Table 1). In refractory ovarian carcinoma, administrations of allogenic MSCs from male donor(s) virally engineered to secret IFN-β were evaluated for their safety in a phase 1 single-center trial (NCT02530047). One study delivered MSCs engineered with an oncolytic measles virus encoding a sodium iodide symporter as treatment for recurrent ovarian cancer (NCT02068794). Another phase 1 clinical trial used allogenic MSCs to target prostate cancer cells, while another used adipose tissue–derived culture-expanded AMSCs against pancreatic carcinoma (NCT04087889). To determine the maximum tolerable dose and safety of genetically modified MSCs expressing GX-051 (IL-12), Other investigators have tested intratumoral injections of the modified cells in patients with head and neck cancer (NCT02079324). Similarly, another study evaluated the safety and tolerability of weekly infusions of autologous BMSCs infected with an oncolytic adenovirus (ICOVIR5) (NCT01983709).

In brain cancer, specifically for recurrent high-grade gliomas (including gliosarcoma, anaplastic astrocytoma, and GBM), an ongoing

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TABLE 2. Preclinical Applications of Mesenchymal Stem Cells in Glioblastoma

Reference, year	Cell line/species	Source of MSC/ species	Experimental species	In vitro/in vivo	Results	Implicated cytokine and factors involved in cross-talk/up-regulated in GBM
Smith et al,[42] 2015	Human U87	Human/primary AMSC	Athymic mice	In vitro/in vivo	Preexposure to GBM in vitro enhanced its migratory potential in vivo	TNF-α/yes
Egea et al,[33] 2011	Human U87	Human/primary BMSC	Athymic mice	In vitro/in vivo	Preincubation of BMSC with TNF enhanced its migratory potential to GBM	TNF-α/yes
Choi et al,[43] 2015	Human/primary GBM	Human/primary AMSC	NA	In vitro	Migratory ability of AMSCs toward BTICs is mediated by the cross-talk of brain cancer cells and MSCs	CXCR4/SDF-1, IL-6R/IL-6, IL-8R/IL-8
Shahrokhi et al,[44] 2014	Murine /4T1 —breast cancer line	Murine/primary isolated from BALB/c mice and modified with TNF/CD40	BALB/c immuno-competent	In vitro/in vivo	MSC (TNF/CD)— suppressed helper T cell type 2, and regulatory T cells. Down-regulation of IL-4, IL-10. Enhanced survival in murine models of subcutaneous breast cancer	T cell TNF-α/CD40
Carrero et al,[45] 2012	NA	Human/primary BMSC	NA	In vitro	BMSC increased the recruitment of leukocytes. Reciprocal recruitment observed on IL-1β stimulation of BMCS	IL-1β
Pacioni et al,[46] 2017	Human/U87 Human/GSC1	Human/primary AMSC	Athymic rats	In vivo	Systemic administration of MSCs colocalized to GBM in vivo	GBM-soluble factors (unidentified)
Pavon et al,[47] 2018	Primary human GBM lines sorted for	Human/primary UC-MSC	Athymic mice	In vitro/in vivo	UC-MSCs migrate specifically toward glioma stemlike cells	MCP-1/CCL2

Continued on next page

TABLE 2. Continued

Reference, year	Cell line/species	Source of MSC/ species	Experimental species	In vitro/in vivo	Results	Implicated cytokine and factors involved in cross-talk/up-regulated in GBM
	CD133 (BTICs/ GSC)				(CD133) in vitro and in vivo	SDF-1/CXCL12
Lourenco et al,[48] 2015	Human/U87	Human/primary BMSC	NA	NA	BMSCs migrate to U87 and knockdown of CXCR4 in BMSC abrogated tumor tropism	CXCR4
Li et al,[5] 2014	Human/ GBM276	Human/ commercial	Athymic mice	In vitro/in vivo	Modified and unmodified AMSCs migrate to glioma in vitro and in vivo and substantially enhance tumor survival	CXCR4
	Human/GBM612	AMSC (Invitrogen R7788-115) expressing human BMP4				
Li et al,[49] 2019	Primary human GBM	Human/primary AMSC	Athymic mice	In vitro/in vivo	Preconditioning AMSCs with TGF increased the homing ability in vitro and in vivo. Knockdown of TGF receptor abrogated AMSC homing	TGF-β
						CXCR4

AMSC = adipose tissue–derived mesenchymal stem cells; BMSC = bone marrow–derived mesenchymal stem cell; BTIC = brain tumor initiating cell; GBM= glioblastoma; GSC = glioma stem cell; IL = interleukin; MSC= mesenchymal stem cell; NA = not available; TGF-β = transforming growth factor β; TNF = tumor necrosis factor; UC-MSC = umbilical cord MSC. For expansion of gene symbols, use search tool at www.genenames.org.

phase 1 trial is evaluating cytokine response, safety, toxicity, and progression-free survival in response to intra-arterial administration of allogeneic human BMSCs carrying oncolytic adenovirus (DNX-2401) (NCT03896568).

Adipose tissue–derived MSCs have been a good source of stem cell therapy, not only for regenerative medicine but also as vehicles for antiglioma cargo to the central nervous system.[31,32] They have previously been reported to selectively migrate in vitro and in vivo to xenograft models of human gliomas and secrete antiglioma cargo such as BMP4,[5,33] resulting in tumor reduction and increased survival. As cells that are endogenously designed to maintain homeostasis, they have an intrinsic capacity to adhere to brain endothelium as well as migrate across the BBB, allowing them to travel systemically and home to areas of high chemotactic and growth factor secretion such as that of GBM,[5,33] making them desirable vehicles for cargo delivery. Studies have validated AMSC safety against human brain tumors without risk of oncogenic transformation.[34,35] Adipose tissue–derived MSCs are a convenient form of cell therapy and have displayed promising potential as primary cellular vehicles compared with NSCs (mainly due to limitations in supply and challenges in acquisition) or BMSCs (which require invasive aspirations and have a relatively low extraction yield, eventually declining in life span on implantation).[36]

Similarly, BMSCs can scavenge surrounding cells and modulate tissue via secretion of suppressive and/or supportive soluble factors mediating the polarization or enhanced activation of macrophages; BMSCs can act as innate immunomodulators in their own right without modification.[37] Bone marrow–derived mesenchymal stem cells have been used in clinical trials for chronic inflammation and autoimmune disorders.[27] Their multipotency, immune privilege, and immunomodulatory capabilities make them a viable source for autologous or allogenic applications. Strategies using BMSCs to increase the infiltration of cytotoxic T cells and enhance natural killer cell surveillance are being investigated, with documented efficacy. Preclinically, AMCSs and BMSCs have been engineered to deliver immunoactivating factors such as TNF-related apoptosis-inducing ligand,[38] IL-12,[39] and IL-18,[40] resulting in the activation of natural killer cells and infiltration of tumor-specific CD4 and CD8 T cells. Furthermore, studies suggest that BMSCs can act as antigen-presenting cells in the presence of IFN-γ by up-regulating MHC class I and II.[41] Primary regulators of MSC tropism to GBM are mediated by soluble factors reviewed in the following section (Table 2).

MECHANISM OF MSC RECRUITMENT AND MICROENVIRONMENT MODULATION

Role of TNF-α

Tumor necrosis factor α is a potent soluble cytokine involved in orchestrating response to systemic inflammation. This inflammatory cytokine is enigmatic in the tumor context, displaying tumoricidal effects against glioma cells as well as tumor-promoting abilities. Tumor necrosis factor α facilitates angiogenesis by up-regulating EGFR,[50] induces immune-cell suppression through NF-κβ and STAT3[51] and down-regulates PTEN tumor suppressor gene in glioma.[52] Paradoxically, TNF-α knockout resulted in larger tumors and reduced overall survival in immunocompetent animals bearing GL261 glioma. Study results suggest TNF is involved in reduced macrophage infiltration in histolopathological examinations, suggesting TNF plays a tumor-suppressive role along with its tumor supportive capabilities.[53] Despite its biological versatility in cancer, TNF-α is enriched in patient-derived human GBM–conditioned media, and preincubation of human AMSCs with TNF-α enhanced AMSCs migration toward glioma in vitro.[42] Macrophages and AMSC migration is recruited, in part, by the secretion of TNF-α from tumor microenvironments.[51,54] Similarly, systemically injected human BMSCs, preconditioned with TNF (50 ng/mL), migrated substantially in vivo in murine models bearing human U87 GBM, measured 72 hours postinjection.[55] Similarly, preincubation of human glioma stem cells (GSCs) with AMSC-conditioned media resulted in the decreased expression of inflammatory IL-6 and IL-8 factors in tumor cells post-incubation. These inflammatory

cytokines are implicated in immunosuppression and function as angiogenic and mitogenic factors, their overexpression confers a worse prognosis in patients with GBM. Analogously, a study documented a marked reduction of IL-8 and IL-6 protein expression in indirect coculture assays of human GBM and AMSCs, revealing that soluble factors released by AMSCs, and the response of GBM to these factors in direct or indirect coculture similarly resulted in a decreased expression of IL-6 and IL-8.[43] Although glioma-secreted factors have been found to recruit AMSCs and macrophages, studies suggest that MSCs display endogenous therapeutic effects against glioma cells, outside the context of cargo delivery.

Similar to AMSCs, BMSCs directly influence dendritic cell activation. In a 4T1 subcutaneous murine breast cancer model, virally engineered BALB/c BMSCs with TNF-α/CD40L exhibited enhanced dendritic cell maturation markers (CD86, CD40, and MHC class II) indicative of antigen presentation.[44] Furthermore, BMSCs cocultured with DCs increased expression of proinflammation cytokines (IL-12, IFN) and decreased anti-inflammatory expression, indicated by the reduction of IL-4, IL-10, and transforming growth factor (TGF).[44] These studies illustrate the ability of BMSCs to engage in mechanisms that facilitate antitumor immunity by potentially enhancing DC activation.

Role of IL-1β

Glioblastomas produce large quantities of IL-1β, which plays a crucial role in glioma aggressiveness and survival.[56,57] To evaluate IL-1β activation—induced changes in glioma, human GBM U251 cell line, corresponding to an aggressive "mesenchymal" subtype, was stimulated with recombinant IL-1β in vitro. Proteomic analysis of the secretome revealed 2 biological nodes enriched in U251 on stimulation: (1) extracellular remodeling mechanisms and (2) cellular communication. Specifically, IL-8 and CCL2, two principal components of monocyte recruitment, were substantially up-regulated.[58] The tumor microenvironment has an inflammatory signature that may favor the movement of MSCs via the up-regulation of surface receptors whose common ligands are expressed by the tumor, such as the receptor for IL-8. A study analyzing the response of human BMSCs on IL-1β stimulation revealed notable up-regulation of cellular mechanisms related to migration, cellular adhesion, host defense, and immunoregulation through NF-κβ.[45] Furthermore, IL-1β treatment enhanced BMSC migration and improved the recruitment of neutrophiles and monocytes in vitro. Consequently, blockade of transcription factor NF-κβ in BMSCs reduced BMSC migration and recruitment of leukocytes. This finding suggests that the influences of immune cell recruitment by BMCSs is mediated via NF-κβ and enhanced by the presence of IL-1β.[45] As mentioned earlier, CCL2 is a monocyte recruitment factor implicated in peripheral immunoresponse, and its up-regulation is enhanced in the presence of IL-1β. When neutralizing antibodies were used to block CXCR2, BMSC migration decreased by 45%, implicating the IL-8/CXCR2 axis in the chemotactic regulation of BMSC migration.[59] Adipose tissue—derived MSCs have also displayed the same tropic behavior toward brain cancer stem cells using an orthotopic human GBM model in athymic rats. Systemically administered AMSCs injected through the common carotid artery and femoral vein were able to extravasate through the disrupted BBB and localize in the brain tumor.[46] Umbilical cord MSCs also display enhanced migration toward glioma. Gliomas secrete high levels of CCL2; UC-MSCs up-regulated chemokine receptor type 2 and CCL2 receptors and displayed directed migratory ability to a specific subset of stemlike brain tumor—initiating cells expressing $CD133^{+}$. It is suggested that chemokine receptor type 2 expressed by UC-MSCs enabled the directed migration toward brain tumor cells secreting CCL2. This finding was corroborated using dose-dependent administration of CCL2 in transwell migrations experiments.[47] Investigators further found iron nanoparticle—labeled UC-MSCs migrate toward brain tumor cells tracked by magnetic resonance imaging. Although UC-MSCs migrated toward the tumor, there was no decrease in tumor size in an immunosuppressed rat model of human GBM. This result may be due to the source of MSCs and isolation protocols, thus highlighting the need for protocol standardization for pre-clinical studies using MSC administration. Despite different sources of MSCs (bone

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marrow, adipose tissue, umbilical cord), migration preference toward glioma is maintained presumably through the expression of receptors on MSCs and associate ligands secreted by the brain tumors.

Role of SDF-1/CXCR4

One mechanism of MSC tropism to tumor is through the CXCR4/SDF-1α axis. This receptor-ligand complex plays a vital role in cell migration and inflammation. CXCR4 is up-regulated in GSCs by 25- to 89-fold compared with noninvasive tumor cells and increases with tumor grade.[60] CXCR4 colocalizes with SDF-1α and is frequently found in regions of angiogenesis, necrosis and degenerative environments.[61,62] CXCR4 is a G protein—coupled receptor expressed on glioma cells, microglia, neurons, and astrocytes. SDF-1α (CXCL12), the ligand for CXCR4, is also found on endothelial cells lining the BBB vessels and is suggested to mediate the adhesion and transcytosis of immune and stem cells into the tissue; it is proposed that the administration of cellular therapies, such as MSCs, cross the endothelium of the BBB via the CXCR4/SDF-1α pathway.[63] Binding of SDF-1 to CXCR4 plays an essential role in MSC trafficking. Substantial reduction of BMSC migration toward the U87 GBM cell line was observed with the use of a CXCR4 receptor antagonist in a 3-dimensional invasion assay and similarly displayed reduced tropism in vivo in a pulmonary metastasis models. Studies suggest that BMSC homing is due, in part, by CXCR4.[48] This axis plays a crucial role in cell recruitment during central nervous system injury and may be an important player in recruiting reparative cells during tissue insult.[64] SDF-1α/CXCR4 destabilizes the integrity of the BBB vasculature, resulting in enhanced recruitment of cells from the circulation.[64] This signaling pathway mediates actin polymerization and pseudopodia formation, allowing for immune and MSC transcytosis across the vasculature to areas where a high concentration of SDF-1 is present. This axis may be one of the cardinal regulators of cell homing to brain tumor microenvironments. A cytokine array profile was conducted to investigate the cross-talk of AMSCs in coculture with GBM. The CXCR4—SDF-1 axis was identified as a major regulator in MSC trafficking toward gliomas.[43] Similar to BMSCs, AMSCs significantly respond to SDF-1 released by glioma cells and have been engineered to overexpress CXCR4 to enhance homing to tumors.

Role of IL-6

Interleukin 6 is a soluble constituent involved in the malignant progression of glioma.[65] Interleukin 6 promotes renewal, invasion, and angiogenesis. In glioma, elevated ligand and receptor expression is associated with poor survival.[66] The survival-promoting actions of IL-6 include suppression of immunosurveillance via the recruitment (and stimulation) of MDSCs and tumor-associated neutrophils. This induction cripples the response of surrounding helper T cell type 1 and cytolytic T cells, ultimately leading to T-cell dysfunction and an inhibition of tumor clearance. Interleukin 6 is notably implicated in GBM, and stimulation of brain tumor cells with IL-6 promotes the top 3 signal transduction pathways involved in gliomagenesis: (1) p42/p44-MAPK (mitogen-activated protein kinase); this pathway is deregulated in approximately one-third of all cancers and is highly involved in the sensing and processing of stress signals,[67] (2) PI3K/AKT, a signaling pathway implicated in enhancing angiogenesis, activating epithelial to mesenchymal transition for increased invasion, and the promotion of metastasis,[68] and lastly, (3) JAK-STAT3 pathway, which blocks tumor recognition by immune cells and promotes cell cycling progression and inhibition of apoptosis.[69] Invasion and migration were enhanced in human GBM lines (T98G and U251), with increased exposure to soluble IL-6 documented by scratch assays and in vivo studies.[70] In other cancers, such as breast, a cytokine screen from conditioned breast cancer media identified IL-6 as a regulator of BMSC migration. Enhanced migration was dose dependent, confirmed by the addition of increasing amounts of IL-6 in the lower chamber of transwell assays with dose-dependent response.[71] Glioma environments are under chronic inflammation, and IL-6 is one of the cytokines highly implicated in the chronic inflammatory phenotype often associated with GBM. Tumor-associated macrophages represent a large bulk of the

nonneoplastic cells in the tumor and are large producers of IL-6. Mesenchymal stem cell migration toward IL-6 production could be exploited for targeted therapy to MDSCs using MSCs as delivery vehicles to transport immune-related cargo to the tumor niche for MDSC suppression or TAM polarization.

Role of TGF-β

The TGF-β family has been extensively linked to several diseases and highly implicated in malignant brain tumors for playing a dual role in regulating brain tumor stem cell activity. This "dual" role comes from its regulatory function in maintaining tissue homeostasis. Pretreatment of AMSCs with TGF-β enhanced the expression of pancreatic cancer stem marker CD144 and NANOG,[72] while other members of the TGF-β family, such as BMP4, can attenuate brain tumor stem cell progression. In a human glioma xenograft model, undifferentiated human brain tumor cells with high $CD133^{+}$, alluding to a more stem phenotype, responded to BMP4 by activating their related receptors and triggering the SMAD signaling pathway to induce differentiation and subsequently decrease tumor burden in vitro and in vivo.[5,73] In order to be classified as an MSC, one inclusion criteria is the presence of the transmembrane TGF-β coreceptor CD105 on the cell surface. The receptor is responsible for mediating the directed homing of AMSCs toward microenvironments with high TGF-β expressions, such as that of gliomas. Preexposure of human AMSCs to TGF-β for 48 hours resulted in increased migration in vitro through a transwell assay and displayed morphological changes in lamellipodia formation in scratch assays. In vivo, locally implanted AMSCs preexposed to TGF-β displayed enhanced migration toward primary human glioma cells. Adipose tissue-derived MSC migration was reduced on TGF-β receptor AMSC knockdown.[49]

CURRENT IMMUNOTHERAPY FOR GLIOMA

Immunotherapy is designed to stimulate the immune system or inhibit mechanisms that promote immunosuppression. Glioblastoma is a "cold tumor" in that it harbors a low mutational load, making it difficult to mount an effective immune response. However, the GBM immune environment primarily consists of MDSCs and regulatory T cells involved in actively suppressing the immune response through PDL1 and CTLA4. Antigen-presenting cells present tumor antigens to the adaptive immune system through CD80/CD86 ligand interacting with CD28 costimulatory receptor on T cells; T-cell stimulation is controlled via CTLA4 an antistimulatory and inhibitory signal that is often overexpressed in GBM and inversely correlated with survival. Competitive binding of CD80/CD86 to CTLA4 (due to its overexpression) dampens T-cell activation and ultimately leads to immunosuppression. Ipilimumab, a CTLA4 inhibitor, has undergone clinical trials with unremarkable results for GBM. Similarly, PDL1 is overexpressed on tumor cells; binding of PDL1 to programmed cell death 1 on leukocytes leads to T-cell suppression and self-tolerance by dampening the inflammatory response. Although programmed cell death 1 checkpoint inhibitors (atezolizumab, nivolumab, pembrolizumab) resulted in augmented T-cell diversity, immunoinfiltration, and increased cytokine production in GBM, a study reported associated molecular alterations and clinical response correlated to tumor evolution, suggesting that timing of administration is crucial for advantageous prognosis.

Despite the limited immunotherapies for patients with GBM, several immunotherapeutic vaccines were able to reach phase 3 clinical trials.[74] Rindopepimut,[75] ICT-107,[76] and DCVax-L[77,78] are at the top of this list for the following reasons. Rindopepimut, also known as CDX-110 or PEPvIII, is a peptide that mimics and targets EGFR variant III (EGFRvIII). EGFR variant III is an active mutant form of EGFR that is exclusively expressed in 25% to 30% of patients with GBM.[79] Rindopepimut is a vaccine that depends on a single immunogenic peptide, which exclusively targets the EFGRvIII neoantigen expression on the tumor cells and limits the risk of toxicities.[74] One of the disadvantages is the heterogeneity of the EGFRvIII expression on the GBM cells, which leads to outgrowth of tumor cells lacking this antigen.[74,79] ICT-107 is a multipeptide vaccine that consists of patient-derived DCs incubated ex vivo with tumor peptides.[76] The proof of

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concept of this vaccine was challenging because similar peptides might not share the immunogenicity between humans and mice, and the gene overexpression might not be the same in mouse glioma models and human tumors.[74,76] Last, but not least, DCVax-L, the longest-acting vaccine in history for GBM,[77] acts by utilizing whole tumor lysate from patient GBM tissue to generate autologous DCs.[80] However, this vaccine is considered challenging because it requires the collection of the patient's tumor tissue and then processing the tissue to activate the autologous DCs.[77,78]

Furthermore, given the impermeable nature of the BBB, the delivery of the targeted agents to the brain is considered challenging. Therefore, several approaches have been considered for transiently breaching the BBB. First, the traditional approach consists of the injection of a hyperosmotic solution before the administration of the therapeutics that induce endothelial cell shrinkage and increased vascular leakage in the brain parenchyma.[81,82] Second, focused ultrasound allows for a substantial increase in BBB permeability via the application of fine-tuned acoustic pressure to the brain that could be utilized for therapeutic delivery. Focused ultrasound produced more desirable results in breaching the BBB when used in combination with microbubbles.[81,83–85] Additionally, photodynamic therapy is an approach that utilizes light irradiation via the use of photosensitive molecules. This application is beneficial in delivering large molecules and nanoparticles, but it is limited to a small area of the brain.[81,86,87] Lastly, the convection-enhanced delivery approach (CED) allows for intratumoral local drug injection via a catheter. Despite the invasive nature of the approach, CED has documented efficacy and safety with several therapeutic agents. Furthermore, in vitro and in vivo studies found that CED might increase the invasiveness of the glioma cell by activating the CXCR4-CXCL12 signaling pathway. This adverse effect can be avoided by coadministration of the CXCR4 antagonist AMD3100.[88–90] Although BBB permeability remains a challenge, therapeutic approaches that exploit endogenous mechanisms to mediate extravasation and migration across the BBB into tissue may prove more effective at targeted delivery to GBM.

LIMITATIONS IN MSC APPLICATIONS

For years, MSC transplant has been regarded as safe with a wide array of therapeutic applications. However, low survival on engraftment and short-term retention have hampered MSC therapeutic efficacy. Cell-cell communication and adhesion play an essential role in the viability and proliferative capacity of stem and stromal cells, such as MSCs. During isolation and engraftment, anchorage-dependent mechanisms are disrupted, and cells undergo a form of programmed cell death referred to as *anoikis*. Poor engraftment is due to loss of extracellular matrix anchoraging at the site of implantation.[91] Strategies to improve cell retention may benefit from strategies that inhibit anoikis (eg, encapsulation of stem cells in biodegradable biomaterial). Although transplant is essential for regenerative medicine, therapeutic strategies utilizing MSCs for cargo delivery or immunoactivation would benefit from the transient effects of delivery, reducing potential risks of stable or chronic persistence of exogenously delivered therapeutic cells in tissue. Additionally, a prevailing theory in MSC biology suggests that MSCs act in a "hit-and-run" fashion and exert their therapeutic function on resident cells for stable tissue repair on arrival. Clinically, isolation methods, ex vivo expansion, route of administration, concentration, and synthetic modifications must be considered for each approach exclusively to harness the full potential of MSC therapy.

Although the dynamics of MSC homing to the site of injury have yet to be fully understood,[32] MSCs display considerable potential as cellular vehicles because they are recruited by cytokines substantially up-regulated and secreted by gliomas. Studies have suggested that the mobilization strategies used by MSCs may be similar to the strategies used by leukocytes to home to areas of insult,[53] and MSCs can travel concomitantly during injury with immune cells to mediate repair. The aforementioned homing process of mesenchymal migration toward inflammation and immune-infiltrated loci has been observed in response to various inflammatory diseases.[37]

FUTURE IMPLICATIONS

Glioblastoma is a highly complex tumor that exploits several mechanisms to evade clinically approved therapeutics. The clinical experience of immunotherapy in current GBM treatment paradigms has revealed modest success, partially due to the cytokine secretion profile that blocks intratumoral cytotoxic T-cell migration and activation, resulting in an immunosuppressive state. Reeducating components of the immune system rather than ablating them may be a more effective approach for targeting the GBM tumor microenvironment. Delivery of cargo that induces immune activation (eg, IL-12, IFN, IL-2,) via MSCs may be a logical therapeutic approach in reactivating tumor surveillance mechanisms. Studies of GBM immunotherapy in immunocompetent animal models or humanized mouse models that accurately predict GBM response or escape to new treatment strategies are needed and may enable new discoveries in elucidating the mechanisms mediating the immunosuppression often associated with GBM.

CONCLUSION

Factors secreted in the tumor microenvironment can have multiple effects such as tumorigenesis, immune cell recruitment, and recruitment of exogenous cellular vehicles. Many of the regulatory mechanisms mentioned in this review skew immune infiltrating cells such as TAM (of the innate immune response) T cells (of the adaptive immune response) toward a type 2 phenotype that is often tumor promoting. These mechanisms can all take place simultaneously, dynamically or bidirectionally within the tumor niche, eventually resulting in immunosuppression. Potential interplay between the immune system and exogenous stem cells must be further examined, and a strategic avenue could explore the possibility of immune reeducation. Soluble factors released by tumors are implicated in MSC recruitment to GBM and can mediate site-specific delivery of a therapeutic cargo. We suggest that this characteristic be harnessed for immunomodulation in GBM by MSC via the delivery of an immune-activating or immune-enhancing cargo.

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Abbreviations and Acronyms: **AMSC** = adipose tissue–derived mesenchymal stem cell; **BBB** = blood-brain barrier; **BMSC** = bone marrow–derived mesenchymal stem cell; **CED** = convection-enhanced delivery; **DC** = dendritic cell; **EGFRvIII** = EGFR variant III; **5-FC** = 5-fluorocytosine; **GBM** = glioblastoma; **GSC** = glioma stem cell; **IFN** = interferon; **IL** = interleukin; **MDSC** = myeloid-derived suppressor cell; **MHC** = major histocompatibility complex; **MSC** = mesenchymal stem cell; **NSC** = neural stem cell; **TAM** = tumor-associated macrophage; **TGF** = transforming growth factor; **TNF** = tumor necrosis factor; **UC-MSC** = umbilical cord MSC

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