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Evasion of the Immune System by Glioblastoma Multiforme: An Obstacle to Achieving Effective Therapies

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Glioblastoma multiforme (GBM), a highly aggressive and malignant form of brain cancer, continues to pose a significant challenge in the field of oncology. Despite ongoing advancements in treatment strategies, the prognosis for GBM patients remains grim, with a 5-year survival rate hovering around 5%. The management of GBM involves multiple therapeutic approaches, including immunotherapy, but optimal treatment outcomes in terms of overcoming tumor recurrence and resistance have not been achieved. A key factor contributing to therapy resistance and the progression of GBM is the tumor's ability to evade the immune system, referred to as immune escape from cancer. This phenomenon reflects the tumor cells' efforts to adapt and survive the body's immune response. The release and expression of molecules like TGF- β , IL-10, PD-L1, and NKG2DL by GBM cells impact the activation, recognition, and elimination of tumor cells by the immune system. Additionally, the involvement of cells such as MDSCs, Tregs, and TAMs plays a role in inhibiting the immune system's function, thereby promoting the development of GBM cells. A better comprehension of GBM's immune escape, supported by technological advances, will significantly aid in the future management of GBM patients' treatment.

Keywords: glioblastoma multiforme, GBM, cancer immunity, immune evasion, immune escape, immunotherapy

Introduction

Glioblastoma multiforme (GBM) is classified as a grade 4 cancer by the World Health Organization (WHO), representing the most lethal form of primary brain tumor with the highest prevalence, accounting for 45% of malignant brain tumors. The majority of GBM patients typically fall within the age range of 45 to 75

years. Regrettably, the prognosis for individuals with glioblastoma is quite bleak, with a 5-year survival rate of less than 5%.¹ The incidence of GBM varies from 0.59 to 5 cases per 100,000 individuals each year, and there is a projected annual increase in the number of GBM cases worldwide.^{2,3}

Histologically, GBMs exhibit clusters of poor differentiated, pleomorphic astrocytes with a significant

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vascularization.⁴ Typically, GBM tumors originate from neural stem cells that can develop into astrocytic or neuronal cell types.⁵

The standard treatment for GBM tumors entails surgical resection along with radiotherapy and chemotherapy using temozolomide (TMZ).⁶ Additionally, steroids like dexamethasone are administered to reduce inflammation caused by peritumoral edema.⁷ However, conventional GBM treatment is not entirely effective because it can impact the body's anti-tumor immune response. The combined use of high-dose TMZ chemotherapy and radiotherapy is known to induce lymphopenia.⁸ Simultaneous administration of TMZ, radiotherapy, and dexamethasone in patients can lead to a persistent decrease in CD4⁺ T lymphocytes, increasing the risk of infection and worsening survival rates.⁹

Advancements in science and technology have led to continuous improvements in cancer treatment. For instance, the utilization of 5-aminolevulinic acid (5-ALA) aims to enhance the imaging of GBM cells during surgery, making tumor removal more effective.¹⁰ Immunotherapeutic strategies, such as immune checkpoint inhibitors, cell-based therapies, and vaccines, are also under development to stimulate the body's immune response against cancer cells.¹¹ Nevertheless, current immunotherapy approaches for GBM still fall short of delivering ideal treatment outcomes. The highly invasive nature of GBM tumors, combined with the immunosuppressive tumor microenvironment (TME) of GBM, often hinders the activation of the anti-cancer immune system, leading to tumor recurrence and therapy resistance.¹²

The involvement of the immune system in tumor surveillance

The human body's immune system possesses a sophisticated network of cells and molecules that collaborate to detect and eliminate foreign or aberrant cells, including cancerous ones. Key components include immune cells like antigen-presenting cells (APCs), B and T lymphocytes, and natural killer (NK) cells, which cooperate to identify and target malignant cells, ensuring the body's tumor surveillance system functions effectively (Figure 1).

Tumor cells release antigens, which are identified and recognized by APC cells. These APCs become activated in order to process and present these tumor antigens to T lymphocytes within the primary lymphoid tissue. This activation induces T cells to infiltrate the tumor region via the bloodstream. Activated T cells, in turn, release immune

signaling molecules like cytokines, which serve to activate and recruit other immune cells, including B lymphocytes, NK cells, and macrophages. Facilitated by their respective receptors, these immune cells recognize and eliminate tumor cells through various mechanisms, such as antibody-dependent cellular cytotoxicity (ADCC) and the release of perforin and granzyme.¹³ When eradicated, tumor cells release further antigens, which bolster the immune response against the tumor and set in motion the subsequent cycle of anti-tumor immunity.¹⁴

Mechanisms of immune evasion in GBM

While the immune system diligently monitors and targets tumor cell growth, GBMs employ various strategies to avoid detection and elimination. These following mechanisms collectively create a microenvironment that hinders the effectiveness of anti-tumor immune responses (Figure 2).

Immunoprivileged Nature of the Brain

The brain possesses distinct immune characteristics, creating an immune-privileged environment that tightly controls the entry of immune cells and antibodies into brain tissue. The access of immune cells, particularly T cells, into the central nervous system is governed by endothelial cells. Specific molecules on the surface of these endothelial cells, such as selectins and integrin ligands, serve to capture circulating T cells.¹⁵ Following this, T cells become activated and migrate, leading to a downregulation of integrins and an increase in matrix metalloproteinases (MMPs) expression. This results in the degradation of the extracellular matrix, permitting other immune cells to enter the central nervous system.¹⁶ Nevertheless, the overall passage of immune molecules and cells into the brain is significantly restricted due to the stringent control of the blood-brain barrier.

The blood-brain barrier (BBB) is a complex structure within the walls of cerebral blood vessels and has a crucial role in selectively regulating the entry of cells and substances from the bloodstream into the brain. A key component of the BBB is the brain's vascular endothelial cells, which are interconnected by tight junctions that govern the permeability of the endothelium. Additionally, numerous efflux transporters are present to eliminate foreign substances and waste products from the brain into the bloodstream. Apart from endothelial cells, the BBB is composed of pericytes and perivascular macrophages that inhabit the basal membrane of BBB, helping to regulate endothelial function and forming an additional cellular

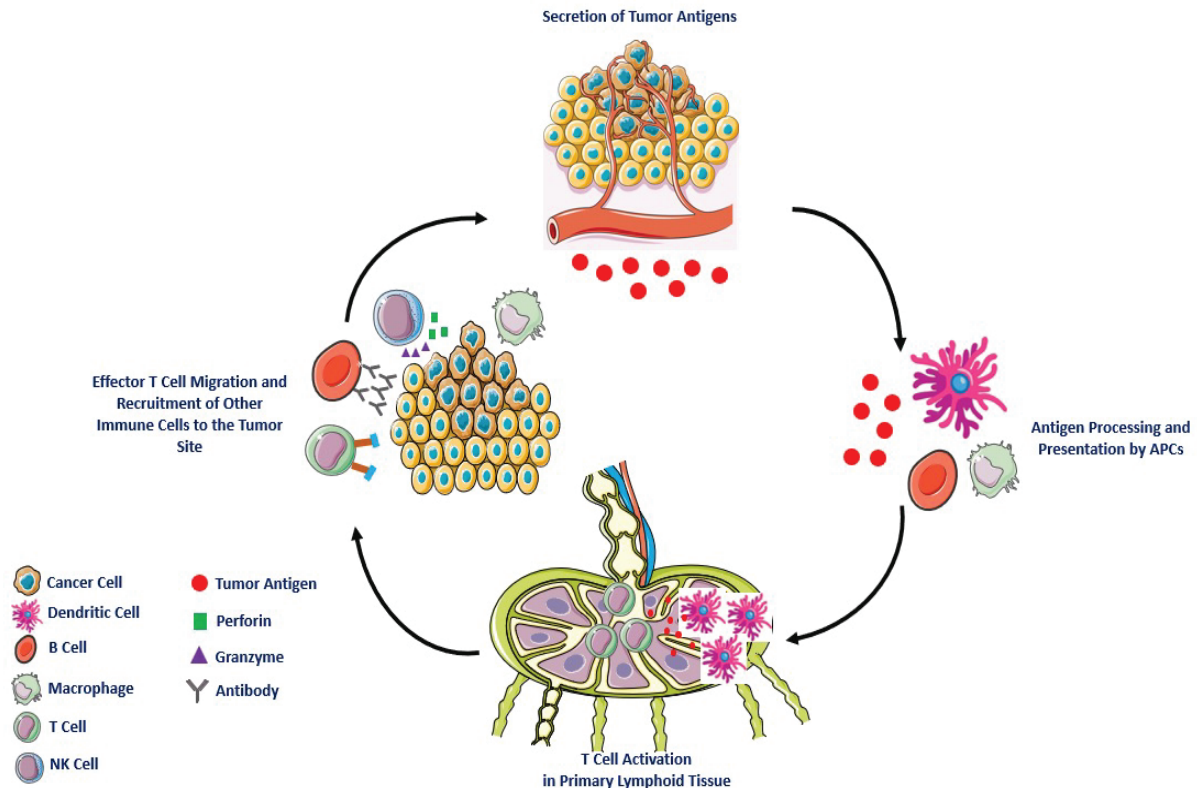


Figure 1. Anti-cancer immunity cycle. (The Figure was partly generated using Servier Medical Art provided by Servier, licensed under a Creative Commons Attribution 3.0 unported license (<https://creativecommons.org/licenses/by/3.0/>)).

barrier around the cerebral vasculature in collaboration with astrocyte cells.^{17,18}

During the development of GBM tumors, the endothelial component of the BBB can be compromised. High metabolic activity of GBM cells can lead to hypoxic conditions and trigger the formation of new blood vessels through angiogenesis.¹⁹ This process damages the tight junctions between endothelial cells, resulting in the creation of new vasculature.²⁰ The inflammatory conditions caused by GBM tumor growth can also weaken the tight junction connections in the endothelium.¹⁶ Despite the weakening of the BBB within the GBM tumor core, the environment surrounding distal GBM tumors still maintains a highly selective permeability. This presents a significant challenge for the immune system to reach tumor cells and limits the accessibility of chemotherapeutic agents and antibodies to target GBM tumors.²¹

Secretion of Immunosuppressive Molecules: Transforming Growth Factor (TGF)- β and Interleukin (IL)-10

GBM cells have the ability to establish an immune-suppressing environment by releasing cytokines like TGF- β

and IL-10, which can hinder the activation and functionality of immune cells. This secretion of soluble factors is often associated with the recruitment of regulatory T cells (Tregs) to the tumor site, further undermining the anti-tumor immune response.

TGF- β is a growth factor that can trigger various cellular responses when it binds to its receptor, including cellular signaling pathways like SMA and MAD gene family homolog (SMAD)2/3, partitioning-defective 6 (PAR6), phosphatidylinositol-3-kinase (PI3K), rat sarcoma (RAS), and TGF- β -activated kinase 1 (TAK1)/TAK1-binding proteins 1 (TAB1)/tumor necrosis factor receptor-associated factor 6 (TRAF6). These pathways can lead to cell growth, differentiation, apoptosis, angiogenesis, and immune responses.²² Intriguingly, TGF- β plays a dual role in tumor development. It initially exerts anti-proliferative effects on various cell types, including tumor cells. In the early stages of tumorigenesis, TGF- β induces apoptosis in tumor cells and inhibits the cell cycle by suppressing c-Myc expression and inducing p21 expression.²³⁻²⁶ As the tumor progresses, TGF- β triggers the epithelial-to-mesenchymal transition (EMT)

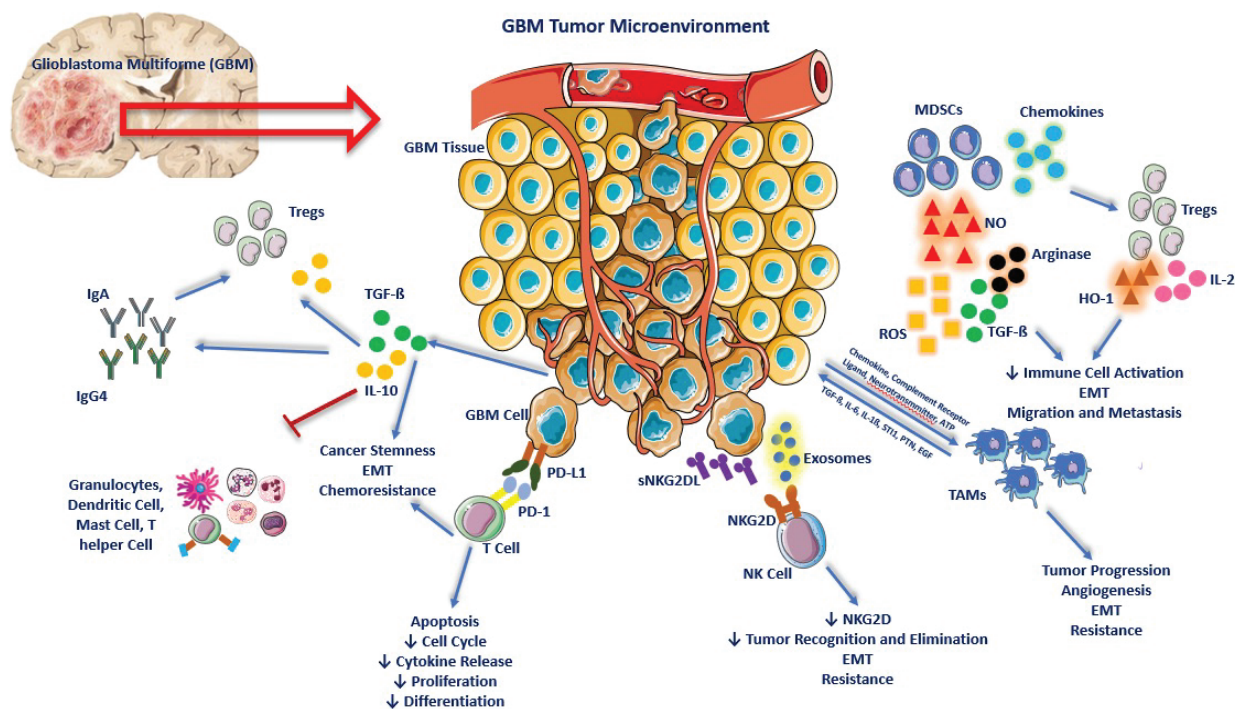


Figure 2. Immune evasion mechanisms of GBM. (The Figure was partly generated using Servier Medical Art provided by Servier, licensed under a Creative Commons Attribution 3.0 unported license (<https://creativecommons.org/licenses/by/3.0/>)).

process, which is crucial for cancer motility, invasion, and metastasis.²⁷

EMT involves altering the cellular structure and morphology from an epithelial to a mesenchymal state, either partially or completely transitioning into a full mesenchymal phenotype. In cases like cancer cells, intact EMT processes empower cells to migrate towards secondary tissues through blood vessels or lymphatic vessels. Various transcription factors, including zinc-finger E-box binding homeobox 1 and 2 (ZEB1, ZEB2), play roles in EMT and carcinogenesis.²⁸ Particularly, ZEB1 regulates the E-cadherin molecule, crucial for epithelial integrity. Activation of ZEB1, as observed in EMT, suppresses E-cadherin transcription, impacting epithelial stability and facilitating tumor cell migration. A correlation between increased ZEB1 expression and decreased E-cadherin expression in patients with prostatic anomalies, a condition often caused by tumors or infection, was reported.²⁹ Additionally, TGF- β -induced EMT may enhance tumor stemness and invasiveness, potentially contributing to cancer resistance against chemotherapy.³⁰ The sensitivity of cancer cells to therapy is influenced by punctuation factors, and genetic alterations in the GBM stem-like cell

(GSC) population lead to tumor recurrence and drug resistance.³¹

IL-10 is essential for immune homeostasis and belongs to a group of anti-inflammatory cytokines that help regulate the immune response during inflammation or tissue injury.³² It prevents pathological conditions resulting from inflammation or autoimmunity.³³

As part of the immune response control, regulatory cells like Treg are activated and recruited, leading to the secretion of IL-10 and TGF- β . Both IL-10 and TGF- β have inhibitory effects on immune cells such as granulocytes, mast cells, dendritic cells, and T helper cells (Th1, Th2, and Th17). Additionally, the release of IL-10 and TGF- β enhances the production of immunoglobulin (Ig)A and IgG4 and the expression of FoxP3, thereby reinforcing the activation of Treg cells and maximizing the suppression of immune responses.³⁴

In the microenvironment of their tiny tumors, GBM cells secrete numerous immune-suppressing factors, including IL-10 and TGF- β , to aid in evading the immune surveillance system. TGF- β , when released by GBM tumors, can reduce the expression of intercellular adhesion molecule (ICAM)-1 and vascular cell adhesion molecule

(VCAM)-1 on endothelial cells, impeding T cell infiltration into the tumor region.³⁵ Furthermore, TGF- β can enhance the activity of matrix metalloproteinase (MMP)2 and MMP9, promoting the motility and invasion of GBM cells into the surrounding brain tissue.³⁶ High messenger RNA (mRNA) expression of IL-10 in GBM tissue was reported in previous study.³⁷ Treg cells that infiltrate the GBM microenvironment can also secrete IL-10, inducing immune tolerance from effector T cells.³⁸ *In vitro* studies indicate that IL-10 secretion not only suppresses the immune system but can also increase the proliferation and migration of GBM cells.³⁹ The immunosuppressive effects of soluble factors like IL-10 and TGF- β hold promise as potential biomarkers for GBM treatment, but further research is needed to explore their full impact.⁴⁰

Programmed Cell Death Ligand 1 (PD-L1) Expression by GBM Tumor

Elevated levels of PD-L1 and programmed cell death protein 1 (PD-1) are commonly observed in cancer patients, particularly those with solid tumors, and are recognized for their role in modulating the immune response against cancer cells. There was an increase in PD-L1 mRNA expression in patients with nasopharyngeal carcinoma (NPC) compared to normal patients. Additionally, PD-1 expression in NPC patients was found to be correlated with the TNM (tumor-node-metastasis) stage, indicating the involvement of PD-L1 and PD-1 in the progression and development of cancer.⁴¹

As well as other cancer types, GBM tumor cells can also elevate the presence of PD-L1 immune checkpoint molecules on their cell surface with its function as immune checkpoint molecules. The interaction between PD-L1 and its receptor, PD-1, can induce exhaustion in immune cells, disrupting the immune response.

PD-1 or cluster of differentiation molecule (CD)279 is a transmembrane protein expressed by various immune cells, including T and B lymphocytes, NK cells, macrophages, and dendritic cells. It plays a crucial role in regulating the immune response. During infections, immune cells that are unresponsive or ineffective against pathogens will display PD-1 on their cell membrane and subsequently become inactive due to interaction of PD-1 with its ligands. Additionally, PD-1 functions to eliminate immune cells that react to self-antigens through the process of immune tolerance.⁴²

As one of the ligands of PD-1, PD-L1 or B7-H1 molecules play a role in modulating the immune system. Generally, PD-L1 is found on the surface of macrophage cells, some T cells and B cells, dendritic cells, and some epithelial cells, particularly during inflammation.⁴² The binding of PD-L1 to PD-1 initiates cellular signaling that suppresses the activity of T cells. This binding leads to the phosphorylation of intracellular domains immunoreceptor tyrosine-based activation motif (ITIM) and immunoreceptor tyrosine-based switch motif (ITSM), recruiting SH2 domain-containing tyrosine phosphatase 2 (SHP2). Activation of SHP2 and phosphatase and tensin homolog (PTEN) then inhibits PI3K and RAS signaling pathways, resulting in cellular responses like apoptosis, cell cycle inhibition, suppression of cytokine release, and inhibition of T cell proliferation and differentiation.⁴³

The capacity of cancer cells to express PD-L1 leads to the suppression of immune cell function, notably T cells. The expression and release of PD-L1 in the microenvironment of microtumors hinder T cell activity and facilitate their evasion from immune responses.^{44,45} Various signaling pathways, including Janus kinase (JAK), PI3K, mitogen-activated protein kinase (MAPK), hedgehog (HH), nuclear factor-kappa B (NF- κ B), and wiggless-type MMTV integration site family (WNT), contribute to the activation of PD-L1 and PD-1 during tumorigenesis. Furthermore, microRNA (miRNA), long non-coding RNA (lncRNA), and cytokines like tumor necrosis factor (TNF)- α and interferon (IFN)- γ are known to stimulate PD-L1 and PD-1 signaling.⁴⁶

GBM tumor cells are recognized for having high levels of PD-L1 expression. Reports indicate that approximately 88% of GBM patient samples express PD-L1 molecules.⁴⁷ Up to 61% of 94 GBM patients had tumor cells expressing PD-L1.⁴⁷ Increased PD-L1 expression is associated with worse patient survival.^{48,49} As PD-L1 is considered a potential therapeutic target in GBM treatment, the combination of anti-PD-L1 antibodies and other immune checkpoint inhibitors is undergoing further evaluation in advanced studies.⁵⁰

In addition to suppressing the activation and response of the anti-tumor immune system, PD-L1 is recognized for its role in controlling the EMT process, a phenomenon associated with the development of resistance to cancer chemotherapy. The regulatory impact of PD-L1 on EMT involves various molecular factors, including mucin 1 (MUC1) and NF- κ B. MUC1, adenosine deaminase that

acts on RNA 3 (ADAR3), and tryptophan hydroxylase 1 (TPH1) can initiate the NF- κ B signaling pathway by influencing upstream factors, subsequently promoting the progression of GBM cells and diminishing their sensitivity to TMZ.⁵¹ Previous *in vivo* and *in vitro* studies revealed that overexpression of PD-L1 not only impacts the malignancy of GBM but also triggers Erk-EMT signaling, potentially contributing to therapeutic resistance.⁵²

Downregulation of Natural Killer Group 2 Member D Ligand (NKG2DL)

One strategy employed by GBM tumors to escape the immune system involves diminishing the recognition capability of NK cells. GBM cells can reduce the expression of ligands associated with one of the NK cell activator receptors, natural killer group 2 member D (NKG2D). This impairment in NK cell activation hampers their surveillance and elimination functions against tumor cells.

The NKG2D receptor is a surface protein found on immune cells, including NK cells. When NKG2D interacts with its ligand, NKG2DL, it triggers intracellular signaling that results in cellular responses like NK cell proliferation and activation. Activated NK cells eliminate target cells through cytotoxicity. In humans, NKG2DL types include MHC class I chain-related protein A/B (MICA/B) and UL16-binding protein (ULBP)1-6. Genetic polymorphism leads to variation in MICA/B and ULBP1-6 molecules, highlighting the immune system's ability to combat various pathogen variants.⁵³

The expression of NKG2DL is regulated by various factors, ranging from transcription to post-translation levels.⁵³ Stress, DNA damage, or infection can trigger cells to increase NKG2DL expression on their surface.⁵⁴ Immune cells with specific receptors for NKG2DL, like NK cells, recognize signals provided by target cells through NKG2DL expression. NKG2D receptors on NK cells bind to NKG2DL on target cells, initiating intracellular signaling pathways with the assistance of DNAX-activating protein 10 (DAP10). The interaction between NKG2D and NKG2DL activates the vav guanine nucleotide exchange factor (VAV)-1, phospholipase C gamma 2 (PLC γ 2), and c-Jun N-terminal kinase (JNK) signaling cascades, leading to NK cell cytotoxicity. Activation of PI3K signaling increases perforin and granzyme secretion by NK cells, while activation of JAK2 and signal transducer and activator of transcription 5 (STAT5) signaling induces cytokine release by NK cells.⁵⁵

In an attempt to evade immune surveillance, tumor cells can reduce NKG2DL expression on their membrane surface through various mechanisms. They can increase the expression of miRNAs, such as miR-20a and miR-93, which inhibit the translation of NKG2DL mRNA into protein. Furthermore, the expression of proteases by tumor cells, like a disintegrin and metalloproteinase domain-containing (ADAM)10 and ADAM17, triggers proteolysis of NKG2DL, resulting in the shedding or degradation of NKG2DL from the tumor cell surface.^{53,56} Shedding leads to soluble NKG2DL (sNKG2DL), affecting NK cell response. Soluble NKG2DL can induce internalization and decreased expression of NKG2D on the NK cell surface.^{53,57} Additionally, tumor cells can secrete NKG2DL into the surrounding microenvironment through exosomes, which can inhibit the recognition process of NK cells in the microtumor environment.⁵⁸

Inducing expression of NKG2DL in GBM tumors through chemotherapy or radiotherapy is a potential avenue for future GBM immunotherapy research. A previous *in vitro* and *in vivo* study demonstrated that TMZ treatment and irradiation can increase gene expression of NKG2DL at mRNA and protein level, both in glioma cell lines and experimental animal models, as well as in GBM patient samples.⁵⁹ Suppression of NKG2DL expression in GBM may be caused by the overexpression of EZH2-92aa protein, which promotes GSC evasion of NK cell recognition and cytotoxicity.⁶⁰ Increased TGF- β expression has a repressive effect on NKG2DL expression in GBM patient samples, indicating a complex interplay of molecular and cellular factors in the microtumor environment that inhibits the anti-tumor immune response.⁶¹ The expression of miRNAs in GBM tumors can also impede the anti-tumor activity of NK cells via NKG2DL signaling. Inhibiting miR-20a, miR-93, and miR-106b can enhance NKG2DL expression and bolster the susceptibility of GBM tumor cells to NK cells.⁶² Nevertheless, there was also an involvement of miRNAs in enhancing the responsiveness of tumors to treatment. The presence of miR-93 was found to impede the autophagy process within the GSC population by inhibiting autophagy regulators, specifically beclin-1 (BECN1), autophagy-related (ATG)5, ATG4B, and sequestosome (SQSTM)1/p62. In the context of tumor development, such as in GBM, it is established that metabolites generated from autophagy are utilized by tumor cells to sustain their energy and nutrient requirements. Furthermore, the expression of miR-93 was observed to heighten the sensitivity of GSCs to

radiation and TMZ administration.⁶³ These study findings suggest a connection between the regulation of the immune system and GBM resistance through miRNAs. Future investigations are warranted to unravel the intricate interplay between these two factors, paving the way for more effective combinations of standard therapy and immunotherapy in patient care.

Immunosuppressive Effects of Immune Cells on the GBM Microenvironment

In addition to manipulating factors that create an immune-suppressive environment, GBM tumor cells can also influence the behavior of immune cells recruited to the microtumor environment. This immunosuppressive microenvironment, partly due to the release of cytokines by tumor cells, can alter the anti-tumor activity of immune cells, causing them to adopt an immunosuppressive phenotype. The involvement of these immunosuppressive cells is a contributing factor to GBM tumor cells' evasion of the immune system.

One such group of immune cells exhibiting an immunosuppressive phenotype in the GBM microtumor environment is myeloid-derived suppressor cells (MDSCs). MDSCs are myeloid cells that function as regulators of the immune system.⁶⁴ MDSCs can suppress T cell function through various mechanisms, including the production of arginase and the secretion of nitric oxide (NO) and reactive oxygen species (ROS).^{65,66} Research data indicates that MDSCs can inhibit the anti-tumor immune response. In patients with tumors, the accumulation of MDSCs is associated with the overproduction of cytokines and growth factors such as granulocyte macrophage-colony stimulating factor (GM-CSF), IL-2, and vascular endothelial growth factor (VEGF).^{67,68} The presence of MDSCs in the tumor surroundings can contribute to increased malignancy and resistance to cancer. MDSCs have the potential to support the processes of EMT, migration, and metastasis in NPC cancers, primarily mediated by cyclooxygenase (COX)-2.⁶⁹ In GBM tumors, the buildup of MDSCs leads to localized immunosuppressive effects.⁷⁰ Monocytic MDSCs are more prevalent in GBM tumors than granulocytic ones.⁷¹ Monocytic MDSCs can release chemokines into the microtumor environment, recruiting regulatory T cells that assist in tumor growth.⁷² The expression of immunosuppressive molecules by MDSCs, such as TGF- β and arginase, further enhances the tumor's suppressive effect on the immune response.⁷⁰

To promote their growth and evade the immune system, GBM tumor cells also exploit the immunosuppressive function of Tregs. Tregs belong to a subset of CD4⁺ T cells that differentiate in the thymus, a primary lymphoid organ. In pathological conditions like infections, Tregs are induced to control the immune response, including suppressing inflammation and preventing autoimmunity.^{73,74} Through the expression of cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) molecules, Tregs can interact with APC cells and inhibit the activation of effector T cells.⁷⁵ Additionally, Tregs can suppress T lymphocytes and APC cells through several other mechanisms, including heme oxygenase-1 (HO-1) expression, upregulation of IL-2, inhibition of IFN- γ , and stimulation of the release of immunosuppressive cytokines that amplify the suppressive effects of Tregs.⁷⁶⁻⁷⁹ These mechanisms of suppression by Tregs may contribute to the inhibition of the immune response in GBM tumors. Studies in GBM patients have shown an increase of Tregs in peripheral blood and among lymphocytes infiltrating tumor tissue compared to normal brain tissue.⁸⁰ The lymphocytes infiltrating GBM tumors, including Tregs, can reduce the expression of CD28 and CD62L molecules, which serve as immune cell co-stimulators, resulting in impaired activation of immune cells and a correlation with tumor progression.⁸¹ Studies on brain tumor patient samples have indicated a positive correlation between Tregs infiltration and WHO tumor grade.⁸² Furthermore, it is known that hypoxic conditions in GBM tumors can stimulate the proliferation of Treg cells and induce the release of cytokines such as soluble colony stimulating factor-1 (sCSF-1), C-C motif ligand-2 (CCL-2), and galectin-3, which play a role in promoting the growth, migration, and invasion of GBM tumor cells.⁸³⁻⁸⁶

In addition to T and B lymphocytes, macrophage cells have been reported to infiltrate GBM tumors as early as 1970.⁸⁷ In high-grade astrocytoma tumors, a significant increase in the number of macrophage cells has been observed, although there is not a clear correlation between the number of lymphoid cells and tumor grade.⁸⁸ GBM tumors also contain cells with a macrophage-like phenotype, such as microglia cells exclusively located in brain tissue and malignant astrocyte cells. These cells, collectively referred to as tumor-associated macrophages (TAMs), infiltrate the tumor tissue due to their expression of similar surface markers, including ionized calcium-binding adapter molecule 1 (IBA1), CD11b, CD68, and human alveolar macrophage (HAM)56.⁸⁹⁻⁹¹ Both TAMs and GBM cells can influence each other to support tumor progression.

GBM cells recruit TAMs through various mediators, including chemokines, complement receptor ligands, neurotransmitters, and adenosine triphosphate (ATP), leading to the accumulation of TAMs in tumor tissue.⁹² Additionally, GBM cells release molecular factors, such as S100 calcium-binding protein B (S100B), arsenite-resistance protein 2 (ARS2), and carbonic anhydrase IX (CAIX), that polarize TAMs into a pro-tumor M2 macrophage phenotype.⁹³⁻⁹⁵ On the contrary, M2 macrophages, also known as TAMs, have the ability to release cytokines like TGF- β , COX-2/STAT, epidermal growth factor receptor (EGFR)/extracellular signal-regulated kinase 1/2 (ERK1/2), SMAD/snail family transcriptional repressor 1 (SNAIL), protein kinase B (PKB)/mechanistic target of rapamycin (mTOR), C-C motif chemokine ligand (CCL)-18, and hypoxia-inducible factor (HIF)-1 α . These cytokines play a role in fostering EMT and contributing to chemoresistance in GBM.⁹⁶ TAMs also contribute to GBM tumor growth and invasion by releasing cytokines like TGF- β , IL-6, IL-1 β , stress-induced phosphoprotein 1 (STIP1), pleiotrophin (PTN), and epidermal growth factor (EGF), stimulating CCL, Toll-like receptor (TLR), and WNT signaling pathways, and secreting exosomes. Furthermore, other molecules released by GBM cells, such as receptor for advanced glycation end products (RAGE), ADAM8, CECR histone acetyl-lysine reader protein 1 (CECR1), SUMO specific peptidase (SSP)-1, and VEGF-A, may promote angiogenesis, supporting tumor progression.⁹⁷

Implications for immunotherapy

The success of GBM therapy is significantly impacted by the presence of the BBB, which can hinder the delivery of therapeutic agents, including antibodies, to the tumor tissue. To address this challenge, various strategies have been developed to enhance the effective delivery of therapeutic agents across the BBB. These approaches encompass direct administration to brain parenchyma or cerebrospinal fluid, serving as routes to bypass the BBB and reach the GBM tumor environment. However, these methods, such as intraparenchymal, intracerebroventricular, or intrathecal injection, are highly invasive and associated with a high risk of infection. Furthermore, direct injection into brain parenchyma or cerebrospinal fluid is hampered by the slow diffusion rate within the dense brain tissue, resulting in ineffective drug distribution to the tumor.⁹⁸⁻¹⁰⁰

Another approach involves disrupting the barrier function of the BBB. Chemical disruption of the BBB, using

hypertonic solutions and endogenous bioactive compounds like bradykinins, or physical methods using ultrasound techniques and microbubbles with increased specificity, is known to induce endothelial cell shrinkage and loosen tight junctions, facilitating the access of therapeutic molecules to the brain.¹⁰¹⁻¹⁰⁴ However, the implications of BBB disruption are not yet fully understood and require further investigation before clinical application. Additionally, the development of drug delivery systems using carriers, such as vesicle-shaped nanoparticles, may aid in bypassing the BBB and reaching the brain parenchyma.¹⁰⁵ These nanoparticles can be linked with molecular markers, like enhanced green fluorescent protein (EGFP)-epidermal growth factor 1 (EGF1) fusion proteins, which selectively recognize tumor-associated receptors, enhancing the targeting efficiency of therapeutic agents.¹⁰⁶ Advances in science and technology have also led to the creation of modified therapeutic antibodies with improved BBB penetration capabilities, such as bispecific antibodies. These antibody molecules can simultaneously target antigens in the tumor environment and transporter components.¹⁰⁷ While these approaches have not yet reached the clinical trial stage, pre-clinical trials in mice with intracranial tumors have demonstrated that anti-CTLA-4 and anti-PD-1 antibodies, combined with poly (β -L-malic acid) biopolymers, effectively bypass the BBB barrier, leading to increased infiltration of CD4⁺ and CD8⁺ T cells into the tumor and improved mouse survival rates.¹⁰⁸

An additional avenue for future GBM immunotherapy options involves targeting pro-tumorigenic cytokines like TGF- β . The administration of Trabectedin, an anti-sense RNA molecule complementary to TGF- β 2 mRNA, to GBM patients with tumor recurrence may improve median survival compared to single chemotherapy treatment, though the difference is not yet statistically significant.¹⁰⁹ Moreover, the administration of GVAX vaccine in combination with anti-TGF- β and anti-PD-1 antibodies has shown increased cure rates and improved anti-tumor effects in mouse models with pancreatic tumors. This combination therapy can reduce the infiltration of regulatory T cells into the tumor area, thus mitigating the immunosuppressive environment in GBM.¹¹⁰

Targeting the PD-1/PD-L1 axis in the GBM microtumor environment is another viable treatment option for patients. Monoclonal antibodies classified as immune checkpoint inhibitors (ICIs), such as pembrolizumab, nivolumab, durvalumab, and atezolizumab, have demonstrated significant efficacy in clinical trials and have gained approval

for treating various types of cancer, including melanoma and lung cancer.¹¹¹⁻¹¹³ In GBM, the effectiveness of anti-PD-1/anti-PD-L1 antibodies is under investigation through pre-clinical studies and clinical trials. Single-agent ICI administration has not resulted in improved patient survival, but neoadjuvant ICI therapy shows promise in terms of anti-tumor effects. This includes reducing immunosuppression in GBM tissue, enhancing local and systemic anti-tumor immune responses, and increasing immune cell infiltration into the tumor.¹¹⁴⁻¹¹⁶ In mouse models with GBM, combining anti-PD-1/anti-PD-L1 antibody therapy with standard TMZ therapy and radiotherapy has improved survival rates, boosted the number of circulating lymphocyte and tumor-infiltrating lymphocyte (TIL), and reduced local immunosuppression, indicating the therapeutic potential of ICI.^{117,118} Nevertheless, some clinical trials have not produced satisfactory results.¹¹⁵ Despite the challenges in the clinical trial phase, the potential anti-tumor effects of ICIs in GBM treatment warrant further exploration through additional studies.

Promoting anti-tumor immune responses via vaccination can serve as a supplementary element in the realm of immunotherapy for GBM. The use of a vaccine, Rindopepimut, which comprises an EGFR variant III (EGFRvIII) peptide conjugate, has been shown to enhance the overall survival rate in GBM patients with EGFRvIII-positive tumors. Nevertheless, the administration of Rindopepimut may lead to tumor recurrence with a notable reduction in EGFRvIII expression compared to baseline. This suggests that targeting individual tumor antigens through vaccination could potentially give rise to tumor variants capable of evading the immune system.¹¹⁹ In response to this concern, multi-peptide vaccines like IMA950 have been developed. Research findings indicate that IMA950 administration is well tolerated and can elicit specific adaptive immune responses against the target antigen, although the detection of specific T cells within the TIL population has not been confirmed. Consequently, the assessment and exploration of vaccine administration in combination with other modalities, such as immune checkpoint inhibitors, are ongoing through further clinical trials.¹²⁰

Recent progress in engineering technology has contributed to the advancement of GBM immunotherapy through chimeric antigen receptor (CAR)-T cell. CAR-T cell therapy involves modifying donor T cells by combining tumor-specific fragments with costimulatory domains, which are then reintroduced into the autologous donor. This

approach allows T cells to express an elevated number of tumor-specific receptors, enhancing their ability to target tumor cells and enabling activation without the need for external costimulatory signals.¹²¹ When CAR-T cell therapy is combined with standard GBM treatments such as TMZ and radiotherapy, a potential therapeutic effect is observed. Despite TMZ and radiotherapy causing lymphodepletion, this condition leads to the persistence of CAR-T cells in the blood circulation of GBM animal models, contributing to an increased survival rate in mice.¹²² CAR-T cells have the potential to restore and enhance the anti-tumor immune response of NK cells, which are suppressed due to the dysregulation of NKG2DL-NKG2D axis. In a GBM GL261 mice model, combining radiotherapy with CAR-T cells expressing NKG2D demonstrated an effect in increasing the survival rate.¹²³ Given the efficacy of CAR-T cell therapy in suppressing tumor growth in pre-clinical trials, it is essential to evaluate its application in patients before implementing it in clinical settings.

Conclusion

The formidable challenge in treating GBM patients stems from the invasive and unresponsive nature of GBM tumors, combined with the immune evasion mechanisms employed by tumor cells. The intricate interplay of molecular and cellular factors within the GBM microtumor environment, including TGF- β , IL-10, PD-L1, NKG2DL, MDSCs, Tregs, and TAMs, alongside the immune selectivity factor of the blood-brain barrier system, creates a highly immunosuppressive and treatment-resistant microtumor environment. Progress in immunotherapy, such as the administration of ICI, vaccination, and the application of CAR-T cell engineering techniques, is anticipated to break through the challenges posed by GBM resistance and recurrence. These advancements not only hold the promise of overcoming treatment hurdles but also aim to enhance the efficacy of standard treatments for GBM patients. Thus, ongoing research into the mechanisms of GBM evasion in the context of therapy is crucial for developing innovative therapeutic approaches that can revolutionize the treatment landscape for aggressive tumors like GBM.

Authors Contribution

KJK and SIW were both involved in conceiving the topic of the manuscript, preparing the manuscript draft, and also designing the figures. All authors took parts in giving critical revision of the manuscript.

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