Glioblastoma (GBM) Immunotherapy: Overcoming the Immunosuppressive Microenvironment and Blood-Brain Barrier (BBB)

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Abstract. Glioblastoma (GBM), the most common and most aggressive type of cancer that originates in the brain, is characterized by its poor prognosis for survival. Immunotherapy has demonstrated significant therapeutic effects in many hematologic malignancies. However, its outcomes in treating solid tumors have been disappointing. The inhibitory microenvironment is one of the most important reasons for the failure of immunotherapy, which is composed of inhibitory immune cells, inhibitory cytokines, chemokines, and the extracellular matrix (ECM). To overcome the challenges of immunotherapy in the solid tumor microenvironment (TME), approaches targeting immunosuppressive cytokines and immune checkpoint molecules are being further investigated. There are also approaches to degrade the ECM, localize the tumor by inhibitory signals, and overcome the BBB. Besides, the development of artificial intelligence (AI) and personalized medicine also shows great potential for overcoming the inhibitory immune microenvironment. By highlighting the recent studies of GBM, this article aims to inspire the development of more effective immunotherapies.

Keywords: Glioblastoma **(**GBM), tumor microenvironment (TME), Blood-Brain Barrier (BBB), Immunotherapy.

1. Introduction

GBM, as a malignant brain tumor, is noted for its aggressive nature and poor therapeutic outcomes. Therefore, it is of utmost importance to develop new and more effective therapeutic strategies. As a novel method, cancer immunotherapy functions by stimulating the patient's immune system to fight against tumors, which shows great potential in the treatment of hematologic malignancies. However, it demonstrates limited therapeutic efficacy against solid tumors. Several challenges may account for this lack of efficacy. Among these, immunosuppressive TME plays a particularly important role. The TME is composed of tumor-related immunosuppressive cells, ECM, cytokines, and chemokines, which suppress tumor immunity and facilitate tumor growth. Besides, the BBB further contributes to the insufficient infiltration of effector cells. In recent years, the rapid development of immunology, molecular biology, and genomics has deepened people's understanding of the mechanisms of GBM, leading to the continuous emergence of new therapeutic targets, concepts, and technologies. This review aims to investigate the impact of the immunosuppressive TME and the BBB on GBM. It also demonstrates some recent research approaches and their limitations, with the hope of providing insights into finding more effective delivery and effector strategies.

2. Immunosuppressive TME

2.1. Suppressive immune cells

Within the TME, various immunosuppressive cells, such as regulatory T cells (Tregs), tumor-associated macrophages (TAMs), and myeloid-derived suppressor cells (MDSCs), contribute to tumor progression and suppress anti-tumor immune responses. Tregs are subsets of $CD4^+$ T cells that share highly similar characteristics with conventional $CD4^+$ T cells but are distinguished by their expression of CD25 and FOXP3. The high-level expression of CD25 competes for Interleukin-2 (IL-2), thereby diminishing the immune efficacy of effector T cells. FOXP3, as a transcription factor, not

only contributes to the maintenance of stemness in glioma cells by modulating the expression of MYB, SOX2, and BEX1, but also recruits Tregs through chemokines such as CCL22[1]. In addition, Tregs can exert immunosuppressive effects both directly and indirectly by expressing high-level inhibitory receptors and secreting immunoregulatory cytokines. Many teams have already explored multiple strategies to reduce the immunosuppressive function of Tregs. CTLA-4 plays a central role in mediating immunosuppressive activities of Tregs. Blocking the CTLA4 molecule reduces the suppressive effects of Tregs and drives DCs to anti-tumor T cell responses. However, it is associated with a high incidence of immune-related adverse events (irAEs). Recently, a type of fully human heavy-chain-only antibodies (HCAbs) against CTLA-4 has been engineered. HCAb 4003-2 is smaller in size. After engineering of the IgG1 Fc domain, it exhibits enhanced antibody-dependent cell-mediated cytotoxicity (ADCC) and a shortened half-life, which may contribute to a reduction in irAEs [2].

A substantial number of MDSCs also accumulate near the GBM, where they exert immunosuppressive effects through the secretion of cytokines, the production of reactive oxygen species (ROS), and the depletion of arginine. Cytokines released by MDSCs can suppress the activity of cytotoxic T lymphocytes (CTLs) while activating and enhancing Tregs' function. The L-arginine depleted by MDSCs is also a critical regulator of T cell survival and antitumor activity. Besides, MDSCs produce a high level of ROS under inflammation, causing DNA damage. ROS can also react with nitric oxide to generate peroxynitrite (PNT), which can nitrate chemokines and prevent cytotoxic T cells from infiltrating tumors. Therefore, MDSCs are effective at immunosuppression. Several drugs targeting MDSCs have been approved by the U.S. Food and Drug Administration. However, due to the phenotypic similarity of MDSCs to normal bone marrow cells, MDSCs' complex immunosuppressive mechanisms, and distinct regulatory pathways of different MDSC subsets, MDSC-targeted therapies are mostly combined with other immunotherapies to exert therapeutic effects. Recent research has identified a specific cancer-associated MDSC subset, termed E-MDSCs, through techniques including single-cell sequencing and gene set enrichment analysis (GSEA). E-MDSCs are recruited to the tumor by stem-like glioma cells [3]. E-MDSCs can markedly inhibit T cell proliferation and exert a pro-tumorigenic effect by interacting with TME. With a deeper understanding of the characteristics and mechanisms of MDSC subsets, more effective therapies against novel targets are expected to emerge. Besides, M2/N2-polarized TAMs and tumor-associated neutrophils (TANs) also play important roles in establishing an immunosuppressive TME.

2.2. Immunosuppressive Cytokines and Chemokines

The immunosuppressive cells mentioned above, and tumor cells release inhibitory cytokines, such as TGF-β and IL-10. Those inhibitory cytokines contribute to the establishment of an immunosuppressive TME, reduce tumor responsiveness to treatments, and facilitate tumor growth and invasion. For instance, TGF-β represents one of the most critical tumor-associated inhibitory factors. It can promote the polarization of macrophages from the M1 to M2 phenotype, and the shift of neutrophils from the N1 to N2 phenotype. It can also induce epithelial–mesenchymal transition (EMT), ECM (ECM) production, and tumor metastasis. Furthermore, the secretion of TGF-β can establish a positive regulatory mechanism. Tregs secrete large amounts of IL-10 and TGF-β, thereby further suppressing $CD8^+$ T-cell function and the maturation of dendritic cells (DCs). The suppressed DCs failed to produce IL-12, but secrete IL-10 instead, which in turn promotes the expansion of Tregs. This mechanism establishes an immunosuppressive feedback loop. Therefore, counteracting TGF-β–mediated immune suppression is also an important strategy for the treatment of solid tumors. A single-chain bispecific CAR-T cell targeting IL-13Rα2 and TGF-β demonstrates significant antitumor efficacy in the treatment of GBM [4].

Chemokines recruit immunosuppressive cells and promote the establishment of an immunosuppressive TME. For example, the interaction between CCR4 and mregDCs promotes Treg activation and the recruitment of CCR4-dependent T cells, thereby establishing a T-mregDC microenvironment. The T-mregDC microenvironment suppresses the migration of mregDCs and

limits tumor antigen presentation, thereby impairing anti-tumor immune responses [5]. At the same time, chemokines can also promote the targeted localization and infiltration of immune cells into solid tumors. Oncolytic viruses engineered to express chemokines can facilitate the homing of CAR-T cells to tumors. Moreover, oncolytic adenoviruses (oAds) armed with the chemokine CXCL11 can reprogram the immunosuppressive TME, enhancing the infiltration of effector T cells, natural killer (NK) cells, and M1-polarized macrophages, while reducing the proportions of Tregs, myeloid-derived suppressor cells (MDSCs), and M2-polarized macrophages [6].

2.3. ECM

The ECM also plays a crucial role in the TME. ECM is primarily composed of collagens, glycosaminoglycans, Proteoglycans, elastin, and non-collagenous glycoproteins, which provide structural support, regulate cell behavior, maintain tissue homeostasis, and facilitate tissue repair and immune regulation under normal physiological conditions. However, these components are often overexpressed in GBM, collectively leading to structural remodeling. The remodeled ECM structure facilitates tumor cell proliferation, migration, invasion, and metastasis. Therefore, degrading the abnormal deposition of the ECM by specific enzymes or eliminating the cells responsible for ECM remodeling represents a promising strategy for the treatment of GBM.

ECM-degrading enzymes, such as hyaluronidases, heparinase, and matrix metalloproteinases (MMPs), can disrupt the integrity of the ECM, thereby enhancing T-cell infiltration. However, it should be noted that excessive degradation of the ECM may increase the risk of tumor metastasis. Cancer-associated fibroblasts (CAFs) are the primary producers of the ECM and play a crucial role in ECM remodeling. fibroblast activation protein (FAP) is overexpressed on CAFs. CAR-T cells targeting FAP can eliminate CAFs [7]. With the depletion of stromal cells, the ECM could be indirectly loosened, thereby improving T-cell infiltration. However, FAP can also promote tumor growth and angiogenesis through multiple mechanisms. If CAR-T cells fail to suppress the pro-angiogenic activity of FAP while depleting CAFs, the risk of tumor metastasis may increase. In addition, since FAP is also expressed in normal tissues, FAP CAR-T cells may induce fatal bone toxicity. To mitigate this toxicity, CAR-T cells can be engineered with different scFvs to make them responsive only to high levels of FAP, thereby reducing toxicity.

The density and thickness of the ECM restrict the penetration and diffusion of oxygen, thereby exacerbating the hypoxic state within the tumor tissue. Hypoxia can inhibit the activation and proliferation of lymphocytes and induce the production of other tumor-supportive molecules, such as Vascular Endothelial Growth Factor (VEGF) and Hypoxia-Inducible Factor (HIF). HIFs are transcription factors that mediate cellular responses to low oxygen levels by regulating the expression of target genes. Hypoxia-sensing CAR-T cells utilize hypoxia-inducible HIFs as regulatory modules, allowing them to be selectively activated and exert cytotoxic effects in hypoxic environments [8]. This approach improves targeting specificity and reduces the risk of off-target toxicity.

3. BBB

In addition to the challenges of the immune microenvironment mentioned above, immunotherapy for GBM faces a unique delivery obstacle: the BBB. The BBB is composed of endothelial cells, pericytes, astrocytic end-feet, and the basement membrane. Its distinctive tight junctions restrict the entry of peripheral substances into the brain, making it difficult for therapeutic agents such as CAR-T cells to reach their targets.

Various strategies have been developed to surmount the delivery challenges associated with crossing the BBB. One approach is to bypass the BBB directly, delivering CAR-T cells locally via intracranial or intraventricular injection [9]. Although a systematic comparison of the toxicity profiles between local and systemic administration of CAR-T cells is currently lacking. From a theoretical perspective, local administration could enhance CAR-T cell infiltration into tumors while reducing

toxicity outside the central nervous system. A transient opening of the BBB can also be achieved through the application of focused ultrasound (FUS) in conjunction with microbubbles (MB).

Under low-intensity pulsed ultrasound (LIPU), microbubbles generate cavitation and mechanical forces that transiently loosen the tight junctions between endothelial cells [10]. Importantly, this process increases the permeability of the BBB without leading to permanent structural disruption. The increased permeability of the BBB significantly improves the efficiency of drug delivery to the brain parenchyma, providing a new strategy to overcome major obstacles in the treatment of brain tumors and neurodegenerative diseases. Moreover, developing small-molecule drugs that can efficiently cross the BBB represents another promising strategy for improving drug delivery to the brain. Researchers have developed pH-sensitive biomimetic nanoparticles (MNPs). Since GBM cells can pass the BBB efficiently, MNPs that camouflage the GBM-cell membrane demonstrate relatively high BBB permeability. Temozolomide (TMZ) and cisplatin (CDDP), as chemotherapeutic agents that have been proven effective in the treatment of solid tumors, can be co-loaded into MNPs, thereby significantly enhancing antitumor efficacy while reducing systemic toxicity [11].

4. Discussion

Several therapies that combine TME-related targets with other validated targets have been developed. However, these combination therapies have yet to achieve sufficiently satisfactory prognostic outcomes. Regarding this, sequencing technology and personalized precision medicine are promising for finding more effective combination therapies in the future. Through high-throughput sequencing technology, novel targets that tailor to specific tumor types can be identified and developed. Whole Genome Sequencing (WGS) can identify a patient's personalized Biomarker. Meanwhile, AI can be applied to analyze complex biological data and predict how someone is going to respond to treatment. Therefore, AI can provide valuable options for selecting target antigens and determining the optimal timing for combining different therapies. Applying AI in immunotherapies to treat cancer overall is one of the biggest trends.

Furthermore, MRI and PET imaging technologies can monitor the distribution of image-guided CAR-T cells in vivo in real time, which allows for the assessment of delivery efficiency. Image-guided CAR-T cell delivery can also be combined with multimodal in vivo tracking to guide CAR-T cells to tumor sites more precisely, thereby enhancing therapeutic efficacy and reducing side effects [12].

5. Conclusion

TME plays a crucial role in the progression and treatment of solid tumors. It is associated with multiple mechanisms, including immune evasion and drug resistance. This review highlights the composition and mechanisms of the TME and discusses related therapeutic strategies and their limitations, which provide insights that may facilitate the development of new cancer therapies. CAR-T therapy represents a highly promising approach for overcoming the challenges posed by the immunosuppressive microenvironment. With the emergence of novel CAR-T cell designs, such as dual-targeted and armored CAR-T cells, CAR-T cells can not only recognize tumor-associated antigens but also modulate the immune microenvironment. There are also novel types of CAR-T cells that rely on chemokine signaling or hypoxia-sensing mechanisms to achieve precise localization, thereby improving their tumor-targeting specificity. For instance, the combination of oncolytic viruses engineered to express chemokines with GD2-targeted CAR-T cells has shown significant therapeutic potential against various solid tumors. In addition, studies have shown that bypassing, disrupting, and designing permeable molecular agents are effective strategies to overcome the BBB.

Although there are many problems to be solved, further research is expected to progressively optimize strategies for overcoming the immunosuppressive TME and the BBB.

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