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Chapter

Immunotherapy for Glioblastomas

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Abstract

Glioblastoma (GBM), a WHO grade IV brain tumor, is an aggressive tumor with poor prognosis; even with current standard care of triple therapy, consisting of surgical resection, chemo and radiation therapy, the patients' median survival time is only approximately 15 months. Recent practice shows that immunotherapy made some progress in some other solid tumors, like melanoma or non-small cell lung cancer. This chapter is going to review some advances in immunotherapy for GBM.

Keywords: glioblastoma (GBM), Blood-brain barrier (BBB), immunotherapy, epidermal growth factor receptor variant III (EGFRvIII), AMG 595 monoclonal antibody, vaccine, rindopepimut, T cells, PD-1 and PDL-1, overall survival (OS), immune checkpoint molecules

1. Introduction

Glioblastoma (GBM), a World Health Organization (WHO) designed grade IV tumor, is an aggressive and most lethal form of brain tumor with extremely poor prognosis. Although there exists a standard triple therapy (surgical, chemo and radiation), the average survival time for patients with GBM is less than 2 years. Due to the presence of blood-brain barrier (BBB), a naturally made special structure of the vascular wall inside brain parenchyma, it allows only smaller molecules get through the BBB into brain parenchyma, which makes it even more difficult for the chemotherapeutic agent to reach the target tumor cells. In addition, due to the infiltration nature of this tumor, surgical resection with GTR (gross total resection) without damage the brain function is almost impossible to archive. Radiation therapy may produce some partial control of the tumor; at the same time, it not only induces radiation necrosis but also causes additional mutation of the tumor. On the other hand, in contrast to mutations of many other solid tumors from other organ systems, most glioma-associated mutations offer slightly better prognosis for the patients, making the target therapy to those mutations less significant and less important. As mentioned above, it is vitally important and necessary to develop a minimally invasive and greatly effective method for treating the glioblastoma. In this regard, novel GBM treatments including immunotherapy are being investigated [1]. Key challenges in glioma-specific immunotherapy as with many other cancers are the limited immunogenicity of the cancer cells and the immunosuppressive environment of the tumor. Although specific antigens have been identified in several cancers, brain tumors, such as GBM, are considered poorly immunogenic [1]. In addition, the tumor's heterogeneity and its consistent mutations may contribute further to its poor immunogenicity [1].

Immunotherapy has garnered increasing support in recent years as a treatment for brain tumors. The immune system has a tremendous capacity for targeting and eliminating tumor cells while sparing normal tissues. Following decades of preclinical development and success in other solid and blood-borne neoplasms, many immunotherapies are now being investigated in patients with GBM. Immunotherapeutic classes currently under investigation in patients with GBM include various vaccination strategies, adoptive T-cell immunotherapy, immune checkpoint blockage, monoclonal antibodies, and cytokine therapy. Trails include patients with either primary or recurrent GBM.

2. Vaccine therapy

2.1 Adoptive T-cell immunotherapy (ALT)

Over the past decade, the FDA has approved emerging immunotherapies for a variety of cancers [2]. At present, many studies have proven that the brain is no longer an immune-exempt organ, and there are many interactions between tumors and the brain immune system [3, 4]. The fully functional T-cell bank plays an important part in maintaining immune surveillance and initiating antitumor immune responses. However, glioblastoma (GBM) is particularly good at destroying antitumor immunity and causing severe T-cell dysfunction. In GBM patients, both local and systemic immunosuppressive disorders impair any possible antitumor response. Woroniecka et al. [5] have analyzed and summarized the categories and molecular mechanisms of T-cell dysfunction in GBMs, including senescence, tolerance, anergy, exhaustion, and ignorance.

Adoptive T-cell therapy for brain tumors has received increasing attention as a breakthrough emerging therapy. Adoptive T-cell therapy refers to engineering specific T cells to target the tumor cells and recognize tumor-specific antigens, and eventually cause tumor cells to die through the immune response. Because T-cell immune response is strong and specific, it can distinguish tumor tissue from healthy tissue, and can target malignant cells to prevent distant metastasis, and T cells can proliferate to maintain therapeutic effect. Currently, T-cell immunotherapy includes three types of tumor infiltrating lymphocytes (TIL), T-cell receptor (TCR), and chimeric antigen receptor (CAR). Among them, CAR T-cell therapy is the only therapy that has made significant progress in clinical application. Chimeric antigen receptor T (CAR T) cell therapy refers to using the patient's own T lymphocytes, which have been re-engineered, loaded with receptors and co-stimulatory molecules that recognize tumor antigens, and expanded into the patient's body after in vitro expansion to identify and attack their own tumor cell. GBM cells can express a variety of antigens, such as human epidermal growth factor receptor 2 (HER2), interleukin 13 receptor subunit α-2 (IL13Rα2), ephrin-A2 (EphA2), and epidermal growth factor receptor variant III (EGFRvIII), which have been successfully targeted using chimeric antigen receptors T cells (CARs-T) in preclinical models [6].

Studies have shown that CAR T cells targeting EGFRvIII play a role in the treatment of GBM, and multiple trials are ongoing or under preparation. A Phase I study involving 10 patients with relapsed GBM demonstrates the safety and feasibility of EGFRvIII CAR T-cell therapy [7]. IL13Rα2-CARsT cells can produce cytokines, including interferon γ (IFNγ) and tumor necrosis factor-α (TNF-α), and display cytolytic activity by generating a pro-inflammatory microenvironment in mice bearing gliomas. Phase I trials (NCT00730613) for recurrent GBM have been completed and promising results have been shown [8]. Another IL13Ra2-targeted CAR

T-cell therapy for patients with recurrent GBM has also shown significant effects [1–7]. Ahmed and colleagues reported a Phase I study involving 17 HER2 + GBM patients treated with HER2-specific CAR-modified virus-specific T cells, which achieved safety, feasibility, and anti-GBM activity endpoint [9].

Although CAR T cells have high therapeutic potential, complex GBM biological characteristics and tumor microenvironment make CAR T-cell therapy also face challenges.

CAR T cells cannot target intracellular proteins, and tumors may shed their targets and escape treatment. There may also be insufficient proliferation of T cells, resulting in treatment that is not durable. Some researchers are engineering and modifying T cells to improve their antitumor efficacy. Interleukin 12 is an effective pro-inflammatory cytokine. Yeku designed a CAR T cell that carries and expresses IL-12, and proved that the CAR T cell has enhanced proliferation ability, decreased apoptosis, and increased cells toxicity, thereby enhancing antitumor efficacy in ovarian peritoneal cancer [10]. Kevin Bielamowicz et al. created trivalent T cells with three specific CAR molecules (trivalent CAR T cells) to overcome the patient's antigenic variability in glioblastoma. Compared with monovalent and bivalent CAR T cells, trivalent CAR T cells mediate powerful immune synapses by forming more microtubule tissue centers between CAR T cells and tumor targets, and show stronger cytotoxicity according to each patient [6].

In the future, with the continuous deepening of research, adoptive T-cell strategies will definitely open up a bright path for GBM immunotherapy.

ALT therapy has now evolved to leverage advances in gene engineering and retroviral delivery. Patient-derived T cells can be engineered with antigen-specific T-cell receptors (TCRs) or tumor-specific chimeric antigen receptors (CARs) to confer target recognition independent of and in addition to naturally occurring TCRs. The best studied of these T-cell modifications are CARs. CARs are synthetic receptors that couple the single-chain Fv fragment of a monoclonal antibody with various T-cell signaling molecules, thus endowing T cells with the antigen-specific recognition of the humoral compartment, the intracellular signaling required for cytotoxicity, and the co-stimulation necessary for sustained activity. As such, CAR T cells recognize target antigens without a need for MHC peptide presentation, circumventing one major mechanism of tumor immune escape-MHC downregulation. CAR T-cell therapy has demonstrated promising results and FDA approval for hematological malignancy is expected shortly [11].

Clinically, adoptive T-cell therapy has demonstrated its effectiveness with CAR-based treatment for CD19C B-cell malignancies. A clinical trial for 11 recurrent GBM patients has demonstrated infusions of autologous adoptively transferred human cytomegalovirus (CMV)-specific T cells increased OS to > 57 weeks, with four patients maintaining no progression throughout the study period [12].

2.2 Peptide vaccine

Peptide vaccination concerns generation of vaccine based on peptide sequences representing a tumor antigen-specific target. Peptide vaccinations offer the advantage of high specificity and ease of antigen generation. Limitations include poor immunogenicity of peptide [1].

Rindopepimut (CDX-110) is a 14mer amino acid peptide that spans the EGFRvIII mutation site conjugated with keyhole limpet hemocyanin (KLH). In a small single-arm Phase II multicenter trail, 18 patients with newly diagnosed GBM completing standard of care therapy were vaccinated with rindopepimut combined with granulocyte-macrophage colony-stimulating factor (GM-CSF) resulting in a median OS of 26 months [4]. Overall, this vaccine was well tolerated with minimal toxicity.

Another randomized Phase II trial including 65 newly diagnosed EGFRvIII-positive patients with GBM was undertaken. Patients again received rindopepimut combined with GM-CSF following tumor resection and TMZ chemoradiation. The median OS was 24.6 months. Randomized Phase III clinical trial currently underway and initial Phase III data showed increased progression-free survival (PFS) and OS from point of diagnosis [1].

3. Monoclonal antibodies to EGFRvIII

The epidermal growth factor receptor (EGFR) gene expression is associated with a malignant phenotype in multiple cancers, like colon cancer, non-small cell lung cancer, head and neck squamous cell carcinoma, and GBM [13].

The EGFR gene amplification has been reported to be the most common genetic alteration in primary GBMs (40–70%) [13]. In approximately about 50–60% of GBMs with EGFR overexpressed, there is a specific type of EGFR gene variant generally called as EGFR variant III (EGFRvIII) [14]. EGFRvIII is the most common result of gene rearrangement after EGFR gene amplification [15–17]. Histone modification of gene enhancer on chromosome 7p12 leads to EGFRvIII formation [18]. Juxtaposition of EGFR exon 1 and 8 forms a novel glycine residue between these two exons. Deletion of EGFR exon 2–7 yields a truncated transmembrane protein receptor that lacks the extracellular ligand-binding domain but retains the constitutional tyrosine kinase activity that stimulates malignant growth [19, 20]. At present, there is no evidence of EGFRvIII expression in wild type human tissues [17, 21–25]. Thus, EGFRvIII serves as a unique tumor-specific antigen and is a candidate for targeted therapy [26]. EGFRvIII can show ligand-independent activity and continuously activate downstream signaling pathway [27, 28], which promotes proliferation, reduces apoptosis, enhances tumor cell xenograft ability, and increases angiogenesis and invasion [22, 27, 29–31].

According to current research, antibodies that target EGFRvIII for the treatment of gliomas include ABT-414 and AMG-595.

Depatuxizumab mafodotin (depatux-m, ABT-414) is a tumor-specific selection antibody drug conjugate (ADC) composed of an anti-EGFR antibody ABT-806 and a potent microtubule inhibitor (MMAF). Studies by Philips et al. have shown that ABT-414 can selectively kill the tumor cells with EGFRwt or EGFRvIII overexpressed tumor in vivo xenograft models and in vitro. ABT-414 combined with radiotherapy and chemotherapy can significantly inhibit tumor growth in vivo [32]. At present, the clinical trials for the treatment of glioma with ABT-414 mainly include NCT02343406, NCT02573324, NCT02590263, and NCT01800695.

AMG 595 is an antibody-drug conjugate comprising a fully humanized, anti-EGFRvIII monoclonal antibody linked to the maytansinoid DM1, a semisynthetic derivative of maytansine. AMG595 binds to EGFRvIII but not native EGFR; after binding, the AMG595-EGFRvIII complex is internalized via the lysosomal pathway, leading to the release of DM1 and mitotic arrest and potent growth inhibition [13]. AMG 595 exhibited favorable pharmacokinetics and is a unique therapy with possible benefit for some patients with EGFRvIII-mutated GBM with limited therapeutic options [26].

4. Immune checkpoint blockade

As early as 1863, Rudolf Virchow reported the inflammatory infiltration in tumor tissues and proposed an important connection between cancer and the

immune system [33]. In the following researches, the concept of "immune checkpoint molecules" was proposed. One of the important physiological functions of the immune checkpoint molecule is to keep the activation of the immune system within the normal range. Dysfunction of immunological checkpoint molecules can lead to immune evasion in many human tumors. Nowadays, immune checkpoint therapies are attracting a lot of attention from scientists who are devoted to cancer treatment.

It has been recognized that coinhibitory receptors on T cells play an essential role in attenuating the strength and duration of T cell-mediated immune responses. These inhibitory receptors are referred to as immune checkpoint molecules, which are responsible for maintaining self-tolerance and preventing autoimmune reactions [34]. To date, the two most intensely investigated coinhibitory molecules are CTLA-4 (that acts early in T-cell activation) and PD1 (that blocks T cell at late stages of the immune response). It has been demonstrated that blockade of CTL4 and PD1 could induce tumor regression and promote long-term survival in mouse glioma models [34].

Among a lot of immune checkpoint molecules, the membrane-bound molecules programmed death 1 (PD-1) and its ligand PD-L1 (PD-1/PD-L1) are the two most popular markers.

4.1 PD-1 and PD-L1

The programmed cell death ligand 1 (PD-L1) protein belongs to the B7 family, and is widely expressed in almost all tumor cells as well as many normal cells. The combination of PD-L1 and PD-1 provides a strong inhibitory signal that inhibits the proliferation, activation, and infiltration of cytotoxic T lymphocytes (CTLs) [35, 36], thereby mediating the immunosuppressive effects of tumors. This is considered to be the major negative regulatory mechanism of CTLs in the cancer microenvironment. More importantly, in addition to binding to PD-1, PD-L1 can also bind to other co-stimulatory molecules such as CD28, CD80, and CTLA-4 in cancer cells [37], which indicates that PD-L1 can mediate a broader and more complex immune regulation mechanism. Therefore, it is important to analyze the expression and cell distribution of PD-L1 in GBM tissues.

Glioblastoma (GBM) creates immune evasion and suppression, thereby evading the body's immune system and promoting tumor growth. Despite standard management composed of the maximal surgical resection with the combination of radiation therapy and chemotherapy, the median survival time of GBM patients is only 12–15 months after diagnosis [38]. At present, it is found that immunological checkpoint proteins can be blocked by related checkpoint inhibitors, thus becoming a viable target for tumor therapy. Therefore, it is very meaningful to explore new immunotherapy to counteract the immunosuppressive effects in GBM, and necessary to explore new immunotherapy to counteract the immunosuppressive effects in GBM.

4.2 Expression and cell distribution of PD-L1 in human glioma tissues

The PD-1 ligand, PD-L1 (also known has B7-H1), has been observed to be expressed in GBMs and GBM-associated macrophages, but the positivity rate in GBMs is controversial and highly variable, probably due to the selections of different antibodies by those researches [34, 39, 40]. Berghoff et al. [41] used a non-commercial anti-PD-L1 antibody, 5H1, showed membranous PD-L1 expression in 37.6% of newly diagnosed and 16.7% of recurrent GBMs, and diffuse/fibrillary PD-L1 expression in 84.4% of newly diagnosed and 72.2% of recurrent GBMs. However, in a study with 1035 GBM specimens using SP142 antibody, the positive rate of PD-L1 was only

19% [42]. Our own data, using the same standard in NSCLC (cutoff value was ≥1% of tumor cells expression), showed PD-L1 (clone number 28-8) expressed in 52% (69/133) of GBMs (see **Figure 1**).

4.3 Prognostic value of PD-L1 in GBM patients

Many studies have investigated the association between PD-L1 expression levels and the prognosis of GBM patients. But the results from different studies are non-conclusive. Nduom et al. [43] discovered that positive PD-L1 expression was associated with a poor prognosis, though this result has limited significance. Two recent studies have suggested that positive PD-L1 immunostaining in human GBM tissue means a poor prognosis [44, 45]. However, Berghoff et al. proposed the PD-L1 was not a negative predictor of survival [41], and Lee et al. [46] found the PD-L1 expression did not appear to be an independent factor for unfavorable prognosis according to multivariate analysis.

Efforts aimed at inhibiting the PD-1/PDL1 pathway have shown more promising results. In a preclinical study using the GL261 glioma mouse model, combination of anti-PD-1 antibodies and radiotherapy doubled median survival and elicited long-term survival in 15–40% of mice compared with either treatment alone [34]. Clinically, pembrolizumab, a PD-L1 antibody has been approved by the FDA, to apply in the treatment of metastatic melanoma and NSCLC. In GBM, nivolumab, another PD-1 antibody, developed for GBM patients is being tested with two clinical trials [34].

A randomized Phase III study aimed at testing nivolumab versus bevacizumab in recurrent GBM patients will also test combination therapy of nivolumab and ipilimumab. Another two Phase I/II trials will analyze the effectiveness of combinatorial pembrolizumab and bevacizumab, and combinatorial pembrolizumab with MRI-guided laser ablation in recurrent GBM patients. In addition, MED14736, a humanized PD-L1 mAb, is currently being tested in clinical trials for GBM patients combined with radiotherapy and bevacizumab [34].

Currently, immunological checkpoint inhibitor drugs associated with PD-L1 for the treatment of glioblastoma are undergoing relevant clinical trials. Nivolumab is a fully humanized IgG4 subtype programmed death-1 (PD-1) immune checkpoint inhibitor antibody that binds with high affinity to PD-1 receptors on T cells and blocks their interaction with PD-L1 and restores T-cell antitumor function. There are several clinical trials of nivolumab for GBMs. The first large-scale randomized clinical trial of Checkmate 143 (NCT 02017717) evaluated the safety and efficacy
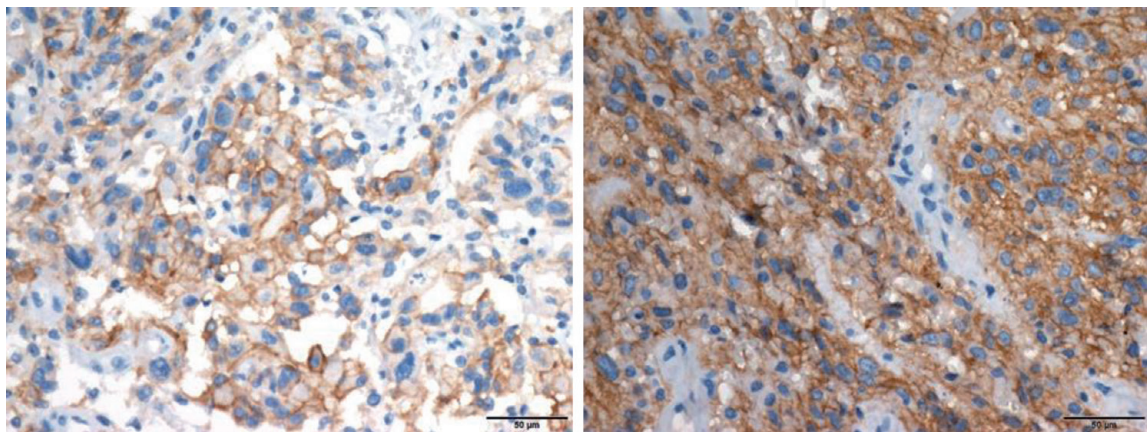

Figure 1.
The positive staining of PD-L1 in tumor cells by immunohistochemical stain. The brown color of the glioma cell membrane indicates the positive staining.

of nivolumab in GBM patients. In addition, the trial included a study comparing nivolumab monotherapy with bevacizumab in patients with recurrent GBM [47]. However, in the third phase of the clinical trial, 369 patients with first recurrence of GBM were recruited and it was found that nivolumab failed to prolong OS in patients compared with bevacizumab [48].

Pembrolizumab is another humanized monoclonal IgG4 anti-PD-1 antibody. Pembrolizumab was evaluated in 29 patients with high-grade malignant gliomas, including overall response rate (ORR) on contrast MRI, characterizing toxicities, progression-free survival (PFS), and overall survival (OS) [49]. Another trial about pembrolizumab showed that this drug is well tolerated, but the anti-PD-1 mono-therapy was not effective for most GBM patients [50, 51].

In addition, other PD-L1 immune checkpoint inhibitor drugs for GBM that are being studied include durvalumab, atezolizumab, pidilizumab, and so on. Durvalumab is a fully humanized immunoglobulin G1k monoclonal antibody that blocks the binding of PD-L1 to PD-1 and CD80, thereby enhancing the identification and killing of tumor cells by T cells. Atezolizumab is a humanized immuno-globulin G1 (IgG1) monoclonal antibody directly targeting PD-L1. It prevents the interaction of PD-L1 with the receptors PD-1 and B7.1 by binding to L1. Currently, three open clinical trials (NCT 01375842, NCT02458638, and NCT03174197) are investigating atezolizumab in GBM patients. Pidilizumab is a humanized immu-noglobulin (Ig) G1 monoclonal antibody directed against human PD-1 to block the combination between PD-1 and its ligands, PD-L1 and PD-L2. NCT01952769 is an ongoing clinical trial evaluating pidilizumab for GBM [52].

In future, besides PD-L1, more immune checkpoint inhibitors will be put into clinical trials to target this highly malignant brain tumor in future. Overall, the combination of various immune checkpoint modulators has shown promising effectiveness in the treatment of some solid tumors. The application of combinato-rial checkpoint modulators in GBM and other tumors therefore requires further investigation into the interplay of co-stimulatory and coinhibitory molecules [34].

5. Conclusion

Although still in its infancy, immunotherapy for cancers has already shown significant effect against some types of malignancy, such as melanoma and lung cancer. Current open clinical trials of immunotherapy for GBM predominantly focus on dendritic cell (DC) vaccines and antibodies targeting immunosuppres-sive checkpoints have achieved promising immune activity and clinical responses. However, durable and sustained response remains rare, highlighting the need for novel promising approaches including gene therapy and combinatorial immuno-therapeutic treatment [12].

Current obstacles for immune therapy for GBM lie in: (1) finding drugs to pen-etrate the BBB; (2) identifying specific, suitable, and immunogenic tumor antigens; and (3) identifying appropriate pre- and post-therapeutic biomarkers to reliably evaluate the treatment effect [34]. Additional research is necessary in the future to overcome those difficulties and identify a good treatment option or options for patients with GBM.

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