The roles and challenges of immunosuppressive cells in TME for CAR-T cell therapy in GBM

Fang Yang*

Department of Xinxiang Medical University, Xinxiang, China

*Corresponding author: sbrown64498@student.napavalley.edu

Abstract. CAR-T cell therapy has achieved remarkable success and effectiveness against hematological malignancy. However, transitions of CAR-T for solid tumor are restricted abruptly due to the tumor micro-environment. Recent studies revealed that various immune cells expressed in TME prevented T cell activation, greatly reducing the efficacy of CAR-T. At present, many clinical studies and trials have been conducted on gliomas, especially glioblastoma, demonstrating that the advantages and disadvantages of applying CAR-T in clinical practice are worth exploring. In this paper, the targets and positive effects of CAR-T for glioblastoma are discussed, as well as the negative effects of T regulatory cells, myeloid-derived suppressor cells, and tumor associated macrophages in tumor microenvironment. Simultaneously, this review also proposes a few suggestions for optimizing the CAR-T therapy for glioblastoma.

Keywords: CAR-T; immunosuppressive cells; tumor microenvironment; glioblastoma; TAMs Tregs MDSCs.

1. Introduction

Malignant tumors of the brain have become one of the alarming diseases due to the damage to the central nervous system and injury to patients' health, especially glioblastoma (GBM). Additionally, with a median survival time of about 14–16 months, they often have a bad prognosis.

At present, the standard therapies for GBM include surgical resection, radiation therapy, and chemotherapy, but the efficacy is not satisfactory enough. However, with the gradual attention paid to intracranial tumors from the public, the cells of origin that harbor mutations that drive the molecular determinants of immunotherapeutic response in GBM have been identified [1]. Moreover, one of the biggest scientific and medical advances in tumor research has been the successful creation of immunotherapy, providing an alternative way of cancer treatments [2].

In recent years, CAR-T has developed into a sophisticated type of adoptive cell therapy that involves introducing an artificially designed CAR molecule into T cells through gene editing and other techniques, endowing T cells with targeted function and then injecting the modified CAR-T cells back into the patient's body [3]. The mechanism of CAR-T cells killing tumor cells does not have MHC limitation. It can be activated by binding to targeted antigens, enhancing the cell's ability to recognize antigen signals, thereby efficiently killing tumor cells.

Because of its superiority over the above treatment methods, Adoptive cell therapy has shown tremendous success in treating patients with hematologic malignancies thanks to T cells created to express tumor-specific chimeric antigen receptors (CARs) [4]. Although immunotherapy for GBM is considered promising, attempts to treat solid tumors have encountered multiple significant challenges. These includes insufficient delivery to the tumor, poor persistence in the body, and a highly immunosuppressed tumor microenvironment [5]. Significantly, Tregs, MDSCs and Tams have some significant effects that greatly reduce the efficacy and prognosis of CAR-T.

Although the difficulties and disadvantages that previous researchers showed might seem undefeated, some inspiring outcomes that can further promote the sustainable development of the treatment of CAR-T still exist on GBM. Different target-antigen combinations being studied on CAR-T represent the abundant targets that can be applied in clinical practice. Among them, the studies on EGFRvIII, HER2, and GD2 are various, and their results are encouraging as well as clinically

significant. This treatment method has achieved definite results. However, it may increase the risks of the potential off-target effect or cytokine storm.

Moreover, during the continuous infection of the host population, the virus accumulates point mutations and undergoes minor antigenic variations. This accumulation of the antigen variation will lead to antigenic drift, which is also a severe phenomenon. Such problems cannot be ignored and urgently need to be solved. However, there are several solutions to improve the effectiveness of CAR-T therapy.

Herein, based on results of previous studies, this review briefly introduces the CAR-T therapy and targets for GBM. Related tumor immunosuppressive factors are highlighted as well. Moreover, this paper illustrates three feasible ways that enable CAR-T to perform the curative effects better and improve the prognosis to some extent.

2. Structure and Optimization Design of CAR Molecules

2.1. CAR Molecular Structure

The CAR molecule includes three main parts: intracellular domain，transmembrane domain and extracellular domain(Fig.1A) [6]. The extracellular domain contains an antigen-binding domain that is usually derived from a single stranded fragment scFv molecule of an antibody. It is composed of the variable light chain (ML) and variable heavy chain (VH) of the antibody, as well as the intermediate Linker region connection. It is then connected to the transmembrane through the Hinge region composed of CD8 and lgG4 molecules.

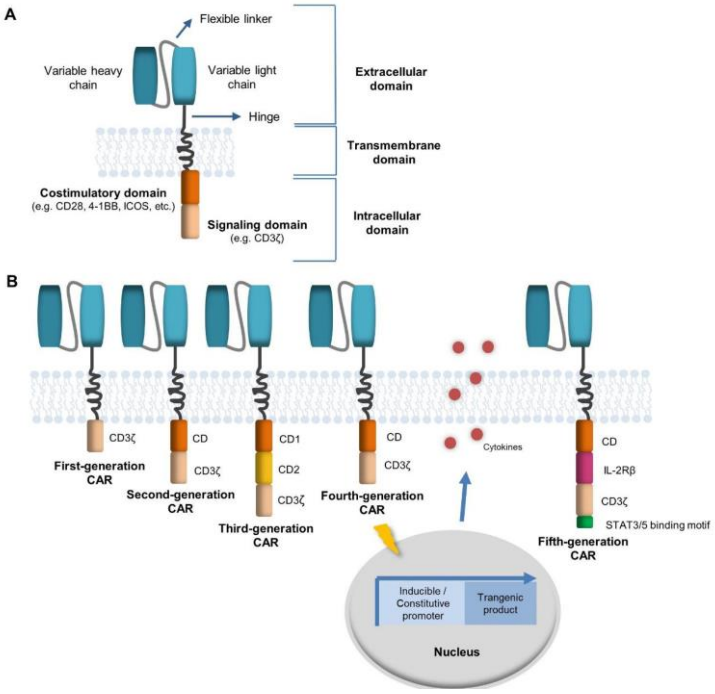

Fig. 1 (Double column figure) Thumbnail demonstration of the development of CAR generations and structure. (A) The structure of CAR molecular. (B) Generations of CARs

2.2. Optimization Design of CAR Molecules

According to the differences in the design of intracellular domains and the introduction of cytokines and ligands, the order of CAR molecules can be divided into five generations (Fig.1B) [6].

First generation: The first report of tumor-targeting CARs combines antibody scFv with CD3ζ. It directly blended into protein and proved that T cells delivering this fusion protein can transfer signals and secrete leukocyte. Due to the inclusion of just one CD3 chain for T-cell signaling and the absence of a co-stimulatory domain, the signal transduction efficiency of the first-generation CAR-T is so low

that its ability of proliferation in vivo is weak with shorter survival time. Ultimately, it failed to achieve the expected clinical application efficacy.

Second generation: In the early 21st century, scientists recognized the importance of co-stimulatory factors because endogenous TCR requires combinations with other co-stimulatory factors to emit strong signals. To enhance the ability of T cells, scientists have added other co-stimulatory molecules (CD28, 4-1BB) to the structure of CAR. This type of CAR has shown astonishing therapeutic effects in targeting CD-19 to treat hematological malignancies. Currently, the CAR-T drugs on the market are all second-generation products, such as Kymarih and Yescarta.

Third generation: Scientists, in order to improve its efficacy, have identified co-stimulators (CD28, CD27, OX40, 4-1BB) and inserted multiple structural domains to help build the third-generation CAR. However, the strength of co-stimulus signals does not vary simply depends on the addition of co-stimulatory factors. Instead, the original signal may be weakened or cannot be transmitted due to alterations in the intracellular domain of the lengths of CAR-T. Therefore, the clinical benefits provided by this method are still disputed.

Fourth generation: In order to enhance the function of CAR-T cells, an Intracellular domain was recommended in the fourth generation of CAR-T that co-expresses some immune regulatory factors, such as cytokines and chemokines, which can trigger signals induced by cytokines or block some signaling pathways that has an impact on the function of CAR-T cells in order to achieve stronger therapeutic effects.

Fifth generation: The fifth generation CAR-T, also known as universal CAR-T, refers to obtaining T cells from healthy volunteers and perform gene editing to knock out relevant genes, and then transfer them into CAR-T cells. The main advantage is that there is no need to obtain T cells from the patient's body for customization as well as achieving spot supply, thereby saving time and costs of treatment. Based on this, many experiments are currently underway.

3. Application and Therapeutic Advantages of CAR-T Cell Therapy based on TME in GBM

3.1. Optimization Design of CAR Molecules

GBM is recognized as the most common tumor with bad prognosis. In the microenvironment, multiple factors work together to suppress immunity. At present, there are fewer antigens with clear therapeutic effects for CAR-T in gliomas, but the number of targets in clinical and related trials is increasing. Among then, the most studied targets by scientists in recent decades are EGFRvIII, HER2, and GD2.

EGFRvIII: Studies have shown that gene amplification and mutations expressing EGFR occur in over half of GBM patients [7]. Specifically, over-expression of the EGFRvIII in GBM ranges from 25% to 81%. Targeted EGFRvIII treatment for GBM in clinical trials has demonstrated the safety and effectiveness of this method, with visible prospects and efficacy.

HER2: HER2 has been used to treat breast cancer. HER2 is a 185KD transmembrane glycoprotein which also belongs to the EGFR family. HER2 is over-expressed in GBM and medulloblastoma. This over-expression of HER2 is related to cell transformation and carcinogenic effects, as well as adverse clinical outcomes. it was found that using trastuzumab alone or in combination with chemotherapy can prolong the survival of primary and metastatic cancers.

GD2: GD2, which is a type of ganglioside found on the outer cell membrane, is compared to the protein antigen of engineered CAR T cells. GD2 is highly over-expressed in various tumor cells, and reported data shows that its expression density on the membrane of neuroblastoma cells can reach 5-10 million molecules/cell. Disialoganglioside GD2 is highly expressed in glioma cells with h3k27m mutation [8]. In addition, the level of circulating GD2 is not sufficient to interfere with its specific monoclonal antibody binding in the circulation.

3.2. Treatment Advantages

Compared with the traditional cancer treatment, immunotherapy can achieve therapeutic effects by enhancing the immune system of the body and clearing tumor cells from the body.

The key to CAR-T cell therapy is to express specially designed CARs through modifying T cells, which are amplified in vitro and injected into the patient's body. By specifically binding to antigens of the targeting cells, CAR can accurately identify and kill targeting cells, thereby achieving the goal of treating tumors. CAR consists of extracellular, transmembrane, and intracellular domains. The extracellular domain is composed of scFv acquired from the light and heavy chain regions of antibodies, which can bind to specific antigens without the involvement of MHC molecules, effectively avoiding immune escape caused by tumor cells' down-regulation of MHC expression levels.

3.3. Negative Effets of immunosuppressive TME

GBM has the characteristics of a highly immunosuppressive TME, blood-brain barrier, and low tumor antigen presentation. Among them, various immunosuppressive factors in the TME are one of the main culprits that make it difficult for immunotherapy to achieve good results in solid tumors. During the progression of tumors, TME transforms into an environment that actively protects tumor cells from immune attacks, thereby avoiding immunotherapy (Fig.2) [9]. Cells of the TME that have been blamed to the function of protecting tumor are Tregs, MDSCs, and TAMs [9]. Due to the presence of immune cells and highly immunosuppressive TME, CAR-T cells directed to solid tumors have been less effective.

Therefore, there is a need to promote intra-tumoral infiltration of CAR-T cells and overcome the effect of TME.

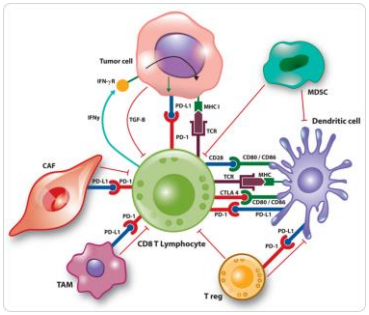

Fig. 2 Tregs, MDSCs, TAMs are tumor-protective cells of the TME.

Tregs: Tregs are effective in sustaining self-tolerance and immunologic homeostasis. The autoimmune and tissue problem can be prevented through their inhibitory function. However, in the tumor micro-environment, Treg cells absorb IL-2 from the surrounding environment, making it inconvenience for effector T cells. Tregs also creates immunosuppressive cytokines, such as IL-10, which can also undergo immune suppression.

MDSCs: MDSC flows from the bone marrow through blood circulation to inflammatory sites and solid tumors. In the inflammatory environment of TME, multiple factors promote MDSC inhibitory activity. Meanwhile, TGF- β and immunosuppressive molecules such as IL-10 can induce activation of multiple signaling pathways in MDSCs, thereby affecting the effectiveness of immunotherapy[10].

TAMs: Due to the influence of hypoxia in TME, M-MDSC rapidly differentiates into TAMs. Macrophages varied from M1 macrophages to M2 macrophages associated with cancer progression [11]. TAM maintains tumor growth by producing growth factors (such as EGF and VEGF), releasing proteases like cathepsin and soluble mediators (such as TGF-βand IL-37) to promote extracellular matrix remodeling.

Other growth factors, alongside with extracellular matrix proteins can also inhibit T cell pathways and tumor cell infiltration to some extent.

4. CAR-T Treatment Strategies for GBM

The complex immunosuppressive TME of solid tumor makes the efficiency of CAR-T cell still unsatisfactory. The presence of dense tumor stroma and immunosuppressive signals constitute immune cells.

The dilemma of CAR-T therapy for solid tumor lies in different ways. These include how to avoid tumor antigen heterogeneity, reverse the immunosuppressive efficacy of TME, improve the sustained action and imbibition ability of CAR-T cells, and reduce potential adverse reactions and drug resistance**.**

4.1.Target Selection

Many types of targets have been studied in previous research, but the boundedness of traditional approaches and immunotherapy are easy to see. Thus, targeting the factors in TME has proved to be a new direction of tumor immunization.

In recent years, more and more experiments have demonstrated the existence of new targets, providing more alternative options for CAR-T targeted treatment of GBM. Meanwhile, multiple experiments have shown that the design of dual target (Bi-CAR-T) refers to parallel and series CAR in clinical practice. The parallel approach requires the simultaneous expression of two antigens on the target cells, which makes it practicable for Bi-CAR-T to have a killing effect. Double targets have more precise targeting effects than single targets. They can avoid toxic side effects such as organ damage caused by a small amount of single expression on normal tissue cells that makes CAR-T being indiscriminate between the enemy and the self. In the series form. the target cells only need one antigen expression to be recognized by CAR-T to produce a killing effect, which has the effect of expanding the killing effect. This method has achieved success in clinical trials for treating various diseases such as non-Hodgkin's lymphoma with high remission rates, minimal side effects, and a good prognosis [12]. This provides references for the application of dual-target CAR-T in the subsequent treatment of solid tumors.

4.2.Combined Medication

In clinical trials, the efficacy of immunotherapy has been demonstrated in treating GBM patients. The emergence of immune checkpoint inhibitors is one of the major breakthroughs in tumor immunotherapy, but single use of immune checkpoint inhibitors often results in poor efficacy or rapid drug resistance. Many experiments have shown that this combination can effectively improve T cell depletion and recruit endogenous immune cells, increasing its anti-tumor effect. At the same time, some combination methods can also overcome tumor antigen heterogeneity and reduce the possibility of antigen escape, making it a feasible and promising solution [13].

In addition, CAR-T cell therapy combined with nanomedicine therapy has developed certain consequences in the study of solid tumor. The plans of tumor lysis or vaccines, chemotherapy, or radiotherapy also offer new strategies for breaking the barrier of CAR-T therapy for solid tumors.

4.3.Introduction of New Co-stimulatory Factors

The synergistic effect of co-stimulatory molecules is a key factor determining the killing intensity, proliferation ability, and persistence of T cells. Therefore, co-stimulatory molecules have been widely used to enhance CAR-T function and become the most effective means clinically. The two most used co-stimulatory molecules are CD28 and 4-1BB. Although both have achieved good clinical therapeutic effects in CAR-T treatment of B-cell lymphoma, the application on solid tumors still has shortcomings. The idea of introducing new co-stimulatory factors and targeting antibodies to these molecules is considered a promising adjuvant cancer immunotherapy.

5. Summary

CAR-T treatment, whether used for hematomas or solid tumors, faces some common challenges, including safety, process complexity, cost, and accessibility. In the field of hematomas, CAR-T has shown remarkable therapeutic effects. However, for the therapy of solid tumor, more challenges mainly focus on tumor heterogeneity, targeting specificity, and immune escape caused by the tumor immune suppression micro-environment. However, some phased breakthroughs have been made. With the iteration of technology and the accumulation of experience, CAR-T treatment for solid tumors will gradually improve its efficacy and feasibility. It can optimize the treatment effect by improving the design of CAR-T, developing new targets and improving treatment processes. At the same time, new gene editing techniques and advances in biological research have also provided more possibilities for CAR-T therapy. I hold a view that with further research and technological progress, more solutions and strategies will emerge, opening a broader path as well as benefiting more patients.

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