

Glioblastoma and the CAR-T cell therapy: A review

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Abstract. Gliomas are the most common and aggressive tumors that develop from the brain. Glioblastomas are also known as grade IV gliomas, indicating them the most aggressive brain tumors. Unlike other cancers that are categorized by stage, gliomas are classified by grade which describes how aggressive the tumor appears under the microscope and its molecular profiles. The grade for gliomas ranges from I to IV, with I is the least aggressive gliomas and IV is the most aggressive one. Glioblastoma originated from glial cells which are a type of brain cells that support and protect brain cells. The common treatments for glioblastomas include surgery, chemotherapy with temozolomide, and radiotherapy. Though patients experience decreases in symptoms and improvement in emotions, the survival rate for the treatments is significantly low. Part of the reason for the situation is the poor efficiency of TMZ due to the medicine penetrating the blood-brain barrier insufficiently. CAR-T cell therapy, an innovative immunotherapy regarded as a self-duplicating drug to treat cancer, is considered to have a better blood-brain barrier penetration, making the therapy a potentially promising treatment for glioblastoma. Besides, the treatment targets and kills tumor cells directly and it is independent of the endogenous immune response, which is greatly repressed in glioblastoma. Unfortunately, due to the restricted number of specific antigens in glioblastoma and their heterogenous expression, results of CART cell therapy for glioblastoma have not indicated clinical benefit. Nevertheless, adjustments can be made in order to improve the CART cell therapy in glioblastoma.

Keywords: Glioblastoma, CAR-T cell therapy, review.

1. Introduction

Glioblastoma is a common primary malignant brain tumor. Patients with glioblastoma experience various symptoms including seizures, drowsiness, changes in personality, memory loss, speech difficulties, and changes in vision [1]. It is an aggressive brain tumor with only approximately 12 months median survival rate and only 3 to 5 percent of the patients live for more than 3 years [2]. Glioblastoma falls into the category of grade IV gliomas [3]. Gliomas are brain tumors corresponding to the glial cells in the brain, including the oligodendrocytes, the astrocytes, and the ependymal cells. They serve as protective cells that support neurons and produce myelin sheath for the neurons in a healthy individual. When a glial cell grows and forms into cancer, it becomes gliomas. Gliomas are categorized into three major types in terms of their phenotype cell features: astrocytomas, ependymomas, and oligodendrogliomas [4]. Different from most cancers, gliomas are also classified into 4 categories based on their grades or aggressiveness of the disease, with grade one being the mildest and grade four being the most severe one [3]. There are huge divergences in terms of aggressiveness as well as treatments between the different grades of gliomas. Grade 1 glioma, which is known as Juvenile Pilocytic Astrocytomas (JPA), is a mild glioma that is pretty common among children. It can be cured by surgical resection alone because of its mildness. Grade II gliomas are classified as low-grade gliomas. They are usually treated with surgeries with radiographic follow-up recurrence afterward. Grade III gliomas, which are categorized as high-grade gliomas, are mostly treated by concomitant chemoradiation, a surgical resection, and radiographic follow-up recurrence. Grade IV glioblastoma, or glioblastomas are the most destructive brain tumors. Glioblastoma patients are usually treated with daycare surgery, radiotherapy, and chemotherapy with temozolomide[5]. Though therapies can heal some of the patients with Glioblastoma, based on the paper published by Rep Pract Oncol Radiotherapy, there was only thirty percent of the patients who have been treated

with safe incision followed by adjuvant temozolomide for six months and chemotherapy had a one year overall survival and two year overall survival was only 6.7% [6]. Although the study shows that the treatments for glioblastoma can reduce the symptoms in some aspects of patient's lives, the survival rate of glioblastoma is still significantly low [7]. Besides, financial instability and social functioning are disturbing among a majority of the patients. Surgical resections of glioblastoma are significantly challenging due to the infiltrating as well as frequently invasive characteristics of glioblastoma tumor cells and the tumor cells are usually situated in areas of the brain that are associated with motor function, control speech, and the senses [5]. Temozolomide is another common treatment targeting glioblastoma yet it is a major contributor to tumor recurrence and resistance because of the widespread exposure of temozolomide [8]. Besides, another major factor of hurdle towards therapies targeting glioblastoma is the blood-brain barrier [9]. Along with other neuronal complexes, blood-brain barriers are responsible for preserving physical and biochemical homeostasis in the brains of healthy individuals. The penetrating composition of glioblastoma tumor is under the protection of the intact barrier. The impenetrability of this barrier was a major hurdle to successful treatment [10].

Chimeric antigen receptor T-cell therapy (CAR T-cell therapy), an innovative and also the customized immunotherapy regarded as the self-duplicating drug to treat cancer, includes T cells that are capable of pass through the blood-brain barrier, filter through the brain, and perform tumor-associated T-cell response, making it a promising treatment for glioblastoma [11]. In cancer immunotherapy, CAR T-cell therapy is a promising treatment compared to other existing therapies such as chemotherapy and radiation, for it targets the tumors more precisely instead of killing healthy cells along with cancerous ones. From the perspective of glioblastoma, this paper aims to explore the development and specific application effects of CAR-T therapy.

2. Development of CAR-T Cell Therapy

It was first demonstrated by Yoshihisa Kowana along with his colleagues in 1987 in Japan [12]. They integrate components of an antibody with a T-cell receptor. Then, in 1989, Israeli immunologists Zelig Eshhar and Gideon Gross elucidated the concept of CAR T independently. Three years later in 1992, a postdoctoral student who later became an immunologist, Michek Sadelain, started applying retroviral vectors, which were genetic engineering technologies that have recently been created in order to introduce genes into T cells to produce cancer-killing drugs [13]. After a year, an Israeli immunologist Zelig Eshhar, modified T cells with the first chimeric molecule. Though it was known as first-generation CARs, but it did not have clinical effectiveness. In the year of 2002, effective CAR T cells were successfully developed. The second-generation were built by the MSK team, are capable of proliferating, surviving, and killing prostate cancer cells. These findings demonstrate the potential of this therapy in treating the prostate cancer. In 2017, the first two CAR-T products, Kymriah and Yescarta were certified by the FDA for adult patients with refractory diffuse large B-cell lymphoma after several lines of systemic therapies and patients who are younger than 25 years old with refractory B-cell precursor acute lymphoblastic leukemia. Afterward, it has been developed progressively for a couple of decades [3]. Up till now, six of the therapies have been approved by the FDA.

3. Structure of Chimeric Antigen Receptor (CAR)

CAR is composed of three major structural domains: the ectodomain, the transmembrane domain, and the endodomain [12]. Ectodomain is the outermost region of CAR which is in charge of antigen recognition. It is often altered to detect tumor antigens. It includes a single-chain variable fragment (ScFv), and a hinge region. The antigen-binding scaffold generally contains a single-chain variable fragment (ScFv), the specificity and affinity of ScFv targeting tumor antigens are the basis for the safety and effectiveness. The difference in the length and composition of the hinge region will affect

the expression, flexibility, signal transduction, and epitope recognition of CAR molecules, and ultimately affect the function of CAR molecules. The transmembrane domain is responsible for connecting the extracellular domain of the CAR molecule with the intracellular domain, thereby transducing the ligand recognition signal into the cell. It is the component of CAR that is responsible for the stability of the receptor and surface expression. The endodomain is the primary composition. The intracellular signaling domain contains a $\text{CD3 } \zeta$ co-receptor with three ITAM as its primary functional unit in order to relay the primary signals. Endodomain also associates with costimulators that are in charge of delivering secondary signals when CAR attaches to tumor antigens such as CD28, CD134, and so on. Endodomain is the component that is modified and allows CAR to evolve. In the past 30 years, this therapy has developed through five generations, with alternations to structure of the endodomain and the number of CMs being applied in the endodomain.

4. Mechanism of Action of CAR T-cell Therapy

It is an immune-boosting medication that is patient-specific, alive, and self-replicating [12]. The anticancer mechanism of CAR T-cells is almost equivalent the signaling pathway of the natural T-cells. After the CAR T-cells are injected into the patient, the cells are able to aim tumor surface antigens through the ScFv. After tumor antigen is attached to the receptor, the T cells go through conformational modifications and are activated, so that to extensive proliferation and differentiation, which are crucial for their cancer-killing functions. CAR T-cells undergo antitumor activities by utilizing a couple of synergistic mechanisms, such as the death ligand–death receptors, Fas binds to the FasL on CAR T-cells, which leads to the death of the apoptotic cancer cell; perforin-granzyme-mediated cytolytic mechanism, activated CAR T-cells release GZM and perforin rapidly from lytic granules, membrane pores are then formed, allows GZMs to enter the cytoplasm and kill the cancer cells; Besides, CAR T-cells also perform cancer-killing activities through other components of the immune system.

5. CAR T-cell therapy in Glioblastoma

Glioblastoma is the most aggressive among brain tumors. The difficulty of penetrating through the blood-brain barrier and glioblastoma's invasive, heterogeneous characteristics make the treatments for the disease significantly challenging. CAR-T cell therapy has been approved by the FDA to treat patients who suffering from a variety of the aggressive cancers, has great potential to overcome the hurdles that other common treatments for glioblastoma.

5.1. Central Nervous System Penetration

The blood-brain barrier is in charge of protecting the brain and maintaining the biochemical homeostasis within the brain. The barrier restricts the transportation of therapeutic drugs significantly, for it limits large molecules as well as small hydrophilic drugs to pass through, leading to great treatment inefficacy [14].

A study published in 2018 performed the first-in-human study of this therapy by injecting autologous T-cells to 10 recurrent glioblastoma patients [15]. The T cells successfully transferred and altered the mutated epidermal growth factor receptor variant III (EGFRvIII) using a CAR. The trial did not show any cytokines release syndrome or off-tumor toxicity, which demonstrated that producing and infusing the modified CART-EGFRvIII cells is safe and practical.

5.2. Primary Immune Response and Antigen Presentation

Tumor mutational load quantifies the number of mutations per megabase accommodated by tumor cells in a specific neoplasm, it is critical for tumor immunogenicity in a variety of cancers [16]. Since gliomas contain less average tumor mutational load than other types of cancers, therapies such as immune checkpoint inhibitors are restricted in glioblastoma for there is not enough neoantigen for T

cells to recognize it and get rid of it as foreign particles. Also, CAR T-cells are able to be modified genetically to specifically recognize a predetermined tumor antigen. This feature reduces the reliance on tumor mutational load for generating an effective anti-tumor immune response. Genetically modified T cells overcome the issue of glioblastoma being restricted by antigen-independent co-stimulatory signaling and antigen-specific T-cell receptor signaling. One of the reasons is that CAR T cells are able to function despite the absence of antigen presentation. Besides, the chimeric antigen receptor contains co-stimulatory domains that do not require stimulation of a primary immune response.

5.3. Glioma Stem Cells

Glioma stem cells represent a group of tumor-initiating cells that are able to differentiate and self-renew [17]. According to a paper published in Clinical Cancer Res, chimeric antigen receptor IL13-zetakine+ CTL was proven to be able to recognize and kill both IL13R α 2pos and IL13R α 2pos GSC tumor differentiated cells. The study also indicates the receptor was capable of terminating glioma initiation within a mouse tumor model. What's more, a study proved that glioma stem cells express tumor-associated antigens including interleukin-13 receptor alpha 2 (IL-13 R α 2) and EGFRvIII. They aim for CAR T cells in glioblastoma.

6. Challenges and Future Directions

CAR T-cell therapies for patients with glioblastoma also face challenges in the tumor microenvironment, marked by immunosuppressive factors such as cytokines, tumor-derived substances, and inhibitory immune cells. The glioblastoma microenvironment contains cytokine networks such as prostaglandin E2, and TGF- β , which inhibit the function of the T-cell [11]. What's more, post-CAR T-cell treated tumor specimens illustrate an influx of Tregs which is a subpopulation of T cells that represses the T-cell responses and are also found in infiltrating lymphocytes in glioblastoma, making the immunotherapy challenging. Besides, the metabolic landscape of glioblastoma, characterized by hypoxia, nutrient deprivation, and low amino acid levels, also limits CAR T-cell therapy. Indoleamine 2,3-dioxygenase (IDO) inhibitor is suggested to be used to prevent IDO from catalyzing the conversion of tryptophan into kynurenine which can; lead to autophagy responses and protein translation shut down in the cells.

Tumor heterogeneity and the loss of antigen pose significant challenges to the long-term efficacy of this therapy in glioblastoma [11]. The targets in glioblastoma are heterogeneous, both between patients and within the same patient. Spatial and temporal variation in antigen expression has been observed, highlighting the need for combinatorial targeting of tumor-associated antigens. Indirect tumor killing and antigen spreading may play a role in overcoming antigen escape, but further research is needed to understand their effectiveness in humans.

7. Conclusion

Glioblastoma is the most acute brain tumor also known as the grade IV glioma, arising from glial cells. Common therapies for glioblastoma these days include surgical resections, chemotherapy, and radiotherapy. Though the treatments illustrate to improve some aspects of patients' lives, they could not cure the disease fundamentally due to the difficulty of removing the tumor, for glioblastoma is pretty infiltrative, as well as the inability of the therapeutic drugs to penetrate through the BBB. By targeting and killing tumor cells directly and bypassing the suppressed endogenous immune response, the therapy offers an advanced approach in the fight against glioblastoma. The therapy is a patient-specific, living, self-replicating immune-boosting drug. A recent trial illustrates that the modified T cells are able to travel through the blood-brain barrier and initiate an immune response without stimulation of a primary immune response. Furthermore, CAR T-cells are also proven to target and kill the glial stem cells, resulting in preventing the incidence of the glioblastoma. However, CAR

T-cell therapies for glioblastoma face challenges in the tumor microenvironment, marked by immunosuppressive factors such as cytokines, tumor-derived substances, and inhibitory immune cells. IDO inhibitors are suggested to be used to improve the situation. Tregs, a subpopulation of T cells found in infiltrating lymphocytes in glioblastoma, also repress T-cell responses. Additionally, the metabolic landscape of glioblastoma, is characterized by hypoxia, nutrient deprivation, and low amino acid levels. Tumor heterogeneity and antigen loss also illustrate significant challenges to the long-term efficacy. What's more, genetically modified T-cell targets in glioblastoma exhibit heterogeneity between patients and within the same patient. The therapy in glioblastoma is still at the beginning stage, yet the results so far illustrate the therapy is a promising potential treatment for glioblastoma, for it is able to overcome obstacles in other common therapies. However, there are quite a few challenges needed to be overcome in order to develop the therapy. It is necessary to further strengthen the evidence-based data and enhance its persuasive power.

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