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REVIEW ARTICLE

Anti-EGFRvIII Chimeric Antigen Receptor-Modified T Cells for Adoptive Cell Therapy of Glioblastoma

Pei-pei Ren[a,#], Ming Li[b,#], Tian-fang Li[c] and Shuang-yin Han[a,*]

[a]*Translational Research Center, People's Hospital of Henan Province, Zhengzhou University, Zhengzhou 450003, P.R. China;* [b]*Departmentt of Neurosurgery, People's Hospital of Henan Province, Zhengzhou University, Zhengzhou 450003, P.R. China;* [c]*The First Affiliated Hospital of Zhengzhou University, Zhengzhou 450003, P.R. China*

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Abstract: Glioblastoma (GBM) is one of the most devastating brain tumors with poor prognosis and high mortality. Although radical surgical treatment with subsequent radiation and chemotherapy can improve the survival, the efficacy of such regimens is insufficient because the GBM cells can spread and destroy normal brain structures. Moreover, these non-specific treatments may damage adjacent healthy brain tissue. It is thus imperative to develop novel therapies to precisely target invasive tumor cells without damaging normal tissues. Immunotherapy is a promising approach due to its capability to suppress the growth of various tumors in preclinical model and clinical trials. Adoptive cell therapy (ACT) using T cells engineered with chimeric antigen receptor (CAR) targeting an ideal molecular marker in GBM, e.g. epidermal growth factor receptor type III (EGFRvIII) has demonstrated a satisfactory efficacy in treating malignant brain tumors. Here we summarize the recent progresses in immunotherapeutic strategy using CAR-modified T cells oriented to EGFRvIII against GBM.

Keywords: EGFRvIII, chimeric antigen receptor, adoptive cell therapy, glioblastoma.

1. INTRODUCTION

Glioblastoma (GBM) is the most common type of primary brain malignancy, accounting for 82% of total malignant gliomas (MGs) [1]. The treatment outcomes of the existing modalities have been disappointing: a median overall survival (OS) about 14.6 months, 2-year survival about 26.5%, and 5-year survival only about 9.8% [2]. The following factors are most likely involved in the resistance to conventional treatments. 1) the blood-brain barrier lowers drug concentrations at GBM sites [3]; 2) the genetic heterogenicity and aberrant signal pathways in GBM make it refractory to many current therapies [4]; 3) tumor-initiating cells existing in GBM may be responsible for chemo- and radiation-resistance [5]; 4) the immunosuppressive microenvironment induced by GBM hinders the efficient anti-GBM-specific immune responses [6].

Mounting evidence shows the advantages of ACT over traditional chemotherapy and other immunotherapy strategies. With rapid advancement of life sciences, we expect that T cells with enhanced specificity and effector function will be developed after genetic modifications [7,8]. A widely-used ACT approach is to generate tumor-specific T cells by introducing chimeric antigen receptors (CARs) into T cells (CAR-T). The accuracy of CAR-T cell therapy relies on a single chain antibody against a tumor specific antigen. EGFRvIII is an ideal target for immunotherapy in GBM and adoptive transfer of CAR-modified T cells targeted EGFRvIII provides a novel therapeutic approach leading to specific elimination of GBM [9].

2. RATIONALES FOR ADOPTIVE CELL THERAPY IN GBM

Immunotherapies for brain tumors include active approaches with cytokine or dendritic cells and passive approaches with adoptive cell therapy or antibodies. The immune system can recognize tumor epitopes as non-self antigen, thus specifically eradicating or temporarily blocking cancer growth. These well-accepted notions are also held true for brain tumors, especially for GBM. The rationale to take use of immune system to attack GBM is based on the premise that its effector and memory functions can be employed to specifically target invasive tumor cells [10]. Several lines of evidence show that brain tumors can elicit potent anti-tumor responses. Previous observations made in an animal model of brain tumor suggested that the tumor-derived antigens can stimulate specific T cells after transporting to cervical lymph nodes [11]. It is well-established that in a rodent model, the enhancement of impaired tumor specific response can eradicate intracranial glioma [12]. As such, the residual tumor foci within brain after surgical removal of primary neoplasm can be completely eliminated after overcoming tumor immunosuppressive environment with effective immunotherapy. These solid scientific observations indicate that the general rules of anti-tumor response elicited by the immune system can be applied to the brain after improvement of GBM immunotherapy.

In GBM immunotherapy, ACT is more feasible than active immunotherapy. ACT allows direct ex vivo manipulation of tumor associated antigen (TAA)-specific cytotoxic T lymphocytes (CTLs) to enhance anti-tumor functions, which cannot be done in vivo [13]. The acquired biologic functions of T cells generated by genetic engineering can disrupt immunosuppressive microenvironment and incite more potent antitumor T cell responses. In contrast, antitumor activities of endogenously activated T cells induced by vaccination are insufficient to suppress tumors because tumor-specific antigens may be self-antigens and tumors have immune evasion mechanisms to avoid immune surveillance system of host. ACT is particularly effective in eliminating residual GBM loci after surgery. Multiple forms of ACT utilizing NK, NKT cells, or T cells transfected with CAR have been explored in preclinical or clinical studies for GBM treatment. Some effector cells have endogenous antitumor properties, while others have been engineered to specifically target a certain GBM antigen. Human NK cells deriving from PBMC transplanted either systemically via tail vein or locally to tumor per se

*Address correspondence to this author at the Translational Research Center, People's Hospital of Henan Province, Zhengzhou University, #7 Weiwu Road, Zhengzhou 450003, China; and Dr. Tian-Fnag Li, The First Affiliated Hospital of Zhengzhou University, #1 Jianshe Road, Zhengzhou 450003, China; Tel: +86 13203710057; Fax: +86 371 65580059;
E-mails: hansyzzu@163.com; tfli@zzu.edu.cn
[#]*Contribute equally.*

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showed robust therapeutic effects in an orthotopic GBM xenograft models through induction of apoptosis of GBM cells in the brain [14]. In similar model, NK cells modified by ErbB2 CAR exhibited potent and specific activity against ErbB2-positive GBM and a marked increase of symptom-free survival upon repeated stereotactic injection of CAR NK cells into the tumor area [15]. These observations made thus far indicate that ACT is a promising approach with a robust anti-tumor potential [16]. With further innovation and refinement of ex vivo T-cell manipulation, ACT may become a mainstream treatment for GBM.

3. ADVANTAGE OF CAR-T CELLS IN TUMOR IMMUNOTHERAPY

Chimeric antigen receptor-engineered T cell is one of the big progresses in ACT research. The unique structure of CAR endows T cell tumor specific cytotoxicity and capability to disrupt immunosuppressive microenvironment in cancers, which helps overcome the issue of immunological tolerance. CARs incorporate a single chain variable fragment (scFv) of a tumor antigen specific antibody and signaling domains of T cell receptor (TCR), thus gaining the specificity of antibody as well as the cytotoxicity of cytotoxic T lymphocytes (CTLs) [17]. CAR-T cells are very valuable in cancer ACT because of its antigen specific recognition, activation and proliferation in an MHC independent manner. Further, adding costimulatory molecules such as CD28 and 4-1BB in the CAR structure significantly enhances T-cell expansion, survival, cytokine secretion and tumor lysis [18]. The unique architecture of CAR allows T cells to bypass many immune escape mechanisms commonly seen in GBM such as down-regulation of the MHC, reduced expression of costimulatory molecules, induction of suppressive cytokines and so on.

The observations from preclinical and clinical studies have revealed a very encouraging therapeutic efficacy of the CAR-mediated immunotherapy in a variety of cancers including hematological malignancies and some solid tumors. To date, the most encouraging clinical observations have been achieved from patients with chronic lymphocytic leukemia (CLL) and lymphoma treated by CD19-orientated CAR T cells [19]. In a pioneer work done by Dr. June's group, two out of the three refractory CLL patients receiving CD19 CAR T cells therapy achieved complete response (CR) and one with partial response (PR). Further, the adoptive transferred CAR T cells demonstrated an excellent ability of cell engraftment (up to 3 log expansion) and tumor cell lysis [20]. This amazing result inspired numerous clinical studies focusing on CD19 for CAR technology. In parallel with the clinical trials in hematological malignancies, CAR-based therapy has also been conducted in solid tumors, including human epidermal growth factor receptor 2 (HER2) for sarcoma, folate receptor-α for ovarian cancer, carcinoembryonic antigen (CEA) for colorectal and breast cancer, and prostate-specific membrane antigen (PMSA) for prostate cancer [21-24]. GBM is ideal for CAR cancer immunotherapy as some of its tumor-associated antigens are not expressed at significant levels on normal tissues, thus decreasing concurrent toxicity. Currently, several Phase I/II studies are on the way. A group of scientists from Baylor College of Medicine for the first time targeted Her2 antigen with cytomegalovirus specific CAR T cells in which 4-1BB was replaced by the CD28 signaling domain [25]. Shortly after this, a new CAR targeting EphA2 antigen was developed and demonstrated an excellent safety profile and effectiveness when treating EphA2 positive MG patients [26]. More recently, T cells expressing IL13Rα2-specific CAR was injected intra-cranially to patients with GBM. The clinical response continued for 7.5 months after CAR T-cell transfer [27].

4. EGFRvIII AS AN IDEAL TARGET FOR GBM IMMUNOTHERAPY

EGFRvIII is the most common mutation of the epidermal growth factor receptor resulting from an in-frame deletion of 267 amino acids in the extracellular domain [28]. This mutant was initially identified from five malignant gliomas after structural analysis of the amplified and rearranged EGFR [29]. Accumulating data demonstrate that it is highly expressed in a large majority of glioblastoma patients as well as patients with other malignancies. Of note, EGFRvIII was found to be commonly expressed on $CD133^+$ glioblastoma cancer stem cell and the $EGFRvIII^+/CD133^+$ defines the population of cancer stem cells (CSC) with the highest degree of self-renewal and tumor-initiating ability. EGFRvIII expression is preserved in tumor sphere culture, but lost in standard cell culture [30]. EGFRvIII functions as a constitutively active tyrosine kinase causing tumorigenesis, invasiveness, resistance to standard therapy, and reduced apoptosis [31]. $EGFRvIII^+$ cells can incite malignant transformation of nearby cells through paracrine signaling of IL6 family cytokines and the intercellular transfer of EGFRvIII positive exosomes [32,33]. A novel sequence with a glycine residue at the fusion junction of extracellular domain creates a tumor-specific and immunogenic epitope that is rarely expressed in the normal tissue. The unique properties of EGFRvIII including a surface neoantigen specifically expressed in malignant cells, a particularly high frequency of expression in GBM and cancer stem cells, and its ability to induce phenotypic transformation toward malignancy. These properties make it an ideal target for immunotherapy of GBM [34,35].

A variety of immunotherapies targeting EGFRvIII for GBM are currently under investigation and they include peptide vaccines, dendritic cell vaccination therapy, monoclonal antibodies, and genetically modified T cells. Rindopepimut, a peptide vaccine approved by the US FDA, elicits EGFRvIII-specific humoral and cellular immune responses. Phase I and II clinical trials have demonstrated significantly higher progression-free and overall survival times (26 months vs 14.6 months) in vaccinated patients with EGFRvIII-expressing GBM tumors [36]. In vivo and human studies have demonstrated peptide-pulsed dendritic cells can induce EGFRvIII-specific cell-mediated immunity and initiate antitumor responses [37]. In terms of antibody therapy, many antibodies specific for EGFRvIII have been shown to be able to elicit antitumor activity via Fc- and Fab-mediated activity [38,39], and the antibodies conjugated with toxins also show significant cytotoxic activity against EGFRvIII-expressing tumors [40]. Rapid progresses made in recent years about genetically engineered T cells urge scientists to utilize CAR-T to specifically target and efficiently kill the EGFRvIII-expressing gliomas cells for GBM treatment [41].

5. EGFRvIII CAR-T CELLS FOR GBM IMMUNOTHERAPY

To date, significant progresses have been made in the preclinical models of ACT using CAR-T cells targeting EGFRvIII, which have expedited the translation of this novel therapy into clinical application. Johnson et al constructed the second-generation CAR using a murine 3C10 single chain variable fragment (scFv) fused with 4-1BB and CD3ζ signaling domains (BBZ). The human T cells from healthy donors transduced with retroviral EGFRvIII CAR were delivered systemically via tail vein in a intracranial xenograft model of GBM [42]. To avoid human anti-mouse antibody (HAMA) responses in future clinical use, humanized 3C10 scFv was also generated and tested in subcutaneous and orthotopic xenograft models of human EGFRvIII positive GBM. Both the murine and humanized scFv from 3C10 demonstrated specific affinity to EGFRvIII and lack of reactivity to wild type EGFR (EGFRwt), and both CAR-T cells significantly delayed tumor progression and effectively contained tumor in mouse models [43]. The EGFRvIII CAR containing ICOS signaling domain gene rated by our group also revealed specific and efficient antitumor effect of T cells against EGFRvIII expressing glioma [44]. Other forms of the EGFRvIII-oriented CARs have also demonstrated their capability to target the EGFRvIII-expressing GBM cells [45,46].

Rosenberg and his colleagues developed the third generation EGFRvIII CAR using scFv from an antibody clone (mAb139) and intracellular signaling domain from CD28, 4-1BB, and CD3ζ. Retrovirus encoding EGFRvIII CAR was prepared to infect T cells from mouse splenocytes and its efficacy was determined in a fully immune-competent mouse model of malignant glioma. EGFRvIII CAR-T cells infused via tail-vein showed a long-term persistence in vivo and the mice gained resistance to rechallenge with EGFRvIII positive tumors [47]. To determine whether the therapeutic effects of EGFRvIII-targeted CAR-T cells are maintained in the context of Standard of Care (SOC) therapy for GBM, Riccione et al performed a temozolomide (TMZ) and whole brain irradiation (WBI)-induced lymphopenia before administration of EGFRvIII-specific CAR T cells in mice bearing EGFRvIII-positive intracranial tumors. Enhanced clonal expansion of adoptive transferred cells and increased overall antitumor response were observed [48].

Most of the current therapies are non-specific and often cause unexpected damage to adjacent healthy brain tissue. In contrast, CAR-T can precisely target tumor cells, thus not only increasing the efficacy but also reducing the concurrent toxicity. From the observations made in preclinical models, we expect a satisfactory success rate in the future clinical application of the EGFRvIII CAR-T cells. EGFRvIII-specific CARs are now being examined in a phase I/II study at the National Cancer Institute for patients with recurrent GBM. In a phase 1 study (NCT02209376), humanized scFv was used in CAR structure and either residual disease after initial resection or first recurrence of $EGFRvIII^+$ GBM patients were recruited. Patients will be enrolled in one of the two cohorts: the residual disease will receive EGFRvIII CAR-T cells after preconditioning with TMZ and WBI, while the recurrent disease will not undergo such pretreatment. In another clinical trial (NCT01454596), the third generation of EGFRvIII CAR was designed and transduced with a retroviral vector to T cells for patients undergoing leukapheresis. Patients will receive a non-myeloablative but lymphocyte depletion using cyclophosphamide and fludarabine followed by intravenous infusion of ex vivo tumor reactive, CAR-transduced PBMC, plus intravenous aldesleukin.

FUTURE DIRECTION AND CONCLUSION

The ever-updating observations provide the proof of principle for the efficacy of this new anti-tumor strategy of GBM. Adoptive cell therapy with EGFRvIII CAR-T cells has demonstrated a great potential in achieving long-term tumor suppression and a reduced mortality associated with GBM. It is expected to play an increasingly important role in the clinical arena. The heterozygosity of GBM, the blood-brain barrier and the local immunosuppressive micro-environment still remains an unmet medical need and warrant further studies. Scientists are designing newer generations of CAR to improve the safety, such as splitting synthetic receptor or inducible suicide gene controlled CAR. In addition, the efficacy will be increased through structure refining, selective T cells subsets or optimized clinical administration regime.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

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