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Chimeric Antigen Receptor Therapeutic Strategies: The Future of Glioblastoma Management

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Abstract

The field of adoptive cellular therapy, using autologous T-cells modified *ex vivo* to specifically target tumor cells prior to being reintroduced to the patient, has become a new focus of research endeavors searching for a novel and efficacious treatment for oncologic disease, including glioblastoma. Chimeric Antigen Receptor (CAR)-T-cells consist of a single chain variable fragment of a monoclonal antibody coupled with extant T-cell intracellular signaling cascade systems using a viral vector *ex vivo*. This provides the advantage of targeting tumor specific surface markers, while minimizing off-target effects and potential toxicity. Additionally, the CAR T-cells bypass the need for MHC-restricted presentation, a system which is frequently down-regulated in tumor cells. Among the surface antigens described as targets for CAR T-cell therapy for GBMs, Epidermal growth factor variant III (EGFRvIII), HER2 (HER2/neu, ERBB2), interleukin-13 receptor α2 subunit (IL-13Rα2), and erythropoietin-producing hepatocellular carcinoma A2 (EphA2) are the leading options for tumor specific surface antigens to target with CAR-T cells. This article reviews history and advantages of CAR-T cell therapies, and discuss future directions.

Keywords: Glioma; CAR-T; EGFRvIII; HER2; IL-13Rα2; EphA2

Introduction

Glioblastoma (GBM) remains the most common primary brain tumor, with a universally poor prognosis. Advances in chemotherapy and radiation treatment protocols have improved survival, but the overall 5-year survival rate remains less than 10%. The use of immunomodulating therapies for the treatment of GBM has been of long interest to researchers and clinicians. The field of adoptive cellular therapy (ACT), using autologous immune cells modified *ex vivo* to target tumor cells prior to being reintroduced to the patient, has become a new focus of research endeavors searching for a novel and efficacious treatment for oncologic disease, including GBM [1]. These therapeutic strategies include a variety of non-specific immune cells, including lymphokine-activated killer (LAK) cells, tumor infiltrating lymphocytes (TILs), γδT cells, antigen-specific cytotoxic T lymphocytes (CTLs), and natural killer (NK) cells. However, the results of these research endeavors have been mixed, and concern remains regarding toxicity in non-tumor tissues. In an attempt to provide more tumor-specific immunomodulatory options, and minimize off-target effects, T-cells have been modified with chimeric antigen receptors allowing for specific targeting of tumor cells in a variety of solid and hematologic malignancies [2-6]

Chimeric Antigen Receptor (CAR) Therapy Overview

The construction of CAR-modified T-cells has been extensively described; briefly, a single chain variable fragment (scFv) of a monoclonal antibody is coupled with extant T-cell intracellular signaling cascade systems using a viral vector *ex vivo*. This provides the advantage of targeting tumor specific surface markers, while minimizing off-target effects and potential toxicity. Additionally, the CAR T-cells bypass the need for MHC-restricted presentation, a system which is frequently down-regulated in tumor cells [7-9].

Potential limitations of CAR-modified T-cells include the need to target tumor surface markers, unlike other ACT therapies such as TCR therapy which can target extra- or intra-cellular tumor antigens. The activation of T-cells through CAR therapy may lead to cytokine release syndrome (CRS), which has been reported in a number of studies, and may range from a mild to a serious complication [10-14].

History of CAR therapies

The initial development of CAR therapies in the late 1980s demonstrated cytotoxicity, which was supported by multiple clinical trials in a range of solid and hematologic malignancies [15]. These early attempts were further refined, creating single costimulatory domain (CD28 or 4-1BB) and double costimulatory domain (CD28 + OX40 or 4-1BB) constructs. These second and third generation CAR avoided a major pitfall of first generation CAR, namely the limited functional lifespan of CAR-T cells and the occurrence of quiescence in the absence of costimulatory signals. Clinical trials have demonstrated significant long-term disease free survival in patients with hematologic malignancies using second generation CAR-T cells [2,16], and both second and third generation CAR-T cells have been shown to be efficacious against murine glioma models [17-22].

CAR therapies for gliomas

There remains great interest in using CAR-T cell therapies to provide a novel adjuvant therapy for management of gliomas that may minimize the significant toxicity to non-tumor tissue that is a significant side effect of standard of care chemotherapy and radiation regimens. However, the microenvironment of GBMs is challenging for successful immune-modulating therapies, as tumor cells suppress the

endogenous immune response systems, including T cell proliferation, CD8+ costimulatory signals, and MHC expansion. These factors have produced obstacles for adaptive cellular therapy to overcome; the MHC-independent nature of CAR-T cells provides an added benefit in the success of this therapy against GBMs (Figure 1).

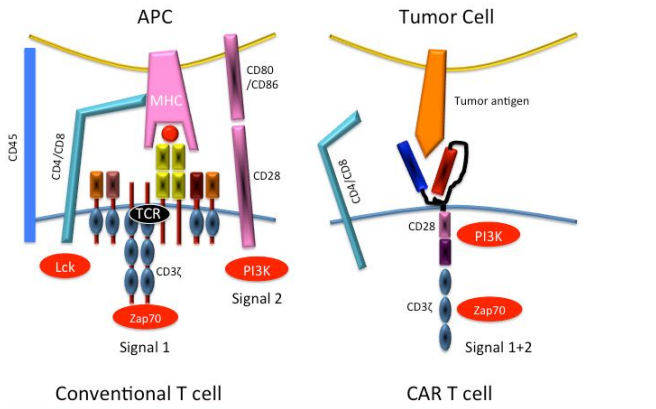

Figure 1: Differences between conventional T cells and CAR-T cells. CAR-modified T-cells consist of a single chain variable fragment (scFv) of a monoclonal antibody coupled with extant T-cell intracellular signaling cascade systems. CAR T-cells bypass the need for MHC-restricted presentation. These early attempts were further refined, creating single costimulatory domain (CD28 or 4-1BB) and double costimulatory domain (CD28 + OX40 or 4-1BB) constructs overcoming the limited functional lifespan of CAR-T cells and the occurrence of quiescence in the absence of costimulatory signals.

Target Surface Markers for CAR-Therapies

EGFRvIII (epidermal growth factor variant III)

EGFRvIII is the most frequently occurring EGFR variant, found in a variety of malignancies, including GBM [23-25]. A deletion of exons 3-6 of the extracellular domain results in a constitutively active tyrosine kinase, producing tumorigenic signals through the RTK/RAS/PI3K pathway; notably, the type III variant is frequently expressed in GBM cells but absent from non-tumor cells, providing an attractive CAR-T cell target that minimizes the risk of off-target cytotoxicity. Our group has previously developed a mouse mAb, 3C10 [26], and its scFv antibody that specifically recognizes the glycine residue of EGFRvIII [27]. We successfully generated human T cells expressing CAR targeting the EGFRvIII antigen using 3C10-scFv (3C10-CAR) [22]. Several CAR constructs targeting EGFRvIII, including 3C10-CAR, showed induction of IFN-γ production when added to EGFRvIII-expressing target cells [21], and stimulated tumor lysis *in vitro* and *in vivo* [22,20]. Humanized 3C10-CAR was subsequently generated to avoid a human anti-mouse antibody response, which restricts the persistence of 3C10-CAR-T cells and may cause anaphylaxis [28]. Phase I clinical trials of EGFRvIII-targeting CAR-T cell therapy for recurrent GBMs are currently underway (NCT01454596, NCT02209376).

Additionally, groups have explored the use of CAR-modified natural killer (NK) cells for treatment of GBM [29].

There are concerns, however, that the administration of CAR-T cells specific only for EGFRvIII may lead to surface antigen loss in the GBM cell population, thereby creating a tumor strain resistant to further therapy. This was seen in patients receiving EGFRvIII peptide vaccines, where 82% of patients had EGFRvIII null tumor cells at the time of recurrence [30].

HER2 (HER2/neu, ERBB2)

HER2 encodes a 185-kDa transmembrane glycoprotein with tyrosine-specific kinase activity, and is overexpressed in approximately 30% of breast cancer patients as well as in several other malignancies including GBM [31]. HER2 overexpression is associated with more aggressive disease and poor prognosis [32]. HER2 overexpression results in increased HER2 heterodimerization with EGFR and HER3; these heterodimers drive proliferation and invasion of cancer cells [33]. In addition, GBMs with low HER2 expression are postulated to arise through anaplastic transformation of low grade gliomas, making HER2 CAR-T cells a potential treatment specifically directed at patients with primary GBM [34].

Additionally, success of these cells in treating medulloblastoma has been demonstrated [6], making HER2-CAR-T cells a putative therapy for multiple CNS malignancies in both adult and pediatric populations.

Concerns remain regarding the safety of HER2-targeted CAR-T cells, as HER2 is expressed at low levels in some normal tissues, notably the lungs. Mortality was reported in a single patient who developed acute respiratory failure immediately following administration of HER2-CAR-T cells [35], which was postulated to be caused by localization of the CAR-T cells to the lungs with a subsequent massive cytokine release.

IL-13Rα2 (interleukin-13 receptor α2 subunit)

IL-13 binds to two receptors: IL-13Rα1 and IL-13Rα2. IL-13Rα1 forms a heterodimer with IL-4R and binds IL-13 ligand, triggering downstream signaling pathways. IL-13Rα2 is a monomer that lacks the signaling chain necessary to trigger IL-13 mediated pathways, and given the high affinity of IL-13 for IL-13Rα2, upregulation of this receptor serves to interfere with appropriate signal regulation, leading to more aggressive and invasive tumors [36,37]; IL-13Rα2 overexpression correlates with poor prognosis [38]. IL-13Rα2 is expressed specifically by tumor cells, although some expression in normal testis tissue has been documented. This makes it an attractive target for immunomodulating therapy, although off-target effects seen with other surface antigens must be anticipated and carefully monitored for in murine models [39-41].

IL-13-zetakine is a chimeric immunoreceptor using membrane-tethered IL-13 E13Y mutein for selective binding to IL-13Rα2. IL-13-zetakine T cells induced T cell proliferation and secretion of IFN-γ and TNF-α, and demonstrated antitumor activity when co-cultured with IL-13Rα2-expressing GBM cells *in vitro*. IL-13-zetakine CAR-T cells have also demonstrated effective targeting or IL-13Rα2 tumor cells in a murine model of human glioma, with regression of tumors and no neurotoxicity in the study [42].

A pilot study of IL-13 zetakine CAR-T cells in patients with recurrent GBMs demonstrated a reduction in IL-13Ra2 expression, with some indications of increased tumor necrosis [43]. Recently, second-generation CAR targeting IL-13Rα2 (IL-13Rα2-CAR)-

transduced T cells were shown higher anti-tumor effect than IL-13-zetakine [44].

However, while issues of off-target effects and toxicity have been minimal in murine models and small clinical trials, questions and concerns remain. One group found that the recognized antigen for a commercially available IL-13Rα2 mAb recognized VCAM-1 not IL-13Rα2, and that the antibody used in many analyses, B-D13, is di-clonal, recognizing both IL-13Rα2 and VCAM-1 [45]. Although the successful results of the IL-13Rα2 CAR-T cell studies remain valid, questions about the immunologic mechanisms and pathways underlying the results may require further investigation

EphA2 (Erythropoietin-producing hepatocellular carcinoma A2)

EphA2 is a member of the Eph family of receptor tyrosine kinases, and binds to the glycosylphosphatidylinositol-anchored ephrin-A ligand. Nearly 90% of GBMs overexpress ephrin, and EphA2 is commonly overexpressed in a variety of malignancies [46,47]. EphA2 exhibits ligand-dependent inhibition and ligand-independent promotion of cell migration and invasion; Akt kinase phosphorylation of EphA2 promotes ligand-independent cell migration, while ERK-RSK signal pathways also regulate this phosphorylation, altering migration and invasion dynamics of tumor cells [48,49], suggesting EphA2 as a target for CAR-T cell therapy.

EphA2 specific CAR-T cells have been developed, and shown to be effective in targeting tumor cells, reducing tumor burden, and minimizing the development of antigen loss in GBM cells [50]. Additionally, this group demonstrated targeting of neurospheres, an *in vitro* surrogate for putative glioma-initiating cells, indicating that EphA2 CAR-T cells may be effective both against the main tumor burden and microinfiltrating cell nests that evade traditional chemotherapy and radiation, and are proposed as the source for recurrence and spread of GBM. A phase I/II clinical trial of EphA2-CAR-T cell therapy for EphA2-positive MGs was initiated in China in 2015 (NCT02575261).

Future Directions

Although limited studies have evaluated the efficacy of CAR-T cell therapies in conjunction with standard of care chemotherapy and radiation [18], this remains an incompletely explored area. The synergistic effect of immune checkpoint inhibitors and CAR-T cells is a promising method for augmenting ACT, making a more efficient and effective oncologic treatment modality [51]. The first trial of this method is ongoing and will evaluate the efficacy of ipilimumab combined with CD19-CAR-T cells against B cell non-Hodgkin lymphoma, acute lymphocytic leukemia, and chronic lymphocytic leukemia (NCT00586391). CAR-T cells engineered to secrete anti-PD-1 antibodies have recently been developed, and were shown to induce regression of renal cell carcinoma *in vivo* [52]. These innovations provide renewed potential for CAR-T cell immunotherapy to successfully treat solid cancers.

Given the propensity of GBM cells to develop treatment resistance through a variety of mutations, the use of multiple CAR-T cell targets simultaneously may be an attractive proposition to ensure efficacy, particularly in patients with recurrent GBMs. Bispecific CAR-T cells targeting HER2 and IL-13Rα2 have enhanced functionality against GBM cells, and provide increased tumor control *in vivo* [53]. While some research exists on CAR-T cells co-expressing receptors for multiple GBM markers, additional work is needed to determine the most efficacious combination of markers (23939024, 27427982).

Additionally, CAR-T cells have been engineered with dual-antigen expression designed to increase specificity [54] and to provide dynamic self-regulation as a safety mechanism [55], however these cells have proved challenging to produce, which may limit large-scale clinical applications.

Another novel variant, synthetic Notch (synNotch) AND-gated CAR has recently been reported [56]. In this system, the synNotch receptor recognizes a tumor-specific antigen, and drives expression of a CAR for a second tumor-specific antigen. This dual-specificity helps minimize toxicity to non-tumor tissue. The synNotch mechanism is independent of CAR/TCR signaling, and rather than triggering T-cell activation, serves to prime the expression of CAR. The AND-gated T cells demonstrated robust therapeutic discrimination *in vivo*, and overcame the problem off-tumor/on-target cross reaction in normal tissue.

It is important to find new tumor-specific antigens to expand the repertoire of CAR-T cell therapies while minimizing toxicity. Tumor-specific carbohydrates and glycolipids are potential novel candidate targets, because CARs (unlike classical TCRs) can recognize structures other than protein epitopes [57]. New approaches to antigen discovery have also focused on the recognition of somatic mutations present in tumor antigens, as mutant peptides may serve as T cell epitopes [58]. Tumor epitopes identified by using whole-exome sequencing analysis with mass spectrometry have revealed immunogenic mutant peptides [59].

Finally, another possible strategy is to create universal donor CAR-T cells. Human T cells in which HLA class I has been genetically deleted in order to evade the immune response provides a source of cells from a single donor can be administered to multiple recipients [60].

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