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Intracerebral delivery of a third generation EGFRvIII-specific chimeric antigen receptor is efficacious against human glioma

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Abstract

Chimeric antigen receptors (CAR)-transduced T cells hold great promise in the treatment of malignant disease. Here, we demonstrate that intracerebral injection with a human, epidermal growth factor receptor variant III (EGFRvIII)-specific, third generation CAR successfully treats glioma in mice. Importantly, these results endorse clinical translation of this CAR in patients with EGFRvIII-expressing brain tumors.

Keywords

Central nervous system neoplasms; Epidermal growth factor receptor; Glioblastoma; Immunotherapy; T-lymphocytes

1. Introduction

Glioblastoma (GBM) is the most common and deadly primary malignant brain tumor, for which there are limited treatment options.¹ Chimeric antigen receptors (CAR) represent a promising technology that redirects T cells to treat tumors via surface antibody-based domains translated in tandem with intracellular T cell signaling moieties. Costimulatory 4-1BB signalling has been shown to significantly improve the ability of CAR-transduced T cells to persist and achieve antitumor T cell responses.² However, such “third generation” CAR have not been tested for efficacy against intracerebral tumors. A mutation of the epidermal growth factor receptor, variant III (EGFRvIII), is frequently expressed on the surface of GBM but is completely absent from all normal tissues.³ Here, we demonstrate

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Conflicts of Interest/Disclosures

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that an EGFRvIII-targeted, third generation CAR is specific and effective against human GBM cells *in vitro* and *in vivo*.

2. Materials and methods

2.1 Cell lines

Human glioma cell lines U87MG and U87MG.ΔEGFR are previously described.⁴

2.2 EGFRvIII CAR retroviral vector and analysis

Human peripheral blood lymphocytes were transduced with EGFRvIII CAR as described.⁵ Cytokine staining for interferon γ (IFN γ) was performed according to manufacturer's instructions (Cytotfix/Cytoperm; BD Bioscience, San Jose, CA, USA).

2.3 In vivo experiments

Efficacy was tested in non-obese diabetic scid gamma mice. Glioma cells (5×10^4) and T cells were implanted intracerebrally as described.⁶

2.4 Statistical methods

Frequencies of IFN γ^+ cells with respect to groups defined by EGFRvIII specificity and peptide blockade were evaluated by two-way analysis of variance with interaction. The Kaplan – Meier estimator was used to generate survival curves, and groups were compared using the generalized Wilcoxon test.

3. Results

3.1 Third generation EGFRvIII CAR is specific for EGFRvIII⁺ glioma

One limitation of potent CAR-based therapies has been lethal toxicity arising from affinity for antigens co-expressed on healthy tissues.^{7,8} Targeting EGFRvIII, however, greatly reduces this risk of autoimmunity. Demonstrating its specificity, EGFRvIII CAR-transduced T cells did not exhibit detectable immunity to EGFRvIII-negative cells above untransduced levels. However, on incubation with U87MG.ΔEGFR, EGFRvIII CAR-transduced T cells yielded a significantly elevated frequency of IFN γ -expressing cells, which was subsequently inhibited in a dose-response fashion by peptide blockade with PEPvIII (Fig. 1).

3.2 Third generation EGFRvIII CAR treat intracerebral tumors

To investigate the ability of EGFRvIII CAR-transduced T cells to treat glioma in the entral nervous system, mice were implanted intracerebrally either with U87MG.ΔEGFR alone, U87MG.ΔEGFR with untransduced (UT) T cells, or U87MG.ΔEGFR with EGFRvIII CAR-transduced T cells at various doses. Mice receiving tumor with UT T cells did not exhibit a significant change in survival compared to mice implanted with tumor alone. However, mice treated with CAR-transduced T-cell doses of 5×10^4 or greater showed a dose-dependent increase in survival (EGFRvIII CAR *versus* UT, $p < 0.001$, log-rank test; Fig. 2), without toxicity to adjacent normal brain upon histological analysis.

4. Discussion

Here, we have demonstrated that intracerebral injection of T cells expressing third generation EGFRvIII CAR can mediate safe, therapeutic responses against EGFRvIII-expressing tumors in the brain. Additionally, soluble peptide blockade was shown to specifically inhibit the functional activity of CAR, and may serve as a potential antidote for CAR targeting less tumor-specific targets.

Our third generation EGFRvIII CAR promises to foster potent T cell function beyond what would otherwise be expected with earlier generation CAR constructs availing fewer costimulatory signaling domains.^{6,9} Together these data provide further rationale for the expedient translation of T cell based therapies for malignant glioma.

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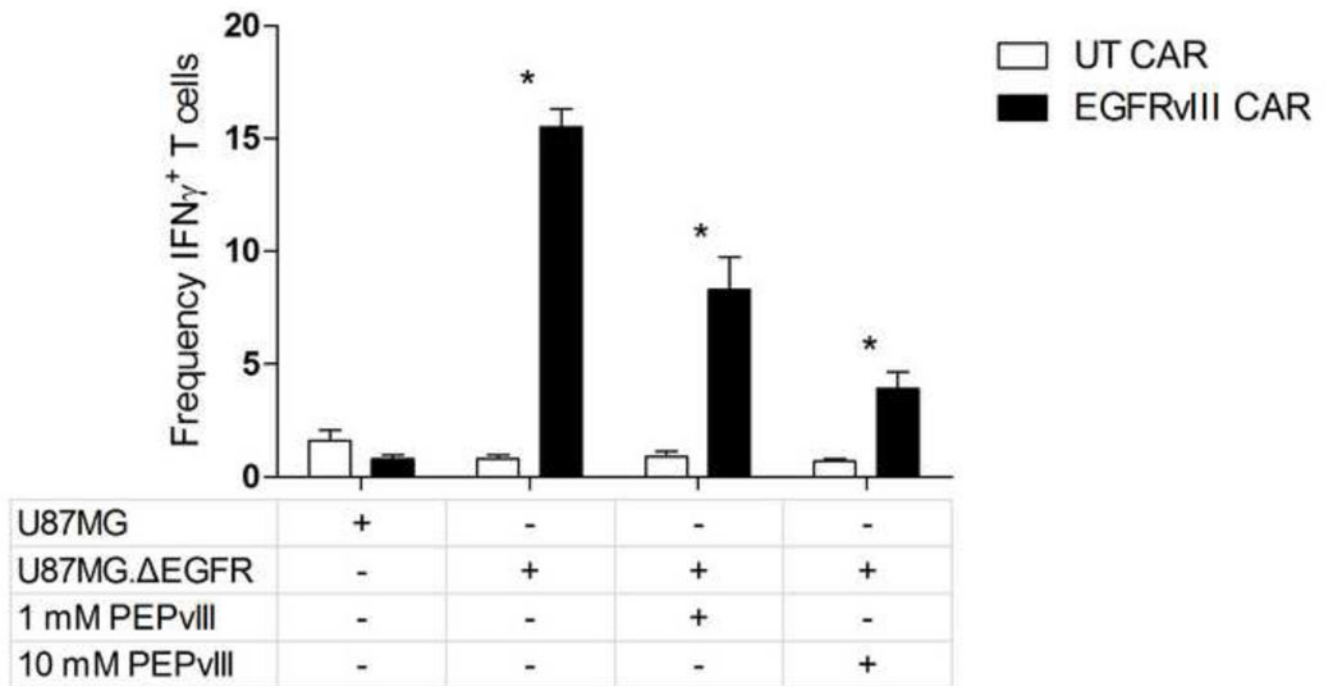


Fig. 1.

Graph showing epidermal growth factor receptor variant III (EGFRvIII) chimeric antigen receptors (CAR) T cell function and antitumor efficacy is specific for EGFRvIII⁺ tumors and is inhibited by soluble PEPvIII blockade. CAR-transduced or untransduced T cells with glioblastoma targets and soluble PEPvIII peptide was performed. Frequency of interferon γ^+ cells was significantly greater in the presence of the EGFRvIII CAR, as well as reduced in a dose-dependent manner in the presence of increasing concentrations of PEPvIII.

* $p < 0.001$.

CAR = chimeric antigen receptors, EGFRvIII = epidermal growth factor receptor variant III, IFN = interferon, UT = untransduced.

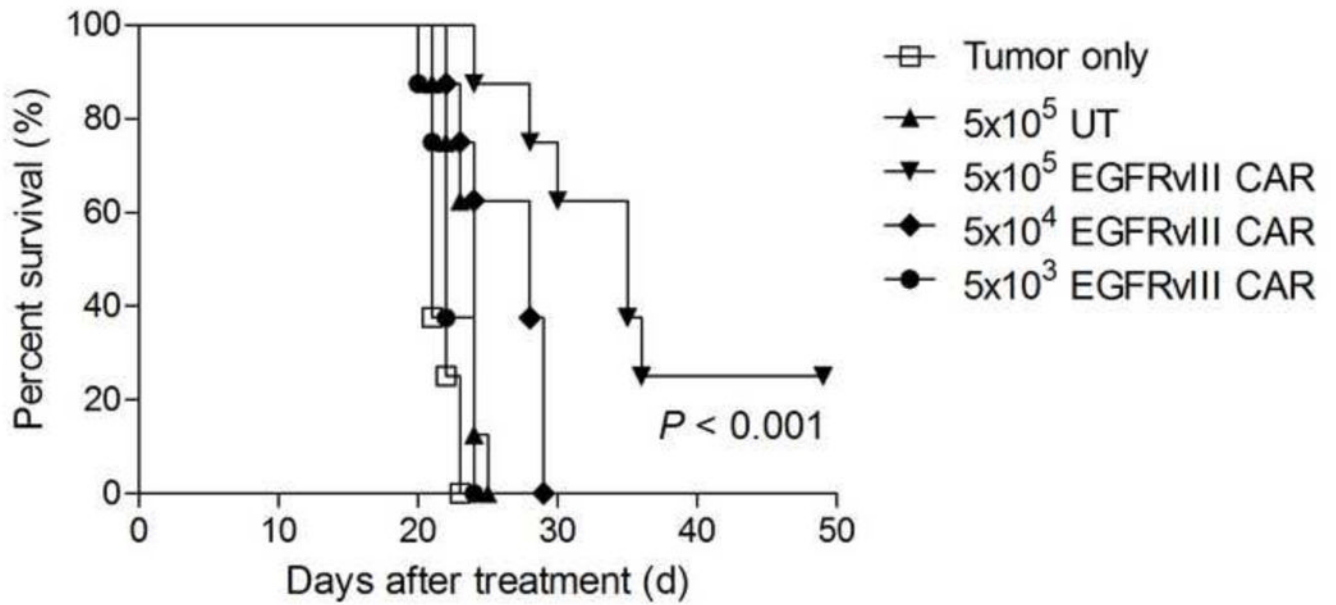


Fig. 2. Graph showing epidermal growth factor receptor variant III (EGFRvIII) chimeric antigen receptors (CAR)-transduced T cell therapy treats intracerebral glioma in a dose dependent manner ($p < 0.001$; generalized Wilcoxon test). EGFRvIII CAR-transduced T cells were implanted intracerebrally. The log-rank test was used to determine statistical significance. CAR = chimeric antigen receptors, EGFRvIII = epidermal growth factor receptor variant III, UT = untransduced.