A novel anti-EPOR CAR-T cell for the treatment of clear cell renal cell carcinoma

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Background

- The efficacy of chimeric antigen receptor (CAR) T cells targeting CD19 in B cell malignancies has made it approved by FDA as an option for patients.
- Application of CAR-T cell therapy in solid tumors remains challenging.
- Erythropoietin receptor (EPOR) is an antigen involved in cell proliferation that is highly expressed in renal cell carcinoma (RCC).
- Preliminary data showed that anti-EPOR monoclonal antibody (mAb) could suppress RCC tumor growth in vitro and in vivo
- **Hypothesis**: Anti-EPOR CAR-T cell with the 3rd generation CAR vector could have the ability to kill RCC cells in vitro and in vivo.

Objective

- To construct vectors containing scFv sequences from EPOR monoclonal antibody and 3rd generation CAR.
- To validate the killing effect of EPOR CAR-T cells on renal cancer cells in vitro and in vivo.

Methods

- Construct the EPOR antigen and immunize mice to obtain monoclonal antibody
- 5'RACE sequencing was performed to obtain the specific scFv sequence

Methods

- CAR-T cells were generated with the 3rd generation CAR and efficacy was evaluated in RCC cell lines under normoxia, hypoxia and EPOR over-expressed condition.
- Cytotoxicity was determined through cytokine release and xenograft mouse model using 786-0 cell line.

Results

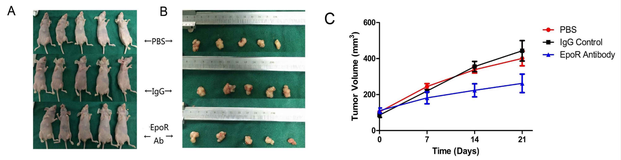


Figure 1. Anti-EPOR monoclonal antibody has the ability to suppress RCC cell growth in vitro and in vivo

Figure 2. Structure of chimeric antigen receptor (CAR). The CAR includes ectodomain, transmembrane domain and endo domain

Results

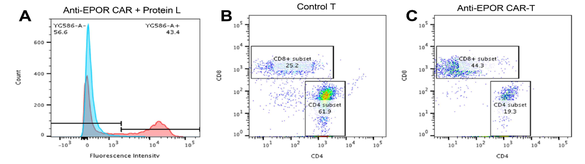


Figure 3. (A) Transduction efficiency detection through Protein L expression. (B) Anti-EPOR CAR-T cell have a higher CD8+ T cell ratio.

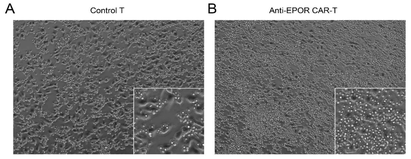


Figure 4. Adherence of EPOR CAR-T cells to RCC cells

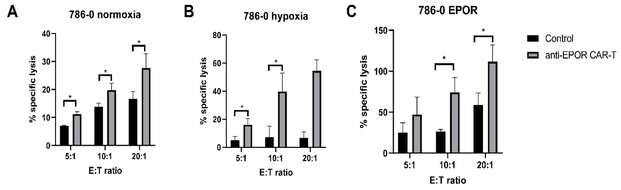
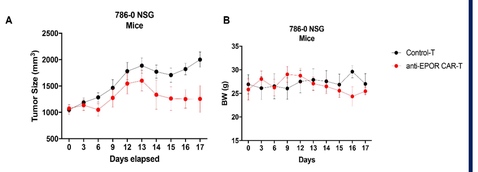


Figure 5. EPOR CAR-T cells were co-incubated with RCC cells for 48 hours at different effector (E): target (T) ratios. Cytotoxicity was measured by lactate dehydrogenase (LDH) release assay. (*: P<0.01)



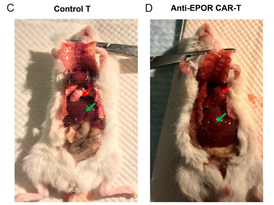


Figure 6. (A-B) Anti-EPOR CAR T cells suppress tumor growth in RCC xenograft mouse model. (C-D) After intravenous injection of anti-EPOR CAR-T cells for 2-3 weeks, adverse effects such as lung consolidation and hepatomegaly were observed on several mice.

Conclusions

- Anti-EPOR CAR-T is promising to be a therapeutic way to inhibit RCC tumor growth.
- Adverse effects need to be overcome with other strategies

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