DUAL TARGETING LIVER CANCER BY GPC3 CAR-T CELLS SECRETING A BISPECIFIC T CELL ENGAGER ANTIBODY FOR AN INTRACELLULAR TUMOR ANTIGEN AFP

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Background GPC3 chimeric antigen receptor T cell (CAR-T) therapy for liver cancer holds great promise. However, the effectiveness of CAR-T cells targeting a single antigen is limited due to the heterogeneous expression of the target antigen in liver cancer. To overcome this challenge, we present an innovative approach utilizing engineered T cells with dual targeting against two highly expressed antigens in liver cancer: cell surface GPC3 and intracellular AFP.

Methods In this study, we engineered GPC3 CAR-T cells (OriC101) to secrete a bispecific T cell engager (BiTE) composed of a TCR mimic antibody targeting AFP-MHC complex (OriA373). This BiTE specifically recognized the AFP-derived epitope (158–166) presented by the HLA-A02:01 molecule (figure 1). The secreted AFP BiTE effectively recruited and redirected both CAR-T cells and other CAR-T T cells towards the AFP/MHC complex on tumor cells.

Results Our findings revealed that the optimized GPC3 CAR-T cells secreting AFP-BiTE (OriC633–05) effectively activated both CAR-T cells and non-CAR-T cells while preserving a robust memory phenotype of CAR-T cells in vitro (figures 2 and 3). Moreover, OriC633–05 demonstrated significantly augmented anti-tumor activity against liver cancer cells with low GPC3 expression, both in vitro and in mouse tumor models (figure 4), surpassing the efficacy of GPC3 CAR-T cells and GPC3 CAR-T cells secreting alternative structures of AFP-BiTE.

Conclusions Hence, by leveraging dual orthogonal cytotoxic modalities with distinct specificities targeting surface and intracellular tumor-associated antigens, we have developed a promising strategy to overcome resistance to CAR-T cell therapy not only in liver cancer but also in other cancer types. Our results, demonstrating the superior activation and memory phenotype of GPC3 CAR-T cells secreting AFP-BiTE (OriC633–05), as well as its significantly enhanced anti-tumor activity against liver cancer cells with low GPC3 expression, and the potential for improved efficacy against liver cancer with high GPC3 expression, highlight the promising potential of this approach (figure 5).

Ethics Approval All animal experiments were approved by the Ethical Committee of East China Normal University.

Abstract 241 Figure 1 (A) Illustration of GPC3 CAR-T/AFP-BiTE Construction: A Schematic Overview. (B) Confirmation of GPC3 and AFP expression on target liver cancer cell line (SK-HEP-1-GPC3-AFP) by flow cytometry. The cell line used for both in vitro and in vivo experiments was consistent throughout the study.

Abstract 241 Figure 2 Cytotoxicity (A) and IFN-γ Production (B) of CAR-T Cells with Varied Structures and Target Cells after 24-Hour Incubation at a 1:1 Ratio.

Abstract 241 Figure 3 (A-B) Flow cytometry analysis was performed to detect the memory/effector phenotype of CAR T cells. In panel A, CAR T cells were cultured in serum-free medium for 3 days in the absence of target cells. In panel B, CAR T cells were co-cultured with target cells for 3 days. (C) The expression of the activation marker CD25 was assessed following co-incubation of CAR T cells and target cells for 3 days.

Abstract 241 Figure 4 (A) Schematic of the liver cancer xenograft model used to investigate the in vivo activity of CAR-T cells. NSG mice were subcutaneously injected with 3x10^6 target cells, and CAR-T cells were injected at specified time points. Each group consisted of n=5 mice. (B) Tumor growth was assessed three times per week following CAR-T cell injection. (C) In vivo toxicity was evaluated by monitoring the mice’s body weights throughout the treatment. (D-E) Peripheral...
blood samples were collected weekly to analyze the levels of IFN-γ (D) and CD3+ T cells (E).

Abstract 241 Figure 5

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