331 FUNCTIONAL ENHANCEMENT OF T CELLS BY THE CO-EXPRESSION OF IL-7 AND DOMINANT-NEGATIVE TGFβRII

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Background The effect of transforming growth factor β (TGF-β) is cell type- and context-dependent. In the tumor microenvironment, TGF-β restricts T cells' expansion, survival, and antitumor efficacy.[1 2] Blocking of the TGF-β signaling enhanced the infiltration and antitumor responses of chimeric antigen receptor-T (CAR-T) cells and improved the cytotoxicity of tumor-infiltrating lymphocytes.[3 4] However, TGF-β signaling is also required for IL-7Rα expression and the abrogation of TGF-β receptor expression led to the failed maintenance of peripheral CD4+ T cells,[5] since IL-7 signaling is essential for homeostasis, persistence, and survival of naïve and memory T cells.[6 7] Therefore we tested whether co-expression of IL-7 and dominant-negative TGFβRII (dnTGFβRII) could enhance the proliferation and antitumor efficacy of CAR-T cells and TILs isolated from primary hepatocellular carcinoma (HCC) tissues.

Methods T cells isolated from peripheral blood mononuclear cells or primary resected HCC tissues were activated by CD3/CD28 stimulation, lentivirally transduced with IL-7 and dnTGFβRII-expressing cassettes, and expanded *ex vivo*. For CAR-T cell preparation, anti-CD133 CAR was additionally transduced. Transgene expression was assessed by flow cytometry. *In vitro*, antitumor efficacy was analyzed by IFN-γ ELISA and real-time quantitative cytotoxicity assay with an IncuCyte instrument. The antitumor efficacy of the engineered T cells was further evaluated in a xenograft immunocompromised mouse model.

Results The expression of dnTGFβRII was analyzed with flow cytometry, and ELISA confirmed the secretion of IL-7 in TILs and anti-CD133 CAR-T cells (figure 1A-C). The genetically modified TILs revealed an increased activation level compared to unmodified TILs and the dnTGFβRII-expressing TILs as determined by IFN-γ ELISA (figure 1D). These engineered TILs also showed more potent and sustained killing efficacy against HCC cell line SK-Hep1 in the presence of TGF-β (figure 1E) *in vitro* and significantly higher tumor control efficacy *in vivo* (figure 1G). Similarly, IL-7 and dnTGFβRII co-expressing CAR-T cells also exhibited increased cytotoxicity against colorectal cancer cells HCT116 and HT-29 in the presence of TGF-β (figure 1F) *in vitro* and higher tumor control efficacy *in vivo* (figure 1H). Interestingly, a membrane-bound fusion protein, IL7:dnTGFβRII, linking IL-7 to the N terminus of dnTGFβRII further improved CAR-T cell proliferation when co-cultured with HT-29 cells. This finding prompts further investigation on this novel chimeric construct.

Conclusions A robust co-expression of IL-7 and dnTGFβRII on both TILs and CAR-T cells has been achieved. The combination of IL-7 signaling enhancement and TGF-β inhibition can synergistically improve T cells' anti-tumor efficacy and their resistance to immunosuppression.

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Ethics Approval All animal studies were conducted at the Ruiye model animal (Guangzhou) Biotechnology Co., Ltd facility as approved by its Experimental Animal Care and Use Committee (approval No. RYEth20220516–1).

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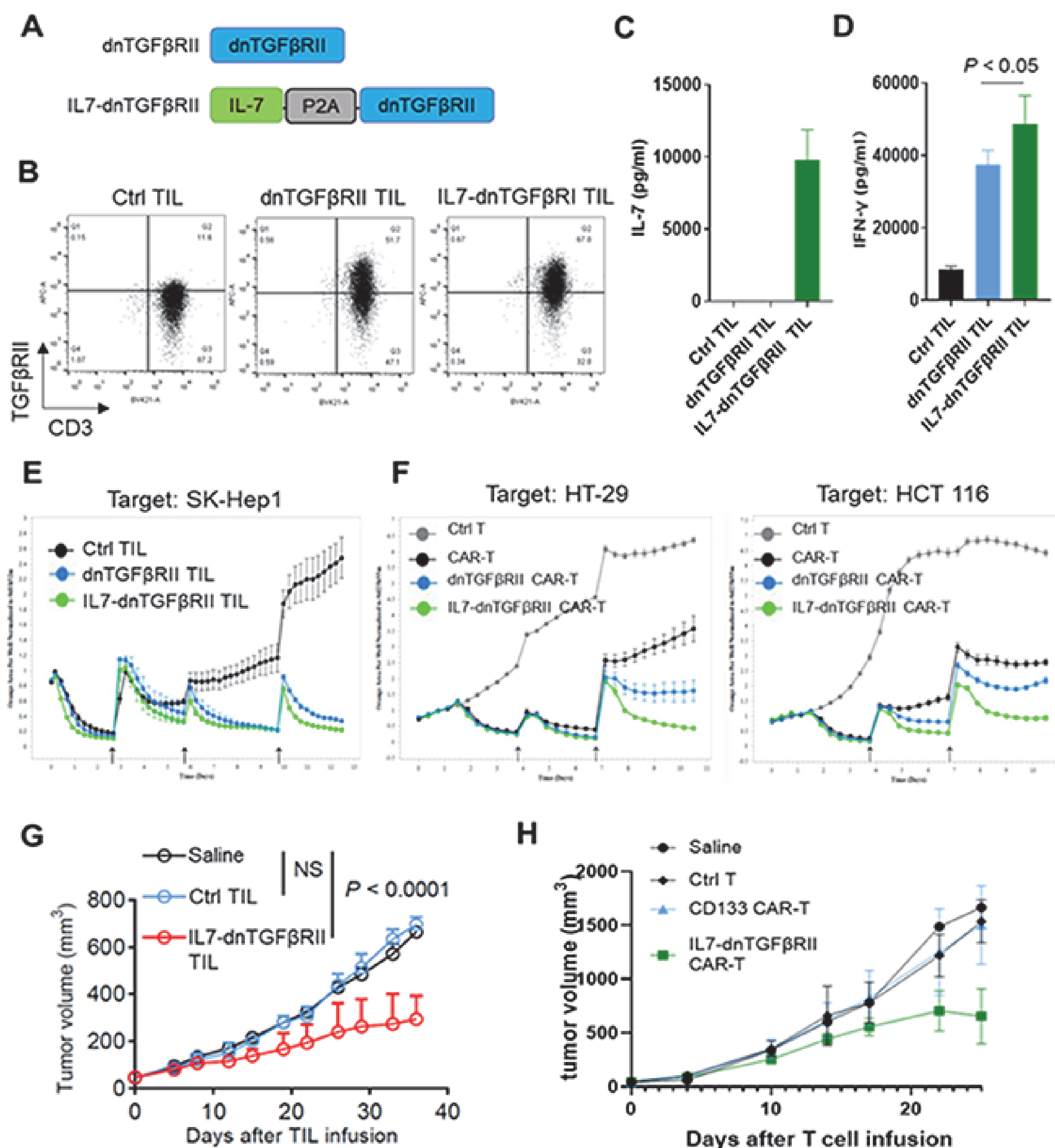

Abstract 331 Figure 1 Co-expression of IL-7 and dnTGFßRII enhanced the antitumor activity of TILs and CAR-T cells. (A) Schematic diagrams of transgene cassettes in lentiviral vectors. (B) Flow cytometry analysis of the surface expression of TGFßRII on TILs transduced by lentiviral vectors with a dnTGFßRII-expressing cassette or with an IL-7 and dnTGFßRII-expressing cassette. (C) IL-7 secretion. Control and engineered TILs were cultured at a density of 1 x 10^6 cells/ml for 96 h and the level of IL-7 released in the medium was measured by ELISA. (D) IFN-y release. Control and engineered TILs were co-cultured with SK-Hep1 cells for 16 h at an E:T of 1:1 in the presence of 10 ng/ml TGF-ß. The level of IFN-y released in the medium was measured by ELISA. (E) IncuCyte real-time quantitative cytotoxicity assay. Control and engineered TILs were co-cultured with mCherry-expressing SK-Hep1 cells at an initial E:T ratio of in the presence of 10 ng/ml TGF-ß. Arrows, time- points of target cell addition for repetitive stimulation of TILs. (F) IncuCyte real-time quantitative cytotoxicity assay. Control and enhanced CAR-T cells were co-cultured with mCherry-expressing HCT 116 or HT-29 cells at an initial E:T ratio of 2:1 in the presence of 10 ng/ml TGF-ß. Arrows, time-points Of target cell addition for repetitive stimulation of T cells. (G) In vivo antitumor efficacy engineered TILs. SK-Hep1 cells were subcutaneously inoculated in NOG immunocompromised mice, and TILs were intravenously infused. (H) In vivo antitumor efficacy enhanced CAR-T cells. SW620 cells were subcutaneously inoculated in NOG mice, and T cells were intravenously infused.

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