CRISPR-MEDIATED INSERTION OF IL-12 INTO THE PDCD1 LOCUS IMPROVES THE ANTITUMOR ACTIVITY OF TCR-T CELLS AGAINST SOLID TUMORS

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Background Interleukin-12 (IL-12) is a powerful immunostimulatory cytokine that has been expressed ectopically in genetically engineered T cells to enhance their antitumor activity. However, the constitutive production of IL-12 by engineered T cells caused severe adverse effects in patients. Thus, to fully harness the immunostimulatory potential of IL-12 while avoiding systemic toxicity, we inserted IL-12 gene into the PDCD1 locus in T cell receptor (TCR)-engineered T cells using the CRISPR/Cas9-based genome editing tool, which allows for IL-12 secretion to be induced strictly in a T cell activation-dependent manner.

Methods As a model TCR, we used a monoclonal TCR that is specific to the NY-ESO-1 (SLLMWITQV) peptide. The PD1-edited NY-ESO-1-specific TCR-T cells were generated by sequential lentiviral transduction and Cas9 RNP/AAV6-based knock-in into human primary T cells. The resulting TCR-T cells were co-cultured with an A375 cell line expressing NY-ESO-1 antigen to evaluate cytokine production, cytotoxicity, and proliferation in vitro. In vivo antitumor activity of the TCR-T cells was investigated in A375 xenograft models using NSG mice.

Results The PDCD1 locus was successfully edited in NY-ESO-1 TCR-T cells by replacing the endogenous PD-1 gene with a single-chain IL-12 transgene, without affecting the viability or expansion of the engineered T cells. Upon recognition of the target cells, the IL-12 transgene was expressed successfully, resulting in the strong phosphorylation of STAT-4 in the TCR-T cells. As compared to control TCR-T cells, these ΔPD-1-IL-12 NY-ESO-1 T cells displayed enhanced in vitro effector function, including increased secretion of IFNγ, TNF, and IL-10 as well as faster tumor cell lysis. In addition, ΔPD-1-IL-12 NY-ESO-1 T cells expanded more robustly after repeated challenges with PD-L1 overexpressing target cells. In xenograft models, ΔPD-1-IL-12 NY-ESO-1 T cells potently eliminated established tumors and demonstrated increased tumor infiltration compared to control TCR-T cells.

Conclusions Using the CRISPR/Cas9 system, we demonstrated that the upregulation of PD-1, an immune-suppressive event in T cells, could be reprogrammed to secrete immunostimulatory IL-12 in TCR-T cells. In both in vitro assays and in vivo mouse xenograft studies, these PD-1-edited TCR-T cells demonstrated enhanced cytotoxic activity. Our approach may offer a novel engineering option for adoptive T cell therapy against solid tumors.