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POSTER ABSTRACTS

703.CELLULAR IMMUNOTHERAPIES: BASIC AND TRANSLATIONAL

Mitochondrial Isocitrate Dehydrogenase Inhibition Enhances CAR T-Cell Function By Restraining Antioxidant Metabolism and Histone Acetylation

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Introduction: The efficacy of chimeric antigen receptor (CAR) T-cell therapy is hampered by relapse in hematologic malignancies and by hyporesponsiveness in solid tumors. Mitochondria are vital for the regulation of memory T-cell formation or exhaustion. A mitochondria-related compound screening was performed and we found that the FDA-approved isocitrate dehydrogenase 2 (IDH2) inhibitor enasidenib enhances long-lived memory CAR T-cell formation, and improves tumor clearance *in vivo*. IDH2 as the key component of the tricarboxylic acid (TCA) cycle, we hypothesized that IDH2 inhibition might promote CAR T-cell persistence by reprogramming the metabolism. The study aims to systematically evaluate the role and the mechanism of IDH2 inhibition on CAR T cells, and explore the application of enasidenib in CAR T-cell therapy.
Methods and Results: To identify mitochondrial components that affect the long-term efficacy of CAR T-cells, we performed an *in vitro* screening using a mitochondria-related compound library based on the enrichment of the $CD62L^+$ CAR T-cell subset, which mainly contains T memory stem cells (T_{SCM}) and central memory T cells (T_{CM}). Among several candidate compounds that promoted memory CAR T-cell formation, enasidenib (ENA), an inhibitor of both wild-type and mutant IDH2 enzymes, was the most effective one. The proportion of CD62L has increased by over 20%. To determine the effect of ENA on exhaustion induced by tonic CAR signaling and tumor antigen stimulation, we measured the expression of inhibitory receptors PD-1, TIM-3, and LAG-3 and the level of apoptosis in freshly expanded CAR T cells and B-ALL encountered CAR T cells. CAR T cells treated with ENA exhibited reduced surface levels of those inhibitory receptors. We measured the production of granzyme B and IFNγ, and tumor rechallenge assay in CAR T cells after ENA treatment to assess the effector function, ENA treated CAR T cells exerted enhanced and sustained cytotoxicity in a tumor rechallenge assay at an extreme E:T ratio of 1:10. These results were observed not only in CD19-41BBζ CAR T cells, but also in CD19-28ζ and GD2-28ζ CAR T cells. CAR T cells expanded in the presence of ENA significantly prolonged survival of recipients after Nalm-6 infusion ($p < 0.0001$), extended *in vivo* ENA treatment further enhanced CAR T-cell expansion and tumor suppression. The median survival was extended from 45 to 67.5 days. Consistent with the effect of ENA treatment, *IDH2* knockdown increased the proportions of the T_{scm}/T_n and T_{cm} subsets, and alleviated CAR T-cell exhaustion. *IDH2*-knockdown CAR T cells had sustained cytotoxicity and higher CD62L expression after rounds of killing B-ALL cells *in vitro*.
To systematically explore the effects of IDH2 inhibition on the metabolism of CAR T cells, relative metabolite amounts was measured using mass spectrometry (MS). IDH2-inhibited CAR T cells exhibited a substantial reduction of metabolites in the TCA cycle, including succinate, fumarate, and malate, and in the glycolysis pathway, including lactate, phosphoenolpyruvate. Mechanistically, IDH2 inhibition reprogram central carbon metabolism of CAR T cells by redirects glucose carbon utilization from glycolysis to the pentose phosphate pathway. In addition, IDH2 limits cytosolic acetyl-CoA level to prevent histone acetylation that promotes memory cell formation.
Significance: Our study indicates that metabolic intervention in CAR T cells with the FDA-approved IDH2 inhibitor enasidenib can advance current treatment, with better tumor eradication and CAR T-cell persistence.

Disclosures No relevant conflicts of interest to declare.

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