

SIRP α DEFICIENT CAR-MACROPHAGES EXHIBIT ENHANCED ANTI-TUMOR FUNCTION AND BYPASS THE CD47 IMMUNE CHECKPOINT

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Background Adoptive macrophage cell therapy represents a novel approach for cancer immunotherapy. Macrophages engineered to express chimeric antigen receptors (CAR-M) have shown promising pre-clinical results against solid tumors by improving tumor clearance, overall survival and facilitating the remodeling of the tumor microenvironment to induce a potent adaptive immune response. CD47 is a well-established macrophage immune checkpoint molecule that is over-expressed on tumor cells. CD47 binds to the macrophage signal regulatory protein α (SIRP α) to limit phagocytosis and macrophage effector functions. In this study we evaluated the impact of CD47 on CAR-M activity and showed that CD47-resistant targeted macrophage cell therapy mediates enhanced anti-tumor activity.

Methods CRISPR/Cas9 was used to deplete the cognate receptor SIRP α from primary human macrophages (>90% efficiency and >90% viability) to increase CAR-M function. To assess anti-tumor activity of CAR-M, *in vitro* co-culture assays were established with an anti-human epidermal growth factor receptor 2 (HER2) CAR and HER2+ tumor cell lines. Macrophage killing and phagocytosis of target cells were quantified in real-time using a genetically encoded fluorophore (to monitor tumor cell growth) or a pH-sensitive dye (to monitor phagocytic acidification). In parallel, phenotypic characterization of surface molecules, cytokine secretion levels, biochemical analysis of downstream signaling molecules and response to purified HER2 and CD47 protein stimulation were evaluated.

Results SIRP α knockout (KO) alone failed to induce tumor phagocytosis and cytotoxicity but enhanced targeted CAR-M anti-tumor activity. This was demonstrated by a reduced time required to kill 50% of tumor cells and a 2-fold increase in phagocytic activity, indicating synergy between SIRP α KO and CAR stimulation. Furthermore, in the absence of SIRP α , enhanced cytokine/chemokine secretion, macrophage polarization, and downstream signaling were observed.

Conclusions We show for the first time the feasibility of generating gene edited primary human CAR macrophages for therapeutic purposes, and demonstrate that SIRP α deletion enhances the targeted anti-tumor activity of CAR-M.

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