DYSFUNCTIONAL IMMUNE SYNPSES RESTRAIN ANTI-DIPG ACTIVITY OF CAR T CELLS

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Background Diffuse intrinsic pontine gliomas (DIPGs) are highly lethal pediatric brain tumors. Thus, there is an urgent need for novel therapeutics. While chimeric antigen receptor (CAR) T-cell therapy has the potential to meet this need, early phase clinical studies with CAR T cells has shown limited anti-tumor activity for brain tumors. T cells require three signals for optimal activity, being (1) T cell activation, (2) costimulation, and (3) cytokines. While numerous investigators have focused on improving signals 2 and 3, the goal of this study was to investigate the role of immune synapse (IS) formation, which is critical for proper T cell activation, in the context of DIPG-targeted CAR T cell therapy.

Methods We generated GRP78-CAR T cells by expressing a 2nd generation CAR with a CD28.z signaling domain, which recognize an endoplasmic reticulum chaperone protein that is broadly expressed on the cell surface of DIPGs. We compared the effector function of GRP78-CAR T cells against U87 glioma and the patient-derived DIPG007 cell line, which are both positive for GRP78. Then we evaluated in vitro CAR T cell effector functions by MTS-based assays (cytotoxicity), repeated stimulation assays (persistence), and Milliplex (cytokine secretion). To gain mechanistic insight into tumor and CAR T cell interaction, we analyzed IS formation by live cell imaging confocal microscopy.

Results Our in vivo studies demonstrated that GRP78-CAR T cells eradicate orthotopic U87 brain tumors but have no anti-tumor activity against DIPG007, despite that GRP78-CAR T cells efficiently killed U87 and DIPG007 cells in vitro. However, the total cytokine production was significantly lower post DIPG007 activation (28 fold) when compared to U87 activation with IFNg, GM-CSF, TNFa, IL-2 and IL-13 being the most significantly suppressed in DIPG setting. In concordance, CAR T cells were only able to expand and retain their cytolytic activity in the presence of U87 cells. When we look at the CART:DIPG007 IS resulted in a significantly lower calcium flux in comparison to CART:U87 synapses. Likewise, the recruitment of lysosomes to CART:DIPG007 IS was significantly diminished. Importantly, we observe the same dysfunctional IS formation regardless of CAR T cell specificity and targeted antigen expression level indicating that the suppressive effect is DIPG-tumor mediated.

Conclusions Our study demonstrates that DIPG tumors suppress CAR T cell effector function by damping T cell activation through dysfunctional immune synapse formation. We are now testing other CARs and genetic engineering approaches directed at improving IS formation to overcome the suppressive effects of DIPGs.