

ABLATION OF THE INHIBITORY RECEPTOR TIGIT ENHANCES CAR-NK CELL FUNCTION IN THE TUMOR MICROENVIRONMENT

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Background: The efficacy of chimeric antigen receptor (CAR)-NK cells that target pediatric solid tumors has been limited, in part, because of tumor microenvironments (TMEs) that include immune-inhibitory and tumor-promoting myeloid derived suppressor cells (MDSCs) and M2 tumor-associated macrophages (M2s). MDSCs and M2s in the TME highly express ligands for the inhibitory receptor TIGIT, highly upregulated on CAR-NK cells during their ex vivo generation. The goal of our study is to overcome TIGIT-mediated suppression of CAR-NK immunotherapy in pediatric TMEs. Current therapeutic options being evaluated in clinical trials targeting the TIGIT axis are antibody-based and are handicapped by poor antibody bioavailability and temporary antibody binding within the TME. To overcome these limitations and improve CAR-NK anti-tumor response, we hypothesized that genetic depletion of TIGIT within CAR-NK cells would result in a profound and durable improvement in the anti-tumor activity of CAR-NK cells in pediatric TMEs.

Materials/Methods: We used a combination of flow cytometry and tumor growth assays in a novel TME culture system to assess the anti-tumor activity of CAR-NK cells in which the TIGIT gene was deleted via CRISPR/Cas9 technology.

Results: Flow cytometric analysis of pediatric neuroblastoma and sarcoma patient tumor samples confirmed high surface expression of TIGIT ligands (CD155 and CD112) on tumor cells as well as intra-tumoral MDSCs and M2s. To define the influence of TIGIT on CAR-NK cell effector functions, we successfully ablated TIGIT using CRISPR/Cas9 to generate stable TIGITKO primary human CAR-NK cells. TIGITKO enhanced CAR-NK cytokine secretion but not degranulation in short-term TME cultures with MDSCs and neuroblastoma tumor. In long-term cultures, TIGITKO CAR-NK cells eliminated neuroblastoma tumor, whereas TIGITwt CAR-NK cells were unable to control tumor growth. In a TME culture system where neuroblastoma and monocytes were first allowed to pre-establish a highly suppressive TME, subsequently-added TIGITKO CAR-NK cells still controlled tumor compared to TIGITwt CAR-NK cells.

Conclusions: Our studies highlight a role for TIGIT in inhibition of CAR-NK cell function and TIGIT deletion as a novel NK cell therapeutic platform to evade TIGIT-mediated immune suppression in the TME. This highlights the potential of gene-edited CAR-NK cells to improve clinical outcomes in children with solid tumors.

Images / Graph / Table

HYPOTHESIS

