Multimodal Analysis of the Interaction Between CD70-Directed Chimeric Antigen Receptor Natural Killer Cells and Target Tumors

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Introduction

- Natural killer (NK) cells are effector lymphocytes of the innate immune system.
- NK cells form conjugates with their targets to induce cytotoxicity through direct contact.
- CD70, the ligand of CD27, is expressed on multiple solid and hematologic cancers. Thus, NK cells transduced to express a CD27 chimeric antigen receptor (CAR) can be used to target CD70-expressing tumor cells.
- This study aims to compare the conjugate formation, cytotoxicity, and the metabolic fitness of CD27 CAR NK cells and nontransduced (NT) NK cells, in order to better understand the interaction between CAR NK cells and their tumor targets.

Methods

Effector cells: NK cells were harvested from UCB units, stimulated with IL-2, and expanded with uAPCs. CAR NK cells were obtained by retroviral transduction with a vector encoding a CD27 receptor and containing an IL-15 transgene.

Fluorescence tagging and imaging: Samples were stained with shown markers and ran through Amnis imaging flow cytometer and LSRFortessa X-20 Cell Analyzer.

Cytotoxicity assay: Standard chromium release assay was performed. Target cells were radiolabeled with chromium-51 and cocultured with NK cells for 4 hours at various effector-to-target ratios. Lysis was then determined by measuring chromium released in the supernatant from dying cells.

Metabolic analyses: The Cell Mito Stress Test and Glycolysis Stress Tests were run on Seahorse XF Analyzer to measure oxygen consumption rate (OCR) and extracellular acidification rate (ECAR), respectively, of NT and CAR NK cells. Liquid chromatography-mass spectrometry (LC-MS) was conducted at the MD Anderson Metabolomics Core.

Results

Immune Synapse Between CAR NK and Tumor Cells

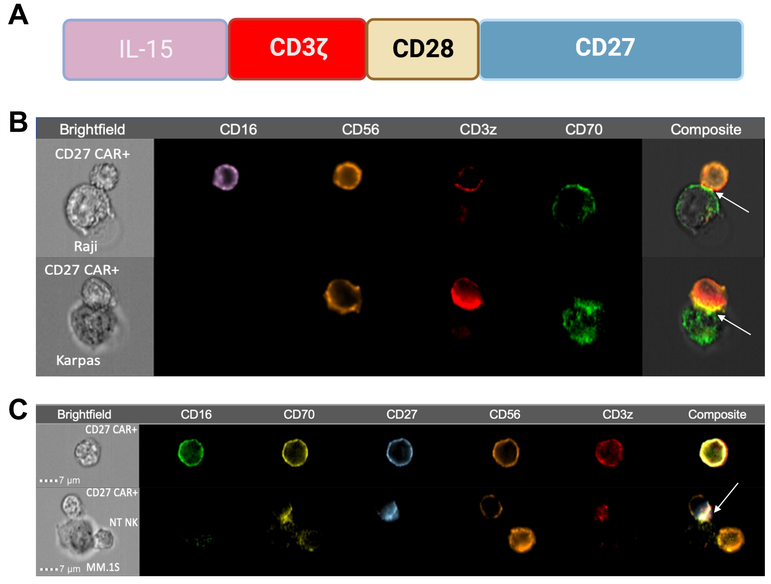


Figure 1. (A) Map of CD27-based CAR construct. **(B)** Formation of CAR NK cell immunological synapse (IS) (shown with white arrows) with CD70-expressing Raji and Karpas lymphoma cell lines. **(C)** CAR NK cell singlet, and the binding of an NT NK and CAR NK cell to a MM.1S multiple myeloma cell. Shown here is the accumulation of CD27 and CD3ζ at the IS formed by the CAR NK cell, but not the NT NK cell.

Cytotoxicity of CAR NK versus CD70+ Tumor Cells

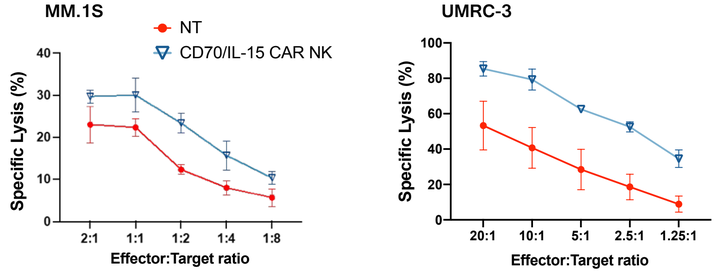


Figure 2. Chromium-51 cytotoxicity assays showing higher killing of CD70+ MM.1S and UMRC-3 cell lines by CAR NK cells than by NT NK cells.

Enhanced Metabolic Fitness of CAR NK Cells

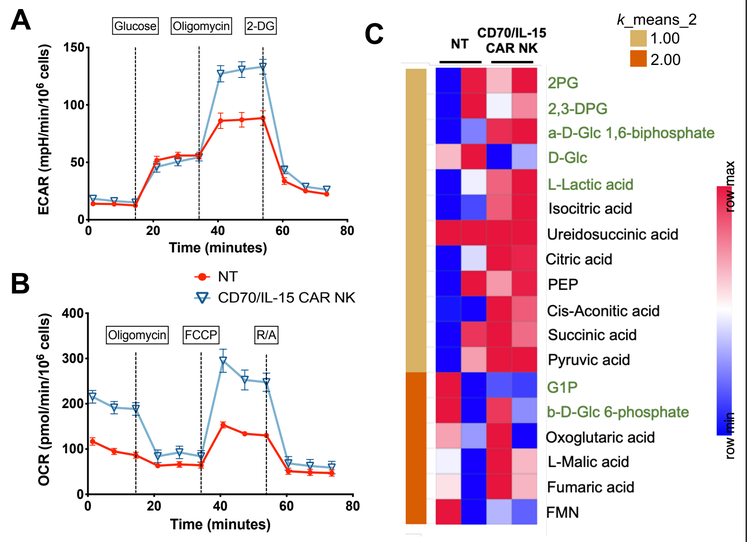


Figure 3. (A) ECAR of NT and CAR NK cells as measured by Seahorse Glycolysis Stress Test. **(B)** OCR of NT and CAR NK cells as measured by Seahorse Mito Stress Test. **(C)** Glycolysis (green) and TCA (black) metabolites of NT and CAR NK cells by LC-MS shown as normalized heat map ranked by *k*-means clustering.

CD70 Downregulation Prevents CAR NK Fratricide

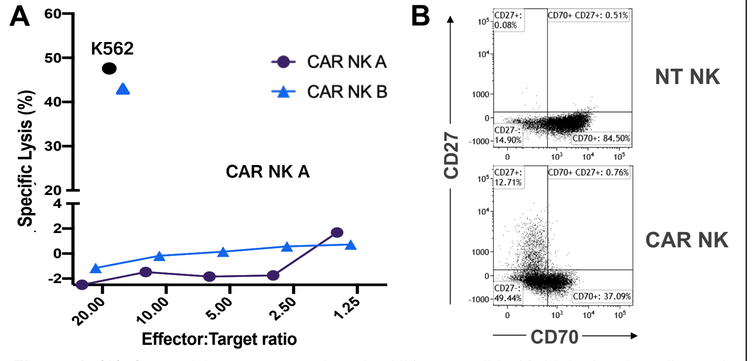
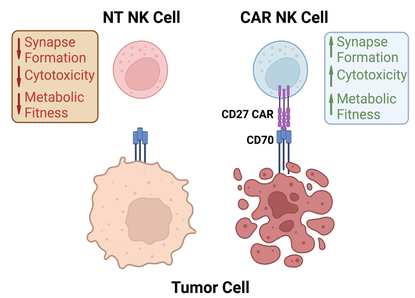


Figure 4. (A) Cytotoxicity assays against the NK-susceptible K562 leukemia cells, and against CAR NK A cells by sibling CAR NK A cells (HLA-A2$^+$ and HLA-A3$^-$) and CAR NK cells of different HLA haplotype B (HLA-A2$^+$ and HLA-A3$^+$) showing lack of fratricide. **(B)** Flow cytometry indicating downregulation of CD70 in CD27-transduced CAR NK cells.

Conclusions

CD27 CAR Empowers NK Cell Function



Schematic depicting CD27 CAR NK cells actively engaging tumor cells, compared to NT NK cells.

Future Directions

- Long-term cytotoxicity and tumor rechallenge assays against CD70+ tumors.
- Short- and long-term apoptosis assays against HLA-mismatched CAR NK cells.
- Metabolic assays and LC-MS of CAR NK cells following coculture with CD70+ tumors.

References

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