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

Dawei Liu,1,2 Pinaki Banerjee, PhD,2 Hind Rafei, MD,2 Paul Lin, MD, PhD,2 Rafet Basar, MD,2 Sunil Acharya, PhD,2 Nadima Upreti,2 Sanjida Islam,2 Yan Cui,2 Katy Rezvani, MD, PhD2

1 Wallace H. Coulter Department of Biomedical Engineering at Georgia Tech and Emory University, Atlanta, GA 30332
2 Stem Cell Transplantation and Cellular Therapy, MD Anderson Cancer Center, Houston, TX 77030

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 CD70, is expressed on multiple solid and hematologic cancers. Thus, NK cells transduced to express a CD70 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 CD70 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 of a vector encoding a CD70 CAR receptor and containing an IL-15 transgene. Fluorescence tagging and imaging: Samples were labeled with shown markers and run through Amnis imaging flow cytometer and LSRSFORTessa 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 Metabo 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

Figure 1. (A) Map of CD70-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 CD70 and CD38 at the IS formed by the CAR NK cell, but not the NT NK cell.

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.

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.

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

Conclusions

CD70 CAR Empowers NK Cell Function

- 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 coulture with CD70+ tumors.

Future Directions

- CD70 CAR NK cells actively engaging tumor cells, compared to NT NK cells.

References


Acknowledgements

This project was funded by the RP210028 CPRIT Research Training Grant. Thanks to the Department of Experimental Radiation Oncology for providing me with the opportunity to partake in the CPRIT-CURE Summer Program. I would like to express my sincere gratitude to Dr. Pinaki Banerjee, Dr. Hind Rafei, Samprit Islam, Toan Bui, Dr. Omar Madoor, and Dr. Katy Rezvani for their incredible mentorship. I would also like to thank everyone in the Rezvani lab for their welcome and dedication to helping increase access. This poster is dedicated to the memory of Dr. Judy Moyes.