CISH gene-knockout anti-CD70-CAR NK cells demonstrate potent anti-tumor activity against solid tumor cell lines and provide partial resistance to tumor microenvironment inhibition

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Introduction

Peripheral blood natural killer (NK) cells are attractive candidates for adoptive cell therapy. NK cells possess innate ability for tumor cell killing and are also amenable to genomic engineering for enhanced functions. Moreover, NK cells possess an inherent capacity for allogeneic, off-the-shelf therapy since, unlike T cells, they are neither HLA-restricted nor known to cause graft-versus-host disease. Cytokine inducible SH2-containing protein (CISH) is a negative regulator of interleukin 15 (IL-15) signaling in natural killer (NK) cells. Here we show the potential application of CISH gene-knockout CAR NK cells targeting CD70 and expressing a membrane-bound form of IL-15. CD70 is an antigen that is aberrantly expressed in a variety of malignant settings, including renal cell carcinoma (RCC), while its expression in normal tissues is restricted to a subset of lymphoid cell types.

Methods

To target CD70 on RCC cells, we generated CD70-CAR NK cells with CISH deletion. Using the CRISPR/Cas9 system, we knocked out CISH expression in isolated peripheral blood NK cells from healthy donors. Since CD70 expression is present on activated NK cells, we also targeted CD70 for CRISPR knockout to avoid fratricide. We then expanded these edited NK cells by using IL-2 and stimulation using NKSTIM, a modified K562 stimulatory cell line expressing membrane-bound form of IL-15 (mbIL-15) and 4-1BBL. IL-12 and IL-18 were added during expansion to drive memory-like NK cell differentiation. We transduced the expanded NK cells to express engineered CD70-targeted CAR and mbIL-15. We assessed CAR expression, NK cell persistence, and NK cell activity against RCC target cells using end-point cytotoxicity assays and IncuCyte

Results

CISH gene-knockout CD70-CAR NK cells could be produced efficiently and exhibited extended persistence in culture. After engineering and expansion, CD70-CAR transduction efficiency was 60-80%. CD70-CAR NK cells displayed potent cytotoxicity against CD70-expressing renal cancer derived cell lines. Interestingly, cytotoxicity assays demonstrated that CISH gene-knockout CD70-CAR NK cells were partially resistant to TGFβ and adenosine inhibition of cytotoxicity. Furthermore, CISH gene-knockout CD70-CAR NK cells maintained their activity during prolonged culture.

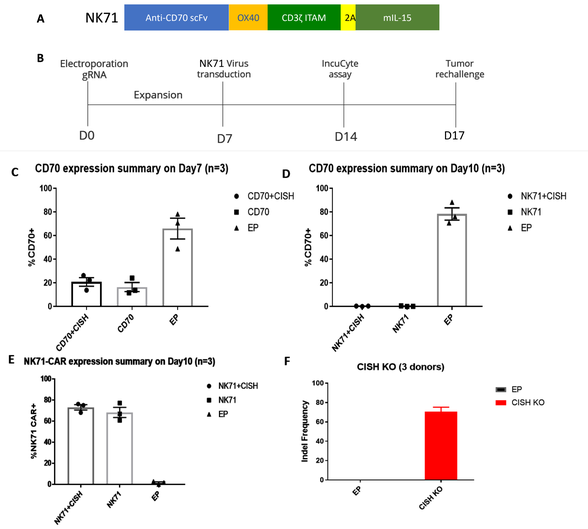


Figure 1. CD70 knockout and CD70-CAR transduction efficiency are consistent across donors. (A) Schematic map of retroviral vector encoding CD70-CAR (NK71) and membrane bound IL-15. (B) Schematic timeline for process of CISH gene-knockout CD70-CAR NK cells. (C) CD70 expression was successfully knocked down from 60-70% to around 20% before NK71 transduction. (D) CD70 expression was completely disrupted 3 days post transduction. (E) NK71 (CD70-CAR) transduction efficiency was 60-80% across 3 donors. (F) Indel frequency of CISH gRNA/RNP based on targeted NGS of primary human NK cells. CISH gRNA shows around 70% deletion efficiency.

Figure 2. CISH knockout increases cytotoxicity and persistence of CD70-CAR NK cells. (A) Cytotoxicity of CISH gene-knockout CD70-CAR NK cells or control NK cells measured against ACHN cells at a 1:2 and 1:4 E:T ratio. At day 3, a second bolus of ACHN was added.
(B) CISH gene-knockout CD70-CAR NK cells maintained enhanced cytotoxicity after 75 days culture.

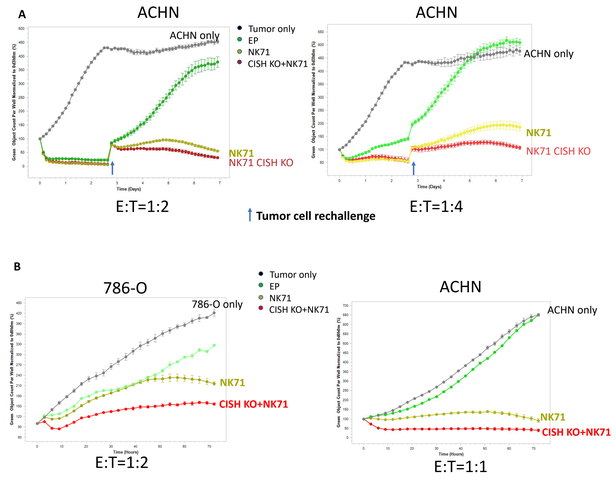


Figure 3. CISH gene-knockout CD70-CAR NK cells partially overcome inhibition of TGFβ on NK71 cells. Cytotoxicity of CISH gene-knockout CD70-CAR NK cells against:
(A)786-O cells or
(B) ACHN cells at a 1:2 E:T ratio in the absence or presence of TGFβ (20ng/ml) via Incucyte.

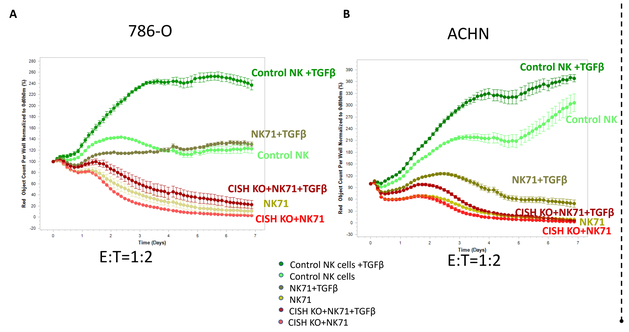
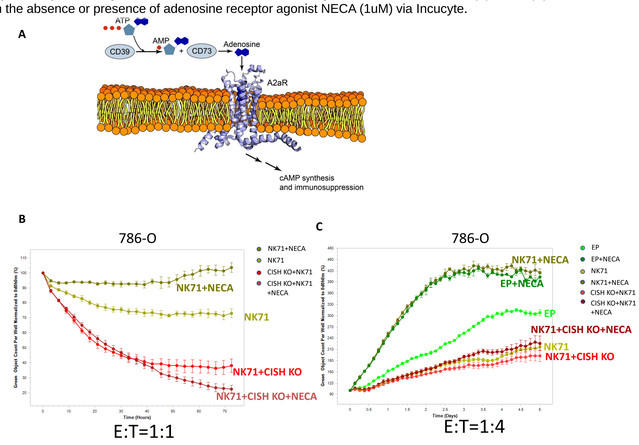


Figure 4. CISH gene-knockout CD70-CAR NK cells may overcome Adenosine Receptor agonist NECA inhibition of NK cell cytotoxicity. (A) Schematic illustration of Adenosine-A2AR pathway triggering cAMP synthesis and immunosuppression (adapted from BPS Bioscience).
Cytotoxicity of CISH gene-knockout CD70-CAR NK cells against 786-O cells at a 1:1 (B) or 1:4 (C) E:T ratio in the absence or presence of adenosine receptor agonist NECA (1uM) via Incucyte.



Conclusion

In summary, we show CISH gene-knockout CD70-CAR NK cells demonstrate potent anti-tumor activity against relevant solid tumor cell lines. CISH knockout adds to the potency and durability to CAR NK cells expressing mbIL-15, and provides resistance to inhibition by key immunosuppressive elements of the tumor microenvironment. These data support the further exploration of CISH gene-knockout CD70 CAR NK cells for clinical application.

References

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