CRISPR-Cas12a Gene Editing Enhances Functional Metabolism of Natural Killer Cells and Enables Tumor Cell Cytolysis in Metabolically Stressful Conditions That Inhibit Effector Cell Function

POSTER 1532

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OBJECTIVE

To evaluate the function of healthy donor-derived CRISPR-Cas12a-engineered natural killer (NK) cells in the nutrient-deprived and metabolically unfavorable tumor microenvironment as a proxy for CRISPR-Cas12a-engineered NK cells derived from induced pluripotent stem cells (iPSCs).

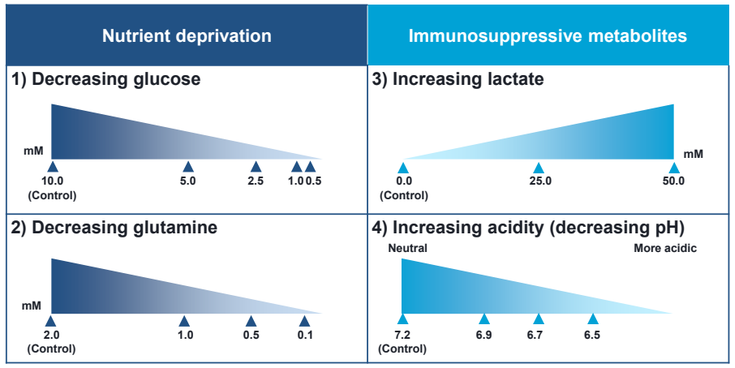
INTRODUCTION

- The tumor microenvironment is nutrient-deprived, due to competition between effector and tumor cells for essential nutrients, and enriched in immunosuppressive metabolites (eg, lactic acid) due to Warburg Metabolism.
- Effective anti-tumor cell therapies must be able to function in the tumor microenvironment.
- NK cells derived from healthy donors or iPSCs (iNK cells) with CRISPR-Cas12a-mediated knockout of cytokine-inducible SH2-containing protein (*CISH*) and transforming growth factor beta receptor II (*TGFBR2*) genes have demonstrated resistance to TGF-β inhibition and increased tumor control.[1–3]

METHODS

- CRISPR-Cas12a-edited *CISH*$^{-/-}$/*TGFBR2*$^{-/-}$ NK cells derived from healthy donors were used as a model for CRISPR-Cas12a-engineered iNK cells.
- NK cells were challenged with SK-OV-3 ovarian tumor spheroids + 10 ng/mL TGF-β in the unfavorable metabolic conditions shown in the figure below, in isolation or combinatorially in a multifactorial matrix, and assayed for tumor cytotoxicity and inflammatory cytokine production.

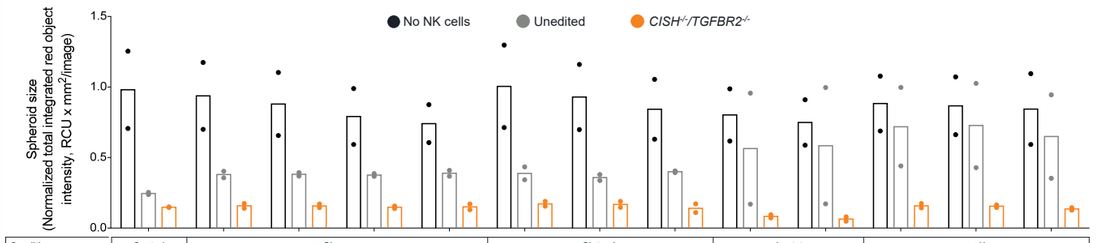
Metabolically unfavorable tumor microenvironment conditions



- The cytotoxicity of NK cells was also assayed with SK-OV-3 ovarian tumor spheroids that were selectively evolved to grow in nutrient-deprived and/or high lactate media.
- The mitochondrial function and fitness of NK cells were assayed using the Seahorse Cell Mito Stress Kit.

RESULTS

Figure 1. *CISH*$^{-/-}$/*TGFBR2*$^{-/-}$ NK cells had enhanced cytotoxicity vs unedited controls in unfavorable metabolic conditions in isolation

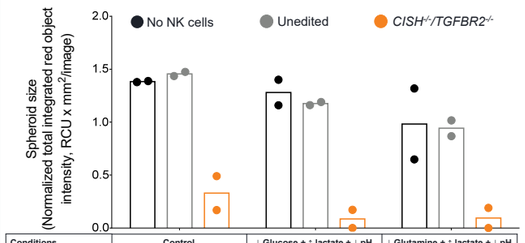


Conditions	Control	↓ Glucose				↓ Glutamine			↑ Lactate		↓ pH		
Glucose (mM)	10.0	5.0	2.5	1.0	0.5	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0
Glutamine (mM)	2.0	2.0	2.0	2.0	2.0	1.0	0.5	0.1	2.0	2.0	2.0	2.0	2.0
Lactate (mM)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	25.0	50.0	0.0	0.0	0.0
pH	7.2	7.2	7.2	7.2	7.2	7.2	7.2	7.2	7.2	7.2	6.9	6.7	6.5

- *CISH*$^{-/-}$/*TGFBR2*$^{-/-}$ NK cells demonstrated greater benefits vs unedited controls in high lactate and acidic microenvironments.

SK-OV-3 tumor spheroids (without TGF-β) at 10:1 E:T; spheroid size measured at 100 hours. n=2 independent experiments from n=2 donors. Gray shading indicates unfavorable metabolic conditions.
CISH, cytokine-inducible SH2-containing protein; E:T, effect:target; NK, natural killer; RCU, red calibrated unit; TGFBR2, transforming growth factor beta receptor II.

Figure 2. *CISH*$^{-/-}$/*TGFBR2*$^{-/-}$ NK cells had enhanced cytotoxicity vs unedited controls in multifactorially unfavorable metabolic conditions

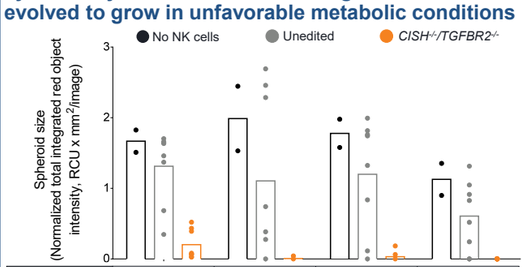


Conditions	Control	↓ Glucose + ↑ lactate + ↓ pH	↓ Glutamine + ↑ lactate + ↓ pH
Glucose (mM)	10.0	0.5	10.0
Glutamine (mM)	2.0	2.0	0.1
Lactate (mM)	0.0	50.0	50.0
pH	7.2	6.5	6.5

- *CISH*$^{-/-}$/*TGFBR2*$^{-/-}$ NK cells also produced higher concentrations of IFN-γ and TNF-α than unedited controls in these conditions.

SK-OV-3 tumor spheroids +10 ng/mL TGF-β at 5:1 E:T; spheroid size measured at 100 hours. n=2 independent experiments from n=2 donors. Gray shading indicates unfavorable metabolic conditions.
CISH, cytokine-inducible SH2-containing protein; E:T, effect:target; IFN-γ, interferon gamma; RCU, red calibrated unit; TGFBR2, transforming growth factor beta receptor II; TNF-α, tumor necrosis factor alpha.

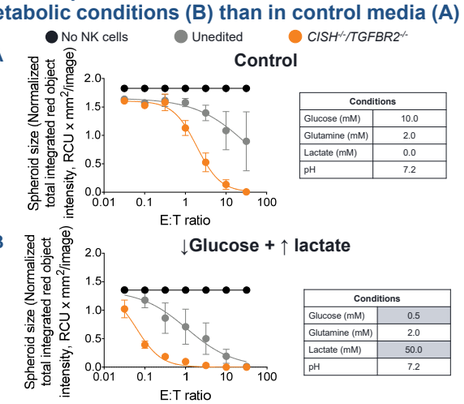
Figure 3. *CISH*$^{-/-}$/*TGFBR2*$^{-/-}$ NK cells had enhanced cytotoxicity vs unedited controls against tumor cells evolved to grow in unfavorable metabolic conditions



EC_{50}: Unedited	35.2	0.6	4.5	1.0
EC_{50}: *CISH*$^{-/-}$/*TGFBR2*$^{-/-}$	1.9	0.0	0.3	0.1
Conditions	**Control**	**↓ Glutamine**	**↑ Lactate**	**↓ Glucose + ↑ lactate**
Glucose (mM)	10.0	10.0	10.0	0.5
Glutamine (mM)	2.0	0.1	2.0	2.0
Lactate (mM)	0.0	0.0	50.0	50.0
pH	7.2	7.2	7.2	7.2

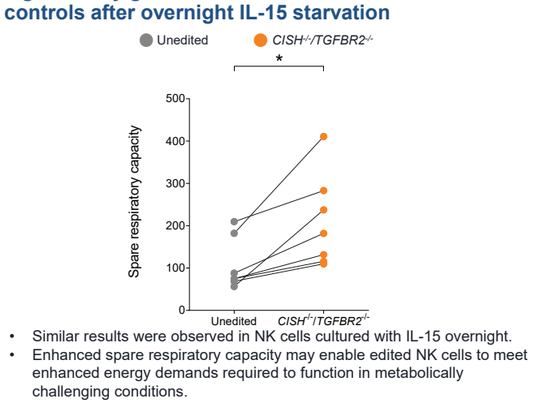
SK-OV-3 tumor spheroids selectively evolved to grow in unfavorable metabolic conditions (gray shading) +10 ng/mL TGF-β at 10:1 E:T; spheroid size and EC_{50} measured at 100 hours. n=2 (no NK cells) or n=8 (unedited or *CISH*$^{-/-}$/*TGFBR2*$^{-/-}$ NK cells) independent experiments from n=2 donors.
CISH, cytokine-inducible SH2-containing protein; E:T, effect:target; NK, natural killer; RCU, red calibrated unit; TGFBR2, transforming growth factor beta receptor II.

Figure 4. *CISH*$^{-/-}$/*TGFBR2*$^{-/-}$ NK cells had a greater cytotoxic potential vs unedited NK cells in unfavorable metabolic conditions (B) than in control media (A)



SK-OV-3 tumor spheroids selectively evolved to grow in unfavorable metabolic conditions (gray shading) +10 ng/mL TGF-β at 100 hours n=2 (no NK cells) or n=8 (unedited or *CISH*$^{-/-}$/*TGFBR2*$^{-/-}$ NK cells) independent experiments from n=2 donors.
CISH, cytokine-inducible SH2-containing protein; E:T, effect:target; NK, natural killer; RCU, red calibrated unit; TGFBR2, transforming growth factor beta receptor II.

Figure 5. *CISH*$^{-/-}$/*TGFBR2*$^{-/-}$ NK cells exhibited significantly greater metabolic fitness than unedited controls after overnight IL-15 starvation



- Similar results were observed in NK cells cultured with IL-15 overnight.
- Enhanced spare respiratory capacity may enable edited NK cells to meet enhanced energy demands required to function in metabolically challenging conditions.

*p<0.05. n=7 independent experiments from n=3 donors. Spare respiratory capacity is a function of mitochondrial mass and fitness. A cell with a larger spare respiratory capacity can produce more ATP and overcome more stress, including oxidative stress.
ATP, adenosine triphosphate; CISH, cytokine-inducible SH2-containing protein; IL-15, interleukin-15; NK, natural killer; TGFBR2, transforming growth factor beta receptor II.

CONCLUSIONS

- CRISPR-Cas12a-edited *CISH*$^{-/-}$/*TGFBR2*$^{-/-}$ NK cells demonstrated superior cytotoxicity and enhanced metabolic function in metabolically unfavorable conditions compared with unedited controls.
- This nutrient-deprived and immunosuppressive metabolic tumor model will be used to evaluate the metabolic and cytotoxic functions of CRISPR-Cas12a-engineered iNK cells with edits targeting metabolic pathways.

REFERENCES

1. Wong KK, et al. SITC Annual Meeting 2020:Abstract 145
2. Borges CM, et al. ASH Annual Meeting 2020:Abstract 1436
3. Moon J-I, et al. ASH Annual Meeting 2020:Abstract 3257

DISCLOSURES

Employees and shareholders of Editas Medicine: S.S., K.W., J.N., A.P., C.M.B.
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