Identification of a novel human immunodeficiency enables mechanistic dissection of a previously unknown regulator of NK and CD8+ T-cell cytotoxicity

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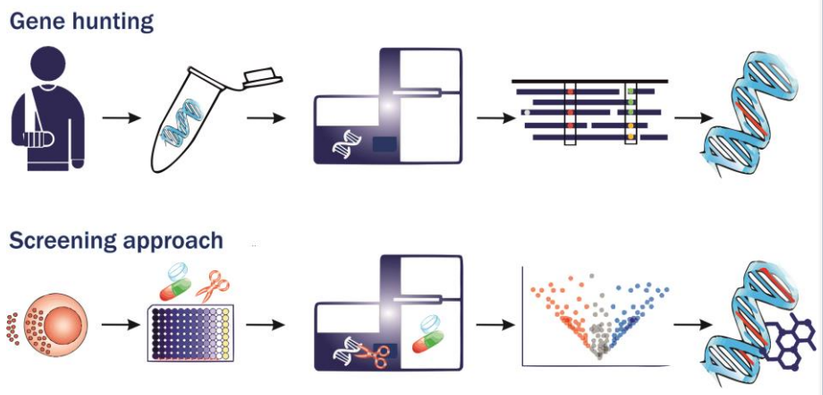
INTRODUCTION

The investigation of genetic inborn errors have greatly enhanced our understanding of disease pathology & immunity. In addition to a targeted single-gene centered disease study we aim to gain systematic understanding of immune function by deciphering the key molecules using high-throughput CRISPR/ drug screens.

AIMS

i. Identification of novel disease-causing gene entities[1]

ii. Functional screening for primary immune-dysfunction[2,3]



High-throughput chemical probing of granule exocytosis

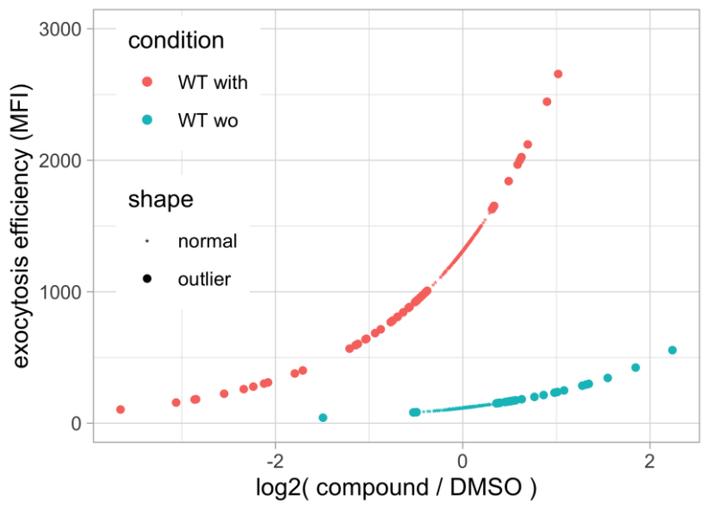


Figure 4. High-throughput granule exocytosis compound screen using NK92 WT with a library of >300 different drugs[3]. NK92 WT with target cells (red), NK92 without target cells (turquoise). Hits are indicated as outliers.

Functional NK-cell deficiencies

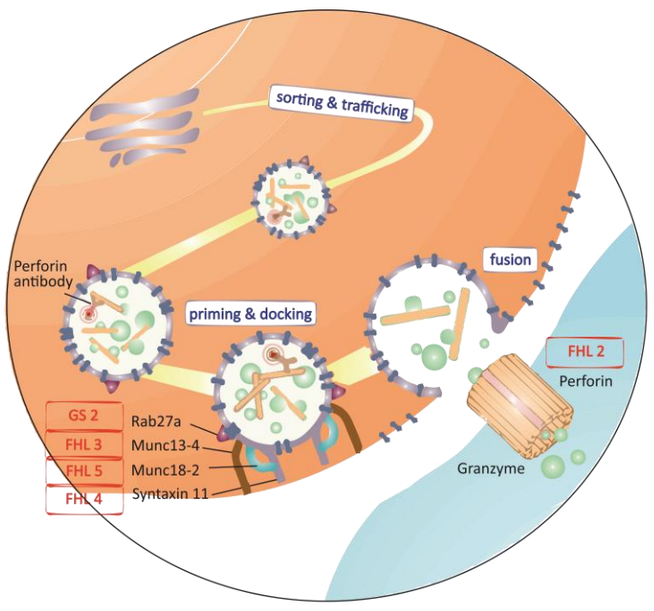


Figure 1. Functional NK-cell immunodeficiencies impairing lytic granule convergence towards target cells and thus hindering the effector cell's cytotoxic activity leading to primary/ familial hemophagocytic lymphohistocytosis (FHL) or HLH-related syndromes[4].

Novel human immunodeficiency with FHL phenotype

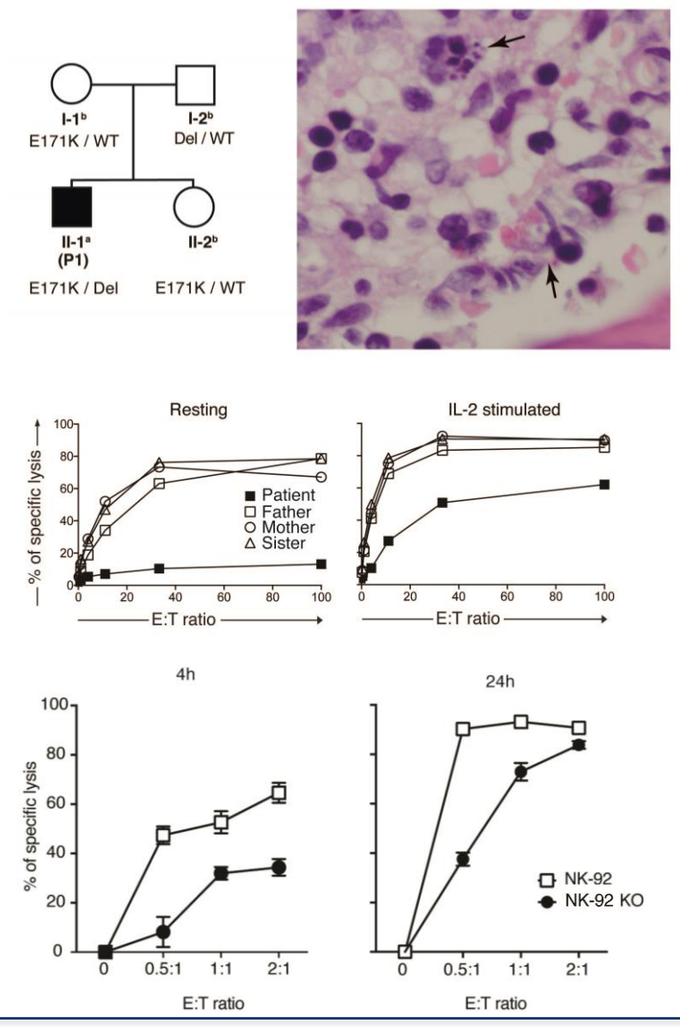


Figure 2. Index patient with novel mutation presenting with diagnostic HLH criteria[5].

Figure 3. Primary NK cells and NK92 KO cell line show diminished cytotoxic activity towards target cells – a hallmark of HLH.

Targeted small-scale knockout screen sets basis for systematic genome-wide approach

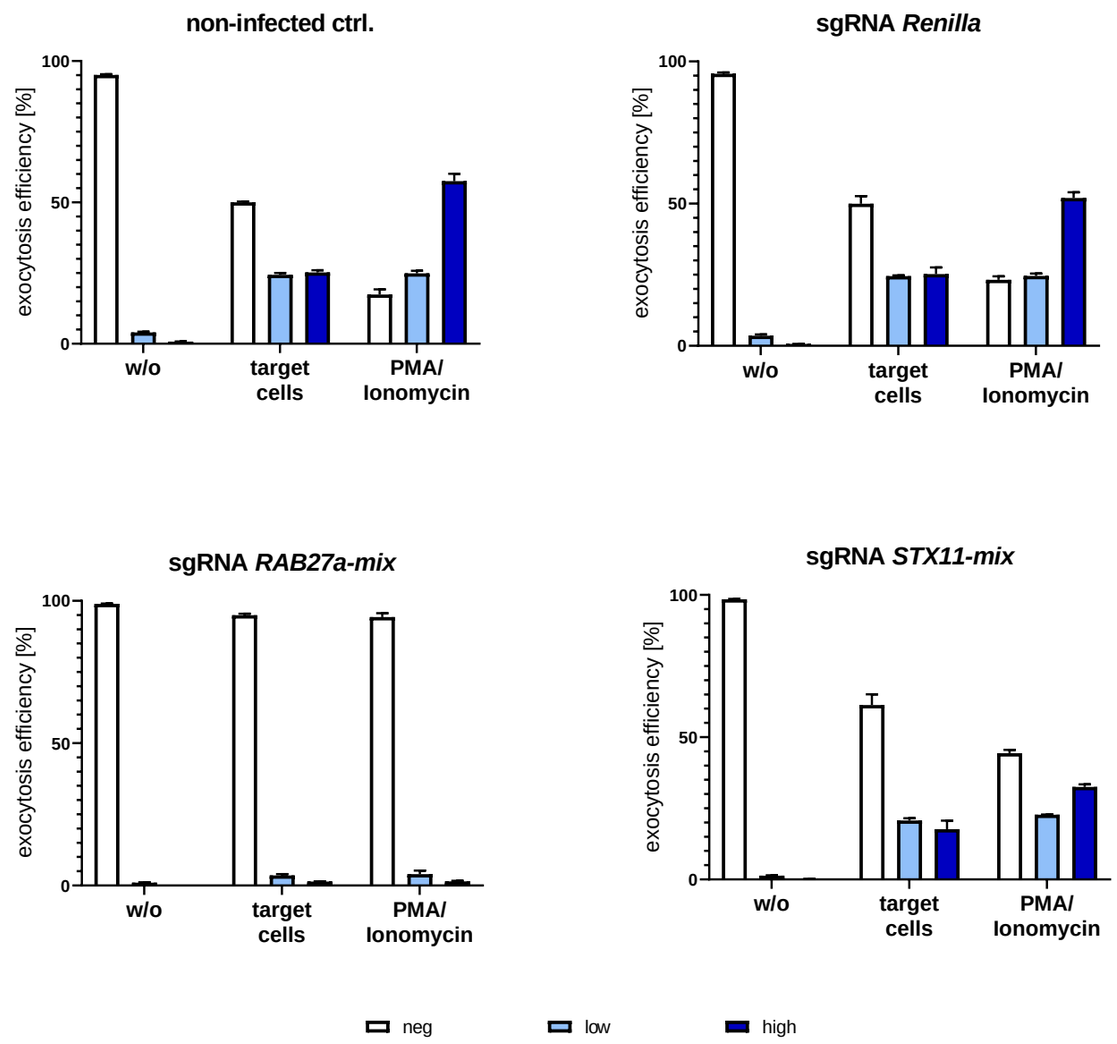


Figure 5. The effect of CRISPR-mediated gene ablation on exocytosis efficiency in NK92 cells upon stimulation with target cells or PMA/Ionomycin.

FUTURE DIRECTIONS

- Use targeted approaches to dissect the cellular pathways & key molecules responsible for rescue & pathology-causing mechanisms
- Probe & validate drug screen hits with help of pathway annotation
- Perform CRISPR screen and validate candidate genes identified

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