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NK cell recognition of malignant cells
– a CRISPR approach to define novel mediators

Akademisk avhandling

Som för avläggande av medicine doktorsexamen vid Sahlgrenska akademin, Göteborgs universitet kommer att offentligen försvaras i Arvid Carlsson, Medicinaregatan 3

Fredagen den 5 april, klockan 9.00

av **Linnea Kristenson**

Fakultetsopponent:
Karl-Johan Malmberg, Universitetet i Oslo, Norge

Avhandlingen baseras på följande delarbeten

I. **Kristenson L**, Badami C, Ljungberg A, Islamagic E, Tian Y, Xie G, Hussein BA, Pesce S, Tang KW, Thorén FB. Deletion of the *TMEM30A* gene enables leukemic cell evasion of NK cell cytotoxicity. *Proc Natl Acad Sci U S A. 2024. Accepted for publication.*

II. Badami C, **Kristenson L**, Svensson F, Kathirkamanathan T, Thorén FB. *BAP1* deletion disrupts IFNγ signaling and sensitizes cancer cells to NK cell cytotoxicity. *In manuscript.*

III. Hussein BA*, **Kristenson L***, Pesce S, Wöhr A, Tian Y, Hallner A, Brune M, Hellstrand K, Tang KW, Bernson E, Thorén FB. NKG2A gene variant predicts outcome of immunotherapy in AML and modulates the repertoire and function of NK cells. *J Immunother Cancer. 2023 Aug;11(8):e007202.* *Equal contribution

IV. **Kristenson L**, Islamagic E, Badami C, Rockstein L, Lind S, Hussein BA, Svensson F, Pesce S, Johansson L, Tang KW, Hellstrand K, Thorén FB. Identification of natural cytotoxicity receptor ligands using a CRISPR-engineered target cell line. *In manuscript.*

SAHLGRENSKA AKADEMIN
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NK cell recognition of malignant cells
– a CRISPR approach to define novel mediators

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Abstract

Natural killer (NK) cells are cytotoxic lymphocytes of the innate immune system, capable of eliminating malignant cells. Their activity is intricately regulated through a balanced interplay between activating and inhibitory receptors that interact with molecules on their prospective target cells. This thesis employed genome editing to delve deeper into genes and molecules that influence these dynamic interactions. A loss-of-function genome-wide CRISPR screen using the leukemic cell line K562 with NK cell cytotoxicity as the selective pressure, unveiled genes impacting target cell susceptibility. *TMEM30A* depletion (**paper I**) rendered target cells partially resistant to NK-cell-induced lysis. Subsequent investigations elucidated its role in phospholipid transport within the plasma membrane. Loss-of-function mutations in *TMEM30A*, observed in certain cancers, upregulated phosphatidylserine on the cell surface enabling interaction with inhibitory NK cell receptor TIM-3, providing protection from NK cells. *BAP1*, another gene identified in the CRISPR screen (**paper II**) was found to support MHC class I expression through involvement in interferon-γ signalling. Depletion of *BAP1* increased target cell sensitivity to NK cells by eliminating the inhibitory signal. The CRISPR/Cas9 technique was further employed to suppress the expression of crucial ligands for activating NK cell receptors. This manipulation allowed the investigation of alternative receptor-ligand interactions and provided a model to decipher the impact of a single nucleotide polymorphism (SNP) in the receptor NKG2D gene on NK cell function (**paper III**). Notably, the identified SNP in the linked gene for NKG2A emerged as the key driver of NK cell function and additionally influenced the clinical outcome of immunotherapy in acute myeloid leukemia. Leveraging this established model cell line, dominantly killed via NKp46, a subsequent genome-wide CRISPR/Cas9 screen was conducted to identify potential ligand candidates for the NKp46 receptor (**paper IV**). In conclusion, CRISPR/Cas9 technology proved to be instrumental in uncovering molecular mechanisms that regulate the interaction between NK cells and their target cells, which may pave the way for therapeutic interventions in cancer.

Keywords: Natural killer cells, CRISPR/Cas9 screen, *TMEM30A*, TIM-3, phosphatidylserine, *BAP1*, AML, immunotherapy, HDC/IL-2, NKG2A, NKp46 ligand

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