Mechanisms underlying human placental CD34+-derived natural killer cell cytotoxicity against glioblastoma

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INTRODUCTION

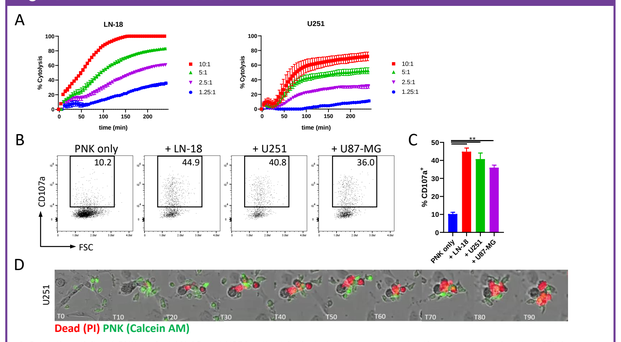
Background

Natural killer (NK) cells are innate immune cells with a critical role in immune surveillance against cell transformation and tumor development. NK cells express an array of unique activating and inhibitory receptors whose aggregate signaling determine the activation of NK cell effector function. Adoptive transfer of NK cells has demonstrated the potential to induce antitumor responses in the clinic.

Celularity has developed a platform for generating cytotoxic NK cells from placental CD34$^+$ cells (PNK cells) for adoptive cancer immunotherapy. Although PNK cells demonstrate cytotoxicity against diverse cancer cell types, their activating mechanisms are little characterized.

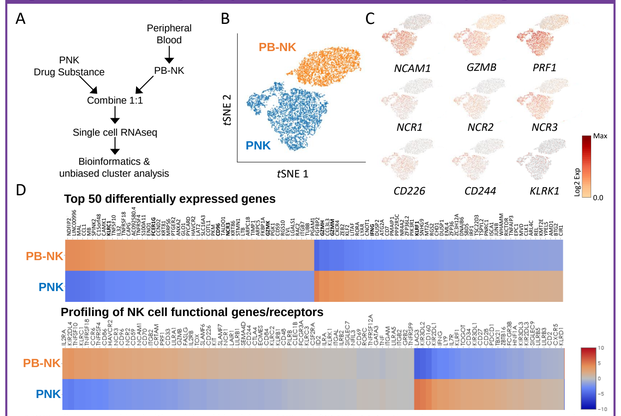
In this study, we explore the contribution of specific signaling pathways and upstream NK cell receptors involved in PNK cell cytotoxicity against glioblastoma multiforme (GBM) cell targets.

Figure 1. PNK kill GBM cells



A Cytolytic activity of PNK against LN-18 and U251 cells at indicated effector to target ratios measured using the xCELLigence platform. **B** and **C** Representative dot plots (**B**) and quantification (**C**) of CD107a expression on PNK cells after exposure to GBM cell lines. Numbers indicate proportion of cells in the parent gate. **D** Calcein AM-stained PNK cells killing U251 cells in culture. Notice dead cells stained by propidium iodide (PI) accumulating over time (T, minutes). n=2, N=3, mean±SD

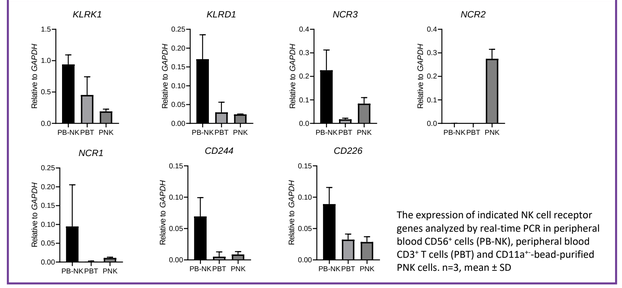
Figure 2. PNK cells highly expression natural killer cell receptor genes



A PNK cells were combined with peripheral blood derived NK cells (PB-NK) at 1:1 ratio and gene expression analyzed on single cell level using 10X Genomics Chromium platform and Illumina sequencing. **B** tSNE plot depicting PNK and PB-NK cells as distinct populations. **C** tSNE plots of selected NK cell specific genes. **D** Top differentially expressed genes and selected NK cell marker genes in PNK and PB-NK.

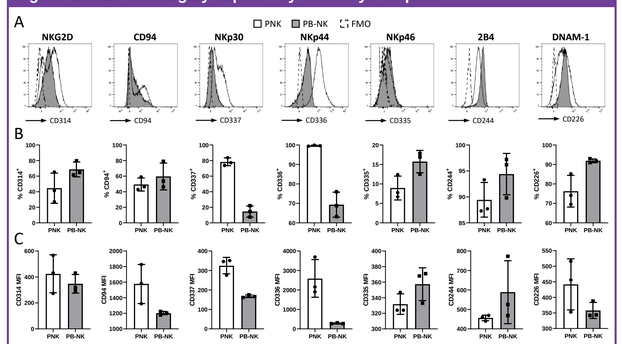
RESULTS

Figure 3. PNK cells highly expression natural killer cell receptor genes



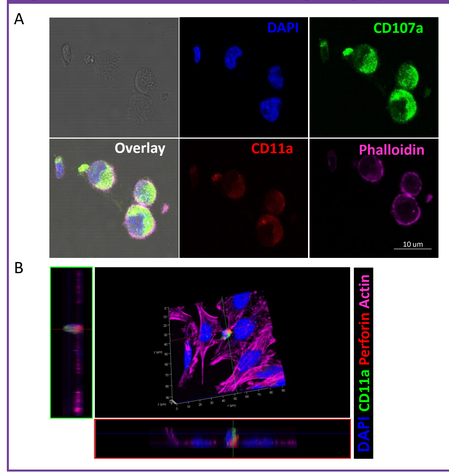
The expression of indicated NK cell receptor genes analyzed by real-time PCR in peripheral blood CD56$^+$ cells (PB-NK), peripheral blood CD3$^+$ T cells (PBT) and CD11a^{++}-bead-purified PNK cells. n=3, mean ± SD

Figure 4. PNK cells highly express cytotoxicity receptors



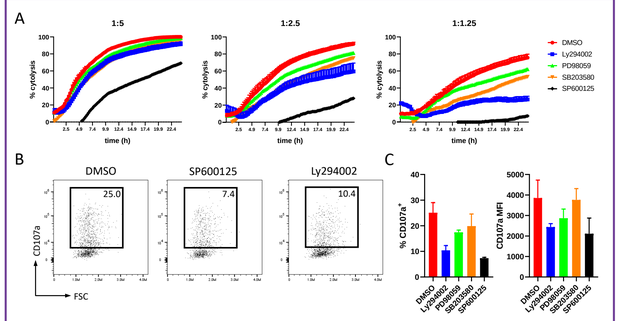
A Representative histograms of indicated NK cell receptor expression on freshly thawed cryopreserved PNK cells and peripheral blood monomuclear cells (PBMC). PNK and peripheral blood NK cells (PB-NK) were gated as live CD45$^+$ CD3$^-$ CD14$^-$ CD19$^-$ CD56$^+$ CD11a$^+$ cells. **B** Proportion of cells expressing the indicated NK cell receptor when compared to fluorescence minus one (FMO) control. **C** Mean fluorescence intensity (MFI) of indicated NK cell receptors. n=3 N=3 mean±SD

Figure 5. PNK cells form an immunological synapse with target cells



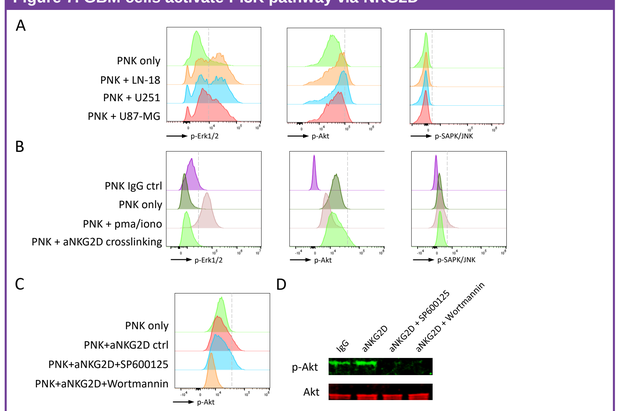
A Immunofluorescence analysis for the indicated markers was performed on cytospins from PNK cells. **B** A confocal microscopy image showing CD11a+ perforin+ PNK cell attached to large U251 tumor cells (purple). Notice the polarization of perforin towards the tumor cell.

Figure 6. JNK and PI3K signaling regulate PNK cytotoxicity against GBM



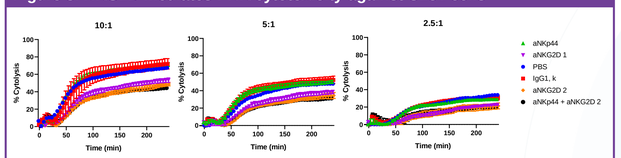
A Cytolytic activity of PNK cells against LN-18 cell line at indicated effector to target ratios measured using the xCELLigence platform. PNK cells were preincubated with indicated small molecule inhibitors. **B** and **C** Representative dot plots (**B**) and quantification of CD107a expression and mean fluorescence intensity (MFI) of CD107a (**C**) on small molecule inhibitor-treated PNK cells after incubating with GBM cell lines. Numbers indicate proportion of cells in the parent gate. n=2, N=3, mean±SD

Figure 7. GBM cells activate PI3K pathway via NKG2D



A-C Representative histograms of phospho flow cytometry analysis of phospho (p-)Erk, p-Akt and p-SAPK/JNK in PNK cells. **A** PNK cells were exposed to GBM cell lines. **B** PNK cells were incubated with anti-NKG2D antibody that was subsequently crosslinked with F(ab')2. **C** PNK cells were incubated with indicated small molecule inhibitors, followed by NKG2D crosslinking. **D** Western blot demonstrating p-Akt and Akt in PNK cells upon NKG2D crosslinking in PNK treated with small molecule inhibitors. n=3

Figure 8. NKG2D mediates PNK cytotoxicity against U251 cells



Cytolytic activity of PNK cells against U251 cell line at indicated effector to target ratios measured using the xCELLigence platform. PNK cells were preincubated with indicated blocking antibodies. n=3

CONCLUSIONS

- PNK cells efficiently kill GBM cell lines in vitro
- Transcriptional and phenotypic characterization demonstrated that PNK cells express the cytotoxic machinery at a high level.
- Contact with GBM cells activates Erk and PI3K signaling pathways in PNK.
- PI3K and JNK pathways in PNK cells mediate cytotoxicity against GBM cell line targets.
- PI3K pathway is activated downstream of NKG2D receptor.
- NKG2D is an important receptor triggering cytotoxicity against GBM cells.

- These data combined with our receptor profiling on PNK cells establish the rationale for further investigating receptor-ligand interactions that directly modulate PI3K and JNK activity.