Understanding Virally Induced NK Cell Memory: HLAs as a Potential Mechanism

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Introduction

Glioblastoma brain tumors and pancreatic tumors have a poor prognosis, with life expectancies of less than a year. Despite constant innovation, current treatment options can only delay the effects of these aggressive cancers.

Natural Killer (NK) cells are effective at killing these solid tumors types in the innate immune system. But alone, they are deficient at breaking into the tumor microenvironment. So, oncolytic viruses (OVs) such as Delta-24-RGD are genetically engineered to penetrate this microenvironment and kill cancer cells. Combination immunotherapy of NK cells and OVs presents a promising treatment option against many types of cancers, including Glioblastomas and pancreatic tumors.

Preliminary Experiment:

Preliminary data from combination immunotherapy of NK cells and oncolytic viruses suggests that infecting pancreatic cancer cells with an OV gives NK cells a **memory-like effect**. This memory boosts the killing capabilities of NK cells.

Methods:

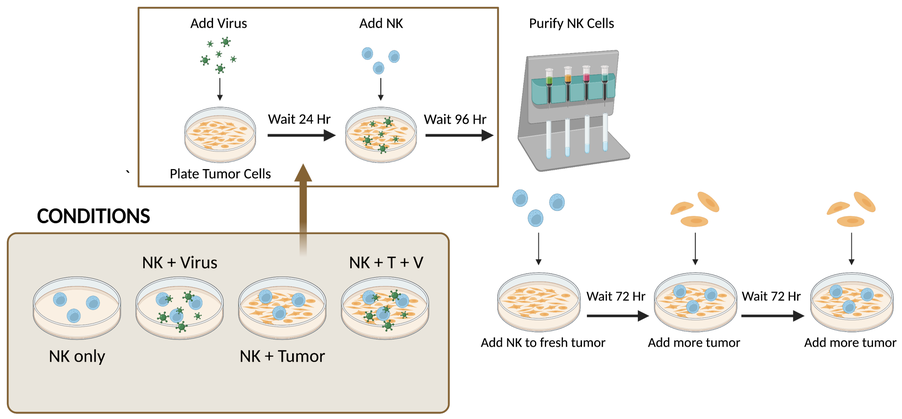


Figure 1. **Re-challenge Experiment Method.** 5 conditions that NK cells incubated and "trained" in: tumor only, NK only (control), NK + virus, NK + tumor, and NK + tumor + virus. NK cells were purified out, and the killing capability of the NK cells were tested on new tumors. The NK cells were rechallenged multiple times.

Results:

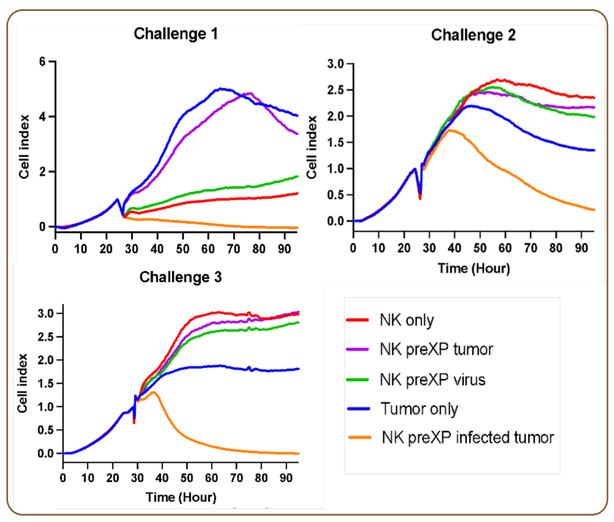


Figure 2. **Re-challenge Experiment Results.** The condition "NK preXP infected tumor" (NK cells pre-exposed to Tumor cells infected with oncolytic virus) had the greatest decrease in cell index and thus greatest killing ability. "NK preXP virus" (NK cells 'trained' with oncolytic virus without tumor cells) was not able to kill with nearly the same capacity as "NK preXP infected tumor", indicating the need for infected tumors.

Experimental Question

Objective: Understand the mechanism behind the memory-like effect of NK cells induced by oncolytic viruses.

Hypothesis: Upregulation of HLA-F and downregulation of HLAs - A/B/C are responsible for the memory in NK cells.

Rationale: HLAs-A/B/C (Human Leukocyte Antigens, immune-regulating cell surface proteins) have an inhibitory interaction with KIRs (Killer Immunoglobin Receptors) on NK cells. HLA-F has been shown to bind to the activating receptor KIR3DS1. These 2 interactions may be responsible for the induced NK memory effect.

Methods:

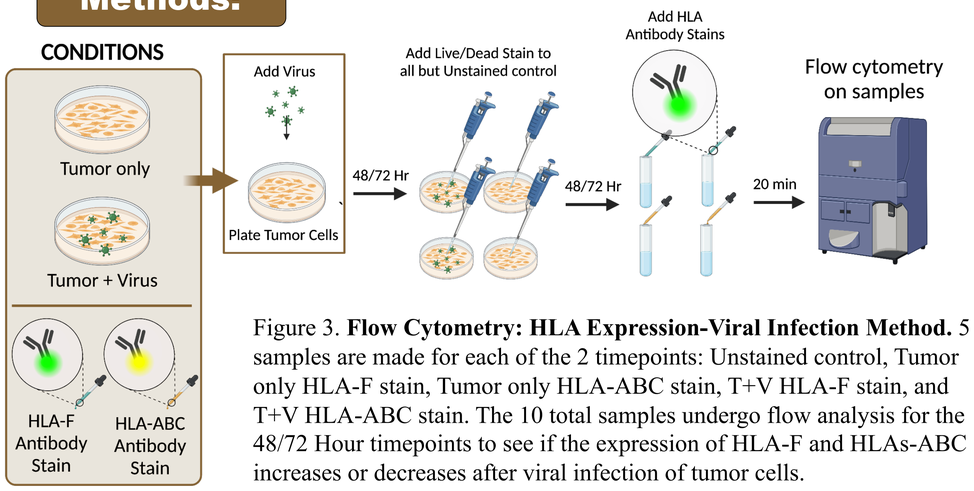
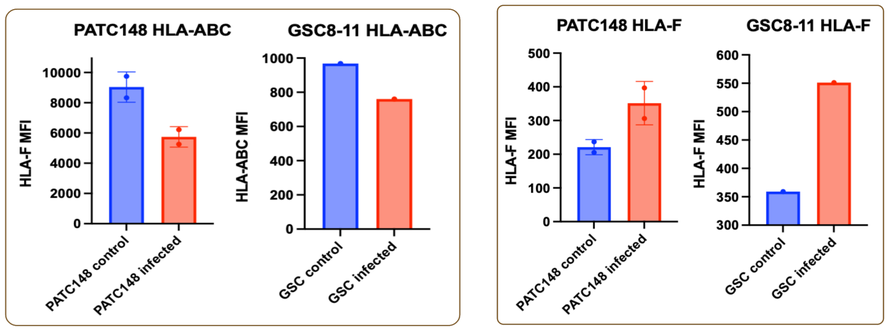


Figure 3. **Flow Cytometry: HLA Expression-Viral Infection Method.** 5 samples are made for each of the 2 timepoints: Unstained control, Tumor only HLA-F stain, Tumor only HLA-ABC stain, T+V HLA-F stain, and T+V HLA-ABC stain. The 10 total samples undergo flow analysis for the 48/72 Hour timepoints to see if the expression of HLA-F and HLAs-ABC increases or decreases after viral infection of tumor cells.

Results:



Figures 4-5. **Flow Cytometry: HLA Expression-Viral Infection Results.** Flow cytometry data shows that from the control to the infected PATC148 (pancreatic tumor cells) and GSC8-11 (glioblastoma stem cell), the infected tumor had a significantly lower MFI (mean fluorescence index) for HLA-ABC. The infected tumors also had a significantly higher MFI for HLA-F.

Methods: (for future experiment)

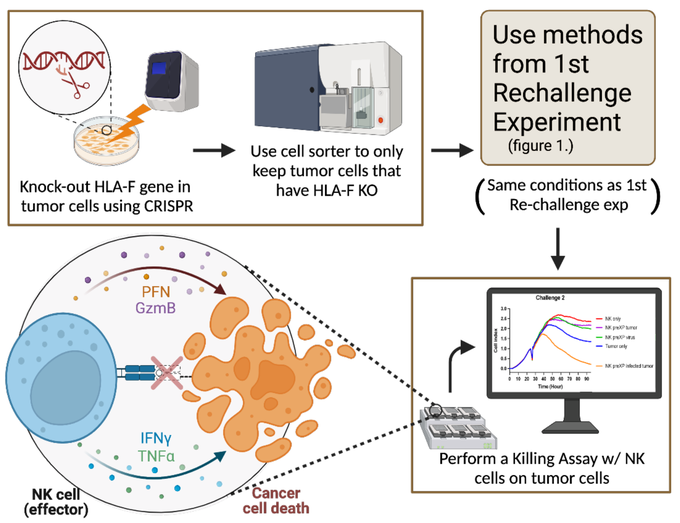


Figure 6. **NK Rechallenge: HLA KO.** Using CRISPR/Cas9, HLA-F gene is knocked out in tumor cells. After, a killing assay is conducted to see if HLA-F is necessary in NK cell memory.
The bottom left image shows an According to the missing-self hypothesis, tumor cells not expressing MHC class 1 molecules (HLAs-ABC) are unable to inhibit NK cell activity, causing NK cells to release perforins and granzymes, killing the tumor cells.

Conclusion

Combination therapy with Natural Killer cells and Delta-24-RGD oncolytic virus grant NK cells a memory-like effect. This memory increases NK cells' ability to kill cancer cells.

This memory may be caused by the interaction between HLAs on tumor cells and KIRs on NK cells.

The **"HLA Expression"** experiment results indicate that HLAs-ABC are downregulated, and HLA-F is upregulated during viral infection of tumor cells. This upregulation and downregulation may cause the NK cell memory.

The **"NK Rechallenge: HLA KO"** experiment is currently being conducted. This will test NK cells on tumor cells that have the HLA-F gene knocked out. Results showing the NK + T + V (HLA-F KO tumor cells) yielding no significant memory increase from normal NK cells would indicate HLA-F as being crucial in NK induced cell memory.

Future Directions:

Performing NK re-challenge experiments with different KO genes:

- HLAs (A-G)
- NK receptors
- Stress ligands
- TLRs

Performing NK re-challenge experiments **in-vivo** with KO gene(s) crucial to NK memory.

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