

Cell-cell interactions between NK and breast cancer cells



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Abstract

Natural killer (NK) cells are a type of lymphocyte being investigated for use in immunotherapy against a number of hematologic malignancies and solid tumors, including neuroblastoma, ovarian, colon, renal, and gastric carcinomas. Their cytotoxicity and rapid killing could make NK cells useful for adoptive therapy, but the susceptibility of target cancer cells to NK response depends on the balance between activating/inhibitory regulatory ligands expressed on the surface of the target cell. In this project, we are studying the interactions between breast cancer and NK cells and evaluating changes in gene expression when the two are co-incubated at the single cell level, using both non-activated and activated NK cells from cancer patients and healthy donors. Tumor cells from patients with breast cancer are dissociated from tissue biopsies and circulating tumor cells (CTCs) are enriched from blood samples of breast cancer patients using a label-free microfluidic device. Negative isolation of NK cells directly from whole blood is being done with demonstrated purity of the recovered and untouched NK cells. Tumor cells and CTCs are incubated with activated NK cells on the Polaris™ platform (Fluidigm®). RNA-Seq analyses are used to compare samples to try to elucidate a more comprehensive understanding of mechanisms involved in the role of NK cells in antitumoral activity.

Background

NK cells represent one of the most efficient cellular mechanisms to recognize and kill tumor cells. With ligands to multiple receptors, NK cells can provide early protection against cancer cells by producing cytokines and chemokines. They also interact with other immune cells and exert direct cytolytic activity. This cytotoxicity and rapid killing could make NK cells useful for adoptive therapy, but the susceptibility of target cancer cells to NK response depends on the balance between activating/inhibitory regulatory ligands expressed on the surface of the target cell. Our main objective is evaluate the interactions between breast cancer and NK cells, focusing on changes in gene expression when the two are co-incubated at the single cell level, using both non-activated and activated NK cells from cancer patients and healthy donors.

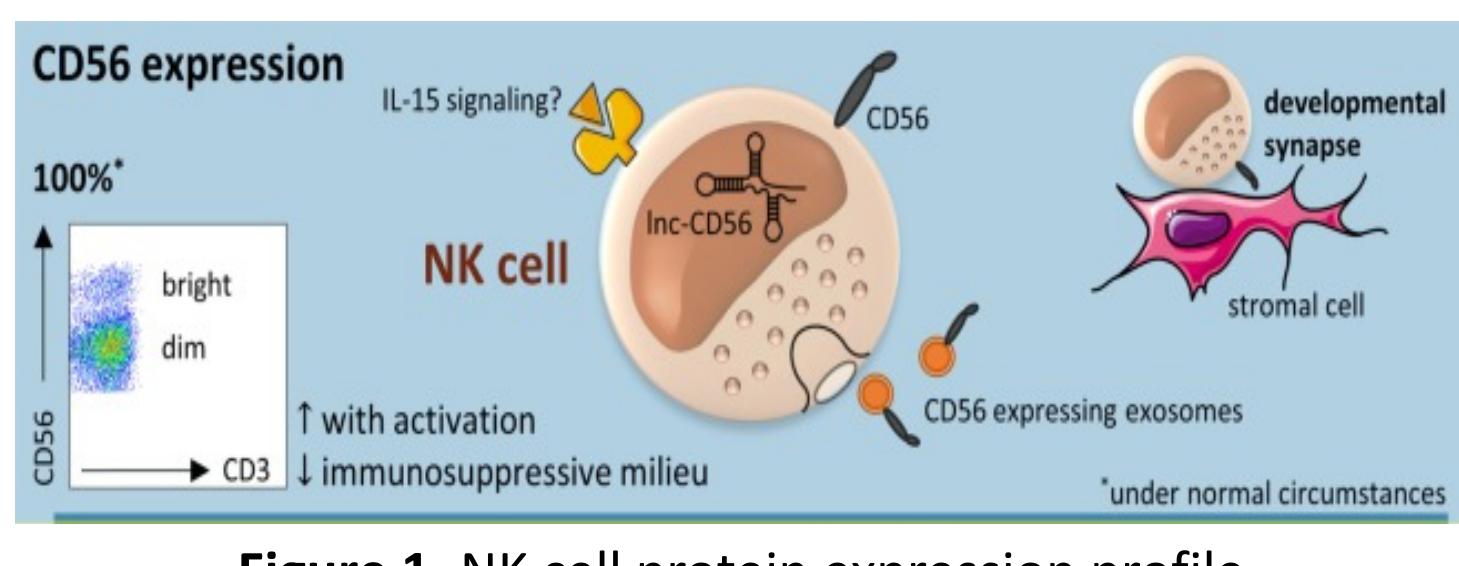


Figure 1. NK cell protein expression profile.

Materials and Methods

NK cell activation time was first established using MDA-MB-231 and NK92 cell lines. Activation was measured at 24 and 48 hours, evaluating the activation markers CD25, CD69 and CD314 using flow cytometry (Sony SH800S) (Table 1).

CD marker	Fluorescent probes
CD56	Alexa Fluor 488
CD25	Brilliant Violet 421
CD69	Brilliant Violet 785
CD314	Alexa Fluor 647

Table 1. CD (cluster of differentiation) molecule markers used to evaluate NK cell activation.

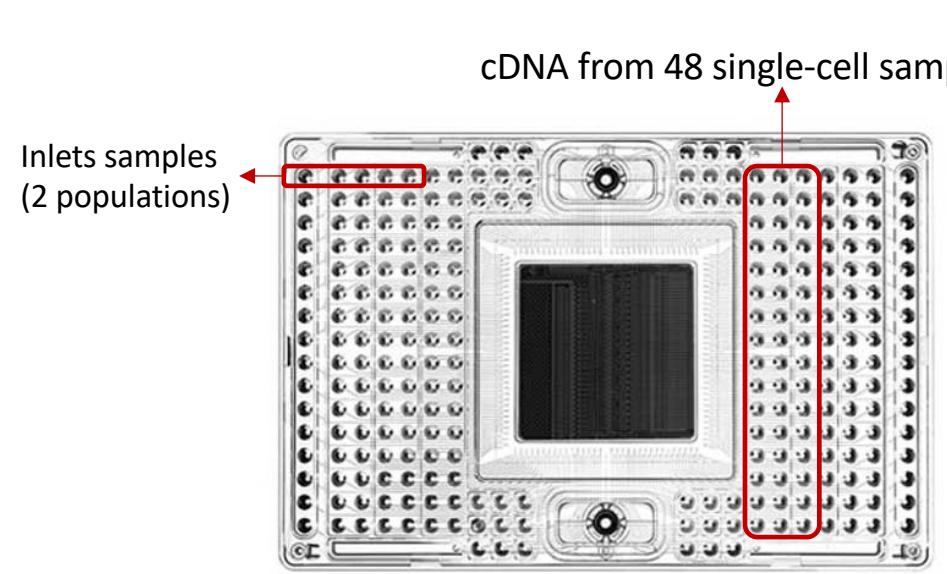


Figure 2. Microfluidic integrated fluidic circuit (IFC) for Polaris system

Materials and Methods (cont.)

Tumor cells from a patient with ER+ breast cancer were dissociated from tissue biopsies using the Tumor Dissociation Kit for human samples (Miltenyi Biotec). NK cells were isolated with negative selection directly from whole blood (EasySep™ Direct Human NK Cell Isolation Kit - STEMCELL Technologies). Purity of the recovered and untouched NK cells was demonstrated using flow cytometry for CD56 and CD3. Single tumor cells were selected, sequestered, and incubated with activated and non-activated NK cells on the Polaris™ platform (Fluidigm®) for a period of 16 hours in an integrated fluidic circuit (IFC) based on biological markers. To test different activation conditions, interleukin-2 (IL-2) was automatically added to the incubation chamber on the IFC. We incubated 48 single cells and/or NK cells under different conditions, including NK cells controls isolated from a healthy donor (Table 2).

IFC-1 using healthy control NK cells

12 tumor cells

12 NK cells

24 tumor cell plus NK cell

IFC-2 using patient NK cells

12 tumor cells

12 NK cells

24 tumor cell plus NK cell

Group	Cells	IL-2 (ng)
1	Tumor only	0
2	Tumor only	50
3	Tumor + NK	0
4	Tumor + NK	0
5	Tumor + NK	50
6	Tumor + NK	50
7	NK only	0
8	NK only	50

Table 2. Amounts of Interleukin 2 used on Polaris experiment.

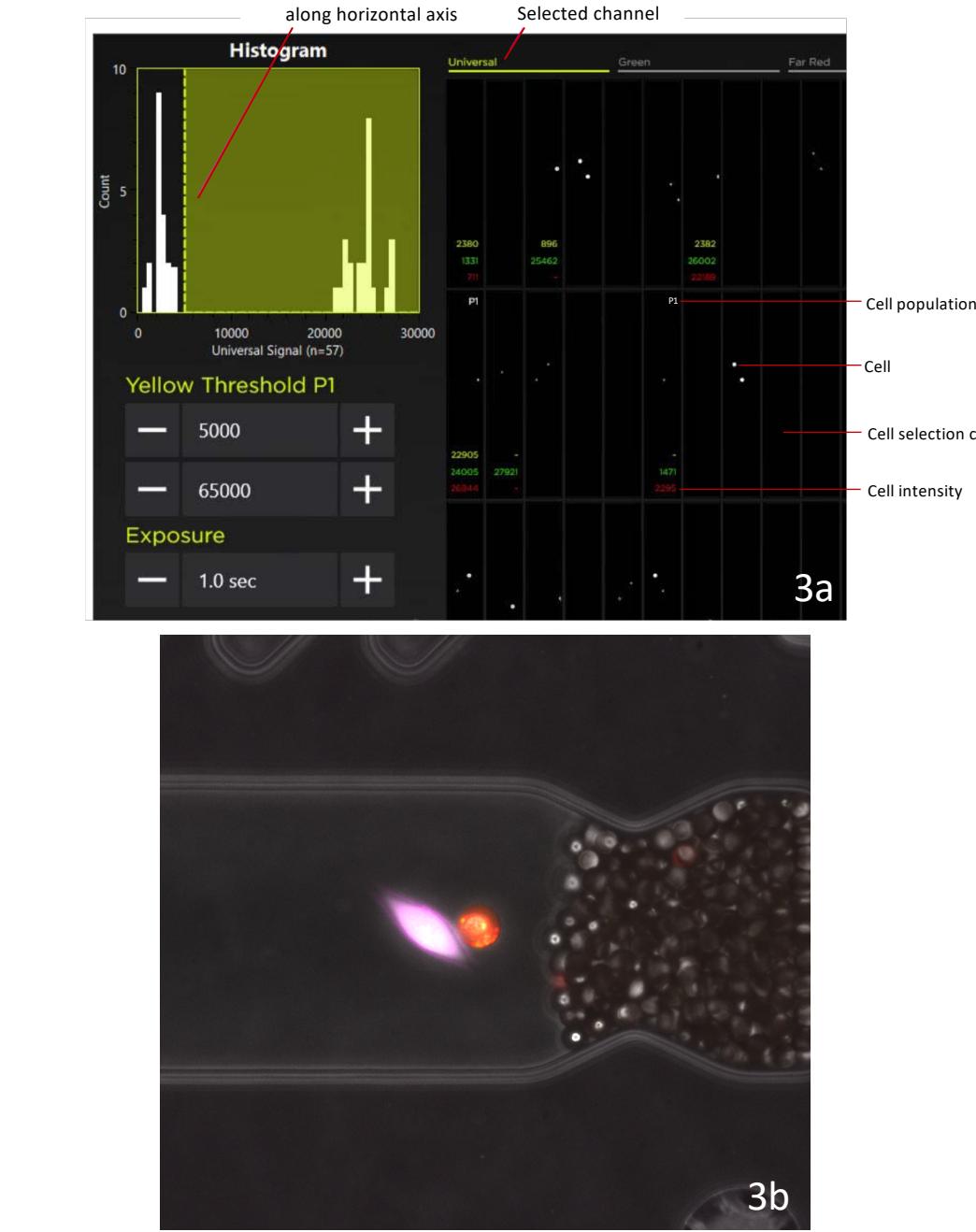


Figure 3. A. Selection of cells; B. Incubation of tumor (violet) with NK cell (red).

Samples were compared using RNA-Seq, and the data were analyzed using a deconvolution bioinformatic approach.

Results

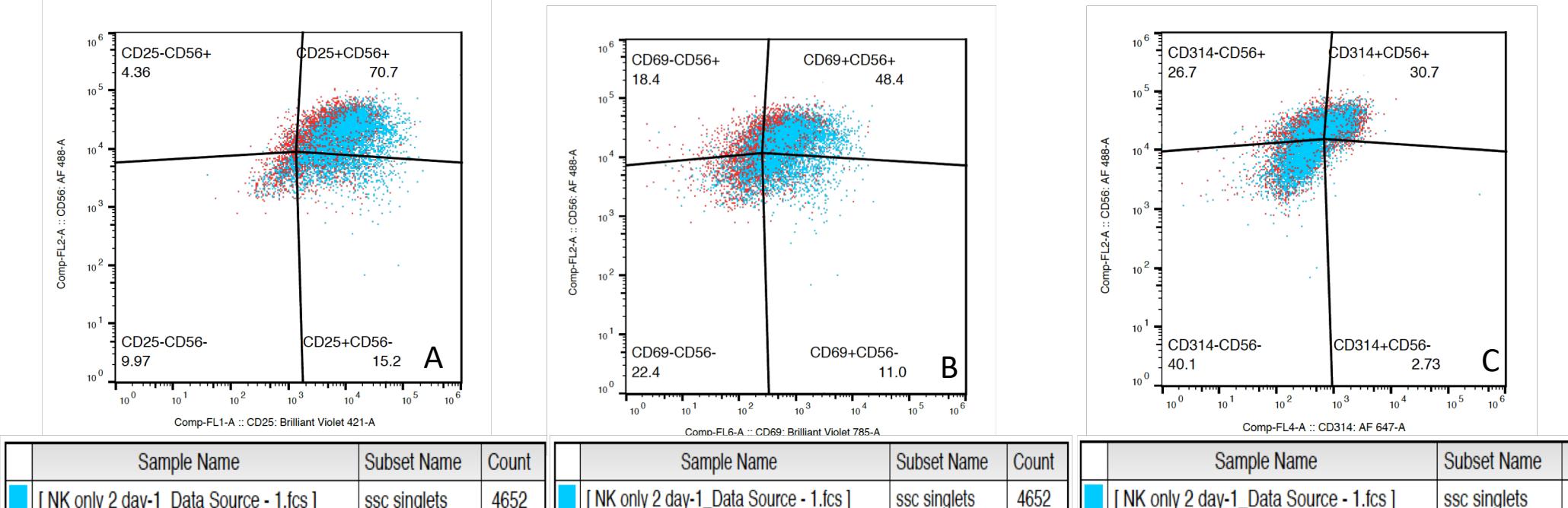


Figure 4. Analysis of NK activation markers by Flow cytometry. A. CD25; B. CD69; C. CD314. NK cells incubated for 24 hrs = red; NK cells incubated for 48 hrs = blue.

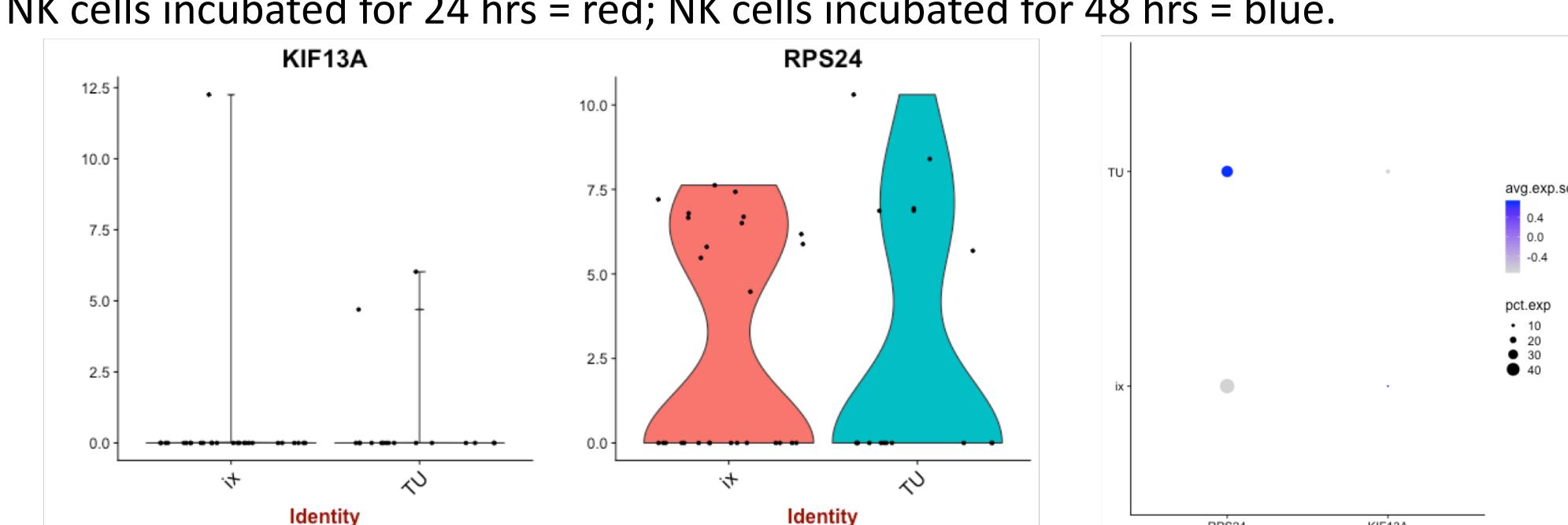


Figure 5. Differentially expressed genes when tumor cells only were compared with tumor cells incubated with NK cells (TU X TU/NK).

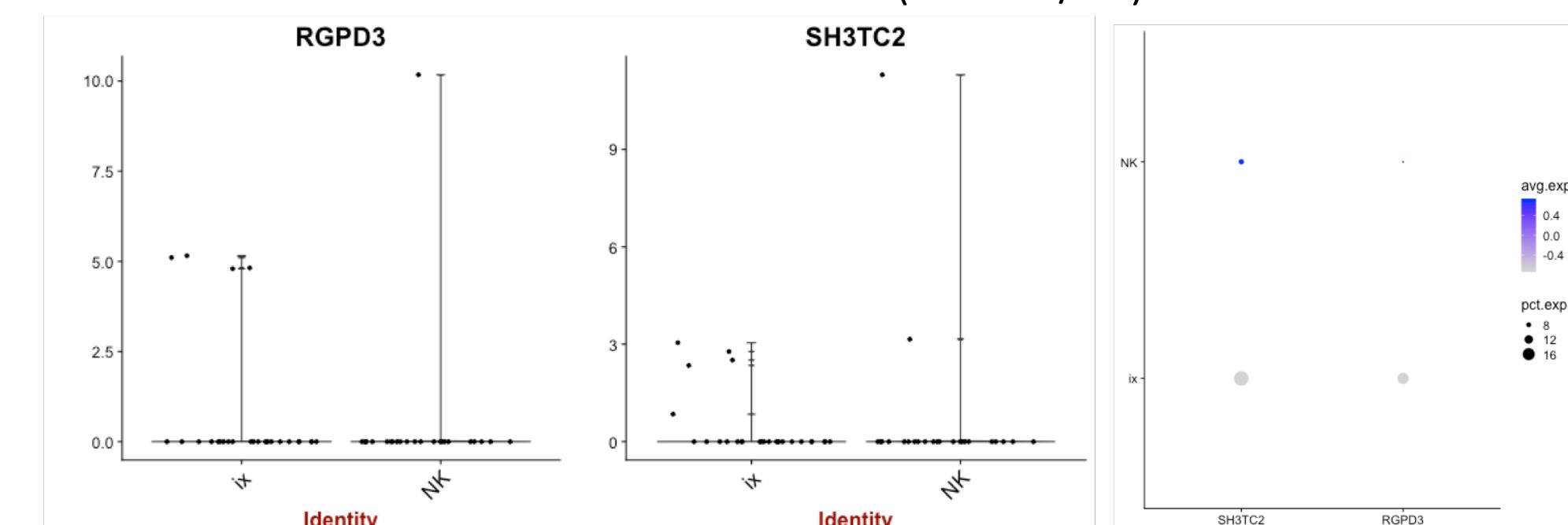


Figure 6. Differentially expressed genes when NK cells only were compared with NK cells incubated with tumor cells (NK X NK/TU).

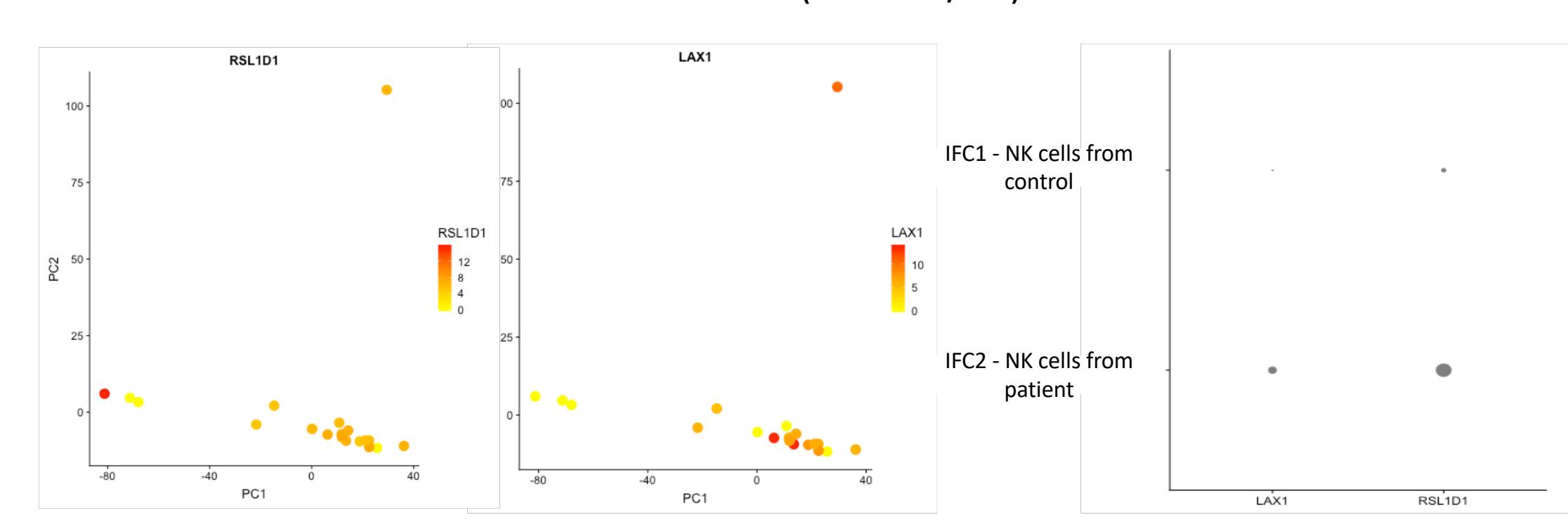


Figure 7. Differentially expressed genes when NK cells from a cancer patient were compared with NK cells from a healthy control.

Analysis and Discussion

- No significant difference was observed between 24 hr and 48 hr IL-2 incubation time for marker CD314 (Fig. 4c).
- Differentially expressed genes when tumor cells were compared with tumor cells incubated with NK cells (TU X TU/NK):

KIF13A - related to clathrin-mediated endocytosis (CME)
RPS24 - encodes a ribosomal protein (S24E family)

- Differentially expressed genes when NK cells were compared with tumor cells (NK X NK/TU):

RGPD3 - involved in nucleocytoplasmic transport, cell cycle progression, spindle assembly and nuclear organization.
SH3TC2 - an adapter or docking molecule (related to neurodegenerative diseases).

- Differentially expressed genes when NK cells from a cancer patient were compared with NK cells from a healthy control.

RSL1D1 - it is a gene related to apoptosis and nucleolar stress.

LAX1 - is a transmembrane adaptor present in all lymphocytes (and a few other types of cells like kidney, gastrointestinal tract and ovarian). Yin et al, 2019 has described this gene as related with higher disease-free survival when overexpressed in patients with ovarian cancer.

Future Directions

- Analyze data for differentially expressed genes between MDA-MB-231 with NK-92 single cells (activated NK cell line) (experiment done):

- 84 samples of tumor (MDA-MB-231)
- 84 samples of NK-92
- 168 MDA-MB-231 + NK-92

This uses single pre-activated NK cells from the NK-92 cell line and tumor cells from a triple negative breast cancer (TNBC) tumor cell line to determine how results differ from the ER+ patient results. We will be following up with tumor cells from TNBC patients.

- Repeat the above experiment to evaluate use of NK cells from patients, when compared with pre-activated NK cell line.
- Investigate NK cell interactions with circulating tumor cells (CTCs) enriched from blood samples of breast cancer patients.
- Analyze the effect of tumor and NK cell proximities - touching versus far apart - in the incubation chamber (Figure 8).

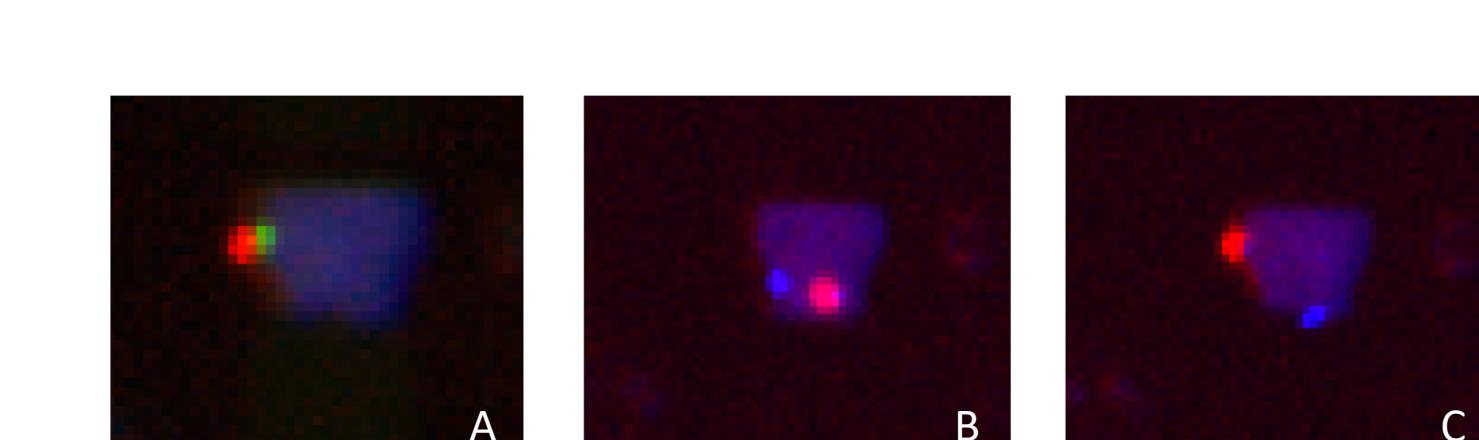


Figure 8. Different proximities of tumor cells (green, blue) and NK cells (red) within the incubation well. A. touching; B. near; C. distant.