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TITLE: microRNA-Based Immunotherapy for Control of Early-Stage Lung Cancer

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1. INTRODUCTION:

Natural killer (NK) cells play an essential role in innate immunity against lung cancer but they can be compromised in the tumor microenvironment due to immunosuppressive factors produced by tumor cells. One such factor is transforming growth factor-beta (TGFb). We recently discovered that TGFb can turn on microRNA (miR)-183 in infiltrating NK cells which then degrades DAP12 that is critical for anchoring activating NK receptors on the cell surface. This event effectively blinds NK cells to the tumor cells. This project introduces a new concept to target miR183 for restoration of NK cells to combat lung cancer. It employs a human xenograft *Nod-scid-IL2Rg^{-/-}* (NSG) mouse model using A549 human lung tumor cells with engraftment of human NK cells in vivo.

2. KEYWORDS:

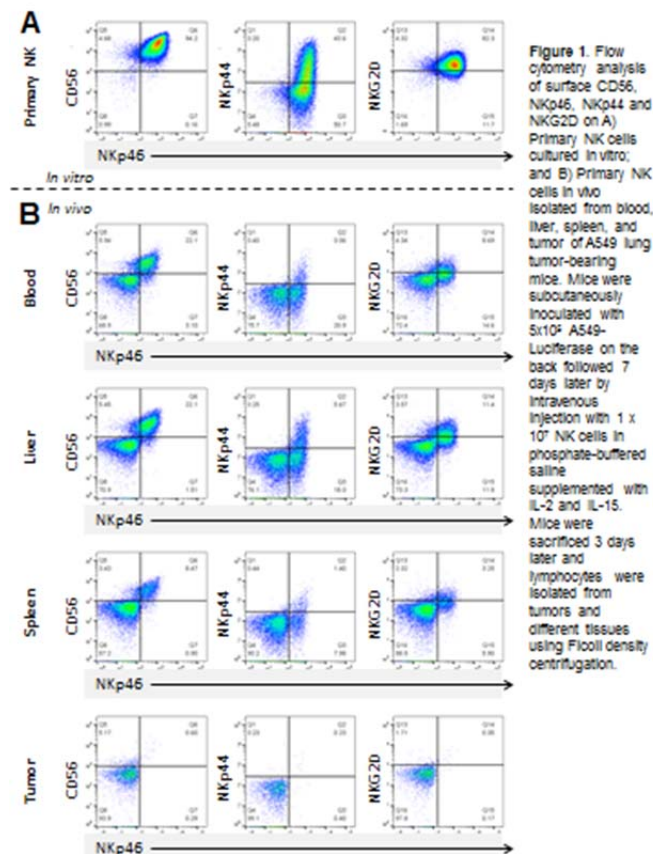
Natural Killer Cell, Transforming Growth Factor-beta, lung cancer, microRNA-183, DAP12, NK receptor, Nkp44, Nkp46, Immunotherapy, Nanoparticle

3. ACCOMPLISHMENTS:

What were the major goals of the project?

The major goals of this project is to test whether removing Natural Killer (NK) cell suppression by blocking microRNA-183 (miR-183) function helps to control early stage lung cancer in a fully humanized xenograft mouse model. These findings help to develop miR-based therapeutics that restores NK cells, which are critical for early immune surveillance against lung cancer.

What was accomplished under these goals?



Month 1-6: Established a robust human xenograft mouse model of lung carcinoma. During the first two months, we established luciferase-expressing human lung tumors in NSG mice. We generated stable luciferase-expressing A549 (A549-Luc) and optimized the tumor implantation in NSG hosts via subcutaneously injection. A549-Luc developed tumors successfully in NSG hosts and mice bearing A549-Luc tumors were the subject of all subsequent studies.

Because healthy human NK cells are critical for the project, we developed and optimized cell isolation, culture and activation of NK cells from whole blood. Next we evaluated NK cells and host interaction by transferring NK cells into A549-tumor bearing NSG host via tail vein injection. Using bioluminescence imaging, we observed that tumors in mice injected with NK cells grew significantly slower than in mice that did not receive NK cells.

At day 28, we harvested tumors, blood and tissues from tumor-bearing mice to analyze for NK presence in the different organs. We found that NK cells pre-cultured in IL2 and IL15 persisted in mouse blood, liver, and spleen as long as one week after being injected. By staining for NK activation markers, such as CD56, NKp46, NKp44, and NKG2D, we found that NK cells were retained all the phenotypes in blood and other organs but a in tumors, suggesting that the tumor microenvironment can suppress NK cell activity (Fig. 1). These results indicate that additional treatments are needed together with the NK therapy against lung cancer.

Month 6-12: Evaluate miR-183 blockade by anti-miR-183 as a new immunotherapeutic strategy.

We hypothesized that TGFb, abundantly produced by tumor cells in the tumor environment, can induce miR-183 up-regulation in NK cells to cause the down-regulation of NK function. Indeed, NK cells treated with TGFb displayed fewer activation markers, quantitated by FLOW staining and western blot, as well as impaired killing function determined by cytotoxic assays. In addition, miR-183 was also at least five times higher in NK cells treated with TGFb compared to untreated controls.

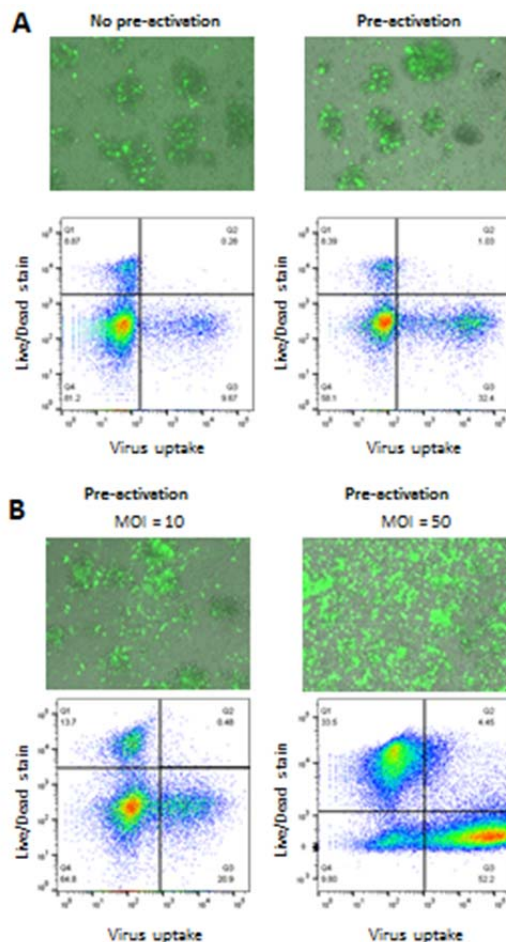


Figure 2. Infectivity of anti-miR183 lentivirus in NK cells. A) NK cells, with or without pre-activation by IL2 and IL15 for 24 hours, were transduced with GFP-tagged lentivirus at MOI=10. B) IL2/IL15 pre-activated NK cells transduced with MOI=10 and MOI=50. GFP-tagged viral uptake was measured by fluorescence microscopy or Flow cytometry. Dead NK cells were gated out by Live/Dead staining.

Because establishing an effective way to block miR-183 function of NK cells is crucial for the study, we have created an anti-miR-183 lentiviral construct and developed different techniques for maximum virus uptake. We noted that actively dividing NK cells stimulated with IL2 and IL15, and high titer lentivirus expressing anti-miR183 were both critical for optimal anti-miR-183 uptake into NK cells, hence maximizing miR-183 blockade. We have spent six months to create anti-miR-183 constructs, producing high titer virus and optimizing the technique of virus uptake into large numbers of NK cells for in vivo delivery into NSG mice bearing A549 human lung tumors.

What opportunities for training and professional development has the project provided?

I learnt a great deal from this award, first in gaining the complex molecular technical training to clone and construct viral vectors and second in

becoming proficient in all the immune assays required to conduct a human NK lung immunotherapy project. What I learnt most was how to plan an experiment and solve each problem as it develops, gaining confidence to think independently. Attendance at the annual meeting of the Am.Assoc. Cancer Research was eye-opening to the myriads of approaches that can be considered to combat cancer, offering me new ideas for my career development.

How were the results disseminated to communities of interest? Nothing to report

What do you plan to do during the next reporting period to accomplish the goals?

In the remaining no-cost extension period, I plan to carry out the final steps to evaluate miR-183 blockade strategy in the human xenograft mouse model. We are in the process to transduce sufficient numbers of NK cells with control lentiviral vector or anti-miR183 construct and inject them intravenously into NSG mice bearing A549 subcutaneous tumors. We will also inject unmanipulated NK cells into mice as additional controls. We expect that we will need to generate sufficient NK cells (for untreated controls, empty vector-containing NK cells, and anti-miR183-containing NK cells) for at least 5 mice per group for each experiment. We intend to repeat it again to obtain statistically-valid data.

4. IMPACT:

What was the impact on the development of the principal discipline(s) of the project?

This project is of high impact because it will establish a new strategy based on microRNA targeting for immunotherapy of cancer.

What was the impact on other disciplines? *Nothing to report*

What was the impact on technology transfer? *Nothing to report*

What was the impact on society beyond science and technology? Nothing to report

5. CHANGES/PROBLEMS: *Nothing to report*

Changes in approach and reasons for change: Nothing to report

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents. *No changes*

Significant changes in use or care of human subjects. *No changes*

Significant changes in use or care of vertebrate animals. *No changes*

Significant changes in use of biohazards and/or select agents. *No changes*

6. PRODUCTS: *Nothing to report*

7.

Website(s) or other Internet site(s): *Nothing to report*

Technologies or techniques: *Nothing to report*

Inventions, patent applications, and/or licenses: *Nothing to report*

Other Products: *Nothing to report*

8. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Name:	<i>Thu Le Trinh</i>
Project Role:	<i>PI</i>
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	<i>12</i>
Contribution to Project:	<i>No change</i>
Funding Support:	<i>No change</i>

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period? *No change*

What other organizations were involved as partners? *Nothing to report*

9. SPECIAL REPORTING REQUIREMENTS *Nothing to report*

10. APPENDICES: *Nothing to report*