NDnano
Notre Dame Nanoscience and Technology

NDnano Summer Undergraduate Research
2022 Project Summary

1. Student name & home university: Francine Graham, University of Notre Dame

2. ND faculty name & department: Donny Hanjaya-Putra, Bioegineering

3. Summer project title: IPSC to LEC Transfection Utilizing ETS2 and ETV2

4. Briefly describe new skills you acquired during your summer research:

I have gained a better understanding of proper Induced Pluripotent Stem Cell (IPSC) care, including expansion and passaging. I have learned how to test for particular cell markers through PCR, FACS, and immunostaining analysis. I also learned about methods of differentiating IPSCs, in addition to the lentiviral transfection that was specifically researched.

5. Briefly share a practical application/end use of your research:

Generation of Lymphatic Endothelial Cells (LECs) allows for advances in wound healing. Using a patient's own tissue, we can provide LECs to facilitate the regeneration of lymphatic vessels. Since the LECs would be derived from the patient's own tissue, there would be no issues relating to matching DNA or possible disease transmission as there would be from using donor cells.

6. 50- to 75-word abstract of your project:

Stem cells have the capacity to become a range of other cells through various processes. Among those processes is utilizing the capacities of lentiviruses to modify the RNA of host cells. Of the existing lentiviruses, ETS2 and ETV2 have been shown to result in endothelial-like cells, which is shown in expression of particular endothelial cell markers. The particular cell marker which this project sought to express was Prox1, an intercellular marker of LECs, which did appear to be present.

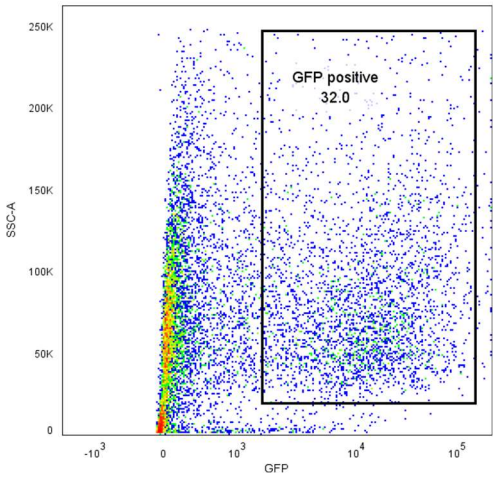
7. References for papers, posters, or presentations of your research:

Efficient Direct Reprogramming of Mature Amniotic Cells into Endothelial Cells by ETS Factors and TGFβ Suppression: Ginsberg, James, and Ding et al.
ETS Family Members Induce Lymphangiogenisis through Physical and Functional Interaction with Prox1: Yoshimatsu, Yamazaki, and Mihira et al.
ETV2 Functions as a Pitoneer Factor to Regulate and Reprogram the Endothelial Lineage: Gong, Das, Sierra-Pagan et al.
The ETS Factor ETV2: a Master Regulator for Vascular Endothelial Cell Development: Oh, Kim, and Park

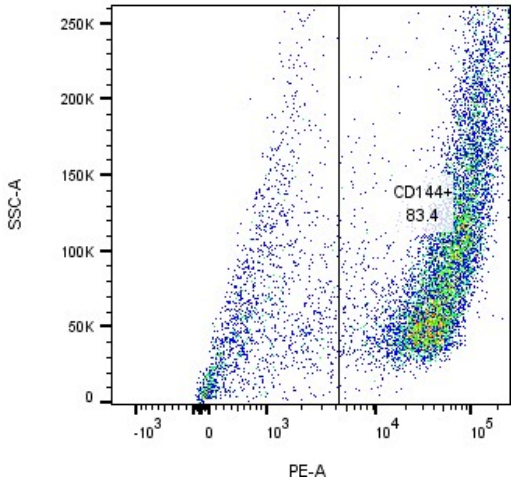
One-page project summary that describes problem, project goal and your activities / results:

This summer, I worked in the Hanjaya-Putra Lab under Sanjoy Saha to test the effectiveness of ETS2 and ETV2 transfection of Induced Pluripotent Stem Cells (IPSCs) into Lymphatic Endothelial Cells (LECs). Currently, while there is no shortage of donor LECs, use of them in wound healing assays is restricted due to the potential for complications when the DNA of the donor does not match that of the patient. There is a possibility of rejection by the body, as well as the potential for disease to be passed from a donor to a patient when not every possible disease can be screened for effectively. Using cells from a patient to address their injury is significantly more likely to avoid both of these potential issues, and so utilizing the capacities of a patient's cells to become IPSCs, the option to differentiate these IPSCs into necessary cells becomes viable. In the particular case of injury, often there is damage to the vascular system, including the lymphatic vascular system, which is able to be repaired via LECs. The goal of this project in specific was to display the intercellular marker Prox1, which is one of the key markers of LECs.

Separate transfections of IPSCs using ETS2 and ETV2 displayed relatively high uptake of ETS2 at 32.0% GFP positive cells and a high expression of the endothelial cell marker CD144 for ETV2 after 48 hours.



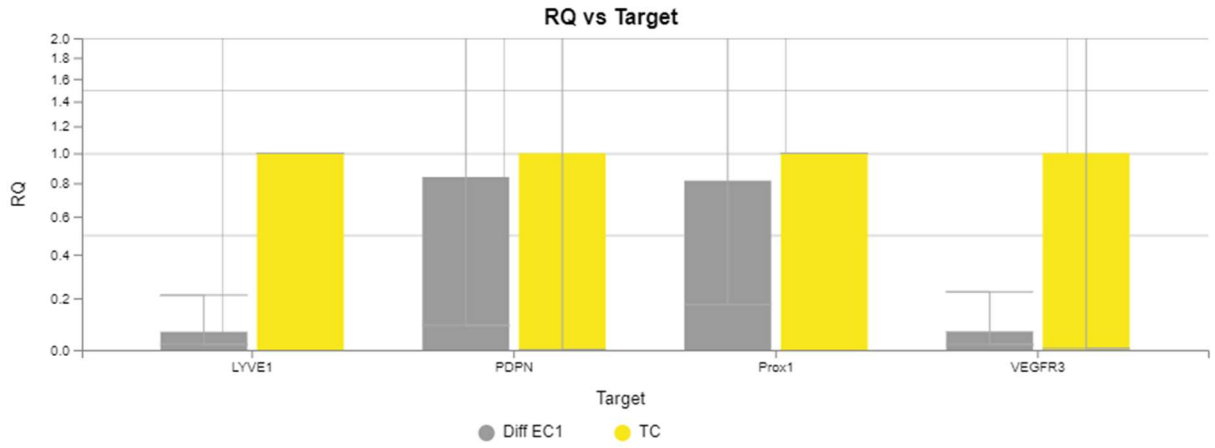
ETS2 GFP treated cells



ETV2 puromycin treated cells

Having selected CD144 positive cells, I then performed PCR analysis to determine the relative expression of four key LEC markers: Prox1, LYVE1, podoplanin (PDN), and VEGFR3.

Compared below are CD144 positive differentiated endothelial-like cells (DiffEC 1) and passage 8 LECs that have been tissue cultured (TC).



These results indicate that the transfection succeeded in causing the cells to express Prox1 and podoplanin to similar levels of passage 8 LECs, but was unable to produce similarly significant levels of VEGFR3 or LYVE1.