The Insulin Producing Cell

STEM Exploration and Engagement Scholars Program

Capstone Project

Undergraduate Research

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Research Background and Overview

I have been working as an undergraduate research assistant in Dr. Daniel Gallego-Perez's laboratory at The Ohio State University since August 25, 2021. During my first semester (autumn 2020) at Ohio State, I immediately pursued this opportunity because my passion for research had been evident in many ways. First, I am type 1 diabetic and have been motivated to do diabetes research since my unexpected diagnosis at the age of 14. Second, I had the opportunity to extensively shadow at Cleveland Clinic's Lerner Research Institute and gain a sound understanding of the laboratory environment, while still in high school. Lastly, also while still in high school, I had the privilege to conduct my own independent genetic research project that yielded multiple district and state honors, as well as a GenBank results publication.

While exclusively searching for research laboratories investigating diabetes, I was immediately referred to Dr. Gallego-Perez's laboratory by the director of Ohio State's Diabetes and Metabolism Research Center. By reaching out and conducting some research of my own, I learned that the Gallego-Perez laboratory uses direct cellular reprogramming to change a common cell type into a specialized cell type with the goal of treating specific diseases. To reprogram a cell, DNA encoding for specific transcription factors are delivered into a common cell via electroporation, a physical DNA delivery mechanism. Once the DNA is translocated inside the common cell, it can be expressed as proteins and manipulate the cell to reprogram from its initial cell type to the specialized cell type of interest.

After considering this and meeting with the team, joining the Gallego-Perez laboratory was an easy decision, for the research perfectly aligned with my passion and previous research experience. I officially joined the Gallego-Perez laboratory in January of 2021. However, due to the pandemic, I was only able to complete online training and attend virtual lab meetings during

my second semester (spring 2021) at Ohio State. Having a summer job already, I began working in the laboratory in August 2021.

Currently, I am working on the Insulin Producing Cell project. The goal of this project is to convert skin fibroblasts into pancreatic β-cells via direct cellular reprogramming. Physiologically, β-cells produce the hormone insulin. Insulin opens the doorway to cellular metabolism in all living cells. Type 1 diabetes is a chronic autoimmune disorder, meaning the immune system spontaneously attacks its own β-cells and eliminates the production of insulin. Without sufficient insulin therapy, metabolism cannot be accomplished. Determining the causation of this autoimmune attack, as well as developing an autonomous insulin therapy, has been unsuccessful. Recently, allogeneic islet cell (i.e., cluster of many pancreatic cells, including β-cells) transplantation has shown great promise in curing type 1 diabetes. However, 0.1 to 1 billion functional β-cells are required to cure a single patient's type 1 diabetes, and we simply do not have access to a cell source that immense[1]. The Insulin Producing Cell project's goal is to combat this problem and meet this need by using the surplus of skin fibroblasts within the human body for its β-cell reprogramming efforts.

Approach and Methodology

The Insulin Producing Cell project began with an in-depth literature review. This search yielded several previously conducted β-cell reprogramming studies, along with studies that investigated biologically important factors in β-cell differentiation. After reviewing these studies, my research supervisor, Luke Lemmerman (graduate fellow in biomedical engineering), identified seven transcription factors for β-cell patterning and three transcription factors for skin cell plasticity that he could justify as potentially essential in the reprogramming of a skin fibroblast to a β-cell[2].

To begin bench work, INS1-green fluorescent protein (GFP) skin fibroblasts were isolated from transgenic mice embryos. These cells are used to observe insulin production via green fluorescence since GFP is under the control of the INS1 (gene for insulin) promoter in this mouse model. These INS1-GFP cells were used throughout.

Since joining the Insulin Producing Cell project, I have been involved in most aspects of the project and have quickly learned several protocols including cell culture, plasmid isolation, in vivo reprogramming, cryosectioning, and immunohistology. Each of these protocols have been crucial to the project's progress.

In my first week, I performed the cell culture protocol, whose purpose was to passage and count the INS1-GFP cells that the project relied on for all of its in vitro aspects. Through my high school experience, I saw cell passaging conducted before, and here it was no different. However, in my experience, I had only ever counted cells manually with a hemocytometer. To my surprise, the Gallego-Perez lab has an EVE automatic cell counter. Upon being trained to use EVE, I was able to count cells in a fraction of the time it used to take me back in high school.

Also early on, I carried out the plasmid isolation protocol to generate the plasmid DNA encoding for the ten transcription factors that we are interested in. Again, my previous research experience

allowed me to quickly master this protocol. The cellular lysis, filtration, and binding steps were almost identical to my previous genetic research project. One difference is that I was able to quantify the amount of plasmid DNA I was isolating after being trained to use the NanoDrop. This was significant to me because I know from experience how important plasmid concentration is during translocation.

After a few weeks in the laboratory, Lemmerman asked me to conduct a comprehensive literature search on β-cell media. We wanted to ensure that the β-cell media we were using was providing the optimal environment for reprogramming. Furthermore, useful β-cells must be responsive in their environment to reach therapeutic capability, and we wanted to ensure we were providing the correct conditions for that as well. As a result of my search, Lemmerman and I discussed and decided not to change our β-cell media for the time being, for most studies were using media similar to ours. My ability to conduct this literature search comes from my previous coursework in Ohio State's biomedical science major and my previous research experience.

Once I was fully settled into the lab, putting in around 10 hours per week, I was the only undergraduate researcher on the Insulin Producing Cell project who was transitioned away from the in vitro research in Fontana laboratory to the in vivo research at the Davis Heart and Lung Research Institute. For this four-week pilot study, I was responsible for six INS1-GFP transgenic mice (2 male, 4 female). In week 1, I executed the nairing protocol, as well as the tissue nanotransfection protocol (TNT). After nairing the mice, Lemmerman and I conducted TNT with the ten-transcription factor cocktail on the upper and lower backs of three of the mice (1 male, 2 female). The other three mice received TNT with a control cocktail. For the next two weeks, I naired and collected three-millimeter skin biopsies from the upper and lower backs of each mouse. On week four, I naired the mice a final time before euthanizing each of them. After

euthanasia, Lemmerman and I harvested twelve-millimeter skin biopsies from the upper and lower backs of each mouse. Furthermore, we harvested the pancreas from each mouse as a positive control to reference later. Through this unique opportunity, I had the chance to learn about research animal care and ethics, as well as improve upon my suturing skills. Lastly, being able to participate in my lab's keynote protocol (TNT) really brought all the work I had been doing full circle.

As a result of how fast I was able to settle in and make major contributions to the Insulin Producing Cell project, I was allowed to take lead on the immunohistology portion of the in vivo pilot study. In October 2021, I applied the cryosectioning and immunohistochemistry protocols to the tissue samples from the in vivo pilot study. In week one with the day 7 samples, I was unable to identify positive fluorescent results that would indicate successful TNT and reprogramming. In week two with the day 21 samples, I was again unable to identify positive fluorescent results. Due to the lack of positive results, I suggested that I resection the samples perpendicular, instead of parallel, to the sectioning chuck. I was concerned by the fact that reprogramming could have been effective, but we were not at the right place or depth in the tissue samples. Lemmerman agreed with my hypothesis. After I completed one cycle of perpendicular sectioning, staining, and imaging, I found fluorescent results that has prompted us to investigate further.

Future Direction

After interpreting the results from our in-vivo pilot study, we have decided to go back to the in-vitro INS1-GFP cellular model. This decision was made because the in-vivo study showed fluorescent results that were potentially promising, but not statistically significant enough to draw clear conclusions. Our plan going forward is to test the significance of each transcription factor in the cocktail individually.

Electroporation of each transcription factor into the INS1-GFP cells will provide a better insight into each factor's role in reprogramming. Quantitative real-time polymerase chain reaction (qRT-PCR) will be heavily utilized to understand the efficiency of integration of our transcription. Understanding and confirming the mechanisms at play, we look to better understand and improve our cocktail. With this understanding, it is our goal to create a cocktail that optimizes functional β-cell reprogramming once we return to the in-vivo mouse model.

Research Significance

This project is significant and different from previously conducted β-cell reprogramming studies in many ways. First, our ten-transcription factor "cocktail" has never been used before; previous studies have only utilized portions of it. Second, many studies have attempted to reprogram skin fibroblasts via indirect cellular reprogramming (e.g., using induced pluripotent stem cells), but reprogramming of skin fibroblasts via direct, non-viral methods are unknown. Lastly, one of our study's goals is to overcome the dangerous tumor susceptibility of induced pluripotent stem cells (iPSCs), for iPSCs that fail to reprogram via indirect reprogramming result in invasive tumors[3]. Successful reprogramming that leads to safe, functional, and responsive β-cells could be an accomplishment that is pivotal in finding a cure for type 1 diabetes.

Works Cited

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