Direct Fibroblast Reprograming with CRISPRa

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Introduction: This project aims to evaluate the use of CRISPR activation (CRISPRa) in converting fibroblast cells to endodermal cells which can then develop into spherical organoids of liver, intestine, and colon progenitors[1][2]. Whole gene insertion and reprogramming with lentivirus has been used before but is time-consuming and expensive [3]. CRISPRa is a powerful gene expression tool that allows one to overexpress specific genes through tailored single guide RNAs (sgRNAs) [4]. SgRNAs direct the CRISPR system to precise genomic locations, which facilitates the reprogramming of human stem cells. Endoderm cells, which generate tissues and cells like those found in the embryo and liver, are invaluable for studying disease mechanisms, understanding embryonic development and facilitating cell differentiation. This approach holds promise for advancing drug discovery and developing novel therapeutic strategies. My work focuses on the early stages of this project: creating sgRNA plasmids for the transcription factors required for liver organoid creation: FOXA3, HNF4a, CDX2 and GATA6 [5].

Methods: To make sgRNA plasmids, oligonucleotides were designed to insert the target sequence into the lentiviral backbone CROPseq-Guide-Puro and CROPseq-Guide-Neo. The oligos were simultaneously phosphorylated and ligated using T4 PNK and T4 DNA ligase buffer, respectively. Concurrently, the CROPseq was digested with BsmBI-v2, dephosphorylated with Antarctic Phosphatase, and finally gel purified using the Zymoclean Gel DNA Recovery Kit (Zymo Research, Irvine, CA). The two resulting constructs were ligated with T4 Ligase, transformed into bacteria, and spread for growth on agar plates. Then a PCR from the resulting colonies was performed, and positive samples sent for Sanger Sequencing (Genewiz, South Plainfield, NJ).

Results: While the Sanger sequences for GATA6 and CDX2 were successful, the Sanger sequence for HNF4a and FOXA3 were not. This may be due to a degraded or contaminated DNA template, issues with the sequencing primer, or a signal imbalance caused by an unequal corporation of florescent dye into the DNA fragments during Sanger sequencing.

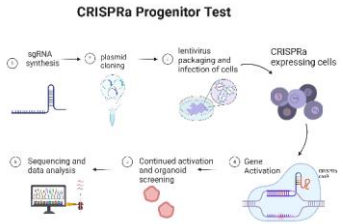

Figure 1: Screening for CRISPRa and its use in creating progenitor cells. Created with BioRender.com.

Conclusions: The anomaly in the DNA sequence has been noted, and the process will need to be repeated to ensure accurate results before further progress is made. Once the sgRNA plasmids are successfully created, lentivirus packing, and infection of fibroblast cells containing the CRISPRa system will be implemented. This will enable the targeting of CRISPRa and activation of the transcription genes. Thus, the ability of CRISPRa to generate liver progenitor cells, which are stem-like cells derived from the endoderm, can be tested. Further work will harness CRISPRa libraries to develop and screen organoid models for disease research and therapeutic applications.

References:

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4. Jiang L. et al. 2022. "CRISPR activation of endogenous genes reprograms fibroblasts into cardiovascular progenitor cells for myocardial infarction therapy." Mol. Ther.; 30(1): 54-74.
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Commented [SG2]: What kind of cells?

Commented [SG3R2]: Is it fibroblast cells?

Commented [SG4]: Why not the HNF4a gene as well?

Commented [SG1]: Can I put a picture comparing the area of the DNA sequence that came back wrong to what it should look like