Name(s)

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Project Number

S0525

Project Title

Generation of MPS IIIA Induced Pluripotent Stem Cells and Correction Using CRISPR Cas9

Abstract

Objectives

The objective of this project was to reprogram Mucopolysaccharidosis (MPS) IIIA human fibroblast cells into induced pluripotent stem cells, then correct the MPS IIIA genetic deficiency using CRISPR Cas9 technology in order to produce therapeutic neural stem cells.

Methods

MPS IIIA human fibroblasts, cell culture media, nucleofector machine and solution, mice DNA, PCR machine, primers and master mix, nuclease free water, pipettes, microscope, a hood. To perform this experiment, MPS IIIA human fibroblasts were grown with cell culture media, then nucleofected with DNA plasmids expressing transcription factors to reprogram them into iPSCs. Mice were genotyped so breeding pairs could produce knockout mice for future use when testing the treatment.

Results

The results showed that after growing the human fibroblasts, they were successfully reprogrammed into iPSCs. Multiple cell confluencies and types of media were used to find the optimal procedure for creating the iPSCs. The mice were successfully genotyped so the knockout genotype mice can be used for future testing of the treatment.

Conclusions

The hypothesis was partially proven because iPSC lines were able to be obtained, however they have not yet been corrected with CRISPR Cas9. After a large volume of iPSC clones is developed, CRISPR will be performed for gene correction. Once tested in mice and proven legitimate, this therapy could treat patients and provide significant insight into future gene therapies.

Summary Statement

I reprogrammed MPS IIIA human fibroblasts into pluripotent stem cells which I will correct using CRISPR Cas9, then inject into mice I genotyped to test the treatment's effectiveness on the fatal genetic disorder MPS IIIA.

Help Received

The lab facility where I had access to equipment and materials was LA Biomed. My mentor was Yewande Pearse. My mentor taught me specific laboratory procedures including feeding/ splitting cells and DNA extractions so I could then independently utilize these procedures in my project.