An estimated 10 million people worldwide have Parkinson's disease (1). Parkinson's disease is a chronic neurological disorder that is characterized by neuron degeneration in the midbrain, leading to progressive involuntary muscle movement. However, the cause of this motor neuron, and its currently incurable. Dopaminergic neuron degeneration leads to a scarce supply of dopamine, which causes the involuntary movements (2). A defining pathological feature of Parkinson’s disease is the aggregation of alpha-synuclein in dopaminergic neurons, which is believed to result in the loss of beneficial alpha-synuclein function. The Engrailed proteins are believed to protect the mesencephalic dopaminergic (mDA) neurons during development and are actively added to protect mDA neurons in Parkinson’s disease experiments. Studying the degeneration of A9 Dopaminergic (DA) neurons in Parkinson’s disease experiments is difficult to do. Therefore, in vitro techniques are used. Stem cells can be derived from human skin cells and then changed into dopaminergic neurons to model Parkinson’s disease. Through my research, I am testing my hypothesis that Engrailed proteins protect neurons from cell death. The Reijo Pera lab has evidence that Engrailed proteins and alpha-synuclein interact physically. We believe Parkinson’s disease may be in part due to high levels of alpha-synuclein having inhibitory effect on the Engrailed proteins. To test this hypothesis, I am overexpressing alpha-synuclein and Engrailed proteins in mDA Neuron cell cultures. I am also testing cell viability and the function of the Engrailed proteins.

**METHODS**

**Lentivirus Overexpression**: Lentiviruses are used as a gene delivery vector. They can fuse to the DNA of the host cell. It is a type of retrovirus that can infect dividing and nondividing cells. Once the cell is infected, the DNA is made through reverse transcriptase. We use a modified lentiviral vector because it can infect and express genes in human cells. By using this method, we successfully overexpressed EN1, EN2, and GFP genes in our cells.

**Alternative Approach**: To overexpress alpha-synuclein, we took the alpha-synuclein protein from bacteria, purified it, and then added it to the cell culture.

**Immunofluorescence General Protocol**: This procedure uses primary and secondary antibodies to tag a protein of interest. After washing cells, a blocking buffer is added to block the site on the membrane. After an hour, the blocking buffer is removed and a primary antibody is added. The primary antibody finds and attaches to the protein of interest. Then to be able to see the protein of interest under a fluorescence microscope, a secondary antibody, that has a fluorescent tag, is added and it attaches to the primary antibody. To tag many different proteins in a cell culture, there are different primary and secondary antibodies that can be added.

**Cell Differentiation**: The degeneration of A9 Dopaminergic (DA) neurons in the substantia nigra pars compacta part of the brain, leads to Parkinson’s disease (PD). Human pluripotent stem cells (hPSCs) can be used to derive DA Neurons for PD regenerative cell replacement therapy, which is one of the most promising PD treatment strategies. I have followed a protocol that forms hPSCs to DA Neurons using small molecule differentiation method. hPSCs originate from floor plate precursors found in the ventral midline of the central nervous system. The culture conditions were optimized by removing this environment floor plate precursor cells, were generated by using small molecule CHIR99021 to activate the Wnt signaling pathway. Then these FP-cells were added to DAneurons with the growth factors BDNF, GDNF, etc. The generated DA neurons will of all DA cell type and they can be derived less than 4 weeks.

**EXPERIMENTAL DESIGN**

There will be three parts to the experiment. First, we hope to find whether or not the Engrailed proteins protect TH neurons from alpha-synuclein. To test this, we will use different genotypes of cells to create 3 different conditions: GFP (control), Engrailed protein-1 (EN-1), and Engrailed protein-2 (EN-2) and then we will add alpha-synuclein protein to those cell culture dishes. We will then create these different conditions and then differentiate the cells into neurons and compare the percentage of TH neurons that survive.

The second part of the experiment will be to see alpha-synuclein inhibits the translational function of the Engrailed proteins. Previous studies show that the Engrailed proteins regulate the translation of Nduf1 and Nduf3 genes. We will test this by adding alpha-synuclein to the cell and using Western Blotting to see the translation of Nduf1 and Nduf3 will change.

The third part of the experiment will be to see alpha-synuclein inhibits the transcriptional regulation function of the Engrailed protein. Engrailed protein is a well-known transcriptional regulator. We will test this by adding alpha-synuclein to the cell cultures and using qPCR to map if there are any changes in the Engrailed proteins binding to the genome.

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**REFERENCES**