

Studying Alzheimer's Risk Factors Using iPSC's

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Abstract

This project focuses on investigating a known Alzheimer's genetic risk factor, which is the allele $\epsilon 4$ of the APOE gene. Our goal is to identify the mechanisms by which the presence of this allele affects astrocyte-neuron interactions by using human induced pluripotent stem cells (iPSC) derived neural cells. We will utilize lentiviruses to force the expression of select transcription factors (TF) which can drive the differentiation of iPSCs into neurons and astrocytes. Due to the scale of this project, we will transduce several cell lines of Alzheimer's disease relevant genotypes with lentiviral vectors that carry specific transcription factors and select, by sub-cloning and then genotyping, the ones that have the integrated virus+TF combination. Knowing that the virus integrates itself into the genome of the cells, we can have control over when the factors need to be expressed. Since making viruses is a time consuming process, using stable cell lines can help alleviate certain issues regarding variabilities between different viral batches and also optimize the differentiation process and duration of the experiments.

Background

-E4 APOE "Risk allele" of apolipoprotein E (APOE) 14% of the population
-E2 APOE- "Protected allele" people with this allele rarely have Alzheimer's. rare, present in 8% of the population
-E3 APOE- "Neutral allele"- Not associated with risk or resistance. present in 78% of the population
There is currently no method to diagnose APOE4 people with Alzheimer's early.
-Astrocytes with APOE4 possess a corrupted form of amyloid beta which damages nerve cells. It causes inflammation in the blood brain barrier, which is a marker in Alzheimer's

Materials and Methods

Cell Lines used- iPSC line HE0019
Plasmids used- Ngn2, RTTA, REV, RRE,
Pmd2G
Maxiprep
Lentiviral Production
Restriction Digestion
PCR
qPCR
Forced expression of neurons
Ngn2 Differentiation

Results

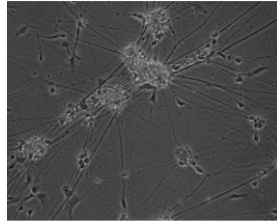


Fig 1. Day 13
Neurons(Plated at
100,000 Cells)

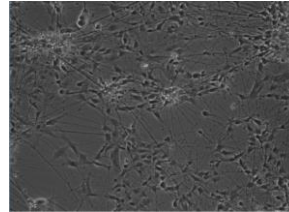


Fig 2. Day 13
Neurons(Plated at
120,000 Cells)

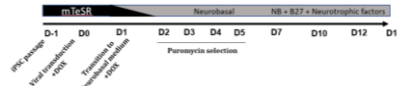


Fig 3. Time
frame for
Neuron
Differentiation

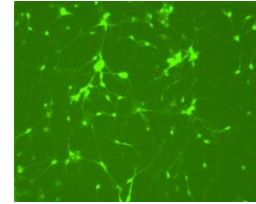


Fig 4. FITC at day 6.
Fluorescence shows
successful Ngn2 infection
which will lead to neurons

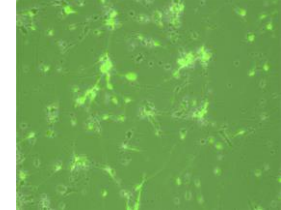


Fig 5. Merged imaging at
day 6 after puromycin
selection shows some
uninfected cells survived

Future Directions

I have been experiencing trouble with completing the full Ngn2 differentiation. I will be retrying this protocol again this month to optimize neuron production and then perform immune to read TUJ and MAP2 markers. Eventually we would like to observe bioactivities of EV's on neurons using exosomes. We will be able to study a mechanism for how the gene works based on this. This will eventually lead to a method for diagnosing Alzheimer's before an individual shows symptoms.

References Cited

- > Citations: Nikitidou E, Khoonsari PE, Shevchenko G, Ingelsson M, Kultima K, Eerlandsson A. Increased Release of Apolipoprotein E in Extracellular Vesicles Following Amyloid- β Protofibril Exposure of Neuroglial Co-Cultures. *J Alzheimers Dis.* 2017;60(1):305-321. doi: 10.3233/JAD-170278. PMID: 28826183; PwCID: PwC5675265.
- > Wang C, Zhang M, Garcia G Jr, Tian E, Cui Q, Chen X, Sun G, Wang J, Arumugaswami V, Shi Y. ApoE-Isoform-Dependent SARS-CoV-2 Neurotropism and Cellular Response. *Cell StemCell.* 2021 Feb 4;28(2):331-342.e5. doi: 10.1016/j.stem.2020.12.018. Epub 2021 Jan 4. PMID: 33450186; PMCID: PMC7832490.

Summary and Conclusions

It is possible to differentiate an iPSC into a neuron by forcing expression of Ngn2 and RTTA. There is still a lot that we need to do with these neurons such as harvesting exosomes and observing bioactivity which we have not been able to do yet. Unfortunately because of this, we will not be able to draw any drastic conclusions yet of how the APOE4 mechanisms fully works yet.