Modeling Parkinson's Disease in a Petri Dish:

EFFECT OF α- SYNUCLEIN ON DOPAMINERGIC NEURONS DERIVED FROM MOUSE iPSCs

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Introduction

Parkinson's disease (PD) is one of the most common neurodegenerative diseases of aging, affecting specific mid-brain dopaminergic (DA) neurons. There is no cure or effective way to slow disease progression, and the causes of the disease remain obscure. Our goal is to generate mouse mid-brain DA neurons for *in vitro* assays. Cellular development of the brain is characterized by specific neuronal linage markers. Beta III Tubulin (TUJ1) positive cells indicate mature neurons and Tyrosine Hydroxylase (TH) positive cells indicate dopaminergic cells. We started with an "off the shelf" kit that required 37 days and produced TUJ1 but no TH positive cells [Ref 1]. We incorporated changes to this protocol from the literature that reduced the time to 20 days and dramatically increased TUJ1 and TH. A Fibroblast Growth Factor (FGF) inhibitor was added during embryoid body (EB) formation. We switched to N2/B27 media made out of fresh ingredients and added Brain-derived neurotrophic factor (BDNF) and Glial cell-derived neurotrophic factor (GDNF) during final maturation. With our new four stage protocol we were able to achieve an increase in our yield of TH positive cells in mouse induced pluripotent stem cells (iPSCs) and mouse embryonic stem cells (ESCs). We also detected midbrain transcription factors FoxA2 and calbindin.

Background

Small molecule inhibitors (Fig 1) of the SMAD and MEK pathways induce a neuronal cell fate. Using growth factors including FGF and Sonic Hedgehog (Shh) we can mimic temporal signaling in brain development (Fig 2). Blocking the Nanog pathway and then promoting the FGF and Shh pathways drive our tail fibroblast deriver iPSC (Fig 3) cultures to a mid-brain dopaminergic neuronal cell fate.

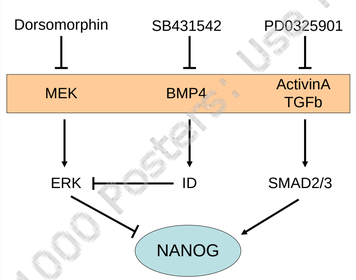


Figure 1. Small molecule inhibitors block exogenous growth factors

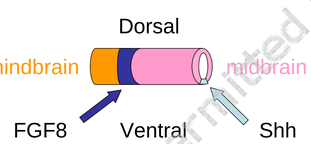


Figure 2. Neural tube signaling provides rational for growth factors

Methods

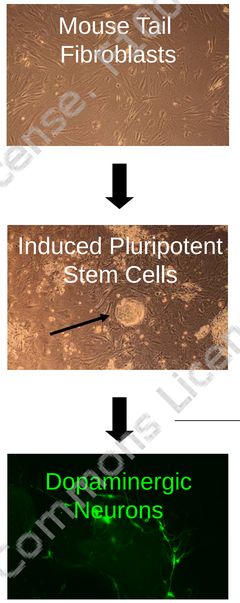


Figure 3. Reprogramming and neuronal differentiation

Protocol	Days in Culture	Media	Media Supplement
Stage I iPSC Expansion	2-4	mESC	LIF
Stage II Embryoid Body	4	mESC	Dorsomorphin SB431542 [Ref 2] PD0325901 [Ref 3]
Stage III Neural Induction	8	N2/B27	FGF8 FGF2 SAG [Ref 4]
Stage IV Neural Maturation	6	N2/B27	GDNF BDNF [Ref 4]

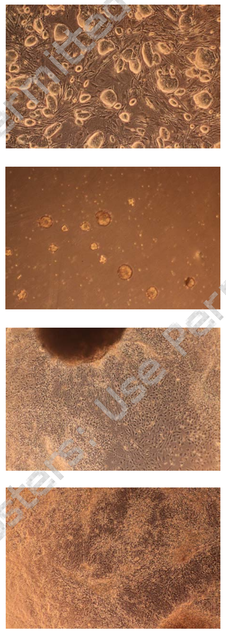


Figure 4. Overview of our four stage differentiation protocol

Results

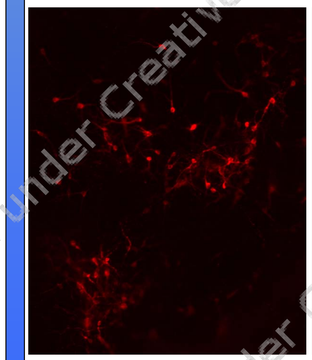


Figure 5. Immunocytochemistry Tyrosine Hydroxylase (red) from the same image as Fig 6

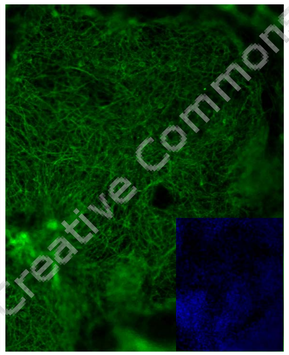


Figure 6. Immunocytochemistry TUJ1 (green) inset Hoechst (blue) all from the same image as Fig 5

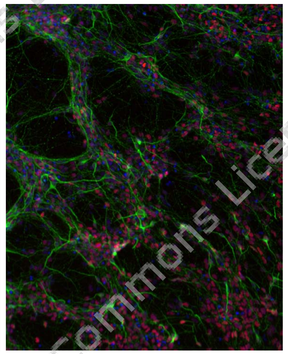


Figure 7. Immunocytochemistry TUJ1 (green) FoxA2 (red)

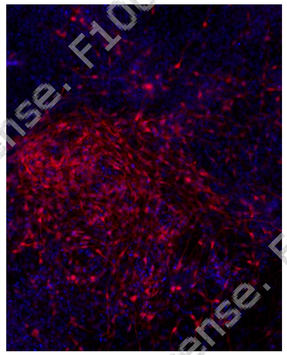


Figure 8. Immunocytochemistry calbindin (red)

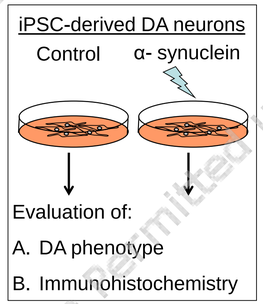
Results and Discussion

With this optimized four stage protocol (Figure 4) we can derive more TH positive cells than our previous five stage protocol. The iPSC expansion Stage I remains the same. For Stage II we added the small molecule inhibitors for two days on a rocker plate. We wanted the embryoid bodies to grow before directing them to a neuronal cell fate. In Stage III, FGF8, FGF2 and a small molecule replacement for Shh smoothened agonist (SAG) were added to direct the cells to a mid-brain fate. We also changed the media from N2 to a 50/50 mix of N2 and B27 media. The original protocol had five stages, Stage III was a Nestin positive expansion stage. We used a Neural Cell Adhesion Molecule (NCAM) magnetic pulldown to help purify neural precursor cells (NPCs). Both of these steps were removed, which shortened the protocol by ten days. We added GDNF and BDNF to the final maturation and now we have a protocol that works on iPSCs and ESCs. Our yield has increased from zero TH positive and a few TUJ1 positive cells to hundreds of TH (Fig 5) and thousands of TUJ1 (Fig 6) positive cells (flow cytometry for quantification is currently underway). The midbrain transcription factors FoxA2 (Fig 7) and calbindin (Fig 8) were also detected.

Future Work

Hypothesis: Extracellular alpha-synuclein is toxic for neuronal cell cultures. DA neurons are more susceptible to toxic effect of alpha-synuclein

Experiment: Challenge DA neurons with recombinant monomeric alpha synuclein under physiological conditions.



References

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