In Vitro Modeling of Parkinson's Disease Using Human Pluripotent Stem Cell-Derived Midbrain Neuron and Microglia Co-Culture With Alpha-Synuclein Fibrils

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INTRODUCTION

Parkinson's disease (PD) is characterized by dopaminergic neuron loss and α-synuclein inclusions, but its underlying molecular mechanisms are still unclear. Microglia play a central role in the progression of PD by orchestrating processes like inflammation and phagocytosis of pathogenic protein. To investigate neuron-glia interactions in the context of PD, we developed STEMdiff™ Midbrain Neuron and STEMdiff™ Microglia Differentiation and Maturation Kits to efficiently generate functional human pluripotent stem cell (hPSC)-derived midbrain neurons and microglia. Our preliminary data show that the STEMdiff™ midbrain system can be used in a PD model where exogenous α-synuclein pre-formed fibrils (PFFs) lead to increased accumulation of phosphorylated α-synuclein. More importantly, these midbrain neurons can be co-cultured with hPSC-derived microglia generated by STEMdiff™ Microglia Kit, creating a model of neuron-glia interactions during neurodegeneration.

METHODS

Midbrain Neuron Differentiation

3 hPSC lines (H1, H7, and STiPS-R038) were maintained in mTeSR™ Plus and differentiated into neural progenitor cells (NPCs) by following the monolayer protocol using STEMdiff™ Neural Induction Medium (NIM) supplemented with SMADi. NPCs were then plated into STEMdiff™ Midbrain Neuron Differentiation Medium. After one week, midbrain neuronal precursors were dissociated and plated into STEMdiff™ Midbrain Maturation Medium and cultured for an additional two weeks. Cell identity was confirmed at the end of the culture period by immunocytochemistry for β-tubulin III (βIIITUB) and tyrosine hydroxylase (TH).

Microglia Differentiation Protocol

hPSCs (H7) maintained in mTeSR™ Plus were differentiated into CD43-expressing hematopoietic progenitor cells (HPCs) using STEMdiff™ Hematopoietic Kit for 12 days and then further differentiated using the STEMdiff™ Microglia culture system for 28 - 34 days. The cells were characterized on day 24 by flow cytometry for CD45 and CD11b expression and by immunocytochemistry by ionized calcium binding adaptor molecule 1 (IBA1) and PU.1 expression.

Co-Culture Experiments

hPSC-derived microglia (H7) were seeded on top of the midbrain neurons (H7 and STiPS-R038-derived) at day 38 at a 1:2 microglia-to-neuronal precursor ratio in STEMdiff™ Midbrain Neuron Maturation Medium supplemented with STEMdiff™ Microglia Maturation Supplement 2 for co-culture experiments (Figure 1). The co-culture was kept for 10 days and fed every 2 - 3 days until it was fixed and stained for βIIITUB.

Co-Culture Protocol

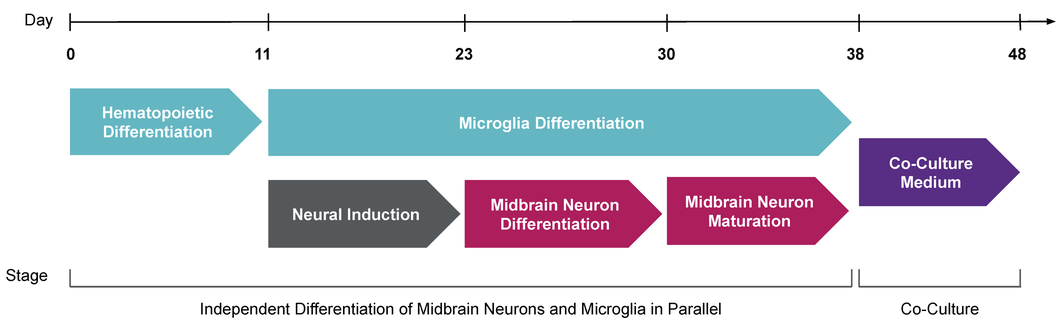

FIGURE 1. Workflow for Co-Culture of hPSC-Derived Midbrain Neurons with Microglia

α-Synuclein Seeding

hPSC-derived neural progenitor cells generated using the STEMdiff™ Neural Induction Kit monolayer protocol were plated into STEMdiff™ Midbrain Neuron Differentiation Medium. Midbrain neuronal precursors were passaged after one week into STEMdiff™ Midbrain Maturation Medium and cultured for 12 days before treatment with 600 nM of α-synuclein PFFs from the wild type (WT) form or the A53T mutant. The A53T mutation in the SNCA gene is linked to an increased risk of familial PD. This mutation affects the α-synuclein protein, potentially leading to abnormal aggregation. WT and mutant PFFs were kindly provided by Thomas M. Durcan's lab and generated following the method found in Feller, Benjamin et al., 2023. In separate experiments, midbrain neurons were co-cultured with microglia generated using the STEMdiff™ Microglia system for 2 days prior to the treatment. The co-culture was then incubated with 600 nM of WT or A53T α-synuclein PFFs added fresh at each medium change. The cells received a total of five treatments as the medium was refreshed every 2 - 3 days. After 14 days of treatment, the cells were fixed and stained with phosphorylated α-synuclein at serine residue 129 (pS129), a pathological post-translational modification of the protein observed in sporadic and genetic forms of PD, as well as βIIITUB, TH, and IBA1 for microglial identity.

RESULTS

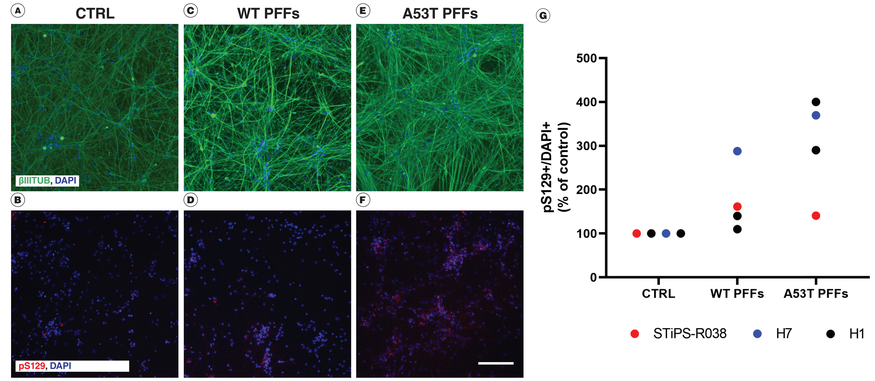

FIGURE 2. Midbrain Neurons Showed Higher Percentage of pS129+ Cells in WT and A53T PFFs Compared to Untreated Control

Representative images of **(A, B)** untreated midbrain neurons, **(C, D)** neurons treated with WT PFFs or **(E, F)** A53T PFFs. Cells were stained with βIIITUB (green), pS129 (red) and DAPI (blue). Scale bar = 100 µm. **(G)** After treatment with WT and A53T PFFs, the number of pS129+ midbrain neurons increased by 1.59 ± 0.60-fold and 5.15 ± 2.39-fold, respectively (mean ± SD; n = 3 cell lines). Data normalized to untreated control. Each data point represents the result of an experimental replicate.

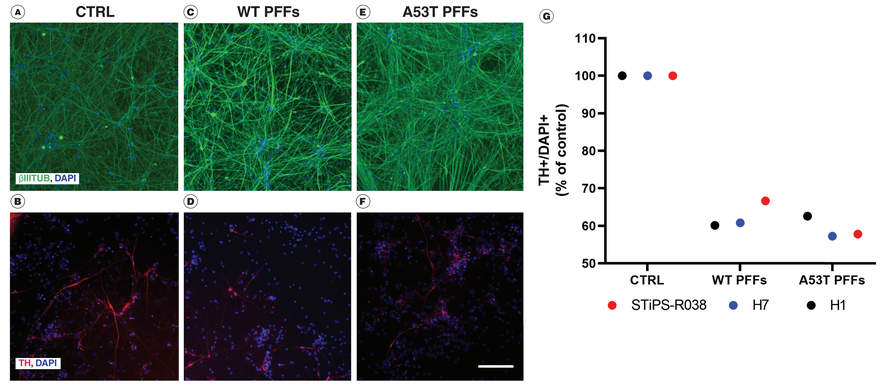

FIGURE 3. Midbrain Neurons Displayed Lower Percentage of Dopaminergic Neurons in WT and A53T Variants Compared to Untreated Control

Representative images of **(A, B)** untreated midbrain neurons, **(C, D)** neurons treated with WT PFFs, or **(E, F)** A53T PFFs. Cells were stained with βIIITUB (green), TH (red), and DAPI (blue). Scale bar = 100 µm. **(G)** Quantification showed the number of TH^+ cells decreased by 0.61 ± 0.16-fold for WT and 0.66 ± 0.09-fold for A53T PFFs (mean ± SD; data normalized to untreated control, n = 1 for H1, H7 and STiPS-R038; each dot represents the result of an experimental replicate).

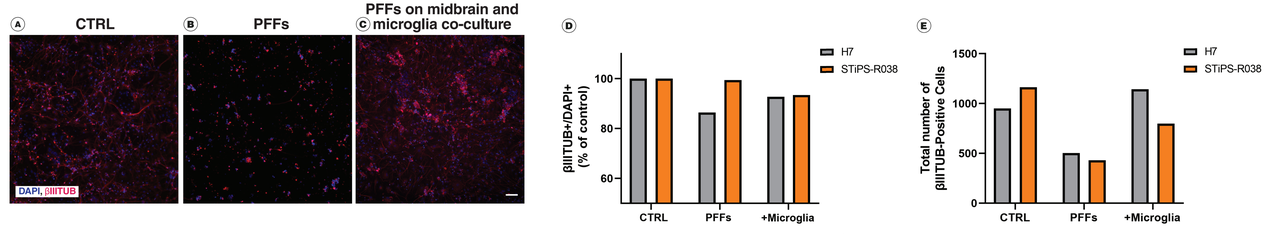

FIGURE 4. Co-Culture of Microglia and Midbrain Neurons Lead to Increased Survival of Total Cells and βIIITUB+ Cells

Representative images of midbrain neurons **(A)** cultured alone, **(B)** treated with 600 nM of A53T PFFs in monoculture **(C)** or in a co-culture with microglia. Cells were stained with βIIITUB (red) and DAPI (blue). Scale bar = 100 µm. **(D)** The addition of A53T PFFs did not affect the percentage of neurons in the culture, but resulted in **(E)** a decreased number of βIIITUB+ cells (467 cells) compared to the untreated control (1057 cells), while this decrease was not observed when the neurons were co-cultured with microglia (971 cells; n = 1 for H7 and STiPS-R038; mean of 2 technical replicates).

Summary

- α-synuclein PFFs promote the accumulation of phosphorylated α-synuclein in hPSCs-derived midbrain neurons while reducing the number of TH+ and βII-TUB+ cells, resembling phenotypes associated with the development of Parkinson's Disease.
- Co-culture with microglia may mitigate this toxic effect, suggesting a protective role of microglia during early stages of PD.
- This in vitro model of PD using α-synuclein PFFs in a co-culture of hPSCs-derived midbrain neurons and microglia provides a valuable tool for studying the molecular mechanisms of disease initiation and progression.