Generation of a Glia-Midbrain Neuron Co-Culture System Derived From Human Pluripotent Stem Cells

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INTRODUCTION

Astrogliosis is a hallmark of Alzheimer's and Parkinson's diseases, and astrocytes and microglia play key roles in both neurodevelopment and neuroinflammation. To investigate neuron-glia interactions in the context of disease modeling, we developed STEMdiff™ Midbrain Neuron and STEMdiff™ Astrocyte Differentiation and Maturation Kits to efficiently generate functional hPSC-derived midbrain neurons and astrocytes. Our data show that the STEMdiff™ astrocyte system can generate a highly pure population of functional astrocytes from hPSCs. More importantly, these astrocytes can be cultured with hPSC-derived midbrain neurons generated by STEMdiff™ Midbrain Neuron kit, which enables various co-culture models for in vitro studies of neurodevelopment and neurodegeneration.

METHODS

Astrocyte and Midbrain Neuron Differentiation and Co-Culture

Undifferentiated ES or iPS cells maintained in mTeSR™ Plus(6 cell lines: 3 ES, 3 iPS) were differentiated into neural progenitor cells (NPCs) following the monolayer protocol using STEMdiff™ Neural Induction Medium (NIM) supplemented with SMADi. The NPCs were either plated into STEMdiff™ Astrocyte Differentiation Medium or STEMdiff™ Midbrain Neuron Differentiation Medium. Astrocytes were passaged every week using the recommended protocol. On the third passage, the medium was replaced with STEMdiff™ Astrocyte Maturation Medium and similarly maintained for an additional two weeks. Midbrain neurons were passaged after one week into STEMdiff™ Midbrain Maturation Medium and cultured for an additional two weeks.Mature hPSC-derived astrocytes were seeded on top of the neurons at an astrocyte-to-neuron cell-type ratio of 2:1 in STEMdiff™ Astrocyte Maturation Medium for 1 day, then the medium was changed to STEMdiff™ Midbrain Neuron Maturation Medium. Cell identity was confirmed using immunocytochemistry for S100β, GFAP, and DCX (astrocytes), or βIIITUB (clone TuJ1) and TH (midbrain neurons).

Calcium Imaging

hPSC-derived astrocytes or hPSC-derived NPCs were treated with 10 µM Fluo-4AM (Thermo Fisher Catalog #F14201) for 30 minutes. The medium was then changed to BrainPhys™ Imaging Optimized Medium. Images were captured continuously at 500 ms intervals using a fluorescence microscope. A vehicle control or 3 µM ATP (final concentration; Sigma-Aldrich Catalog #A6419) was added to the medium during imaging. Two embryonic stem (ES) and three induced pluripotent stem (iPS) cell lines were used in Ctrl and ATP-treated astrocytes. One ES and one iPS cell line were used in ATP-treated NPCs.

Multi-Electrode Array (MEA) Recording and Analysis

Midbrain neuronal precursors were plated and differentiated to midbrain neurons directly in a 48-well CytoView MEA plate (Axion Biosystems; M768-tMEA-48B), with 16 recording electrodes in each well. A 5-minute recording of spontaneous neuronal activity was acquired at 37°C under a 5% CO2 atmosphere using an MEA system (Maestro, Axion Biosystems). To measure neuronal activity, mean firing rate (MFR) was calculated based on the activity recorded from 16 electrodes for each condition. For all recordings, a Butterworth band-pass filter (200 - 3000 Hz) was applied, and the adaptive threshold spike detector was set at 6X standard deviation. Recordings were analyzed using AxIS (2.5.1) analysis software.

Alpha-Synuclein Seeding

Alpha-synuclein (wild type) pre-formed fibrils and monomers were first dissolved in PBS as a 5 mg/mL stock solution and then diluted directly in STEMdiff™ Midbrain Neuron Maturation Medium to make a working concentration of 600 nM. This solution was made fresh each time before feeding. hPSC-derived midbrain neurons were obtained using STEMdiff™ Midbrain Neuron Differentiation Kit and cultured for 14 days using STEMdiff™ Midbrain Neuron Maturation Kit in a 24-well plate seeded at 50,000 cells/cm2. On day 15, half of the medium from each well was changed with complete STEMdiff™ Midbrain Neuron Maturation Medium containing 600 nM of alpha-synuclein. This medium replacement was repeated every third day for 14 days. Cells were then washed and fixed with 4% PFA before being stained for P-Ser129 ASYN antibody (Abcam Catalog #ab51253).

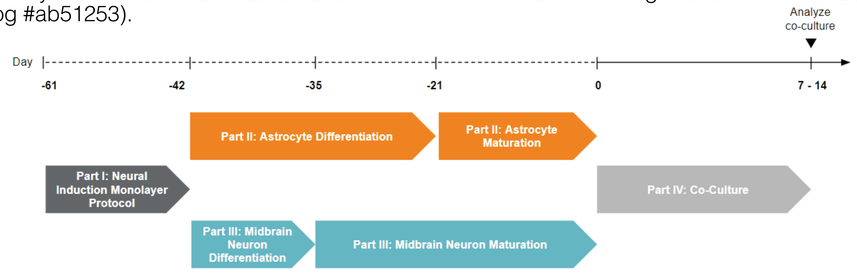

FIGURE 1. Workflow for Co-Culture of hPSC-Derived Midbrain Neurons with Astrocytes

RESULTS

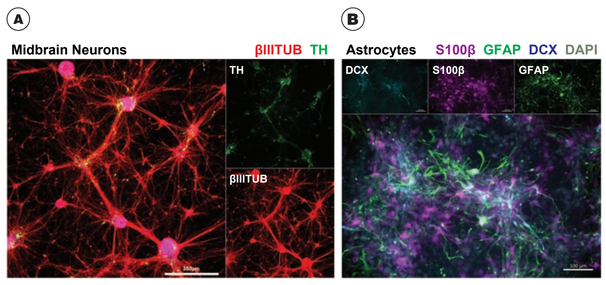

FIGURE 2. NPCs Efficiently Differentiate into Midbrain Neurons and Astrocytes and Express the Relevant Markers

(A) Representative image of midbrain neurons stained with βIIITUB (red) and TH (green) taken at 20X magnification. The resulting cultures from STEMdiff™ Midbrain Neuron Differentiation and Maturation Kits contain neuron populations that are > 90% βIIITUB-positive (red) and > 15% TH-positive (green). Scale bar = 350 µm **(B)** Representative image of astrocytes stained with DAPI (gray), GFAP (green), S100β (magenta), and DCX (cyan) taken at 20X magnification. The resulting cultures from STEMdiff™ Astrocyte Differentiation and Maturation Kits contain a highly pure population of astrocytes, which are > 60% GFAP-positive (green) and > 70% S100β-positive (magenta), with fewer than 15% neurons (DCX-positive cells, cyan). Scale bar = 100 µm.

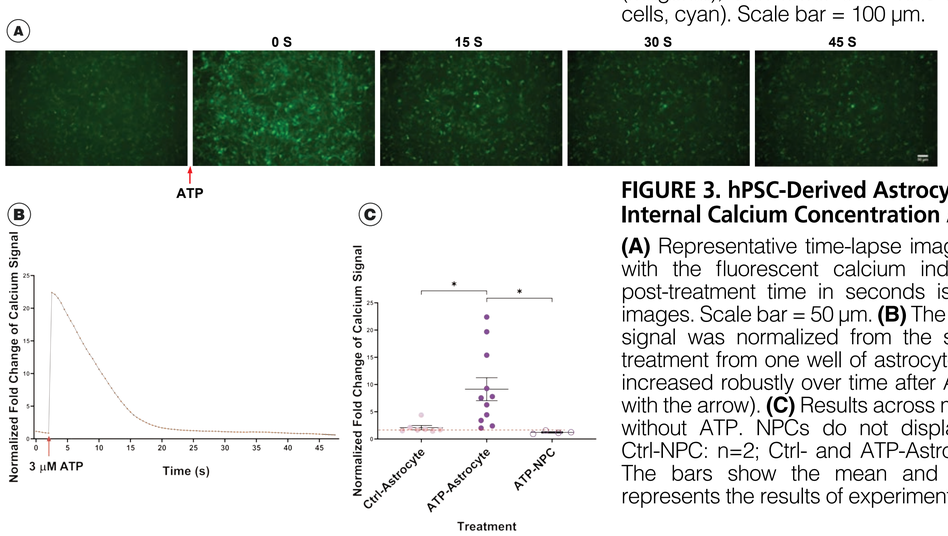

FIGURE 3. hPSC-Derived Astrocytes Modulate Internal Calcium Concentration After ATP Treatment

(A) Representative time-lapse image series of astrocytes with the fluorescent calcium indicator Fluo-4AM. The post-treatment time in seconds is indicated above the images. Scale bar = 50 µm. **(B)** The fold change of calcium signal was normalized from the signal baseline before treatment from one well of astrocytes. The calcium signal increased robustly over time after ATP treatment (labeled with the arrow). **(C)** Results across multiple cell lines. Ctrl is without ATP. NPCs do not display an ATP response. Ctrl-NPC: n=2; Ctrl- and ATP-Astrocyte: n=5 * $p < 0.05$. The bars show the mean and SEM, and each dot represents the results of experimental replicates.

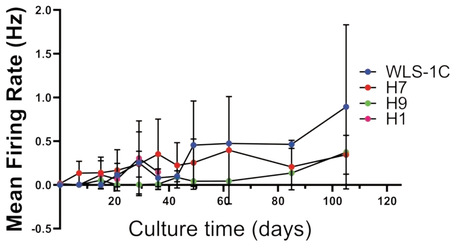

FIGURE 4. Midbrain Neurons Derived Using STEMdiff™ Midbrain Neuron Differentiation and Maturation Kits Develop Spontaneous Neuronal Activity Over Time

MFR was assessed following midbrain neuron differentiation in 4 cell lines. There was a gradual increase in MFR for neurons cultured over time, up to 0.85 ± 0.33 Hz on day 100 (n = 4; mean ± SEM, 3 replicates per line).

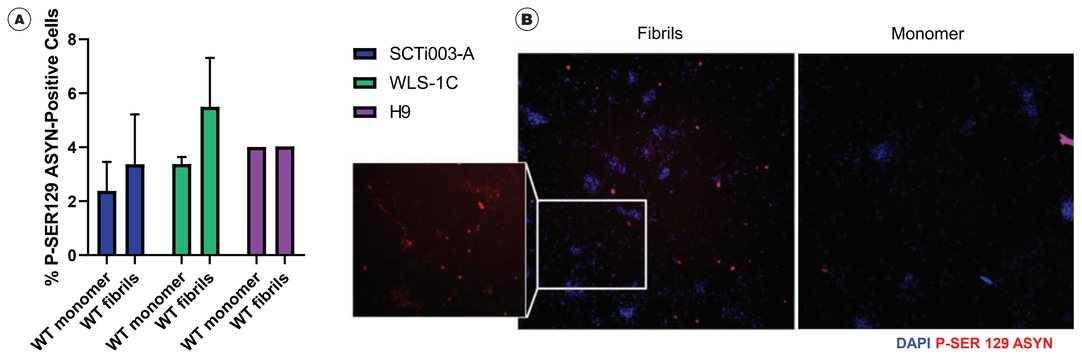

FIGURE 5. Midbrain Neurons Show Higher %P-Ser129 ASYN+ Cells in Fibril Compared Monomer-Treated Condition

(A) Quantification of the % P-Ser129 ASYN+ cells in the monomer-treated group and the fibril-treated group in 3 cell lines (n = 3, mean ± SD, 3 replicates for SCTi003-A and WLS-1C, 1 replicate for H9). **(B)** Representative images of midbrain neurons treated with alpha-synuclein monomer (right) or treated with alpha-synuclein fibrils (left) stained with P-Ser129 ASYN for phosphorylated alpha-synuclein (red) and DAPI for nuclei (blue). Magnified view shows positive P-Ser129 staining in cell bodies. Scale bar = 350 µm.

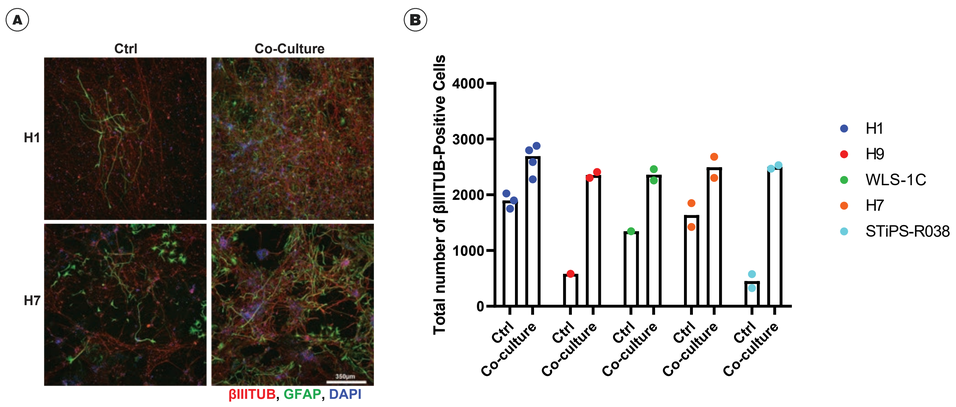

FIGURE 6. Co-Culture of Astrocytes and Midbrain Neurons Leads to Increased Survival of βIIITUB-Positive Cells

(A) Representative images of midbrain neurons cultured alone (left) or in a co-culture with astrocytes (right) stained with βIIITUB for neurons (red) and GFAP for astrocytes (green). Scale bar = 350 µm. **(B)** Quantification of βIIITUB-positive cells in the control (neurons only) and co-culture group (n = 6, each dot represents the results of experimental replicates). Numbers are % βIIITUB-positive over total DAPI in a tiled image. Scale bar = 350 µm.

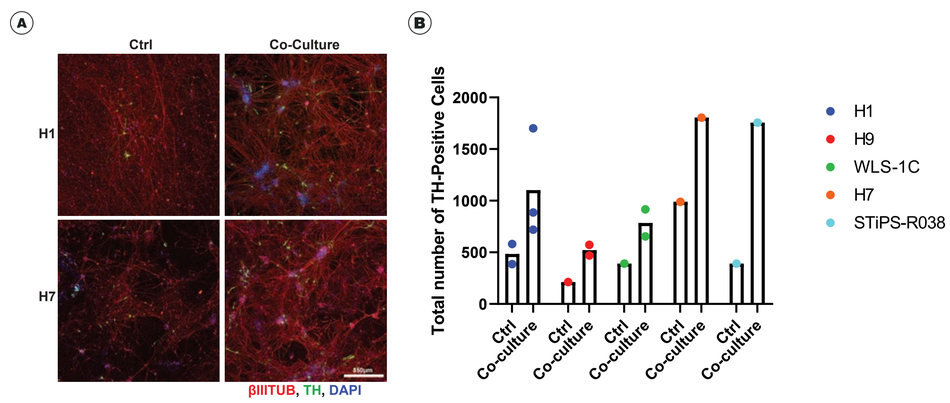

FIGURE 7. Co-Culture of Astrocytes and Midbrain Neurons Leads to Increased Numbers of TH-Positive Cells

(A) Representative images of midbrain neurons cultured alone (left) or in a co-culture with astrocytes (right) stained with βIIITUB for neurons (red) and TH for midbrain dopaminergic neurons (green). Scale bar = 350 µm. **(B)** Quantification of the TH-positive cells in the control (neurons only) and co-culture group (n = 6, each dot represents the results of experimental replicates). Numbers are % positive over total DAPI in a tiled image. Scale bar = 350 µm.

Summary

- The STEMdiff™ astrocyte and midbrain neuron system can generate functional hPSC-derived astrocytes and midbrain neurons
- Culturing midbrain neurons with astrocytes increased the number of βIIITUB-positive and TH-positive cells in the co-culture group compared to the control group
- Co-culture could lead to better survival and maturation of the neurons in vivo, making this a suitable model for studying glia-midbrain neuron interactions as part of neurodegeneration studies