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

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INTRODUCTION

Glial cells, such as astrocytes and microglia, play key roles in neurodevelopment and neurodegeneration. Studies using human pluripotent stem cell (hPSC)-derived neurons in the absence of glia do not fully represent physiological processes within the mammalian brain, or allow investigation into the complex glia-neuron interactions in disease modeling. Therefore, we developed STEMdiff™ Astrocyte kits for in vitro modeling. 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 forebrain neurons and microglia generated by STEMdiff™ Forebrain Neuron and Microglia kits, which enables various co-culture models for in vitro studies of neurodevelopment and neurodegeneration.

METHODS

Astrocyte Differentiation: hPSCs maintained in either mTeSR™1 or TeSR™-E8™ were differentiated into astrocytes following either the embryoid body (EB) AggreWell™ protocol (Figure 1A) or the monolayer protocol (Figure 1B) using STEMdiff™ Astrocyte Differentiation and Maturation Media. The cells were characterized by S100β (Dako GA504), DCX (Aves DCX), and GFAP (Biolegend 644701) expression by immunocytochemistry. The cytokine treatment was performed with a vehicle control, 30 ng/mL TNF-α, 30 ng/mL TNF-α and 3 ng/mL IL-1α, or 30 ng/mL TNF-α and 30 ng/mL IL-1β in STEMdiff™ Astrocyte Differentiation Medium for 24 hours.

Calcium Imaging: hPSC-derived astrocytes or hPSC-derived NPCs were treated with 10 µM Fluo-4AM (Thermo Fisher Catalog #F14201) for 30 minutes. Then the medium was 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.

hPSC-Derived Astrocyte and Forebrain Neuron Co-Cultures: hPSC-derived forebrain neurons were derived using STEMdiff™ Forebrain Neuron Differentiation Kit and cultured for 7 days using STEMdiff™ Forebrain Neuron Maturation Kit. Mature hPSC-derived astrocytes were seeded on top of the neuronal precursors 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™ Forebrain Neuron Maturation Medium.

hPSC-Derived Astrocyte, Forebrain Neuron, and Microglia Tri-cultures: hPSC-derived microglia were generated using STEMdiff™ Microglia Differentiation Kit for 24 days. Astrocytes were seeded on top of forebrain neuronal precursors at a 1:1 ratio. The next day, microglia were seeded at a 1:2 microglia-to-neuron ratio in BrainPhys™ hPSC Neuron Kit with STEMdiff™ Microglia Supplement 2.

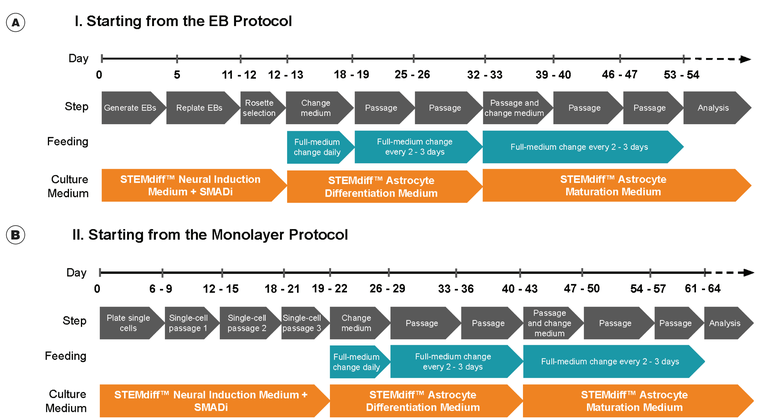

FIGURE 1. Workflows for hPSC-Derived Astrocyte Differentiation and Maturation

RESULTS

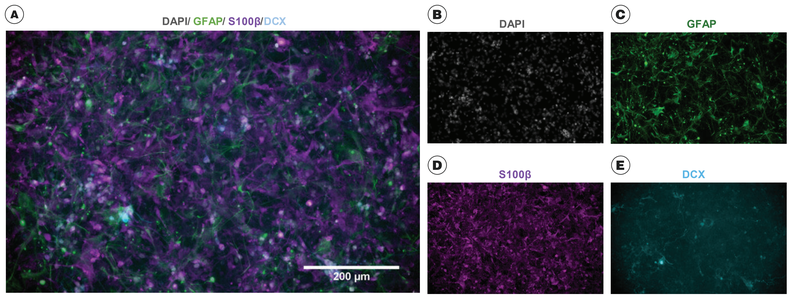

FIGURE 2. hPSC-Derived Astrocyte Cultures Have Low Levels of Neuron Populations

(A) Representative image of astrocytes stained with **(B)** DAPI (grey), **(C)** GFAP (green), **(D)** S100β (magenta), and **(E)** DCX (cyan) taken at 20X. The resulting cultures from STEMdiff™ Astrocyte Differentiation and Maturation kits contain a highly pure population of astrocytes, which are **(C)** more than 60% GFAP-positive (green) and **(D)** more than 70% S100β-positive (magenta), with **(E)** fewer than 15% neurons (DCX-positive cells, cyan). Scale bar = 200 µm.

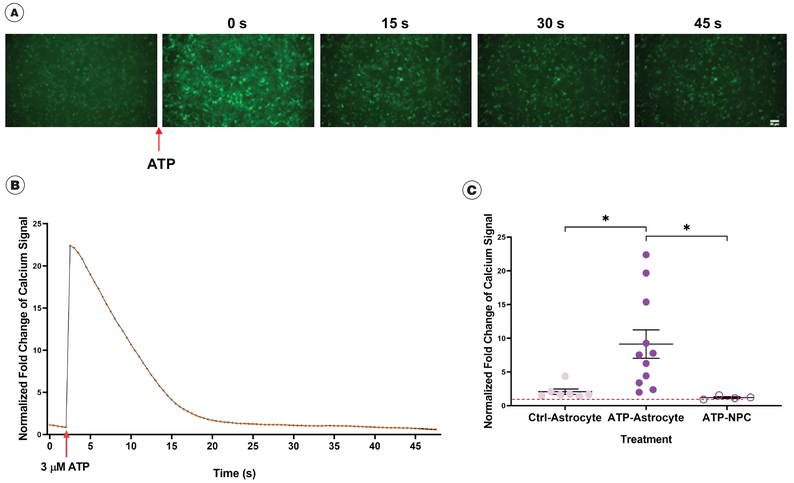

FIGURE 3. hPSC-derived Astrocytes Modulate Internal Calcium Concentration After ATP Treatment

(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 normalized fold change of calcium signal 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. 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. *: $p < 0.05$. The bars show the mean and SEM and each dot represents the results of experimental replicates.

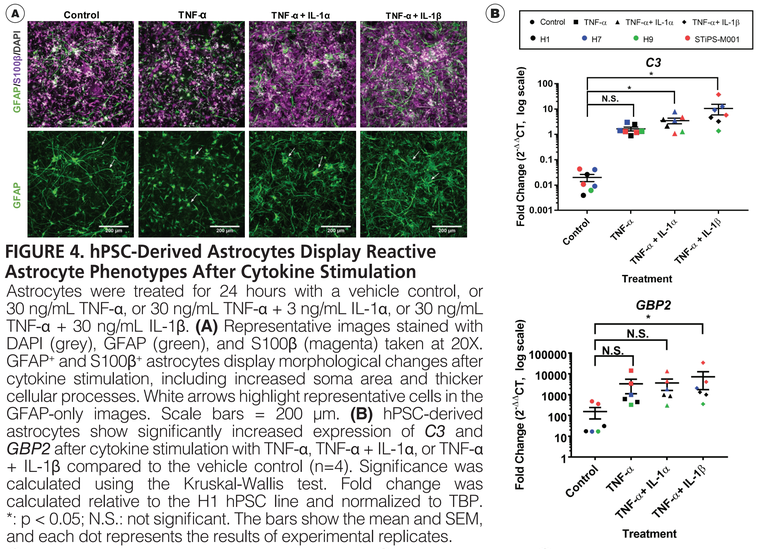

FIGURE 4. hPSC-Derived Astrocytes Display Reactive Astrocyte Phenotypes After Cytokine Stimulation

Astrocytes were treated for 24 hours with a vehicle control, or 30 ng/mL TNF-α, or 30 ng/mL TNF-α + 3 ng/mL IL-1α, or 30 ng/mL TNF-α + 30 ng/mL IL-1β. **(A)** Representative images stained with DAPI (grey), GFAP (green), and S100β (magenta) taken at 20X. GFAP+ and S100β+ astrocytes display morphological changes after cytokine stimulation, including increased soma area and thicker cellular processes. White arrows highlight representative cells in the GFAP-only images. Scale bars = 200 µm. **(B)** hPSC-derived astrocytes show significantly increased expression of *C3* and *GBP2* after cytokine stimulation with TNF-α, TNF-α + IL-1α, or TNF-α + IL-1β compared to the vehicle control (n=4). Significance was calculated using the Kruskal-Wallis test. Fold change was calculated relative to the H1 hPSC line and normalized to TBP. *: $p < 0.05$; N.S.: not significant. The bars show the mean and SEM, and each dot represents the results of experimental replicates.

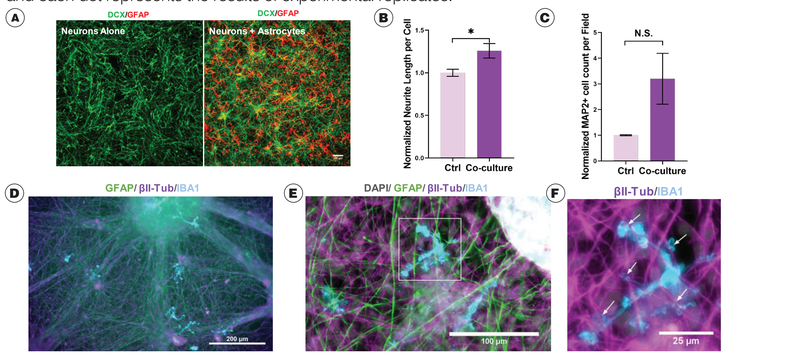

FIGURE 5. hPSC-Derived Astrocytes Can Be Cultured with hPSC-Derived Forebrain Neurons and Microglia for In Vitro Modeling

(A) Representative images of forebrain neurons cultured alone (left) or in a co-culture with astrocytes (right) stained with DCX for neurons (green) and GFAP for astrocytes (red). Scale bar = 100 µm.
(B&C) Statistical data of **(B)** neurite length per cell and **(C)** MAP2-positive (mature neuron) cell count in the control (neuron only) and co-culture group. *: $p < 0.05$; N.S.: not significant; Ctrl: n=4; Co-culture: n=5. The bars show the mean and SEM. **(D-F)** Representative images of tri-cultures stained with IBA1 (cyan), GFAP (green), and βIII-Tub (magenta) taken at **(D)** 20X and **(E&F)** 63X. **(E&F)** The microglia integrate among the astrocytes and neurons and display an unactivated ramified morphology with extended processes. 21-day-old tri-culture displays multiple somatic junctions between a microglial cell and neighboring neurons labeled with white arrows. Scale bars = **(D)** 200 µm, **(E)** 100 µm, and **(F)** 25 µm.

Summary

- The STEMdiff™ astrocyte system can generate functional hPSC-derived astrocytes
- Neural cells generated using STEMdiff™ Astrocyte, Forebrain Neuron, and Microglia kits can be used together for co-culture models