New Tools for the Generation of Differentiated CNS Cell Types from Human Pluripotent Stem Cells: STEMdiff™ Neuron, Dopaminergic Neuron and Astrocyte Kits

Vivian M. Lee, Leon Chew, Justin Yoon, Sam Lloyd-Burlan, Allen C. Eaves**, Terry E. Thomas, and Sharon A. Lees*
*STEMCELL Technologies Inc., Vancouver BC, Canada; **Terry Fox Laboratory, BC Cancer Agency, Vancouver BC, Canada

Abstract

Mature neural cell lineages derived from human pluripotent stem cells (hPSCs) are important in vitro tools for modeling human central nervous system (CNS) development and disorders. However, most published differentiation protocols for generating neurons and glial cells are often inefficient and difficult to reproduce. We have previously demonstrated that STEMdiff™ Neuronal Induction Medium (NIM) and AggreWell™-800 can be used to consistently produce CNS-type neuronal progenitor cells (NPCs) from multiple hPSC lines at high efficiencies. Here we present data demonstrating downstream differentiation protocols for generating mature neurons, dopaminergic neurons or astrocytes, using six new STEMdiff™ kits, each consisting of a basal medium and supplements. To generate each mature neural cell type from hPSC-derived NPCs, we employ a two-step protocol employing lineage-specific STEMdiff™ Differentiation Kits followed by STEMdiff™ Maturation Kits. NPCs were generated from hPSCs with the standard STEMdiff™ NIM embryoid body protocol and subjected to the specific differentiation procedures described below. For generating mature neurons or dopaminergic neurons, STEMdiff™ Neuron Differentiation Medium or STEMdiff™ Dopaminergic Neuron Differentiation Medium, respectively, was applied after re-plating and selection of neural rosettes. After 7 days (mixed neurons) or 10-13 days (dopaminergic neurons), the medium was removed and replaced with STEMdiff™ Neuron Maturation Medium or STEMdiff™ Dopaminergic Neuron Maturation Medium. We obtained 90–95% Tyro-1+ cells (n=2) using the STEMdiff™ Neuron Kits, and 15–20% tyrosine hydroxylase-positive dopaminergic neurons with the STEMdiff™ Dopaminergic Neuron Kits (n=2). For differentiating hPSC-derived NPCs to astrocytes, STEMdiff™ Astrocyte Differentiation Medium was applied after neural rosette selection, for 3 weeks. The differentiation medium was then removed and replaced with STEMdiff™ Astrocyte Maturation Medium to mature astrocytes over 30–60 days. The majority of the cells in the differentiating STEMdiff™ Astrocyte Differentiation Medium (2D and 3D) exhibited star-shaped astrocytic morphology. In summary, our results demonstrate that the six new STEMdiff™ Differentiation and Maturation Kits promote highly efficient differentiation of hPSC-derived NPCs into mixed neurons, dopaminergic neurons and astrocytes. These new STEMdiff™ kits complement our existing STEMdiff™ Neural System workflow and provide an integrated solution for modeling neurological diseases using hPSCs.

Overview of the STEMdiff™ Neural Workflow

The STEMdiff™ Neural System is a set of tools specifically designed to support research on human pluripotent stem cells (hPSCs) differentiation to neural cells. We have previously described the generation, expansion, differentiation, characterization and cryopreservation of neural progenitor cells (NPCs) from hPSCs. Here we present new tools for downstream differentiation of hPSCs to NPC neuronal subtypes and astrocytes and provide robust protocols that enable researchers to efficiently and reproducibly generate these cell lineages.

Summary

6 new STEMdiff™ Neuron kits for differentiation of hPSC-NPCs into neuronal subtypes and astrocytes.
- Optimized for the differentiation of NPCs generated using the STEMdiff™ Neuronal Induction Medium embryoid body protocol.
- Integrate seamlessly into the hPSC to Neural differentiation workflow.
- Enable reproducible generation of mature neural cell types from multiple human ES and iPS cell lines.

Generation of Mature Neurons from hPSCs

A two-step protocol is employed for the production of mature mixed neurons from hPSCs. NPCs maintained in mTeSR™ were used to generate embryoid bodies (EBs) and cultured in STEMdiff™ Neuronal Induction Medium (NIM), EBs were harvested and placed on day 5 to form colonies containing neural rosettes. Neural rosettes were isolated (day 11) and replated in STEMdiff™ NIM. One day after rosette selection (day 12), STEMdiff™ NIM was replaced with STEMdiff™ Neuron Maturation Medium (NMM) and left in culture for 7 days. Tyro-1+ cells were then passaged and seeded in STEMdiff™ Neuron Maturation Medium (NMM); hPSC-neurons were cultured in STEMdiff™ NMM for 14 days or longer for neuronal maturation.

Generation of Mature Dopaminergic Neurons from hPSCs

A two-step protocol is employed for the production of mature dopaminergic neurons from hPSCs. NPCs maintained in mTeSR™ were used to generate embryoid bodies (EBs) and cultured in STEMdiff™ Neuronal Induction Medium (NIM), EBs were harvested and placed on day 5 to form colonies containing neural rosettes. Neural rosettes were isolated (day 11-12) and replated in STEMdiff™ NIM. One day after rosette selection (day 12), STEMdiff™ NIM was replaced with STEMdiff™ Dopaminergic Neuron Induction Medium (DINM) until rosette selection. Neural rosettes were isolated (day 11-12) and replated in STEMdiff™ DNM for another 7 days. Cells were then passaged (day 18-19) and replated in STEMdiff™ Dopaminergic Neuron Maturation Medium (DMM) for 5 days. Next, STEMdiff™ DINM was removed and replaced by STEMdiff™ Dopaminergic Neuron Maturation Medium 2 (DMM 2); neurons were cultured for 14 days or longer in DMM for maturation.

Generation of Mature Astrocytes from hPSCs

A two-step protocol is employed for the production of astrocytes from hPSCs, NPCs maintained in mTeSR™ were used to generate embryoid bodies (EBs) and cultured in STEMdiff™ Astrocyte Differentiation Medium (ADM) one day after rosette selection (day 12-13) and kept in culture for an additional 21 days; cells were passaged twice during this period when cultures were confluent (~7 days between passages). Either on day 32 or 39, cells were passaged and plated in STEMdiff™ Astrocyte Maturation Medium (AMM). Astrocytes were cultured in this medium for up to 14 days or longer. A longer maturation period is desired, cells were passaged and replated in STEMdiff™ AMM when cultures were confluent (usually every 6-7 days).

Table 1: Quantitative RT-PCR analysis of hPSC-derived mature neurons after 14 days in STEMdiff™ Neuron Maturation Medium. Transcripts for TuJ1 (green, red and blue), NeuN (green) and the glutamatergic neuron marker VGLUT1 (green, red and blue) were normalized to untransfected neural progenitor cells and shown as fold changes. Neurons express glutaminergic neuron marker VGLUT1, NeuN also expresses a neuronal type marker NeuN and is negative for astrocytic markers GLAST and ALDH1L1.

Table 2: Quantitative RT-PCR analysis of hPSC-derived dopaminergic neurons after 14 days in STEMdiff™ Dopaminergic Neuron Maturation Medium. Transcripts were normalized to untransfected neural progenitor cells and shown as fold changes. Neurons express dopaminergic neuron markers TH and VMAT2. Neurons also express the Tyro-1+ marker (green) and VGLUT1 (blue) markers. Similar results were obtained using hPSCs (data not shown).

Table 3: Quantitative RT-PCR analysis of hPSC-derived astrocytes after 56 days in STEMdiff™ Astrocyte Maturation Medium. Transcripts were normalized to untransfected neural progenitor cells and shown as fold changes. Astrocytes express astrocyte markers S100 beta, GLAST1 and ALDH1L1. Astrocytes did not express neuronal lineage markers, ASCL1, NeuN1 and NeuN2.

Figure 1: Schematic for the generation of mixed neurons from hPSCs. A two-step protocol is employed for the production of mature mixed neurons from hPSCs. NPCs maintained in mTeSR™ were used to generate embryoid bodies (EBs) and cultured in STEMdiff™ Neuronal Induction Medium (NIM), EBs were harvested and placed on day 5 to form colonies containing neural rosettes. Neural rosettes were isolated (day 11) and replated in STEMdiff™ NIM. One day after rosette selection (day 12), STEMdiff™ NIM was replaced with STEMdiff™ Neuron Maturation Medium (NMM). After 14 days or longer for neuronal maturation, Tyro-1+ cells were then passaged and seeded in STEMdiff™ Neuron Maturation Medium (NMM); hPSC-neurons were cultured in STEMdiff™ NMM for 14 days or longer for neuronal maturation.

Figure 2: Morphology and marker expression of hPSC-derived mature neurons after 14 days in STEMdiff™ Neuron Maturation Medium. Neurons displayed Tyro-1+ (green, red and blue), TH (green), VMAT2 (green, red and blue) and the glutaminergic neuron marker VGLUT1 (green, red and blue). These neurons also express the neurotransmitter GABA (green, blue). Scale bars in all panels = 100 μm.

Figure 3: Schematic for the generation of dopaminergic neurons from hPSCs. A two-step protocol is employed for the production of mature dopaminergic neurons from hPSCs. NPCs maintained in mTeSR™ were used to generate embryoid bodies (EBs) and cultured in STEMdiff™ Neuronal Induction Medium (NIM), EBs were harvested and placed on day 5 to form colonies containing neural rosettes. Neural rosettes were isolated (day 11) and replated in STEMdiff™ NIM. One day after rosette selection (day 12), STEMdiff™ NIM was replaced with STEMdiff™ Dopaminergic Neuron Induction Medium (DINM) until rosette selection. Neural rosettes were isolated (day 11-12) and replated in STEMdiff™ DNM for another 7 days. Cells were then passaged (day 18-19) and replated in STEMdiff™ Dopaminergic Neuron Maturation Medium (DMM) for 5 days. Next, STEMdiff™ DINM was removed and replaced by STEMdiff™ Dopaminergic Neuron Maturation Medium 2 (DMM 2); neurons were cultured for 14 days or longer in DMM for maturation.

Figure 4: Morphology and marker expression of hPSC-derived dopaminergic neurons. A Phase-contrast image of hPSC-derived dopaminergic neurons after 14 days in STEMdiff™ Dopaminergic Neuron Maturation Medium. Neurons displayed typical neuronal morphology and astrocytic markers TH and VMAT2. Neurons produced high purple (98% TuJ1+; red) and express tyrosine hydroxylase (TH, green). The same image (B) showing the TH (green) and DAPI (blue) channels. Similar results were obtained using hPSCs (data not shown). Scale bars in all panels = 100 μm.

Figure 5: Schematic for the generation of astrocytes from hPSCs. A two-step protocol is employed for the production of astrocytes from hPSCs. NPCs maintained in mTeSR™ were used to generate embryoid bodies (EBs) and cultured in STEMdiff™ Neuronal Induction Medium (NIM), EBs were harvested and placed on day 5 to form colonies containing neural rosettes. Neural rosettes were isolated and replated in STEMdiff™ NIM on day 11-12, STEMdiff™ NIM was removed and replaced with STEMdiff™ Astrocyte Differentiation Medium (ADM) one day after rosette selection (day 12-13) and kept in culture for an additional 21 days; cells were passaged twice during this period when cultures were confluent (~7 days between passages). Either on day 32 or 39, cells were passaged and plated in STEMdiff™ Astrocyte Maturation Medium (AMM). Astrocytes were cultured in this medium for up to 14 days or longer. A longer maturation period is desired, cells were passaged and replated in STEMdiff™ AMM when cultures were confluent (usually every 6-7 days).