Rapid, High-Efficiency Differentiation of Motor Neurons from Human Pluripotent Stem Cells

Jin-Yuan Wang[1], Jeanne Chan[1], Allen C. Eaves[1,2], Sharon A. Louis[1], and Erin Knock[1,3]

[1]STEMCELL Technologies Inc., Vancouver BC, Canada; [2] Terry Fox Laboratory, BC Cancer, Vancouver BC, Canada; [3] Simon Fraser University, Vancouver BC, Canada

INTRODUCTION

Human motor neuron (MN) diseases include devastating disorders such as amyotrophic lateral sclerosis (ALS) and spinal muscular atrophy (SMA). A reliable human MN model is critical to uncover disease mechanisms. Therefore, we developed the STEMdiff™ Motor Neuron kits for in vitro modeling. Our data show that the STEMdiff™ Motor Neuron System can generate a highly pure population of motor neurons from human pluripotent stem cells (hPSCs) in two weeks. These hPSC-derived motor neurons can be further matured in maturation medium and cultured with hPSC-derived myotubes generated by the STEMdiff™/MyoCult™ Myotube System. We also found that these motor neurons can be cultured with hPSC-derived microglia generated by the STEMdiff™ Microglia kits. Taken together, our STEMdiff™ Motor Neuron System enables various in vitro models for the study of motor neuron diseases.

METHODS

Motor Neuron Differentiation: hPSCs maintained in either mTeSR™1 or mTeSR™ Plus were aggregated to embryoid bodies (EBs) with STEMdiff™ Motor Neuron Differentiation Kit in an ultra-low attachment or an AggreWell™400 plate (Figure 1). On day 9, EBs were dissociated into single cells and replated for adherent culture. On day 14, the cells were either matured using the STEMdiff™ Motor Neuron Maturation Kit or assessed by immunocytochemistry and qPCR for the indicated markers.

hPSC-Derived Motor Neuron and Myotube Co-Cultures: hPSC-derived myotubes were generated sequentially using STEMdiff™ Myogenic Progenitor Supplement Kit, MyoCult™-SF Expansion Supplement Kit, and MyoCult™ Differentiation Kit, as directed by their respective workflows. hPSC-derived motor neurons were then seeded at 2.6 x 10^4 cells/cm^2 on top of hPSC-derived myotubes in STEMdiff™ Motor Neuron Maturation Medium.

hPSC-Derived Motor Neuron and Microglia Co-Cultures: hPSC-derived microglia were generated using STEMdiff™ Microglia Differentiation Kit for 24 days, as directed by the kit workflow. Microglia were then seeded at a 1:2 microglia:neuron ratio in BrainPhys™ hPSC Neuron Medium with STEMdiff™ Microglia Supplement 2.

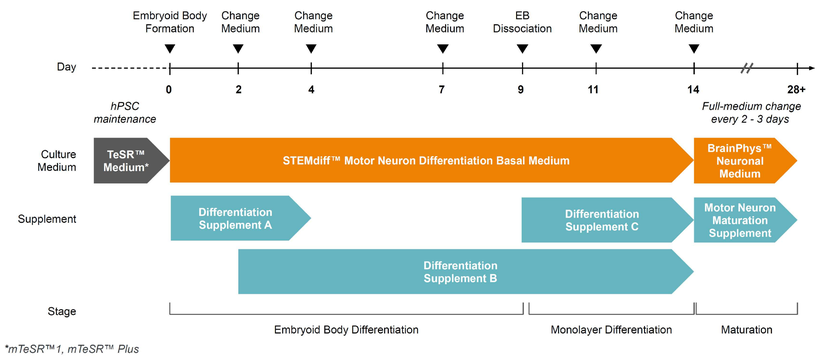

FIGURE 1. Workflow for hPSC-Derived Motor Neuron Differentiation and Maturation

RESULTS

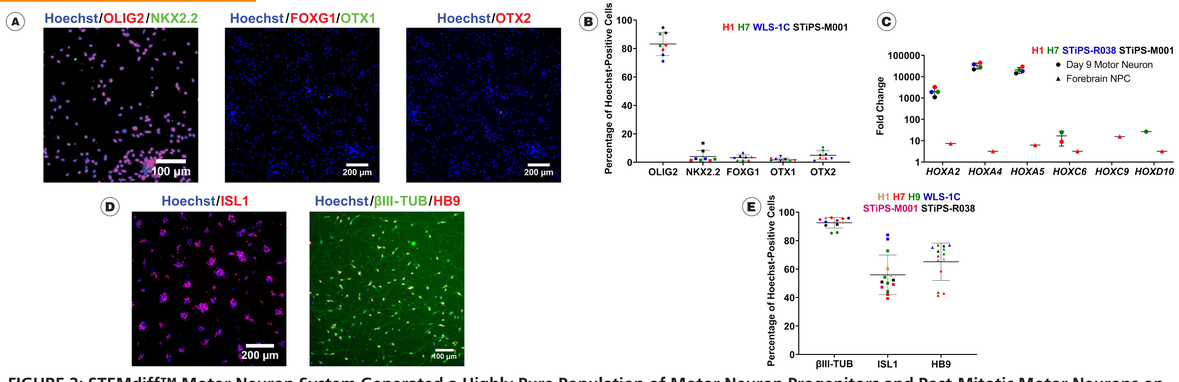

FIGURE 2: STEMdiff™ Motor Neuron System Generated a Highly Pure Population of Motor Neuron Progenitors and Post-Mitotic Motor Neurons on Day 9 and Day 14

(A) Representative images of day 9 motor neuron progenitors stained with Hoechst, OLIG2, NKX2.2, FOXG1, OTX1, and OTX2. **(B)** Quantification of **(A)** showed > 60% OLIG2 positive cells and < 10% NKX2.2, FOXG1, OTX1, OTX2 positive cells in the culture on day 9 (n = 4). **(C)** qPCR analysis of day 9 motor neuron progenitors showed high expression of HOXA5 (n = 4). hPSC-derived forebrain progenitor cells (forebrain NPCs) were used as control. **(D)** Representative images of day 14 post-mitotic motor neurons stained with Hoechst, class III β-tubulin (βIII-TUB), insulin gene enhancer protein 1 (ISL1), and motor neuron and pancreas homeobox 1 (HB9). **(E)** Quantification of **(D)** showed > 80% βIII-TUB-positive cells and > 40% ISL1- or HB9-positive cells in the culture on day 14 (n = 6).

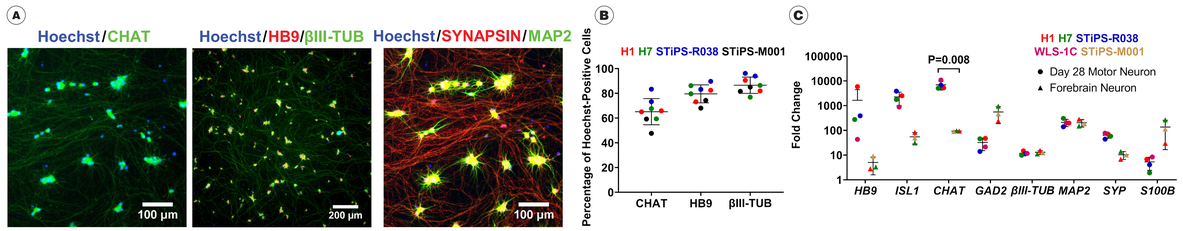

FIGURE 3: hPSC-Derived Motor Neurons Can be Further Matured in STEMdiff™ Motor Neuron Maturation Medium

(A) Representative images of motor neurons after 14 days in STEMdiff™ Motor Neuron Maturation Medium. Cells were stained with Hoechst, CHAT, HB9, βIII-TUB, SYNAPSIN, and MAP2. **(B)** Quantification of **(A)** showed > 80% βIII-TUB-positive cells and > 40% CHAT- or HB9-positive cells in the culture after 14 days in the maturation medium (n = 4). **(C)** qPCR analysis of cells after 14 days in the maturation medium. We used hPSC-derived forebrain neurons as the control. CHAT expression was significantly higher in the hPSC-derived motor neurons compared to forebrain neurons (motor neuron: n = 4, forebrain neuron: n = 3, P = 0.008, unpaired t-test).

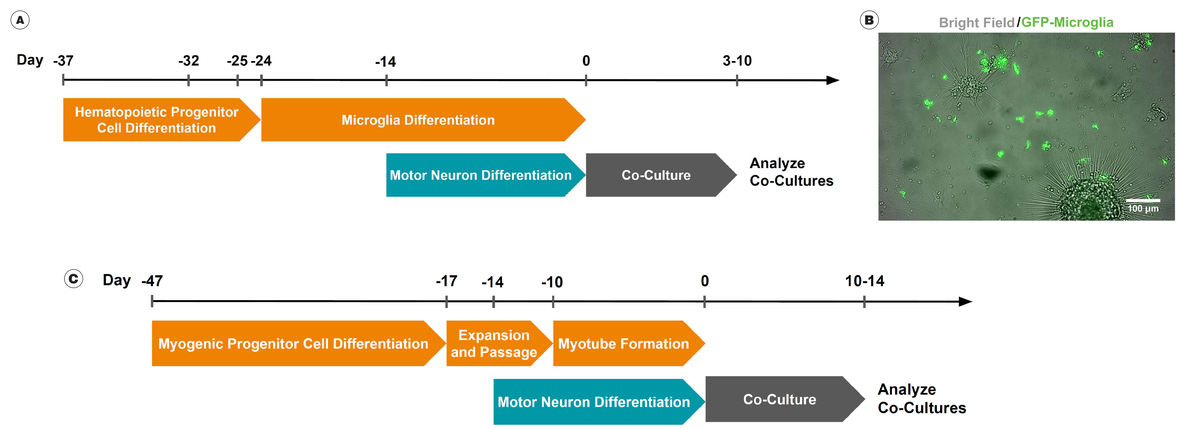

FIGURE 4: hPSC-Derived Motor Neurons can be Cultured with hPSC-Derived Myotubes and Microglia for in Vitro Modeling

(A) Workflow for co-culture of hPSC-derived motor neuron and microglia. **(B)** Representative image of motor neuron-microglia co-culture. The microglia were derived from a GFP-positive cell line. **(C)** Workflow for co-culture of hPSC-derived motor neuron and myotube. **(D)** Representative images of motor neuron-myotube co-culture stained with ISL1 (blue), AChR (green), and βIII-TUB (red). Arrows indicate the in vitro neuromuscular junctions.

Summary

- The STEMdiff™ Motor Neuron System can generate highly purified hPSC-derived motor neurons in two weeks.
- STEMdiff™ Motor Neuron kits can be used together with STEMdiff™ Microglia kits or the STEMdiff™/MyoCult™ Myotube System to generate these relevant cell types for in vitro co-culture models.