The Downstream Differentiation Potential Of Human Induced Pluripotent Stem Cell-derived Neural Progenitor Cells To Forebrain Neurons And Astrocytes

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INTRODUCTION

Neural progenitor cells (NPCs) generated from human pluripotent stem cells (hPSCs) are used extensively for studying human nervous system development, modeling neurological disorders, and screening for therapeutic molecules. NPCs are characterized by their capacity to expand and generate the major differentiated cell types of the central nervous system (CNS), such as neurons and astrocytes. Cryopreserved CNS-type NPCs can serve as a reproducible starting point and provide flexibility and consistency for downstream differentiation. We have developed a protocol to scale up production of highly pure human induced pluripotent stem cell (hiPSC)-derived NPCs from the hiPSC SCTi003-A cell line, using the STEMdiff™ SMADi Neural Induction Kit. Thawed SCTi003-A-derived NPCs maintained in STEMdiff™ Neural Progenitor Medium can be expanded for several passages while retaining NPC phenotype. As expected, NPCs differentiated immediately after thaw towards forebrain neurons displayed a highly pure population of neurogenic cells, and steadily displayed an increase in off-target gliogenic cell population after each passage. Conversely, gliogenic differentiation to astrocytes immediately after thaw resulted in a pure population of astrocytes, which was consistent for up to 10 passages. Overall, we have generated highly pure, expandable, and multipotent hiPSC-derived NPCs suitable for large-scale neural research and screening applications, and investigated their downstream differentiation potential over time to assess the acceptable windows to successfully generate desired neurogenic or gliogenic derivatives.

METHODS

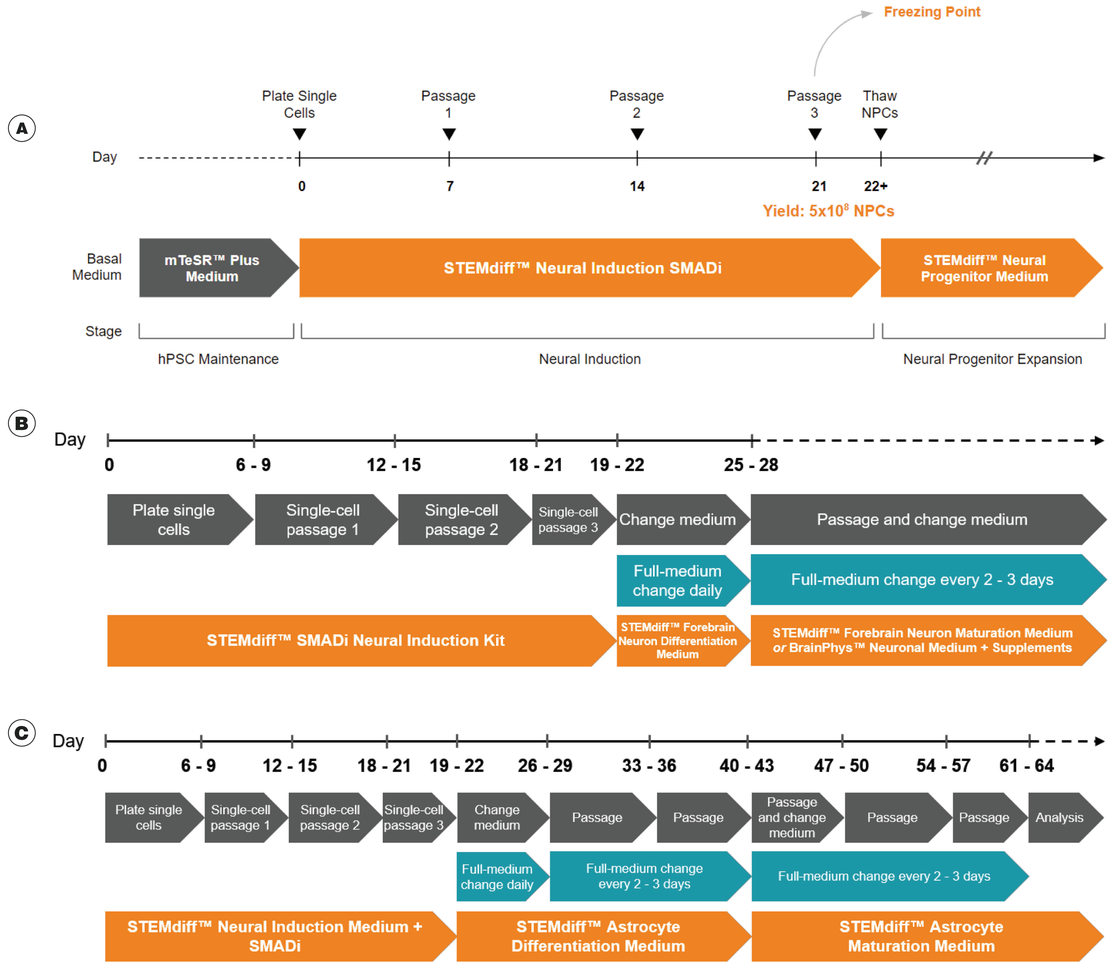

FIGURE 1. STEMdiff™ Protocol Workflows

(A) STEMdiff™ Neural Induction Monolayer Protocol. SCTi003-A cells maintained in mTeSR™ Plus were harvested and plated as single cells at 2 x 10^5 cells/cm2 in STEMdiff™ Neural Induction Medium with daily full-medium changes. On days 7 and 14 of expansion, cultures were passaged and plated down into the same conditions in T-75 flasks for scale-up. The fold-expansion per passage results in a final output of over 27 NPCs per input PSC, resulting in over 5 x 10^6 NPCs per batch. On day 21, cultures were passaged as single cells and cryopreserved for future use in downstream applications.
(B) STEMdiff™ Forebrain Neuron Protocol. Freshly thawed or passaged SCTi003-A-derived NPCs were plated in STEMdiff™ Forebrain Neuron Differentiation Medium as single cells at 1.5 x 10^5 cells/cm^2 with daily full-medium changes. On day 7, cultures were passaged and seeded into STEMdiff™ Forebrain Neuron Maturation Medium as single cells at 8 x 10^4 cells/cm^2 with half-medium changes every 2 - 3 days for an additional 14 days.
(C) STEMdiff™ Astrocyte Protocol. Freshly thawed or passaged SCTi003-A-derived NPCs were plated into STEMdiff ™ Forebrain Astrocyte Differentiation Medium as single cells at 1.5 x 10^5 cells/cm^2 with daily full-medium changes. On days 7 and 14, cultures were harvested and cultured in the same conditions. On day 21, cultures were passaged and plated down into STEMdiff™ Astrocyte Maturation Medium as single cells at 1.5 x 10^5 cells/cm^2 with full-medium changes every 2 - 3 days for an additional 3 passages.

NPC differentiation, freezing, and expansion: SCTi003-A cells were harvested from mTeSR™ Plus cultures and plated at 200,000 cells/cm^2 in STEMdiff™ Neural Induction Medium + SMADi + 10 µM Y-27632 onto human embryonic stem cell (hESC)-qualified Corning® Matrigel®-coated plates. On days 7 and 14 of expansion, cultures were passaged and plated down at 200,000 cells/cm^2 onto new Matrigel®-coated plates/flasks. The fold-expansion per passage results in a final output of over 27 NPCs per input hPSC. On day 21, cultures were passaged and cryopreserved at a density of 1.3 x 10^6 cells/mL in 1 mL of STEMdiff™ Neural Progenitor Freezing Medium and stored at -196°C for ≥ 24 hours before thawing. This process is reproducible and has been reliably performed for at least 3 independent batches. The thawed SCTi003-A-derived NPCs were plated in complete STEMdiff™ Neural Progenitor Medium and further expanded for up to 10 passages. Cultures were fixed and characterized by immunostaining for neural progenitor markers SOX1, PAX6, and neural marker βIIITUB on day 22 (post-thaw) as well as after 5 passages in STEMdiff™ Neural Progenitor Medium.
Forebrain and astrocyte differentiation: Cryopreserved SCTi003-A-derived NPCs were thawed and plated into either STEMdiff™ Astrocyte Differentiation Medium or STEMdiff™ Forebrain Neuron Differentiation Medium and cultured following their respective workflows and maturation mediums. Astrocyte and forebrain neuron cell identity was confirmed through immunocytochemistry staining for glial markers S100 calcium-binding protein B (S100B) and glial fibrillary acidic protein (GFAP), or neurogenic markers class III β-tubulin (βIIITUB) and doublecortin (DCX) on day 14 in STEMdiff™ Forebrain Neuron Maturation Medium or day 28 in STEMdiff™ Astrocyte Maturation Medium. ICC images were quantified using ImageXpress® Micro Confocal system.

RESULTS

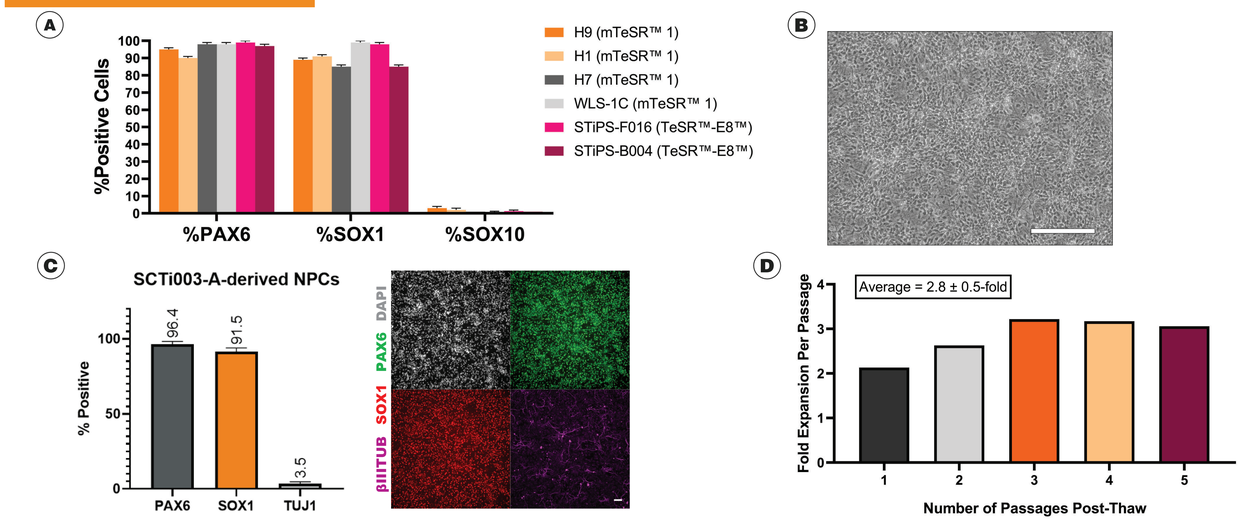

FIGURE 2. SCTi003-A Cells Were Able to Differentiate into Neural Progenitor Cells

(A) STEMdiff™ SMADi Neural Induction Kit typically generates NPCs with > 90% of cells expressing CNS-type markers PAX6 and SOX1 (n = 6 cell lines). **(B)** The resulting SCTi003-A-derived NPCs displayed the expected small, teardrop-shaped morphology. **(C)** The majority of SCTi003-A-derived NPCs expressed neural progenitor markers PAX6 (green; 96.4 ± 3.4%) and SOX1 (red; 91.5 ± 2.4%) , and very few cells expressed neuronal marker βIIITUB (violet, TUJ1; 3.5 ± 1.3%; mean ± SEM; n = 4). **(D)** On average, SCTi003-A-derived NPCs expanded by 2.8 ± 0.5-fold per passage (mean ± SEM), resulting in an overall fold increase of > 170-fold increases over 5 passages. Scale bars = 100 µm.

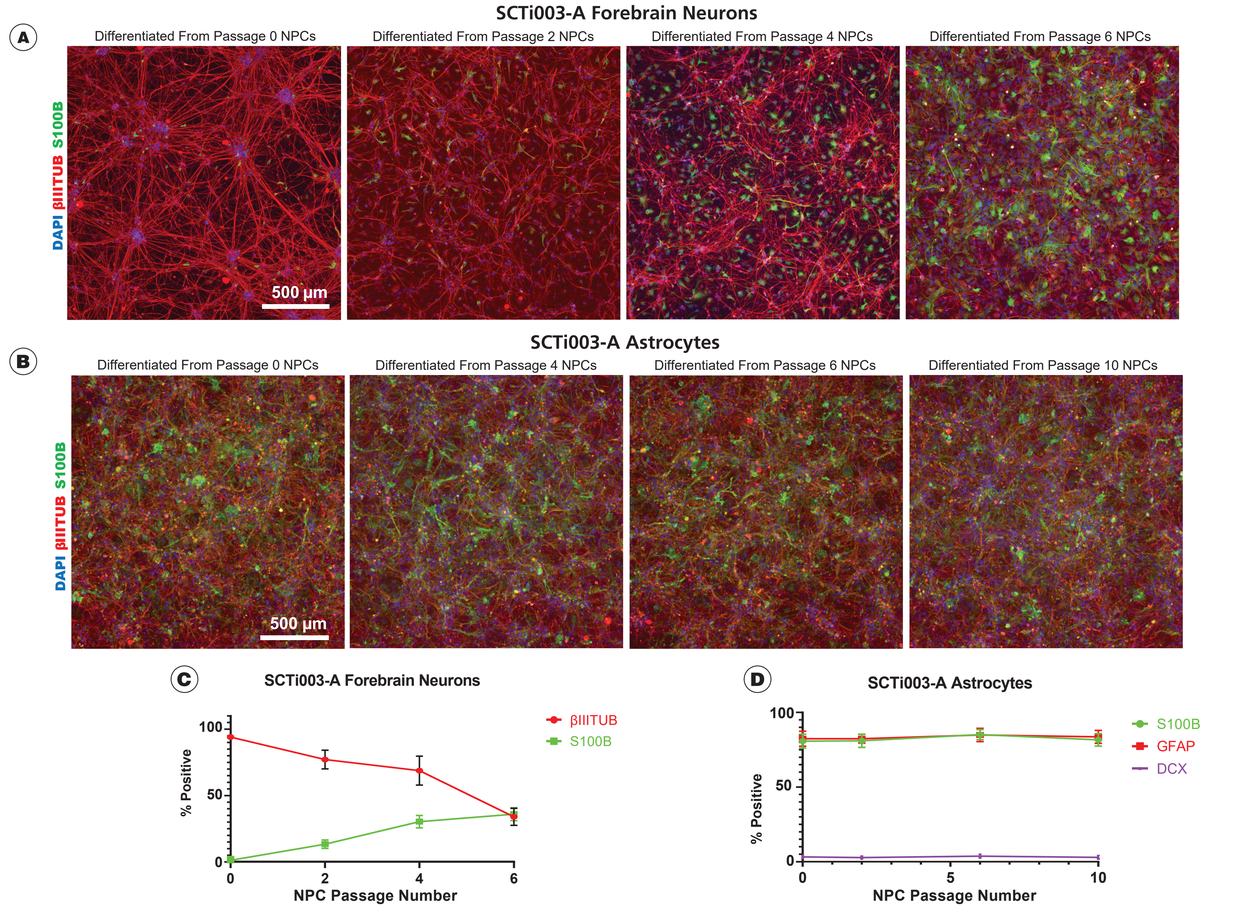

FIGURE 3. SCTi003-A-Derived NPC Differentiation Potential After Passaging Decreases to Forebrain Neurons, but Remains Consistent to Astrocytes

SCTi003-A-derived NPCs were differentiated either immediately after thawing (p0) or after several passages (p4, p6, and p10) to **(A)** forebrain neurons or **(B)** astrocytes using the STEMdiff™ Forebrain Neuron Kit or STEMdiff™ Astrocyte Kit, respectively. Fluorescence microscopy images display a decrease in neuronal differentiation capacity after increased NPC passage number, but maintaining consistent astrocyte differentiation capacity. **(C)** NPCs differentiated to forebrain neurons immediately after thaw displayed high neurogenic differentiation, as indicated by the high ratio of neuronal:glial marker expression (94.0 ± 0.8% βIIITUB+; 1.3 ± 0.7% S100B+; mean ± SEM; n = 4). The number of βIIITUB+ cells steadily declined after each passage (34.0 ± 6.4% after 6 passages), while the number of S100B+ cells increased (36.0 ± 4.9% after 6 passages; mean ± SEM; n = 4). **(D)** NPCs differentiated to astrocytes immediately after thaw resulted in a pure population of GFAP+ (82.4 ± 5.0%) and S100B+ cells (80.8 ± 4.6%), with very few cells expressing DCX (3.2 ± 1.1%; mean ± SEM; n = 4), which were retained for up to 10 passages.

Summary

- We have generated highly pure, expandable and multipotent hiPSC-derived NPCs suitable for large-scale neural research.
- SCTi003-A-derived NPCs express high levels of neural progenitor markers PAX6 and SOX1, and can be expanded for multiple passages for scale up.
- Forebrain neuron differentiation potential for SCTi003-A-derived NPCs is highest directly post-thaw, with differential potential decreasing steadily with each subsequent passage.
- Astrocyte differentiation potential remains high regardless of the number times the cells are passaged.
- Findings on differentiation potential of NPCs align with what is reported in the field.