Efficient Differentiation of Human Pluripotent Stem Cells to Neural Crest Cells

Alym P. Moosa[1], Terry E. Thomas[1], Allen C. Eaves[1,2] and Sharon A. Louis[1], and Vivian M. Lee[1]

[1]STEMCELL Technologies Inc., Vancouver BC, Canada; [2]Terry Fox Laboratory, BC Cancer Agency, Vancouver BC, Canada

INTRODUCTION

Neural crest cells (NCCs) are multipotent stem cells that arise during vertebrate embryonic development. NCCs are formed at the neural plate border, then delaminate from the neural tube, migrate to various locations, and give rise to a wide array of derivatives including the craniofacial skeleton, peripheral and enteric nervous systems, pigment cells, as well as many other cell types and organs. Neural crest cell dysfunction can result in birth defects, for example cleft/lip palate and Hirschsprung's disease; furthermore, neuroblastomas and melanoma are cancers that originate from neural crest lineages. Using NCCs derived from human pluripotent stem cells (hPSCs) to model NCC development and diseases is valuable because obtaining human NCCs is very difficult. Here we describe the STEMdiff™ Neural Crest Differentiation Medium and protocol, which promote efficient and reproducible differentiation of hPSCs to multipotent, $SOX10^+CD271^+$ NCCs with low levels of neuroectodermal $PAX6^+$ cells.

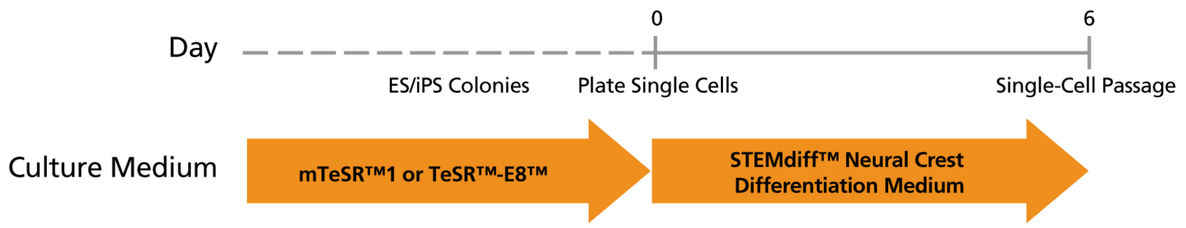

FIGURE 1. The STEMdiff™ Neural Crest Differentiation Kit Workflow
NCCs are produced after 6 days of culture in STEMdiff™ Neural Crest Differentiation Medium. Further expansion using single-cell passaging is possible for up to 3 passages using STEMdiff™ Neural Crest Differentiation Medium or Mesencult™ ACF-Plus Medium, depending on the desired downstream application.

METHODS

Neural Crest Differentiation: Undifferentiated hPSCs maintained in either mTeSR™1 (6 lines: 3 ES, 3 iPS) or TeSR™-E8™ (3 lines: 2 ES, 1 iPS) were dissociated and plated at 2 x 10^5 cells/cm^2 on Corning® Matrigel®-coated 24-well plates in STEMdiff™ Neural Crest Differentiation Medium containing 10 µM Rho-kinase inhibitor (Y-27632 or ROCKi) for one day, followed by daily full medium changes (without ROCKi). On day 6, differentiation was assessed by immunostaining for neural crest markers (SOX10, CD271(p75), TFAP2, HNK1(CD57), and FOXD3) and neuroectodermal marker PAX6.
Peripheral Neuron Differentiation: Peripheral neuron differentiation was induced by passaging neural crest cells at day 6 into conditions used in a published peripheral neuron differentiation protocol (1). Briefly, cells were cultured for 2 days with medium supplemented with 10 ng/mL FGF2 and 10 ng/mL EGF, then cultured for up to 14 days with medium supplemented with 10 ng/mL BDNF, 200 µM ascorbic acid, 10 ng/mL GDNF, 10 ng/mL NGF, 10 ng/mL NT-3, and 0.5 mM cAMP. Cultures were fixed on day 12 and characterized by immunochemistry.
Osteogenic and Chondrogenic Differentiation: Chondrocyte and osteoblast differentiation was induced by passaging neural crest cells at day 6 into MesenCult™-ACF Plus Medium. Cells were expanded in MesenCult™-ACF Plus Medium for 3 passages prior to differentiation using MesenCult™-ACF Chondrogenic Differentiation Kit or MesenCult™ Osteogenic Differentiation Kit (Human) as per the Product Information Sheets (available at www.stemcell.com). Cells were fixed at day 21 of chondrocyte differentiation and day 35 of osteoblast differentiation then stained.

RESULTS

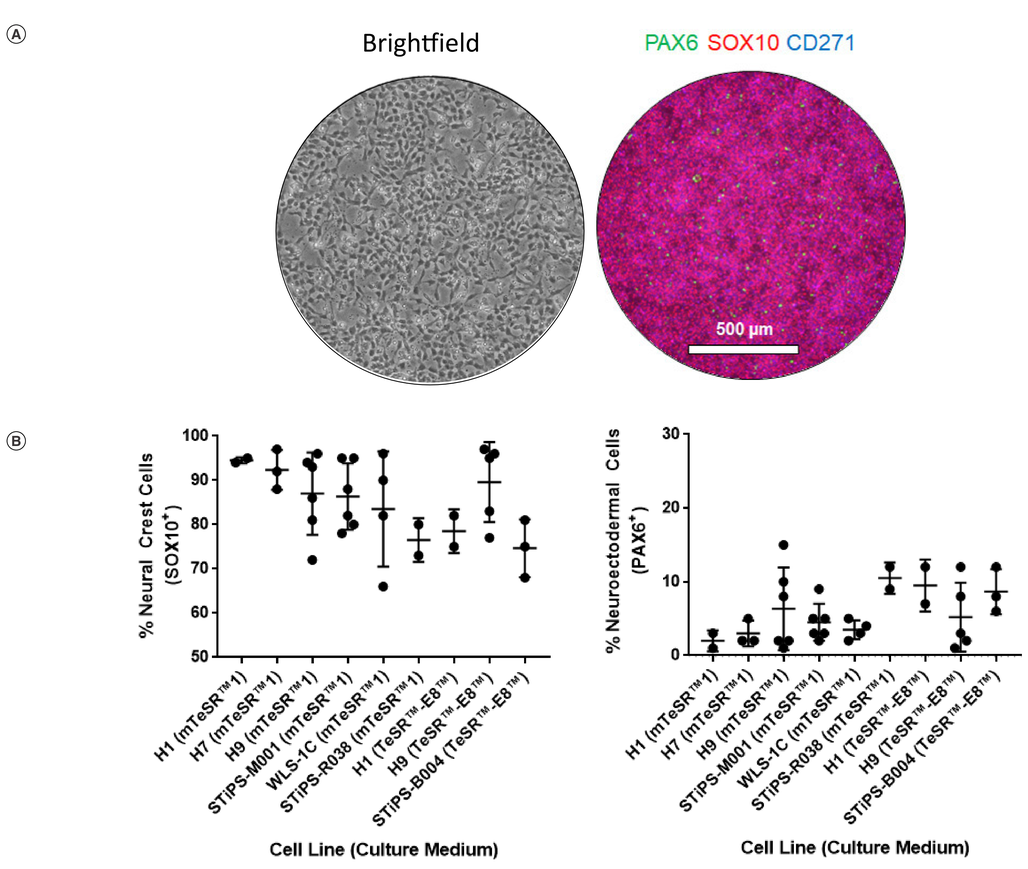

FIGURE 2. STEMdiff™ Neural Crest Differentiation Medium Promotes Neural Crest Differentiation with High Purity (> 70% SOX10+) Across Multiple ES and iPS Cell Lines
(A) Representative brightfield and immunocytochemistry images showing NCC morphology at day 6. The NCC cultures display a phase-dark morphology, are positive for neural crest markers SOX10 and CD271, and contain few $PAX6^+$ neuroectodermal cells. **(B)** Quantification of the percentages of $SOX10^+$ and $PAX6^+$ cells. Efficient conversion of hESC and hiPSC lines maintained in either mTeSR™1 or TeSR™-E8™ into $SOX10^+$ positive NCCs (85.5 ± 1.6%; mean ± SEM; n = 9) with very low levels of $PAX6^+$ neuroectodermal cells (5.6 ± 0.7%; mean ± SEM; n = 9) was observed. Numbers are % positive over total DAPI in a tiled image. Dots show the results of individual experiments.

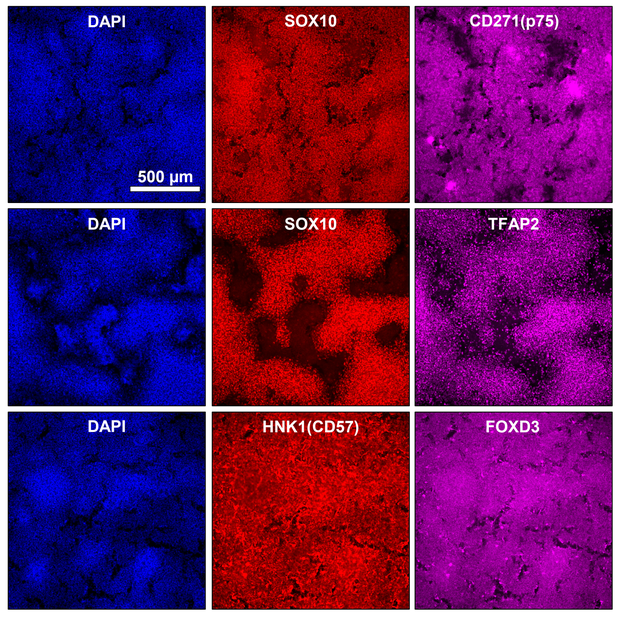

FIGURE 3. NCCs Derived Using STEMdiff™ Neural Crest Differentiation Medium Express Typical Neural Crest Markers
Differentiation was assessed by immunostaining for neural crest markers SOX10, CD271, TFAP2, HNK1, and FOXD3 at day 6. Representative 10X images are shown from the differentiation of the STiPS-M001 cell line cultured in mTeSR™1. Rows shown are the results of individual experiments.

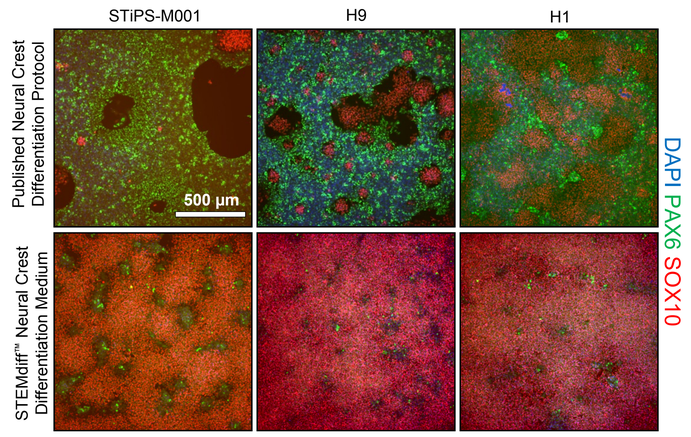

FIGURE 4. STEMdiff™ Neural Crest Differentiation Medium Results in More $SOX10^+$ NCCs and Fewer $PAX6^+$ Neuroectodermal Cells than Previously Published Protocols
Neural crest differentiation as described in Figure 1 was carried out using a published neural crest differentiation medium (top row) that relies on WNT pathway activation (3 µM CHIR) (2), or using the optimized STEMdiff™ Neural Crest Differentiation Medium (bottom row). Differentiation was assessed by immunostaining for SOX10 or PAX6 at day 6. Representative 10X images are shown for one iPS (STiPS-M001) and two ES (H9 & H1) cell lines. All hPSC lines were previously maintained in mTeSR™1.

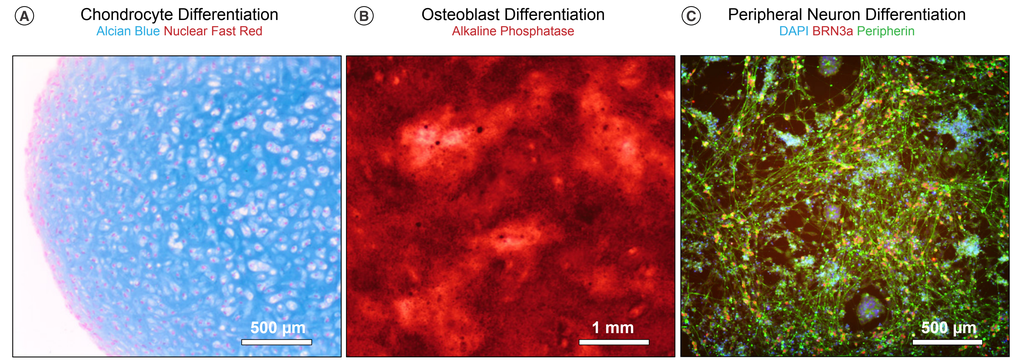

FIGURE 5. hPSC-Derived Neural Crest Cells are Multipotent and able to Differentiate into Chondrocytes, Osteoblasts, and Peripheral Neurons
(A) Representative 10X image of a chondrocyte pellet stained with Alcian blue/Nuclear Fast Red showing deposition of cartilage around the cells. A total of 4 hPSC lines were successfully differentiated to this lineage (STiPS-M001 is shown here). **(B)** Representative 4X image of an osteoblast culture showing high levels of alkaline phosphatase-positive mineral deposition. A total of 4 hPSC lines were successfully differentiated to this lineage (STiPS-M001 is shown here). **(C)** Representative 10X image of peripheral neurons after 12 days of differentiation. The peripheral neurons displayed the expected neuronal morphology with high peripherin (green) expression in the cell body and along the axons. A proportion of these cells also expressed BRN3a (red) in the nucleus (blue). A total of 6 hPSC lines were successfully differentiated to this lineage (STiPS-M001 is shown here).

Summary

STEMdiff™ Neural Crest Differentiation Medium:

- Efficiently generates $SOX10^+$, $CD271^+$, $TFAP2^+$, $HNK1(CD57)^+$, $FOXD3^+$ neural crest cells from hPSCs in 6 days with very low levels of PAX6+ neuroectodermal cells
- Converts multiple hESC and hiPSC lines maintained in either mTeSR™1 or TeSR™-E8™ into $SOX10^+$ neural crest cells
- Increases the efficiency of $SOX10^+$ neural crest cell generation compared to typical published methods[1,2]
- Generates neural crest cells that are multipotent and able to differentiate into downstream derivatives such as chondrocytes, osteoblasts, and peripheral neurons

References:
Lee G et al. (2010) Nat Protoc 5(4): 688–701.
Leung A et al. (2016) Development 143(3): 398–410.