Development of defined conditions for expansion and dopaminergic neuron differentiation of iPSC-derived neural progenitor cells

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Abstract

Human induced pluripotent stem cells (iPSCs) have the capacity to differentiate into all of the somatic cells types and hold great promise for both regenerative medicine and drug discovery. A need for better tools to address neurological disease modeling and neuro-toxicology screening exists. We have developed a scalable process that allows for the generation of large quantities of neural progenitor cells (NPCs) derived from normal and Parkinson's disease iPSC lines, along with a serum-free defined NPC expansion medium and dopaminergic differentiation medium.

To validate the process of NPC derivation, we generated NPCs derived from iPSCs that were reprogrammed with the Sendai virus from the following sources: human foreskin fibroblasts (HFF-1), human CD34$^+$ cells, and human fibroblasts from a patient with Parkinson's disease. Compared to Parkinson's disease patient-derived NPCs, both normal NPC lines demonstrated greater proliferative capacity. Moreover, our CD34$^+$ cell-derived NPCs possessed better tri-lineage differentiation efficiency than that of fibroblast-derived NPCs although all three types of NPCs were capable of differentiating into dopaminergic neurons, astrocytes, and oligodendrocytes.

To validate our NPC expansion and dopaminergic differentiation media, we tested three types of NPCs derived from iPSCs of various origins as described above as well as three lineage-specific reporter NPC lines (MAP2p-NanoLuc®-HaloTag®, DCXp-GFP, and GFAPp-NanoLuc®-HaloTag®) generated by targeting a NanoLuc®-HaloTag® or GFP construct to the C-terminus of the MAP2, DCX, or GFAP genes via zinc finger nucleases. Regarding morphology, proliferative capacity, and expression of NPC markers, the NPC expansion medium performed well for all six types of NPCs. Importantly, the expanded NPCs maintained the capacity to differentiate into dopaminergic neurons, astrocytes, and oligodendrocytes. Furthermore, the dopaminergic neuron differentiation medium was validated for the efficient differentiation of six types of NPCs into dopaminergic neurons. Through this study we have developed a portfolio of NPCs along with an NPC expansion medium and dopaminergic neuron differentiation medium. Collectively, these provide a complete solution for the expansion and differentiation of NPCs and comprise a powerful tool for neurodegenerative disease modeling and drug screening.

Methods

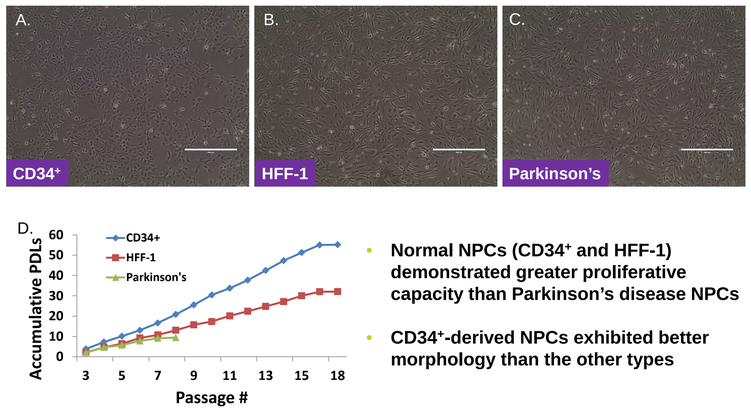
Derivation and expansion of NPCs from normal and Parkinson's disease iPSC lines

Normal iPSC lines derived from HFF-1 cells (ATCC® No. ACS-1019™), CD34$^+$ cells (ATCC® No. ACS-1031™), and Parkinson's disease cell line (ATCC® No. ACS-1013™) were expanded in iPSC culture medium (ATCC® No. ACS-3002) prior to the generation of embryoid bodies (EBs). NPCs were then derived from EBs cultured in defined neural induction media and expanded in serum-free NPC expansion medium prior to tri-lineage differentiation.

Tri-lineage differentiation of NPCs

Expanded NPCs were seeded on Cell Basement Membrane (ATCC® No. ACS-3035™)–coated plates and cultured with dopaminergic differentiation medium for 21 days with media changes 3 times per week prior to immunocytochemistry (ICC) analysis of Tuj1 and TH (Tyrosine Hydroxylase) expression. For astrocyte differentiation, NPCs were treated with astrocyte induction media (XCell Science) for 17 days and then cultured in astrocyte maturation media (XCell Science) for up to 45 days before ICC with GFAP antibodies. For oligodendrocyte differentiation, NPCs were treated with glial cell restrictive medium (GRM) containing NS21, bovine insulin, progesterone, putrescine, sodium selenite, human transferrin, T3, human EGF and retinoic acid for 10 days and finally treated with GRM in the absence of retinoic acid for up to 60 days before ICC with O4 antibodies.

Results



- **Normal NPCs (CD34$^+$ and HFF-1) demonstrated greater proliferative capacity than Parkinson's disease NPCs**
- **CD34$^+$-derived NPCs exhibited better morphology than the other types**

Figure 1. A-C) Morphology and D) growth curves of NPCs derived from CD34$^+$, HFF-1, or Parkinson's iPSC lines, respectively (10×).

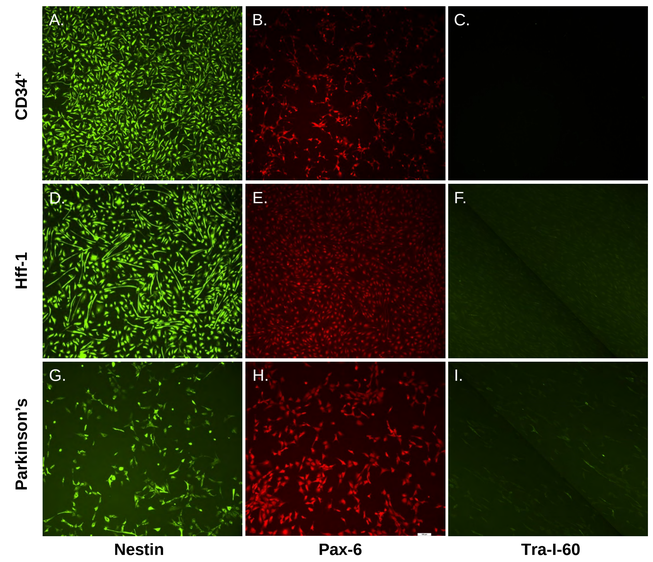


Figure 2. NPCs derived from A-C) CD34$^+$, D-F) HFF-1, or G-I) Parkinson's iPSC lines expressed Nestin and Pax-6 NPC markers and were negative for the Tra-I-60 iPSC marker (10x)

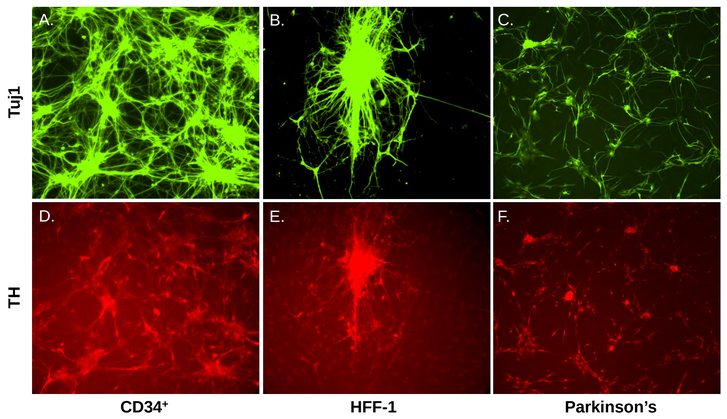


Figure 3. Dopaminergic neuron differentiation of NPCs derived from A and D) CD34$^+$, B and E) HFF-1, or C and F) Parkinson's iPSC lines (20x). CD34$^+$-derived NPCs possessed a higher capacity for differentiation into TH$^+$ dopaminergic neurons than HFF-1 and Parkinson's NPCs.

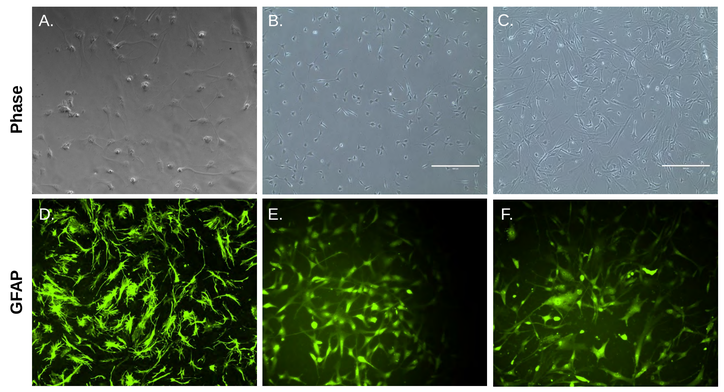


Figure 4. Astrocyte differentiation of NPCs derived from A and D) CD34$^+$, B and E) HFF-1, or C and F) Parkinson's iPSC lines (20x).

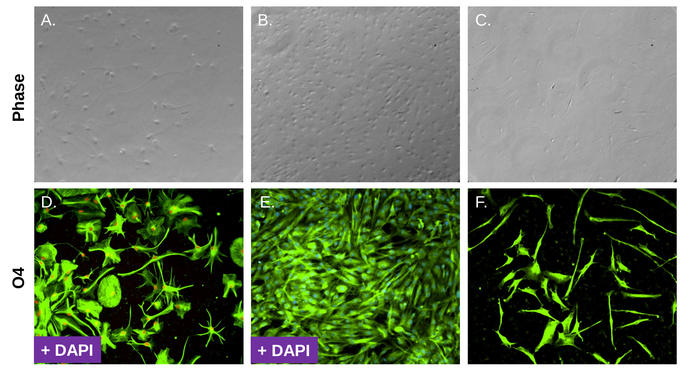


Figure 5. Oligodendrocyte differentiation of NPCs derived from A and D) CD34$^+$, B and E) HFF-1, or C and F) Parkinson's iPSC lines (20x).

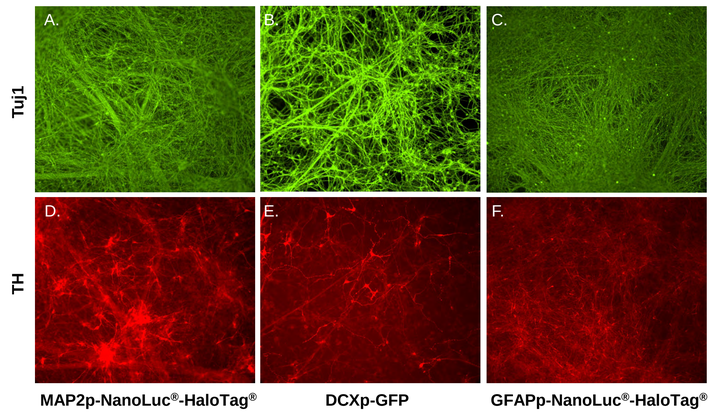


Figure 6. Dopaminergic neuron differentiation of A and D) MAP2p-NanoLuc®-HaloTag®, B and E) DCXp-GFP, and C and F) GFAPp-NanoLuc®-HaloTag® reporter lines (20x). All three reporter NPCs demonstrated high efficiency of differentiation into TH$^+$ dopaminergic neurons after treatment of dopaminergic neuron differentiation medium for 21 days.

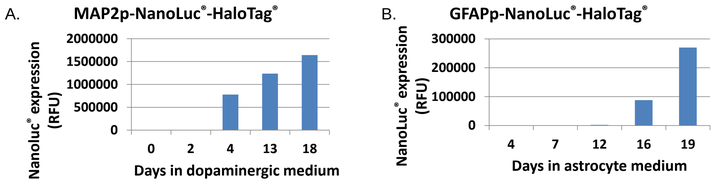


Figure 7. Expression of the luciferase reporter during A) dopaminergic or B) astrocyte differentiation of genome edited NPC reporter lines

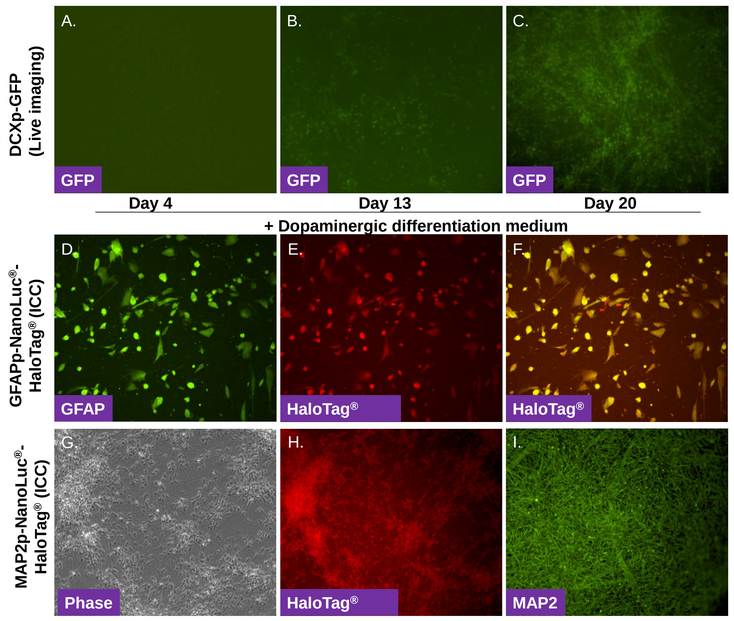


Figure 8. Expression of A-C) GFP or D-H) HaloTag® reporter during dopaminergic or astrocyte differentiation of DCXp-GFP, GFAPp-NanoLuc®-HaloTag®, or MAP2p-NanoLuc®-HaloTag® reporter lines (20x). Expression of GFP or HaloTag® in three NPC reporter lines was detected during directed differentiation into dopaminergic neurons or astrocytes.

Summary

- We have developed a process enabling the generation of an unlimited supply of normal or Parkinson's disease NPCs from ATCC iPSCs and have optimized culture conditions for the expansion and tri-lineage differentiation of NPCs.
- Starting materials of iPSCs play an important role in the morphology, proliferative capacity, and differentiation potential of NPCs.
- Compared to HFF-1 and Parkinson's fibroblast-derived NPCs, the CD34$^+$-derived NPCs exhibited a better proliferative capacity and greater efficiency of differentiation into dopaminergic neurons, astrocytes, and oligodendrocytes although fibroblasts-derived NPCs also had a potential to be differentiated into tri-lineages.
- Three NPC reporter lines generated by zinc finger nuclease technology expressed GFP, NanoLuc®, or HaloTag® during lineage specific differentiation. These NPCs could be used for monitoring neural differentiation processes in real-time.
- ATCC NPC expansion and dopaminergic differentiation media have been validated in six types of NPCs.
- This complete package of NPC products, which includes multiple types of NPCs and NPC maintenance and differentiation media, provides a powerful tool for disease modeling and drug screening