Comprehensive gene expression analysis and neurotoxicity testing of human iPSC-derived neural progenitor cells and neurons

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Abstract

Human induced pluripotent stem cells (iPSC)-derived neural progenitor cells (NPCs) and neurons are an attractive in vitro model to study neurological development, neurotoxicity and to model diseases. However, there is a lack of validated normal and Parkinson’s NPCs and media that support differentiation into multiple types of neurons for disease modeling, drug screening, and toxicity screening. Here, we investigated the expression of genes associated with the differentiation of NPCs during three weeks in dopaminergic differentiation media. Expression of both TuJ1 early neuron and TH dopaminergic neuronal genes was significantly increased in a time-dependent manner (p < 0.05) in three types of NPCs tested. Furthermore, expression of genes associated with glutamatergic (vGLUT1, vGLUT2), GABAergic (GABA) and motor neuronal markers was significantly increased in ACS-5003 and ACS-5007 NPCs during dopaminergic differentiation. To validate that our NPCs and dopaminergic neuronal differentiation media are suitable for drug screening, we conducted neurotoxicity screenings in three types of NPCs and NPCs-derived neurons using Flattest™ cell viability reagent (ATCC®-301014) assay and high content imaging analysis. We found that paclitaxel and vincristine significantly induced neurotoxicity (p < 0.001) in both ACS-5003 and ACS-5001 NPCs while piperine didn’t induce any significant neurotoxicity in all types of NPCs tested. Furthermore, amiodarone and chlorhexidine at 10 or 100 µM significantly decreased cell viability (p < 0.001) in both ACS-5003 and ACS-5001 NPCs. Cisplatin and hydrotetraxine at 100 µM significantly induced neurotoxicity in ACS-5003 NPCs, but not in ACS-5001 NPCs. Furthermore, amiodarone didn’t induce any significant neurotoxicity in ACS-5003 NPCs. The neurotoxic effect of paclitaxel was observed in both ACS-5003 and ACS-5001 NPCs in a time-dependent manner during neuronal differentiation. However, expression of GABA mRNA reached a peak at week 2 in ACS-5001 Parkinson’s NPCs (p < 0.05, ***p < 0.001 vs. day 0, Two-Way ANOVA).

Methods

Dopaminergic neuron differentiation: Normal human NPCs (ATCC® ACS-5007™) were seeded in CellMatrix Basement Membrane Gel (ATCC® ACS-3004™) and cultured in NPC expansion media (ATCC® ACS-3035™) overnight prior to treating NPCs with dopaminergic differentiation media (ATCC® ACS-3034™) for up to three weeks.

qRT-PCR: RNA was extracted from ACS-5003, ACS-5007, and ACS-5001 NPCs treated with the dopaminergic differentiation media for 0, 1, 2, 3 weeks. qRT-PCR analysis of neurotoxicity screening

Results

Figure 1. Early neural marker. TuJ1 mRNA increased significantly in ACS-5003, ACS-5007, and ACS-5001 NPCs at day 0 (p < 0.05, "p < 0.01, "p < 0.001, **p < 0.001, Two-Way ANOVA).

Figure 2. Dopaminergic neuronal marker. TH increased significantly and reached maximum level by the end of three weeks of differentiation in three types of NPCs (p = 0.05, "p < 0.01, ""p < 0.001 vs. day 0, Two-Way ANOVA).

Figure 3. Glutamatergic neuronal markers. mRNA encoding GLS2 and vGLUT2 increased significantly in time-dependent manner during neuronal differentiation in ACS-5003 and ACS-5007 NPCs. However, expression of GLS2 and vGLUT2 reached a peak at week 2 in ACS-5001 Parkinson’s NPCs (p < 0.05, ***p < 0.001 vs. day 0, Two-Way ANOVA).

Figure 4. GABAergic neuronal markers. mRNA encoding GABA significantly increased in ACS-5003 and ACS-5007 NPCs in a time dependent manner during dopaminergic differentiation. However, expression of GABA mRNA reached a peak at week 2 in ACS-5001 Parkinson’s NPCs (p < 0.05, ***p < 0.001 vs. day 0, Two-Way ANOVA).

Figure 5. Motor and cholinergic neuronal markers. LIMH mRNA increased significantly in ACS-5003 and ACS-5007 NPCs while CHAT mRNA increased significantly in ACS-5003 and ACS-5001 NPCs during dopaminergic differentiation (p < 0.05, "p < 0.01, **p < 0.001 vs. day 0, Two-Way ANOVA).

Figure 6. Immunocytochemistry using TuJ1, TH, GLS2, vGLUT2, and CHAT antibodies in ACS-5007 NPCs differentiated into neuronal cells using ACS-5000-5007 NPCs was seeded in Cell Matrix Basement Membrane Gel-coated 24-well plates, respectively, at a seeding density of 10,000 cells/cm² and treated with dopaminergic differentiation media for three weeks prior to adding 10 µM of paclitaxel, capsaicin, or chlorhexidine for two days.

Figure 7. Neuritotoxicity testing of ACS-5001 NPCs treated with paclitaxel, chlorhexidine, amiodarone, and chlorhexidine at 1, 10, or 100 µM for two days. Paclitaxel and vincristine at 1, 10, or 100 µM significantly induced neurotoxicity (p < 0.001) in both ACS-5003 and ACS-5001 NPCs. Furthermore, amiodarone and chlorhexidine at 10 or 100 µM also significantly decreased cell viability (p < 0.05) in both ACS-5003 and ACS-5001 NPCs. Cisplatin and hydrotetraxine at 100 µM significantly induced neurotoxicity in ACS-5003 NPCs, but not in ACS-5001 NPCs (p< 0.05, *p < 0.01, **p < 0.001 vs. DMSO control, Student’s T-test). Similar neurotoxicity was observed in ACS-5007 NPCs (data not shown).

Figure 8. Dose-response curves for cell viability of ACS-5003, ACS-5007 NPCs treated with capsaicin for two days. The neurotoxic effect of capsaicin was similar in both types of NPCs. IC50s of capsaicin in ACS-5003 and ACS-5007 NPCs was 0.9 µM and 1.6 µM, respectively (n=2).

Summary

• Human iPSC-derived NPCs and neurons are an appealing resource for in vitro disease modeling, toxicity screening and drug screening.
• ATCC has developed five types of normal non-reporter, normal lineage-specific GFP or Nanolux-HaloTag reporter NPCs.
• ATCC normal and Parkinson’s NPCs have the potential to be differentiated into dopaminergic, GABAergic, glutamatergic, motor, and cholinergic neurons after treatment with ATCC Dopaminergic Differentiation Media.
• ATCC NPCs and Parkinson’s NPCs have been validated for drug screening with several neurotoxins by using a viability assay and high content imaging assay.
• Paclitaxel is very toxic to NPCs with an IC50 of <1 µM, but not in NPC-derived neurons.
• ATCC NPCs, NPC Expansion Media, and Dopaminergic Differentiation Media are suitable for studying neurological development and neurotoxicity screening.
• For a complete list of NPCs and other media types, please visit the ATCC website (www.atcc.org/neuro).

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