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 cell (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 NPC lines 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 (ATCC® ACS-3004™). Known early neuron markers, MAP2 and Tuj1 genes reached peak expression at two weeks while expression of dopaminergic neuronal genes (TH, Nurr1, VMAT2, AADC) was significantly increased in a time-dependent manner ($p < 0.05$) in two types of normal NPCs (ATCC® ACS-5003™ and ACS-5007™). Furthermore, expression of genes associated with GABAergic (GABRB3) and glutamatergic (vGLUT1, vGLUT2, GLS2) neurons was also induced and peaked at the end of three weeks. This shows that ATCC NPCs and dopaminergic differentiation media are capable of producing GABAergic and glutamatergic neurons, in addition to dopaminergic neurons. Noticeably, there was significant induction of motor neuron gene (EN1, LIM3, Hb9) expression in ACS-5003 NPCs while there was significant induction of cholinergic (ChAT) neuron gene expression in ACS-5007 NPCs, but not in ACS-5003 NPCs during dopaminergic differentiation. To validate that ATCC NPCs and dopaminergic neuron differentiation media are suitable for drug screening, we conducted neurotoxicity screenings in the two types of NPCs and NPC-derived neurons by using a resazurin viability assay and high-content imaging analysis. We found that paclitaxel, a microtubule-stabilizing chemotherapeutic agent, significantly induced neurotoxicity ($p < 0.001$) in the two types of NPCs evaluated, but not in NPC-derived neurons. Vincristine, amiodarone, and chlorhexidine significantly decreased viability of both NPCs and neurons, whereas piperine, cisplatin, and hydroxyurea did not induce any significant neurotoxicity in neurons. This study demonstrates that ATCC iPSC-derived NPCs and dopaminergic differentiation media are suitable for studying neurological development and neurotoxicity screening.

Methods

Dopaminergic neuron differentiation: Normal human NPCs (ATCC® ACS-5003™; a non-reporter line) and a MAP2- NanoLuc® HaloTag® reporter NPC line (ATCC® ACS-5007™) were seeded in CellMatrix Basement Membrane Gel (ATCC® ACS-3035™)-coated 12-well plates at a seeding density of 10,000 cells/cm^2 and cultured in NPC expansion media (ATCC® ACS-3003™) overnight prior to treating NPCs with Dopaminergic Differentiation Media (ATCC® ACS-3004™) for up to 3 weeks.

qRT-PCR: RNA was extracted from ACS-5003 and ACS-5007 NPCs treated with the dopaminergic differentiation media for 0, 1, 2, or 3 weeks and cDNA was synthesized for qRT-PCR analysis of neuronal gene expression by using TaqMan® probes (Thermo Fisher Scientific, Inc.) shown in Table 1.

Table 1. TaqMan® probes used for gene expression analysis

Type of neurons	Name of TaqMan probes
Early neurons	MAP2, TUBB3 (Tuj1)
Dopaminergic neurons	TH, Nurr1, VMAT2, DAT, AADC
Glutamatergic neurons	GLS2, vGLUT1, vGLUT2
GABAergic neurons	GABRB3 (GABA)
Motor neurons	EN1, LIM3, HB9
Cholinergic neurons	ChAT

Neurotoxicity assay: ACS-5003 and ACS-5007 NPCs were seeded in CellMatrix Basement Membrane Gel-coated 96-well plates at a seeding density of 40,000 cells/cm^2 and cultured in NPC expansion media for two days prior to adding multiple neurotoxicants at 1 µM, 10 µM, or 100 µM for two days. Neurotoxicity was assessed by using resazurin viability dye. To examine neurotoxicity of neurons using resazurin and high-content imaging methods, ACS-5003 and ACS-5007 NPCs were seeded in Cell Matrix-coated 96-well and 24-well plates, respectively, at a seeding density of 10,000 cells/cm^2 and treated with dopaminergic differentiation media for three weeks prior to adding various concentrations of drugs for two days.

Results

Figures 1-5. Fold induction of different neuronal markers mRNA during three weeks of neuronal differentiation of ACS-5003 and ACS-5007 NPC lines using dopaminergic differentiation media (ATCC® ACS-3004).

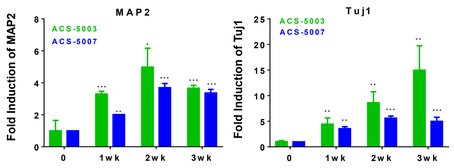

Figure 1. Functional neuronal markers. MAP2 and Tuj1 (TUBB3) mRNA increased significantly in ACS-5003 and ACS-5007 within a week (wk) of differentiation down the neuronal pathway (n=1, *p < 0.05, **p < 0.01, ***p < 0.001, Two-Way ANOVA).

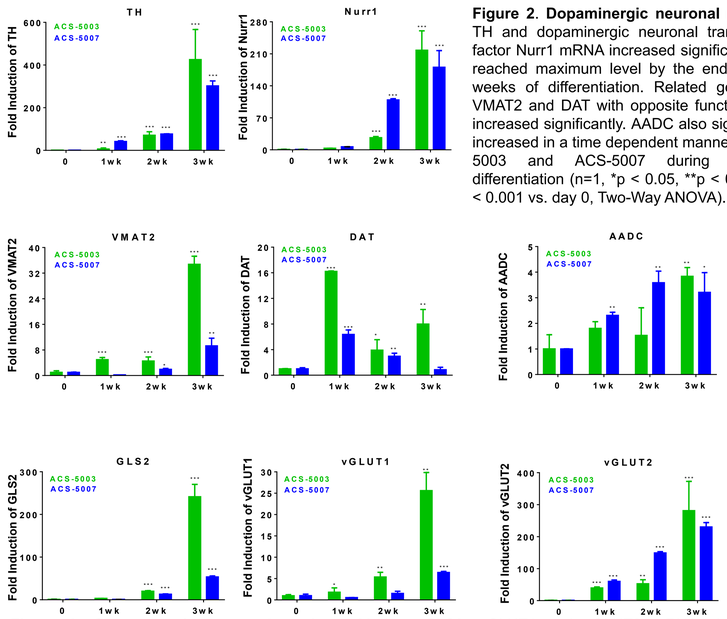

Figure 3. Glutamatergic neuronal markers. Markers GLS2, vGLUT1 and vGLUT2 mRNAs increased significantly in a time dependent manner during neuronal differentiation in ACS-5003 and ACS-5007 NPCs (n=1, *p < 0.05, **p < 0.01, ***p < 0.001 vs. day 0, Two-Way ANOVA).

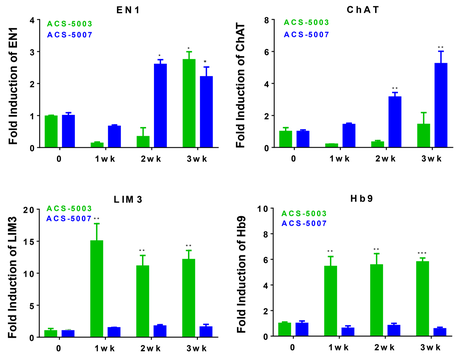

Figure 4. Motor and cholinergic neuronal marker. There was a significant increase in EN1, LIM3, and Hb9 mRNA in ACS-5003 while ChAT and EN1 mRNA increased significantly in ACS-5007 NPCs during dopaminergic differentiation (n=1, *p < 0.05, **p < 0.01, ***p < 0.001 vs. day 0, Two-Way ANOVA).

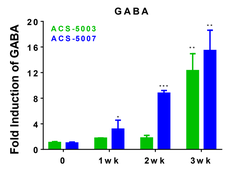

Figure 5. GABAergic neuronal marker. GABA mRNA increased significantly in ACS-5003 and ACS-5007 NPCs in a time-dependent manner during neuronal differentiation (n=1, *p < 0.05, **p < 0.01, ***p < 0.001 vs. day 0, Two-Way ANOVA).

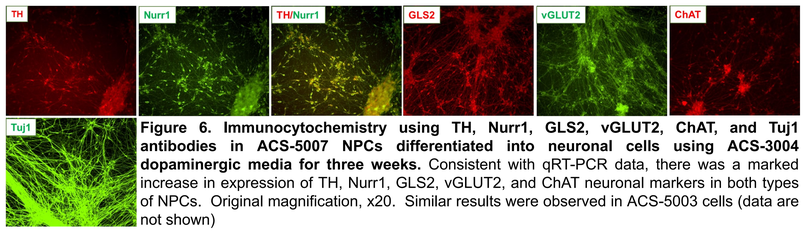

Figure 6. Immunocytochemistry using TH, Nurr1, GLS2, vGLUT2, ChAT, and Tuj1 antibodies in ACS-5007 NPCs differentiated into neuronal cells using ACS-3004 dopaminergic media for three weeks. Consistent with qRT-PCR data, there was a marked increase in expression of TH, Nurr1, GLS2, vGLUT2, and ChAT neuronal markers in both types of NPCs. Original magnification, x20. Similar results were observed in ACS-5003 cells (data are not shown)

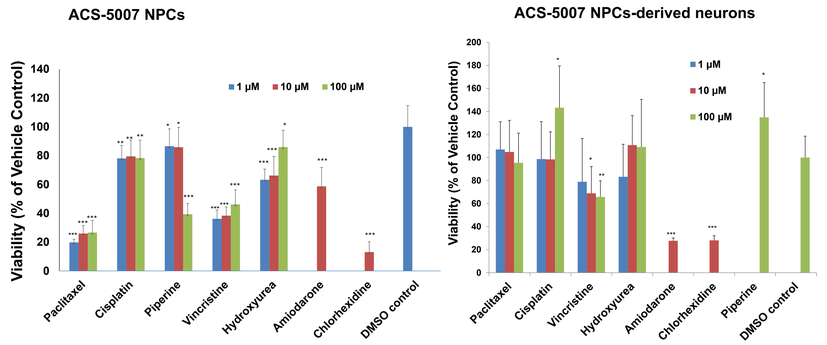

Figure 7. Dose-response curves for cell viability of ACS-5007 NPCs and ACS-5007 NPC-derived neurons treated with paclitaxel, cisplatin, piperine, vincristine, chlorhexidine, amiodarone, and hydroxyurea for two days. Paclitaxel, cisplatin, piperine, and hydroxyurea significantly induced neurotoxicity ($p < 0.05$) in NPCs, but not in NPC-derived neurons. Vincristine, chlorhexidine, and amiodarone significantly decreased viability ($p < 0.01$) of both NPCs and neurons (n=3, *p < 0.05, **p < 0.01, ***p < 0.001 vs. DMSO control, Student's T-test). The only concentration tested for amiodarone and chlorhexidine was 10 µM. Similar neurotoxicity was observed in ACS-5003 cells (data not shown).

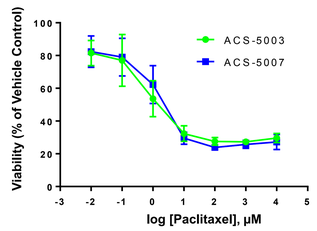

Figure 8. Dose-response curves for cell viability of ACS-5003 ACS-5007 NPCs treated with paclitaxel for two days. The neurotoxic effect of paclitaxel was similar in both types of NPCs. IC_{50} of paclitaxel on ACS-5003 and ACS-5007 NPCs was 0.9 µM and 1.6 µM, respectively (n=2).

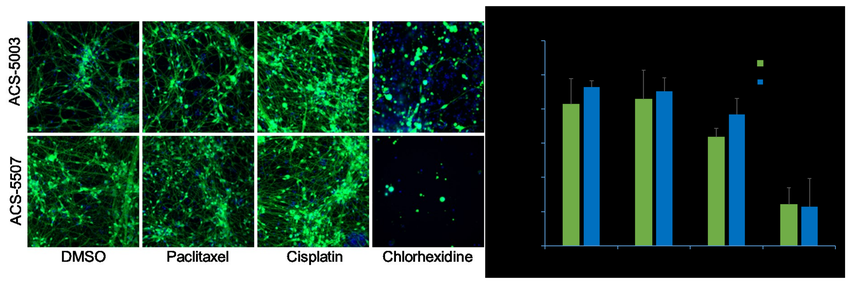

Figure 9. high-content imaging analysis of neurotoxicity of 10 µM paclitaxel, cisplatin, and chlorhexidine for two days in ACS-5003 and ACS-5007 NPCs-derived neurons stained with Calcein Green AM, and Hoechst 33342. Consistent with the viability data, 10 µM chlorhexidine significantly inhibited the neurite outgrowth (n=2, **p < 0.01 vs. DMSO, Student's T-test). Original magnification, x20.

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 and lineage-specific GFP or Nanoluc-HaloTag reporter NPCs.
- ATCC normal and reporter-labeled 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 NPC-derived neurons have been validated for drug screening with several neurotoxicants by using a resazurin viability assay and high-content imaging assessment.
- Paclitaxel is very toxic to NPCs with an IC_{50} 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 cell types and media options, please visit the ATCC website (www.atcc.org/neuro).