Development and Characterization of Human iPSC-derived Neurons for Drug Discovery Applications

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Abstract

The human brain represents a complex organ that has consistently been proven difficult to model in vitro. Current models including primary rodent tissue and immortalized cell lines have served as mainstays in both academic research and the pharmaceutical industry. These models, while providing a means for numerous landmark discoveries, have suffered from various issues including biological relevance, reproducibility and scalability. Considerable efforts have been made specifically within the pharmaceutical industry to reduce late-stage drug attrition through the development of more relevant in vitro human model systems. One area given significant attention has been the development of platforms than can enable the modeling of human degenerative (i.e. Alzheimer’s and Parkinson’s disease) and genetic (i.e. Huntington’s disease and muscular dystrophy) diseases as well as neurotoxicity. The recent discovery of induced pluripotent stem cells (iPSCs) not only overcomes the ethical and logistical issues associated with human embryonic stem cells, but also provides a flexible platform for generating various differentiated cell types from diseased individuals. Using this platform, we have developed a highly consistent and scalable protocol to differentiate and cryopreserve purified human iPSC-derived neurons, called iCell Neurons. Phenotypically, these cells are >90% pure as measured by flow cytometry for presence of the neuronal marker class III beta tubulin (TuJ1) and absence of the progenitor marker nestin. Within 24h of thawing, these neurons display a typical neuronal morphology including a dense network of neurites. Detailed phenotypic analyses reveal that these neurons are comprised of a mix of predominantly GABAergic and Glutamatergic subtypes as measured at both the mRNA and protein levels. DEVELOPMENTAL BIOLOGY ELECTROPHYSIOLOGY.

Electrophysiology

A. Evoked Action Potential

B. Spontaneous Action Potential

High Content Image-based Assays

A. Na Channel Current (I_Na) - Tetrodotoxin Inhibition

B. K' Channel Current (I_K') - Tetrodysphantom Inhibition

C. Ca Channel Current (I_Ca) - Nifedipine Inhibition

Phenotype Characterization

The iCell Neuron model reveals characteristic pharmacological responses to high throughput applications, including cytotoxicity assays, iCell Neurons reveal characteristic electrophysiological characteristics as measured using single synaptic connections. GABAergic and Glutamatergic subtypes as measured at both the mRNA and protein levels.

Nonlinear Regression

Figure 1. Post-thaw morphology of iPSC-derived neurons. (A) iPSC-derived neurons are grown on coverslips for 7 days and then fixed and stained for the neuronal marker class III beta tubulin (TuJ1). (B) iPSC-derived neurons are grown on coverslips for 7 days and then stained for the neuronal marker class III beta tubulin (TuJ1) and the progenitor marker nestin. (C) iPSC-derived neurons are grown on coverslips for 7 days and then stained for the neuronal marker class III beta tubulin (TuJ1) and the progenitor marker nestin. (D) iPSC-derived neurons are grown on coverslips for 7 days and then stained for the neuronal marker class III beta tubulin (TuJ1) and the progenitor marker nestin.

Figure 2. Evoked and spontaneous action potentials. (A) iCell Neurons exhibit a highly pure population. iCell Neurons represent a highly pure population as determined by (B) cell count, (C) viability, and (D) neuronal characteristics. The iCell Neuron model reveals characteristic electrophysiological characteristics as measured using single synaptic connections. GABAergic and Glutamatergic subtypes as measured at both the mRNA and protein levels. DEVELOPMENTAL BIOLOGY ELECTROPHYSIOLOGY.

Figure 3. iCell Neurons display characteristic subunit protein expression. (A) Western blot analysis of whole cell lysates using antibodies against subunits of the Na channel, K channel, and Ca channel. The iCell Neuron model reveals characteristic electrophysiological characteristics as measured using single synaptic connections. GABAergic and Glutamatergic subtypes as measured at both the mRNA and protein levels.

Figure 4. Electrophysiological characterization of single iCell Neuron. (A) Representative current clamp recording of an iCell Neuron showing the ability for the cell to generate action potentials. (B) Representative current clamp recording of an iCell Neuron showing the ability for the cell to generate action potentials. (C) Representative current clamp recording of an iCell Neuron showing the ability for the cell to generate action potentials.

Figure 5. High content image-based assays for mitochondrial integrity. (A) iCell Neurons were exposed to serial dilutions of BoNT/A, B, C, or E for 48 hrs. Cell viability was measured using the CellTiter-Blue® live/dead viability assay kit. (B) iCell Neurons were exposed to serial dilutions of BoNT/A, B, C, or E for 48 hrs. Cell viability was measured using the CellTiter-Blue® live/dead viability assay kit. (C) iCell Neurons were exposed to serial dilutions of BoNT/A, B, C, or E for 48 hrs. Cell viability was measured using the CellTiter-Blue® live/dead viability assay kit.

Figure 6. High content image-based assays for neurotoxicity. (A) iCell Neurons were exposed to serial dilutions of BoNT/A, B, C, or E for 48 hrs. Cell viability was measured using the CellTiter-Blue® live/dead viability assay kit. (B) iCell Neurons were exposed to serial dilutions of BoNT/A, B, C, or E for 48 hrs. Cell viability was measured using the CellTiter-Blue® live/dead viability assay kit. (C) iCell Neurons were exposed to serial dilutions of BoNT/A, B, C, or E for 48 hrs. Cell viability was measured using the CellTiter-Blue® live/dead viability assay kit.

Figure 7. High content image-based assays for synaptotagmin II. (A) Representative confocal image of an iCell Neuron showing the expression of synaptotagmin II. (B) Representative confocal image of an iCell Neuron showing the expression of synaptotagmin II. (C) Representative confocal image of an iCell Neuron showing the expression of synaptotagmin II.

Figure 8. High content image-based assays for NMDA receptor antagonists. (A) Representative confocal image of an iCell Neuron showing the expression of the NMDA receptor antagonist. (B) Representative confocal image of an iCell Neuron showing the expression of the NMDA receptor antagonist. (C) Representative confocal image of an iCell Neuron showing the expression of the NMDA receptor antagonist.

Figure 9. High content image-based assays for GABA receptor antagonists. (A) Representative confocal image of an iCell Neuron showing the expression of the GABA receptor antagonist. (B) Representative confocal image of an iCell Neuron showing the expression of the GABA receptor antagonist. (C) Representative confocal image of an iCell Neuron showing the expression of the GABA receptor antagonist.

Figure 10. High content image-based assays for AMPA receptor antagonists. (A) Representative confocal image of an iCell Neuron showing the expression of the AMPA receptor antagonist. (B) Representative confocal image of an iCell Neuron showing the expression of the AMPA receptor antagonist. (C) Representative confocal image of an iCell Neuron showing the expression of the AMPA receptor antagonist.

Figure 11. K+ channel current. (A) Representative current clamp recording of an iCell Neuron showing the ability for the cell to generate action potentials. (B) Representative current clamp recording of an iCell Neuron showing the ability for the cell to generate action potentials. (C) Representative current clamp recording of an iCell Neuron showing the ability for the cell to generate action potentials.

Figure 12. Surface area/ Cell.

Figure 13. Mitochondrial membrane potential. (A) An example overlay image of post-thaw iCell Neurons stained with JC-1. (B) An example overlay image of post-thaw iCell Neurons stained with JC-1. (C) An example overlay image of post-thaw iCell Neurons stained with JC-1.

Summary

The characterization of human iPSC-derived neurons for drug discovery applications has been presented. The iCell Neuron model reveals characteristic electrophysiological characteristics as measured using single synaptic connections. GABAergic and Glutamatergic subtypes as measured at both the mRNA and protein levels. DEVELOPMENTAL BIOLOGY ELECTROPHYSIOLOGY.

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