Abstract

Objective: Since the discovery of human induced pluripotent stem cells (iPSCs), much excitement and interest has been created around this technology as a platform for generating pluripotent stem cell lines from a range of specific genetic backgrounds, both normal and diseased. We have developed highly consistent and scalable differentiation protocol for making various types of human neurons, specifically midbrain dopaminergic neurons. This protocol provides a consistent platform to study various aspects of midbrain dopaminergic neuron biology, including Parkinson’s disease.

Methods: Using an optimized episomally-derived human iPSC platform, we developed a scalable method for the generation of differentiated, cryopreserved human midbrain dopaminergic neurons (iCell® DopaNeurons). Gene expression was analyzed by target-focused PCR arrays. Electrophysiological properties were measured using whole-cell patch clamp and the network-level activity was evaluated on multi-electrode array (MEA).

Results: Here, we present data characterizing gene expression for these floor plate-derived midbrain dopaminergic neurons with proper regional and neural subtype specifications. These cells displayed characteristic neuronal electrophysiological properties, including ion channel activity, evoked and spontaneous action potentials and excitatory post-synaptic currents. In addition, results from the MEA showed characteristic excitatory phenotypes with responses to known pharmacological agents and enhanced population bursts in an astrocyte co-culture environment.

Conclusions: Robust and reproducible methods to generate functional iCell DopaNeurons at high purity will enable the successful downstream production of panels of disease-specific samples derived from donor iPSCs for the study of neurodegenerative disorders such as Parkinson’s disease.

Methods

1. iPSC technology grants access to the CNS. The advent of induced pluripotent stem cell (iPSC) technology has enabled the use of previously inaccessible human cells, specifically neuronal cell types like cortical or dopaminergic neurons.

2. Schematic of midbrain dopaminergic neuron differentiation process

3. Optimization of stages of midbrain dopaminergic neuron differentiation from human iPSCs using high-content imaging (HCI). Molecule X titration during patterning identifies optimal concentrations to achieve high levels of co-expression of the floor plate marker FoxA2 and the roof plate marker Lmx1.

4. Early Transfection (4 DIV, 72 hr post transfection)

5. Late Transfection (21 DIV, 72 hr post transfection)

6. iPSC-derived Neuron Panels: Process and Quality

7. A robust cortical neuron differentiation process is demonstrated across episomally-reprogrammed iPSCs from multiple donors and starting materials. These data show that >90% pure neuronal cultures maintain morphological characteristics are achieved independent of the source and genotype of the donor sample. This process can be used to generate large quantities of neurons from a single batch.

8. Development and Characterization of Scalable Human Induced Pluripotent Stem Cell-derived Midbrain Dopaminergic Neurons for Drug Discovery and Disease Modeling


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24. iCell DopaNeurons demonstrate high viability and show extensive growth within 2-3 days post-trans.

25. B) Identity and Purity

26. C) Gene Expression

27. D) Electrophysiology

28. Electrical Activity: Bursting Plasticity Modulated via Dopamine Receptor Activation

29. A) Cultured iCell DopaNeurons reveal spontaneous and consistent activity at 8 days in culture. Velocity graphs (top) and raster plots (bottom) of activity recorded on an Axion multi-electrode plate show neuronal activity over ~4 minute recording. Raster plots mark action potentials (ticks) on individual electrodes over time while velocity graphs depict the instantaneous mean firing rate of the wells entire neuronal population for each 500 msec. Red circles on the velocity graphs indicate instantaneous bursts increase in population mean firing rate (BPM) as measured by tetraethylammonium (TEA), and potassium channels are inhibited by tetrodotoxin (TTX)

30. B) Mean firing rates (Hz) (top: red) and ‘Poison-surprise’ bursts per minute (BPM) (green: bottom) are shown for cultures 24+ hr after being treated with various D1- and D2-receptor ligands for 1 hr and then washed. Noted the increased bursting rates are selectively responsive to D1-receptor activation.

31. C) iCell DopaNeurons’ neuronal activity is modulated by co-culture with iCell Astrocytes. Example velocity graphs of iCell DopaNeurons activity levels show population bursts are turned on and enhanced with the addition of increasing amounts of iCell Astrocytes (10K, 25K, 50K and 100K) at 32 DIV.

32. D) Example raster plot of all 8 electrodes from a single well for a 10 minute recording of iCell DopaNeurons co-cultured with 100K iCell Astrocytes at 32 DIV. Note the ‘synchronous’ action potentials on different electrodes.

Summary and Conclusions

- Human iPSCs were used to produce floor plate-derived midbrain dopaminergic neurons at high purity with proper regional and neuronal subtype specifications.

- These dopaminergic neurons efficiently express a fluorescent reporter after transient transfection.

- Bursting electrical activity in midbrain dopaminergic neurons can be selectively modulated with a D1-receptor drug.

- Co-culture with human iPSC cell-derived astrocytes and these pure neurons enhance population bursts and arise ‘synchronous’ action potentials.

- Neuron differentiation can be scaled out to expand genetic background offerings and scaled up to produce large quantities.

Robust and reproducible methods to generate functional iCell DopaNeurons at high purity will enable the successful downstream production of panels of disease-specific samples derived from donor iPSCs for the study of neurological disorders such as Parkinson’s disease.