Characterization and Functional Applications of Human iPS Cell-derived Midbrain Dopaminergic Neurons

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Introduction

Human Donor
Induced Pluripotent Stem (iPS) Cells
Terminally differentiated cell types

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.

What is a Dopaminergic Neuron?

- Dopaminergic (DA) Neuron: producer of dopamine; found in different regions in the CNS with the highest concentration in the midbrain.
- Dopamine: role in voluntary movement and a broad array of behavioral processes such as mood, reward, addiction, and stress.
- Midbrain DA Neurons: located in the substantia nigra compacta (SNc) and the ventral segmental area (VTA); send fibers to other regions in both sides of the brain.
- Parkinson’s Disease (PD): caused by selective degeneration of the SNc DA neurons.

Differentiation Protocol Development

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Based upon work published in the literature (Kriks et al., 2011), coupled with additional differentiation and workflow improvements, an optimized midbrain dopaminergic neuron differentiation protocol was established. This resulted in the ability to produce large-scale cryopreserved DA neurons.

Optimized Protocol

High content imaging (HCl) serves as a useful tool for optimizing critical stages of midbrain dopaminergic neuron differentiation from human IPS cells. Data shown here (left) shows the titration of a critical component (Molecule X) used during patterning to achieve high levels of co-expression of the floor-plate marker FoxA2 and the roof-plate marker Lmx1.

Cell Type Characterization

High Viability and Expected Morphology

iCell DopaNeurons are highly viable, have significant neurite outgrowth within 2-3 days post-thaw, and maintain high purity for extended time in culture.

High Purity Determined by Multiple Methods

Gene expression time course as measured by qPCR indicates that most genes are expressed at very similar levels over a 4-6 week period. An adult human substantia nigra RNA was included as a control for comparison. Relative expression versus GAPDH is depicted. Results lower than 1x10-6 (gray shaded box) are considered to be below background or negative for expression.

Patient-Derived iPS Cells: Models for Parkinson’s

Leveraging the power of iPSC technology, CDI is building out a Disease & Diversity Panel, which includes terminally differentiated cell types from donors of diverse ethnic and disease-specific populations. Not only will there be more "controls", but there is also a heavy focus on neurological diseases, including Parkinson’s Disease-related targets such as LRRK2 and alpha-synuclein.

Functional Application Data

DA neurons were cultured on 48-well MEA plates coated with PEI/laminin and their electrical activity was analyzed at Day 8 following treatment with different compounds. In this example, velocity graphs depicting instantaneous mean firing rate levels over time (binned at 500 msec) were generated with the iCell NeuroAnalyzer before and after drug addition. Stimulation with apomorphine resulted in increased excitatory and connectivity levels (ie. # of bursts and bursting peak heights) after another day in culture. Co-application of a D1 antagonist abolished the induced response of APO, suggesting homeostatic receptor modulation following dopamine receptor stimulation. Control cultures displayed no change over time.

Summary

Here we demonstrate the differentiation of midbrain dopaminergic neurons from human IPS cells. Cell type-specific characterization data, as well as various application data are also presented. Robust and reproducible methods to generate DA neurons at high purity, coupled with the development of functional cell-based assays with “normal” cells, will enable the successful downstream production of panels of disease-specific samples derived from patient IPS cells for the study of neurological disorders such as Parkinson’s Disease.

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