Advancing Drug Discovery for Parkinson's Disease Through Development of HTS Assays Using Industrialized Human iPSC-derived Cell Models

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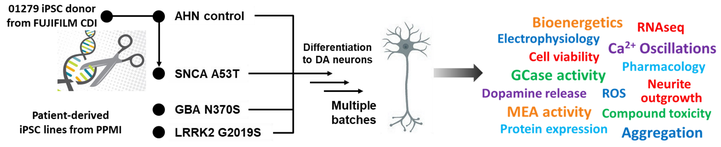
Abstract

Induced pluripotent stem cell (iPSC) technology has opened the possibility of taking somatic cells from virtually any human donor and converting them into virtually any cell type imaginable. With the help of the Parkinson's Progression Markers Initiative (PPMI), as part of The Michael J. Fox Foundation (MJFF), we generated iPSC lines from patients with Parkinson's disease (PD) carrying known risk-associated gene mutations (LRRK2 G2019S and GBA N370S) and clinical data supporting symptoms of PD. Additionally, we have engineered a SNCA A53T mutation from the isogenic iPSC donor. We then performed large-scale differentiations of these four (4) iPSC lines into biologically relevant midbrain dopaminergic neurons (i.e., iCell® DopaNeurons) to facilitate PD-focused assay development and drug screening. All cells showed similar marker expression and neuronal purity characteristic of dopaminergic neurons. We then seeded these cells into multiple assay formats, specifically investigating neuronal activity (multielectrode array), neurite outgrowth and degeneration, cell death, calcium signaling, bioenergetics and metabolism (Seahorse), alpha-synuclein accumulation, and GCase activity. We confirmed that known GBA-modulating compounds, such as cyclodextrin, modify the reduced GCase activity observed in iCell DopaNeurons containing PD-relevant mutations. Because the etiology of dopaminergic neuron cell death in PD is complex and involves multiple factors, this study shows that characterization and testing of different models in parallel is a worthwhile approach to improve understanding of disease progression and mechanism. Finally, the functional performance and consistency of iPSC- derived dopaminergic neurons demonstrate their potential use in drug screening and therapeutic validation.

Parkinson's Progression Markers Initiative

THE MICHAEL J. FOX FOUNDATION FOR PARKINSON'S RESEARCH



Characterization of iPSC-derived Dopaminergic Neurons

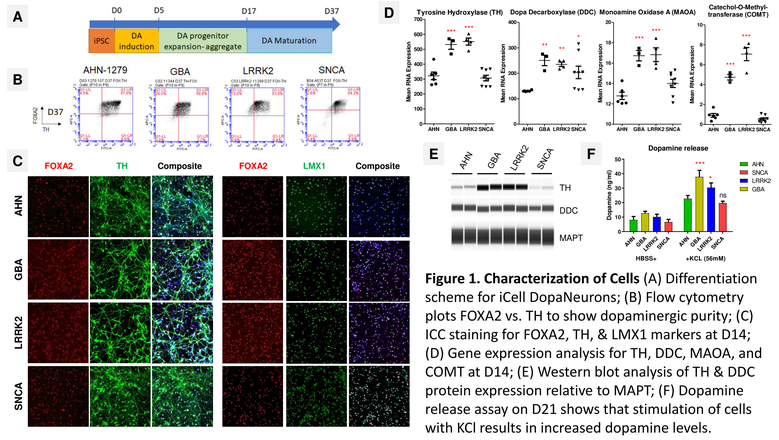


Figure 1. Characterization of Cells (A) Differentiation scheme for iCell DopaNeurons; (B) Flow cytometry plots FOXA2 vs. TH to show dopaminergic purity; (C) ICC staining for FOXA2, TH, & LMX1 markers at D14; (D) Gene expression analysis for TH, DDC, MAOA, and COMT at D14; (E) Western blot analysis of TH & DDC protein expression relative to MAPT; (F) Dopamine release assay on D21 shows that stimulation of cells with KCl results in increased dopamine levels.

GCase Enzyme Activity Assay

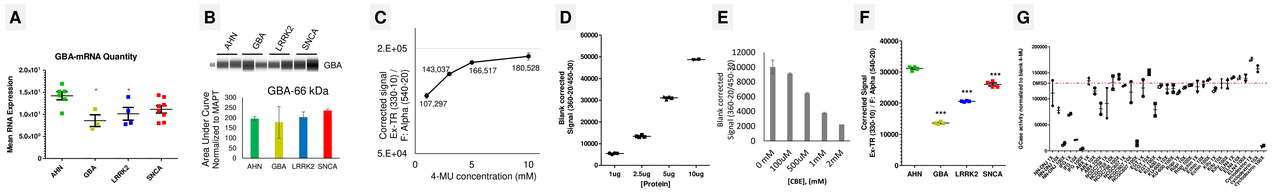


Figure 5. Quantitation of GBA expression levels by (A) RNAseq and (B) Western blot. (C) Cellular GCase activity was measured by 4-MU assay and resulted in (D) linear correlation between amount of protein lysate and enzyme activity. (E) CBE is a specific inhibitor of GCase. (F) All mutant lines showed decreased activity compared to AHN; (G) Screen for compounds that modulate GCase activity was run in LRRK2 G2019S iCell DopaNeurons. Cyclodextrin showed a 30% increase in signal.

PD-relevant iCell DopaNeurons are Highly Active on MEA

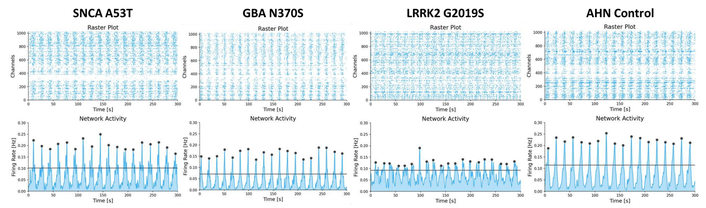


Figure 2. Neuronal Network Activity on HD-MEA. PD-relevant iCell DopaNeurons were co-cultured with iCell Astrocytes on 6-well high-density multi-electrode array (HD-MEA) plates from MaxWell Biosystems. Recordings were conducted on a MaxTwo system, and raster plots show the synchronous network activity detected by 1020 electrodes on Day 14. Approximately 400,000 iCell DopaNeurons and 100,000 iCell Astrocytes were plated on PEI/laminin-coated surface. These data show human iPSC-derived cells are highly functional and show spontaneous electrical activity on MEA.

Delayed Network Bursting in LRRK2 G2019S iCell DopaNeurons

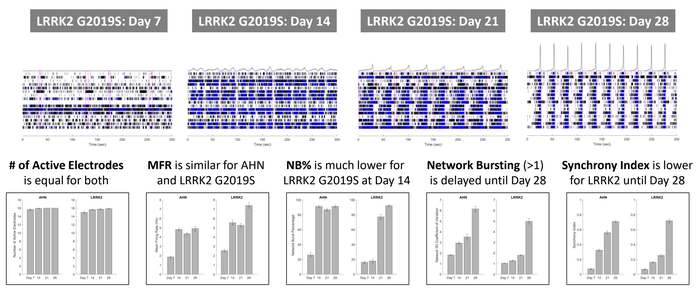


Figure 3. Development of Network Activity Over Time. Raster plots taken at different timepoints in culture (Day 7, 14, 21, & 28) show evolution of network activity for LRRK2 G2019S iCell DopaNeurons maintained on a CytoView MEA plate. Compared with AHN iCell DopaNeurons as a control, the # of active electrodes and mean firing rate (MFR) are similar, but time to organized and synchronous network bursts is delayed for LRRK2. Data were obtained on a Maestro Pro MEA system using n=16 wells per condition. Experiments are ongoing to track cultures out even longer.

Calcium Oscillation Assay with 3D Neurospheres

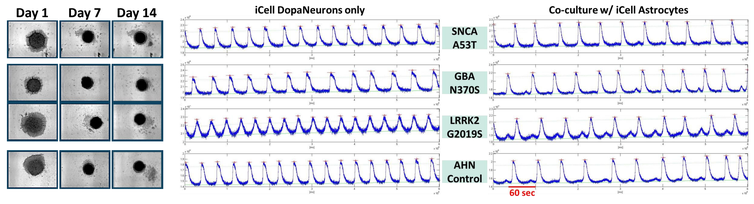


Figure 4. Spontaneous Activity from Cells in 3D Cell Culture. PD panel of iCell DopaNeurons were cultured in 384-well ULA spheroid plates (Sbio) with or without iCell Astrocytes (20K-25K cells per spheroid). These data examine the different Ca^{2+} oscillation phenotypes at Day 14 and the impact of co-culture vs. mono-culture. The advantage of this modular approach to build your own "iCell NeuroSpheres" is that you can tweak cell type ratios and have control over the spheroid composition.

Bioenergetic Analysis of PD iCell DopaNeurons

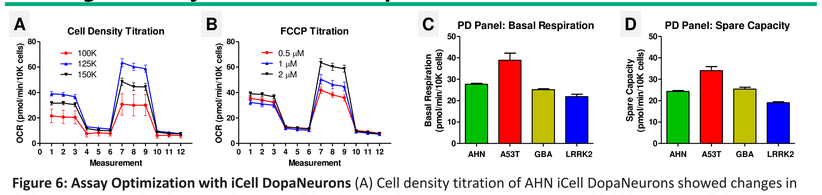


Figure 6: Assay Optimization with iCell DopaNeurons (A) Cell density titration of AHN iCell DopaNeurons showed changes in OCR assay signal on Day 21 on the Seahorse XF Pro Analyzer. Recommended cell density is 125,000 cells/well. (B) Titration of FCCP uncoupler on Day 21 resulted in the highest maximum respiration at 2 µM. PD panel of iCell DopaNeurons were then assayed with 1 µM Oligomycin, 2 µM FCCP and 0.5 µM Rot/Ant A on Day 14. SNCA A53T (**red**) showed the highest (C) basal respiration and the largest (D) spare capacity. Both GBA and LRRK2 came in lower for these metabolic metrics.

Neurite Outgrowth Analysis of PD iCell DopaNeurons

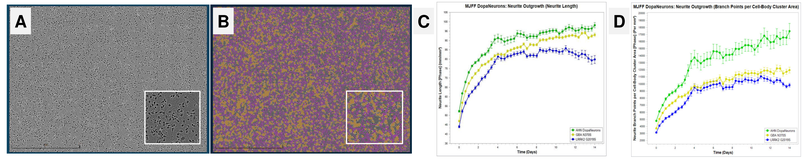


Figure 7. Tracking Neurite Outgrowth (A) Phase image (10X) from Incucyte SX5 of AHN iCell DopaNeurons at Day 14 and (B) Neurite (**magenta**) + cell-body cluster (**orange**) analysis masks from the neurite outgrowth module. Images from AHN (**green**), GBA (**yellow**), and LRRK2 (**blue**) [no SNCA A53T data here] were analyzed to provide metrics for (C) neurite length and (D) branch points over time. Cells were plated at a density of ~70K cells/well on PLO/laminin-coated 96-well plate and cultured in iCell DopaNeurons maintenance medium.

Disease-relevant LRRK2 Pharmacology

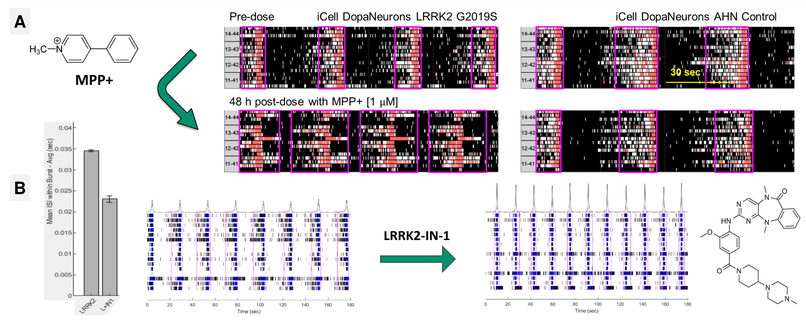


Figure 8: Pharmacological response on MEA. LRRK2 G2019S and AHN iCell DopaNeurons were cultured on a CytoView MEA plate until Day 28. Cells were treated with (A) a low dose of MPP+ (1 µM) for 48 h, resulting in disorganization of network bursts relative to control. It has been suggested that LRRK2 G2019S mutation increases susceptibility to this neurotoxin. (B) Exposure to the inhibitor, LRRK2-IN-1 (2 µM), for 5 days resulted in a significant change in bursting architecture. *Mean ISI within a Burst* measures burst intensity and smaller values indicate more intense bursts. This response is evident visually in the post-dose raster plot with more single-channel electrode bursts (in **blue**) and sharper peaks in the histogram.

Summary

These data demonstrate the utility of donor-derived LRRK2 + GBA and engineered A53T dopaminergic neurons across multiple assays utilized for HTS assays.

The Parkinson's Disease Panel of iCell DopaNeurons display:

- Lot-to-lot consistency in purity and performance across multiple assays
- Altered baseline MEA activity and calcium signaling
- Reduced GCase activity and altered metabolic profiles

Cryopreserved dopaminergic neurons from disease-relevant backgrounds provide a biologically-relevant and reproducible system to expedite facilitate drug discovery and therapeutic development.

iPSC lines for the LRRK2 and GBA mutations are part of the Parkinson's Progression Markers Initiative (PPMI) iPS cell bank.