Characterization of the autophagic-lysosomal pathway in Parkinson's disease using patient iPSC-derived dopaminergic neurons containing a *LRRK2* G2019S mutation

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INTRODUCTION

Parkinson's disease (PD) is the second most common neuro-degenerative disorder worldwide, with more than 4 million people over 50 affected by the disease, with a projection for the number of individuals with PD to double by 2030. PD is characterized by specific dopaminergic neurodegeneration within the SNpc region of the midbrain, an important regulator of voluntary movements. Dopaminergic neuron cell death in PD is complex and combinatorial, with impairments in multiple cellular pathways impacting mitochondrial function, endosomal//lysosomal protein degradation, alpha-synuclein and tau aggregation, and neuro-inflammation.

The *Leucine-rich repeat kinase 2* (LRRK2) gene was identified as the most common genetic cause for familial autosomal dominant PD. Genetic risk factors, such as mutations in LRRK2, have been shown to impact kinetics of the autophagic-lysosomal pathway (ALP), which is suggested to contribute to PD-associated protein accumulation and aggregation. Human-relevant *in vitro* models using patient-derived induced pluripotent stem cells (iPSCs) offer an accessible avenue for understanding the mechanisms for these genetic mutations as well for development of therapeutics against this debilitating disease.

In this study, we evaluated the expression and cellular distribution of ALP-associated proteins in commercially available iPSC-derived dopaminergic neurons (iCell® DopaNeurons) generated from both apparently healthy normal donors (AHN) and donors diagnosed with Parkinson's Disease and harboring a LRRK2 G2019S mutation as well as LRRK2 G2019S/G mutation corrected control iCell® DopaNeurons. Disease iPSCs were obtained from the Parkinson's Progression Markers Initiative (PPMI), part of The Michael J. Fox Foundation (MJFF). Using high-throughput imaging we quantified endosome and lysosome protein expression using an array of highly specific antibodies from Cell Signaling Technology (CST), including LAMP1, COX IV, LC3, and Cathepsin B.

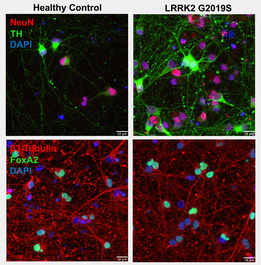

Figure 1: Identification of iCell® DopaNeurons. Donor derived dopa-minergic healthy, WT neurons and cells harboring a LRRK2 G2019S mutation were stained with NeuN and Tyrosine Hydroxylase (**upper panel**) or β3-Tubulin and FoxA2 (**lower panel**).

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METHODS

- iPSC-derived dopaminergic neurons (iCell® DopaNeurons: Control #R1032, LRRK2 G2019S #R1234, LRRK2 G2019S/G mutation corrected control #R1243) were acquired from FUJIFILM Cellular Dynamics and grown according to protocol. Cell were fixed in methanol at 14*DIV.*
- Recombinant monoclonal antibodies against various cellular markers and CNS cell types were analyzed in patient iPSC-derived dopaminergic neurons by immunofluorescence utilizing the CST Immunofluorescence Protocol. Antibodies used have previously been validated in mouse brain tissue (Figure 2).
- Images were captured at 630X using the Operetta CLS HCA system in confocal mode.
- Image quantification was performed using Harmony high-content analysis software, Mitochondria Analyzer [5] in ImageJ, as well as Cell Profiler [3].

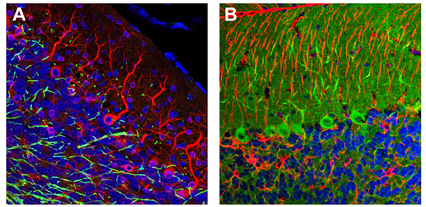


Figure 2: Antibody validation in mouse brain tissue. (**A**) Myelin Basic Protein (green), β3-Tubulin (red) and Lamin A/C (blue) are detected in mouse cerebellum by immunofluorescence. (**B**) LC3A/B (green), GFAP (red) and DAPI (blue) are also detected in mouse cerebellum by immunofluorescence.

Table 1:

Key Antibodies Used	Catalog #
β3-Tubulin (E9F3E) Mouse mAb	45058
LAMP1 (D2D11) XP® Rabbit mAb	9091
LC3A/B (D3U4C) XP® Rabbit mAb	12741
Cathepsin B (D1C7Y) XP® Rabbit mAb	31718
NeuN (E4M5P) Mouse mAb	94403
FoxA2/HNF3β (D56D6) XP® Rabbit mAb	8186
Tyrosine Hydroxylase (E2L6M) Rabbit mAb	58844
COX IV (3E11) Rabbit mAb	4850
Myelin Basic Protein (E9P7U) Mouse mAb	83683
Lamin A/C (4C11) Mouse mAb	4777
GFAP (GA5) Mouse mAb	3670

Assessment of lysosomes in iCell® DopaNeurons

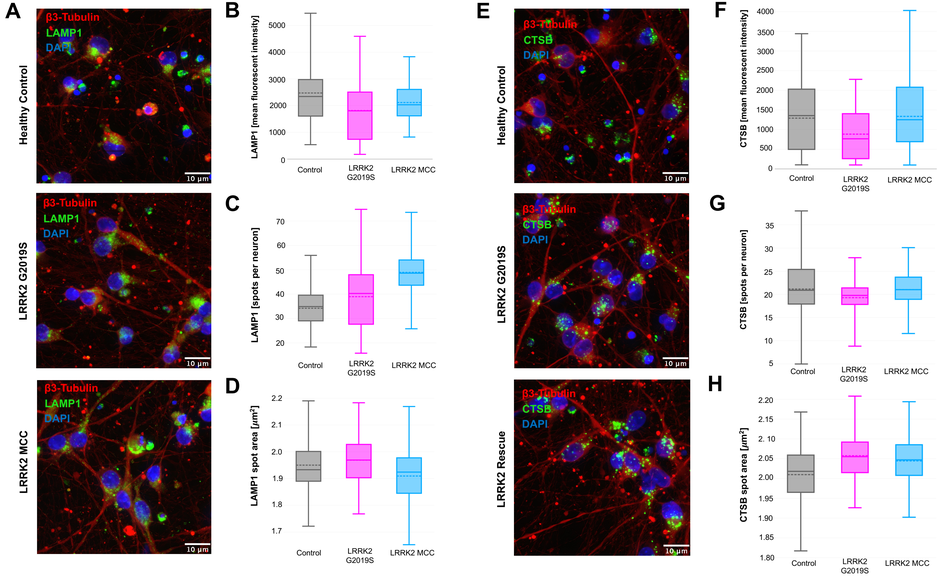
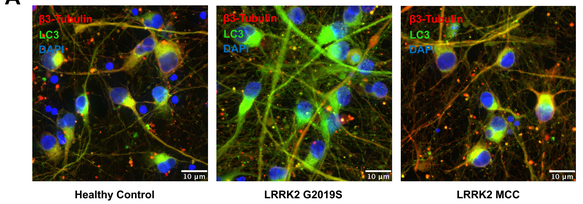


Figure 3: Lysosomal proteins imaged in iCell® DopaNeurons. Lysosomal proteins LAMP1 (**A**) and Cathepsin B (**E**, CTSB) were imaged in healthy control iCell® DopaNeurons, neurons harboring a LRRK2 G2019S mutation as well as LRRK2 mutation corrected control (MCC) neurons. The images were quantified for mean fluorescent intensity (**B, F**), spots per neuron (**C, G**) and spot area (**D, H**).

Neurons with LRRK2 G2019S mutation display increase in LC3 signal



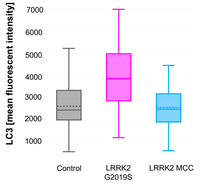


Figure 4: Quantification of LC3 staining. iCell® DopaNeurons were stained with LC3 (**A**) and the the mean fluorescent intensity was quantified (**B**). The increase in signal in LRRK2 mutated neurons was rescued LRKK2 mutation corrected control (MCC) cells.

LRRK2 G2019S does not change mitochondria morphology

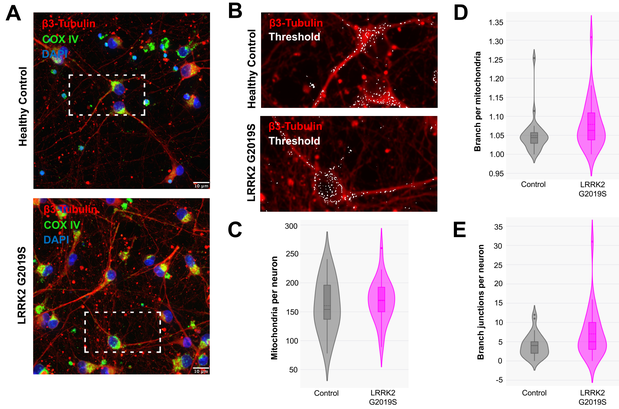


Figure 5: Mitochondria morphology assessment in iPSC-derived neurons. iCell® DopaNeurons were stained with COX IV (**A**) and a threshold was applied (**B**) to quantify mitochondrial parameters. No change was found for number of mitochondria per neuron (**C**), branches per mitochondria (**D**) and branch junctions per neuron (**E**).

CONCLUSIONS

- We have developed a portfolio of rabbit and mouse monoclonal antibodies for organelle and CNS markers that can help assess cellular processes in PD
- We show that these antibodies can be used for specific labeling of components of the lysosomal and autophagic pathway, allowing quantification of these in iCell® DopaNeurons
- We show that LC3 expression is increased in primary iPSC-derived dopaminergic neurons harboring a LRRK2 G2019S mutation, which can be rescued by WT LRRK2 expression
- We further found that mitochondria number and gross morphology was not affected by the LRKK2 G2019S mutation
- We show that human iPSC-derived dopaminergic cells are a relevant model for PD and that iCell® DopaNeurons are compatible for high-throughput screening and analysis using antibodies against disease-relevant targets
- We continue to develop a comprehensive portfolio of high quality monoclonal antibodies to further characterize disease-associated processes and understand the cellular changes in neuronal and glia cells to accelerate new discoveries that lead to better understanding, diagnosis, and treatment of disease in neurodegenerative diseases, such as PD.

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