Parkinson disease-related phenotype characterization of A53T alpha-synuclein iPSC-derived dopaminergic cultures

Aptuit SL, an Evotec Company, Vienna, Italy

INTRODUCTION
Parkinson disease (PD) is a progressive neurological disease caused by selective loss of dopaminergic neurons in the substantia nigra. Although the majority of PD cases are sporadic, familial PD mutations provide a valuable tool for understanding and modeling basic pathophysiological mechanisms. We used MyCell® DopaNeurons carrying the A53T mutation in the SMA gene (A53T DaNs) and healthy isogenic control (WT DaNs) to investigate disease-relevant phenotypes including: α-synuclein (syn) accumulation, mitochondrial dysfunction, and calcium dysregulation.

RESULTS

Analysis of sSYN in WT and A53T DaNs cultures with Western Blot and immunofluorescence

Reduced mitochondrial membrane potential in A53T vs WT DaNs cultures

Detection of sSYN in WT and A53T DaNs cultures with Western Blot and immunofluorescence

Different pattern of spontaneous Calcium oscillations in WT and A53T DaNs cultures

Conclusions

- In this study, an extensive phenotypic characterization of iPSC-derived DaNs carrying a PD-relevant mutation provided a valuable tool for modeling disease-related PD pathophysiology.

Methods

- DaNs cultures (A53T and WT) were initiated according to manufacturer’s instructions in DISH-coated (Gallo CellLine dishes, Stemcell, Canada) 96-well microplates using HEK 293 cells (IBA, Germany) as a feeder layer. Following transfection, cells were cultured in Stemsharkâ® Neuro1 medium (Stemcell, Canada) supplemented with a cocktail containing 10% FBS and L-glutamine until >90% confluence.

- Western Blot analysis was performed to investigate the protein expression of α-synuclein in A53T and WT DaNs cultures.

- Immunofluorescence analysis was performed to determine the localization of α-synuclein in the cytoplasm and nuclei of the cells.

- Membrane potential was assessed by using TMRE fluorescence microscopy.

- Calcium oscillations were analyzed using a FLIPR assay in 96-well plates, with a FLIPOr software for Peak analysis.

- A53T DaNs were compared to WT DaNs in terms of protein expression, immunofluorescence localization, and membrane potential.