INTRODUCTION

Parkinson’s disease (PD) is a neurodegenerative disease caused by the progressive loss of dopamine-producing neurons (DAergic neurons). Genetic mutations affecting DAergic neurons account for about 15% of PD cases, providing a valuable tool for studying basic pathophysiological mechanisms of the disease. One of the most relevant mutations in the PD population is the SNCA gene, which causes the production of a mutated form of alpha synuclein (α-syn), resulting in α-syn aggregation. It has been reported that PD patient neurons display a ~30% reduction of mitochondrial complex I activity. Rotenone is known to reduce mitochondrial complex I activity. Therefore, testing Rotenone administration in vitro could reveal PD effect on α-syn mitochondrial functionality.

AIM

The aim of this work is the identification of PD-relevant phenotypes for in vitro drug testing to be applied in drug-discovery programs for Parkinson’s Disease.

RESULTS

WT and AS3T dopaminergic cultures differentiation in 384 well format

Reduced mitochondrial membrane potential in AS3T vs WT dopaminergic cultures

CONCLUSIONS

In this study, an extensive phenotypic characterization of iPSC-derived DAergic neurons carrying a PD-relevant mutation (A3T) was performed in parallel to aogenic healthy control (WT). Moreover, acute and chronic Rotenone administration on WT or A3T was used to induce mitochondrial stress. Key cellular features related to PD pathogenesis were explored:

- mDAn differentiated in 384 well plates express neuronal marker MAP2 and DA-specific marker tyrosine hydroxylase (TH) and SYN.
- Comparing WT and A3T, an accumulation of αSYN was detected in A3T after 28 days in culture. A3T cells display a differentiation of spontaneous Ca2+ oscillations compared to WT cells, potentially revealing a dysregulation of mitochondrial homeostasis (FLIPR 384 well assay).
- A reduced mitochondrial membrane potential was detected in A3T compared to WT in vitro. The identification of mitochondrial Ca2+ release mechanism (Ca2+-dependent ATPase) and neuronal apoptosis resulted in neurodegeneration and neuronal death. Rotenone administration led to reduction of cell viability and MMP (apoptosis treatment). The stability of SYN was compensated by induction of other mitochondrial complexes and of glutathione.

The above-mentioned assay can provide a panel of markers suitable for testing potential therapeutics acting on different PD pathophysiological mechanisms.

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