CHARACTERIZATION OF NEURONAL DIFFERENTIATION OF iPS CELLS FROM PARKINSON’S DISEASE PATIENTS

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ABSTRACT

Parkinson’s disease (PD) is the second most common neurodegenerative disorder. Its pathologic hallmark is the functional loss of dopaminergic neurons and the appearance of Lewy Bodies, intracellular amyloid aggregates constituted mainly by the protein α-Synuclein (aSyn). Although the majority of PD cases are sporadic, some familial mutations are known and are can be correlated with early onset forms of PD. In this context, we studied the neuronal differentiation of induced pluripotent stem cell (iPSC)-derived neural progenitor cell lines [1], that were obtained from skin biopsies of patients with a triplication of the SNCA gene, the most common LRKK2 point mutation (G2019S) and age matched healthy controls.

The iPSC cell lines derived from fibroblasts were first committed into a neuronal lineage and then differentiated into neurons [2]. In addition, astrocyte assisted differentiation was also tested since its presence better approximates the natural environment of neurons. In order to identify affected cellular processes, antibodies against different neuronal markers were used to compare the differentiation process of all lines by immunohistochemistry and to analyze the extension of neurites outgrowth. Oxidative stress susceptibility was tested using the ROS sensors H₂DCFDA and MitoTracker CM-X-H₂-Ros, while mitochondrial fitness was assayed with JC-1 probe.

Preliminary results suggest aSyn triplication lines have difficulties in differentiating that can be partially ameliorated by astrocyte-conditioned media. Supplementation with specific factors or drugs may be required in order to achieve a high yield of dopaminergic neurons, particularly in the cases with the impaired differentiation phenotype. The LRKK2 mutant lines exhibit other properties, including higher levels of oxidative stress.

To complement these results, new tools are being developed to infect differentiated cells with lentiviruses that induce the expression of fluorescent proteins, free or targeted to specific subcellular locations. This help to examine specific cellular processes and organelle such as mitochondrial ROS stress response, synaptic vesicles and mitochondrial distribution and dynamics, and intercellular contacts. We anticipate that these investigations will assist in the identification of initial molecular triggers leading to neurodegeneration associated with the genetic alterations characteristic of PD.