Triallelic stem cell model of Parkinson's disease reveals epistatic interaction between PRKN and GBA

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Résumé

Mutations in *GBA* are known to be the most common genetic risk factor for developing Parkinson's disease. With up to 30% of Parkinson's disease patients carrying *GBA* mutations, the need for a targeted therapy for these patients is increasing. *GBA* is encoding the lysosomal enzyme glucocerebrosidase, which is implicated in lipid metabolism and α-synuclein degradation. When mutated, glucocerebrosidase is commonly misfolded and redirected to the proteasome for degradation. At cellular levels this results in lysosomal dysfunction, α-synuclein accumulation and ultimately to death of the dopaminergic neurons. The E3-ubiquitin ligase Parkin, encoded by *PRKN*, has been proposed to be implicated in the degradation of mutated glucocerebrosidase.

In our study, we investigated the relationship between glucocerebrosidase and Parkin in iPSC-derived neurons from Parkinson's disease patients harbouring mutations in *GBA*, *PRKN* or both genes. Pharmacological rescue of glucocerebrosidase via Ambroxol, genetic correction of *GBA* via CRISPR-Cas9 and modulation of Parkin levels were used to dissect the specific contribution of each gene to the cellular phenotype.

We evaluated α-synuclein homeostasis in patient-derived neurons harbouring the heterozygous N370S mutation in *GBA* under different levels of expression of Parkin. The overexpression of Parkin resulted in a decrease of glucocerebrosidase levels and consequently to an increase of α-synuclein levels. On the other hand, when silencing Parkin, the levels of glucocerebrosidase were enhanced, resulting in a decrease of intracellular α-synuclein. To deepen our understanding of this relationship in a more physiological model, we evaluated α-synuclein levels in iPSC-derived neurons from a Parkinson's disease patient harbouring both a loss of Parkin and the heterozygous N370S mutation in *GBA*. These neurons presented conserved levels of glucocerebrosidase while intracellular α-synuclein levels were reduced compared to control cells. When rescuing glucocerebrosidase via pharmacological or genetic strategies, the intracellular levels of α-synuclein were increasing.

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Our results show that Parkin modulates glucocerebrosidase levels which subsequently impacts α-synuclein levels. Therefore, the inhibition of the ubiquitination of glucocerebrosidase in order to reduce α-synuclein levels may be a potential novel pharmacological target for the treatment of *GBA*-associated Parkinson's disease.

Mots-Clés: glucocerebrosidase, parkin, iPSC, neurons, CRISPR Cas9