Christiane Olesky

Abstract

Thesis supervisor: Prof. Dr. Rejko Krüger

Institution: Luxembourg Centre for Systems Biomedicine (LCSB)

Studying digenic Parkinson's disease in a stem cell model carrying mutant p.N409S in the *GBA1* gene and the homozygous deletion of exon 3 *PARK2*

Christiane Oleksy (1), Zoé Hanss (1), François Massart, Giuseppe Arena (1), Ibrahim Boussaad (1), Rejko Krüger (1,2,3)

1) Translational Neuroscience, Luxembourg Centre for Systems Biomedicine (LCSB), University of Luxembourg, 2, Avenue de l'Université, 4365 Esch- sur-Alzette, Luxembourg

2) Transversal Translational Medicine, Luxembourg Institute of Health (LIH), Strassen, Luxembourg

3) Parkinson Research Clinic, Center Hospitalier de Luxembourg (CHL), Luxembourg

Background: Variants in the *GBA1* gene encoding the lysosomal enzyme β-glucocerebrosidase (Gcase) are the most common genetic risk factor for developing Parkinson's disease (PD) [1]. Moreover, homozygous deletions in the Parkin gene (*PARK2*) have been associated with early onset autosomal recessive forms of PD [2]. **Objective**: We aim to study the combined effect of the *GBA1* p.N409S mutation and a complete loss of Parkin based on a patient-based stem cell model of a carrier of a heterozygous *GBA1* p.N409S mutation and a homozygous deletion of *PARK2* exon 3. **Methods**: Midbrain dopaminergic neurons were generated as described previously [3]. Enzymatic activity measurements within the lysosomal enriched fraction were performed using a fluorometric readout [4]. Mechanistic pathways were studied with inhibitors of the proteasome (MG132) and of translation (Cycloheximide). Alpha-synuclein protein levels were analyzed by Western Blot. **Results**: Our results revealed an epistatic interaction between *PARK2* and *GBA1*. In contrast to what is expected in *GBA1* mutation carriers, lower intracellular monomeric α-synuclein protein levels were observed in the double mutation carrier compared to healthy controls. Proteasomal inhibition in the nerve cells led to increased Gcase protein levels in *GBA1* p.N409S single mutants only. Moreover, our data suggest that GCase stability is the lowest in the presence of mutated GCase species and wild-type Parkin compared to mutated Gcase in the absence of Parkin and compared to healthy controls. In addition, we found that protein- and relative mRNA levels of binding immunoglobulin protein (BiP), an endoplasmic reticulum (ER) chaperone activated upon ER stress were increased in *GBA1* p.N409S single mutants and not in GBA-Parkin double mutation carriers. Lastly, we found a decrease of approximately 50% of Gcase activity in mutant *GBA1* p.N409S midbrain dopaminergic neurons compared to healthy controls. **Conclusions**: Our results suggest that mutant Gcase protein may be protected from proteasomal degradation in neurons when co-occuring with loss of Parkin compared to

GBA1 p.N409S carrying neurons with physiological Parkin levels. Moreover, a loss of Parkin co-occuring with mutant *GBA1* p.N409S showed beneficial effects on endoplasmic reticulum stress compared to *GBA1* p.N409S single mutation carriers with physiological Parkin levels. Increased levels of even mutant Gcase may therefore be still beneficial and potentially lead to a decrease in α-synuclein protein to a level similar to control. Therefore, pharmacological modulation of Parkin function may be an option to treat GBA-PD at least due to p.N409S mutations.

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