EVALUATION OF TOXICITY IN SHSY5Y CELLS STABLY OVEREXPRESSING LRRK2

E. Lobbestael, V. Daniëls, V. Baekelandt, J.-M. Taymans

Molecular and Cellular Medicine, Catholic University Leuven, Leuven, Belgium

Introduction: Mutations in leucine rich repeat kinase 2, LRRK2, are the most common known cause of genetic Parkinson's disease. Transient overexpression of mutant LRRK2 can cause toxicity in cell culture and primary neurons; however recent reports suggest that this depends on experimental setups. Also, this acute cellular toxicity contrasts with findings from LRRK2 transgenic mice which generally do not display neuronal cell death.

Aims and methods: We aimed to examine the relationship between LRRK2 overexpression and toxicity in SHSY5Y neuroblastoma cell line for its potential use as a LRRK2 cellular toxicity model. We used lentiviral vectors to obtain overexpression of wild-type and pathogenic LRRK2 or fragments of LRRK2. Lentiviral vector mediated overexpression results in more physiological expression levels and higher efficiency compared to transfection. These cell lines were extensively investigated for toxicity effects under basal and oxidative stress conditions using lactate dehydrogenase release and TUNEL as readouts.

Results: Acute overexpression of 3xflag-tagged LRRK2 domains, larger fragments or full length LRRK2 did not lead to reduced viability in SHSY5Ys compared to controls. Moreover, we were able to culture cell lines with stable overexpression of these proteins (WT and pathogenic mutants as 1441C and 2019S) for more than two months without adverse effects on cell growth or viability. The further characterization of these cell lines is ongoing.

Conclusion: Cellular toxicity in SHSY5Ys does not depend on LRRK2 overexpression alone but probably involves other variables. Further development of a LRRK2 dependent toxicity assay in SHSY5Ys may therefore focus on a multi-hit approach.