INVESTIGATION OF LRRK2 SIGNALLING PATHWAYS IN A TETRACYCLINE-INDUCIBLE CELL EXPRESSION SYSTEM

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Parkinson's Disease (PD) is a common neurodegenerative disorder characterised by a variety of movement abnormalities. Mutations in the Leucine-Rich Repeat Kinase 2 (LRRK2) gene are commonly linked to autosomal dominant late-onset PD. Many of the LRRK2 mutations cluster in regions containing the GTPase and kinase domains. The most common mutation G2019S, is located in the kinase domain but little is known about LRRK2 regulation, its physiological substrates or signalling pathways. We have established tetracycline-inducible stable cells overexpressing full-length native wild-type, G2019S and kinase dead (D1994A/D2017A) mutants to address these issues. Optimising the concentration of tetracycline and length of treatment resulted in comparable levels of LRRK2 expression between the different cell lines. LRRK2 kinase activity was confirmed by immunoprecipitating LRRK2 protein and performing in vitro kinase assays. The LRRK2-inducible cells were then used to identify which signalling pathways are activated upon LRRK2 expression. We examined both gene expression changes using microarray studies and protein expression changes using SILAC (stable isotope labelling by amino acids in cell culture) based quantitative mass spectrometry. Results from both studies will be presented.