INVESTIGATION OF PD-ASSOCIATED PHOSPHORYLATION MECHANISMS: IDENTIFICATION AND VALIDATION OF LRRK2 SUBSTRATES

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LRRK2 (leucine-rich repeat kinase 2) plays a major role in the pathogenesis of Parkinson’s disease (PD) as mutations in LRRK2 (G2019S and R1441C) are thus far the most common known cause of autosomal dominant late-onset PD and represent a genetic risk factor in sporadic PD. Although a role for LRRK2 in PD progression is supported by genetic linkage, the underlying mechanism leading to disease progression is not known. Discovery of cellular substrates for LRRK2 will have significant implications since the biological activities of LRRK2 are likely evoked through phosphorylation of its endogenous targets.

In this communication, we describe identification of LRRK2 substrates by incubation of a protein array (ProtoArray®️, Invitrogen) with LRRK2 (WT, G2019S and the kinase deficient D1994A) in the presence of AT33P. On the array, 28 substrates were phosphorylated by WT and/or G2019S LRRK2 but not in the presence of LRRK2 carrying the D1994A mutation. Since artificial kinase-substrate interactions might occur, additional validation steps are required. Therefore, candidate substrates are further validated via in vitro kinase reactions and co-expression with full length LRRK2 in HEK293 cells to verify whether the interaction can be observed in a cellular environment. Analysis of the latter is performed by immunoprecipitation combined with 33P metabolic labeling or phospho specific antibodies.

Further investigation of these substrates will define their potential to be used as pharmacodynamic readout and/or provide more insight in LRRK2-driven pathological phosphorylation mechanisms.