LRRK2 Cell Biology and Parkinson’s Disease

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LRRK2 Cell Biology and Parkinson's Disease

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Research Abstract

Parkinson’s disease (PD) is a common neurodegenerative movement disorder characterized by the selective loss of dopaminergic neurons of the substantia nigra pars compacta and the appearance of Lewy bodies whose major component is fibrillar alpha-synuclein. It is unclear why neurons degenerate in PD. While typically a sporadic disease, there are multiple genetic causes. Mutations in eight genes cause familial forms of PD, including alpha-synuclein, parkin, DJ-1, PINK1, leucine-rich repeat kinase 2 (LRRK2), ATP13A2, EIF4G1 and VPS35. Mutations in the LRRK2 gene cause autosomal dominant familial PD that is clinically and neurochemically

similar to sporadic disease. The neuropathology associated with LRRK2-linked disease is heterogeneous with classical nigral neuronal degeneration and gliosis but with either alpha-synuclein, tau or ubiquitin-positive inclusion pathology. Typically, however, LRRK2 mutations predominantly cause alpha-synuclein-positive Lewy body pathology. The significance of protein aggregation for neurodegeneration occurring in LRRK2 PD brains is not known. The LRRK2 gene encodes a 2527 residue protein that contains GTPase and kinase domains. At least in vitro, LRRK2 can function as both a kinase and a GTPase. A number of putative substrates for LRRK2 have been identified in vitro but LRRK2-mediated substrate phosphorylation in cells or tissues has not yet been confirmed. LRRK2 can bind to GDP/GTP via its GTPase domain and can hydrolyze GTP. GTP binding is critical for normal LRRK2 kinase activity whereas enhancing GTP hydrolysis diminishes kinase activity. Thus, LRRK2 is a GTPase-regulated protein kinase. Disease-associated mutations in LRRK2 have variable effects on GTP binding, hydrolysis and kinase activities of LRRK2 and it is not known which enzymatic activity is important for neuronal degeneration induced by LRRK2 mutations. Mutant forms of LRRK2 can induce neuronal cell death in primary cultures in a manner dependent on intact GTP binding and kinase activity, whereas in vivo the expression of the common G2019S variant of LRRK2 promotes progressive nigrostriatal dopaminergic neuronal degeneration in rodents. The contribution of enzymatic activity and the mechanisms underlying LRRK2-dependent neurotoxicity are poorly understood.

It is not yet known how enzymatic activity contributes to LRRK2-mediated neurodegeneration in animal models. The contribution of GTPase activity to LRRK2-mediated neuronal damage in vivo has not yet been explored. Thus, abnormal enzymatic activity and protein aggregation pathways are implicated in the pathogenic actions of familial LRRK2 mutations but formal proof of their contribution to LRRK2-dependent neurodegeneration in vivo is lacking. Accordingly, experiments are proposed to understand LRRK2 cell biology and its contribution to the pathogenesis of PD. We propose to (1) explore the contribution of GTPase activity to LRRK2-induced neuronal degeneration in vivo, (2) examine the requirement of a-synuclein for mediating LRRK2-induced neuronal toxicity in primary cultures and in vivo, and (3) characterize the LRRK2-dependent phosphorylation of putative substrates in vitro and in vivo, and explore the contribution of substrate phosphorylation and expression to LRRK2-induced neuronal toxicity.

In Aim 1 we will explore the contribution of GTPase activity to LRRK2-induced neurodegeneration. Neuronal toxicity induced by the expression of mutant LRRK2 is prevented by impairing GDP/GTP binding, and we have recently demonstrated that the GTPase domain of LRRK2 is sufficient for inducing toxicity in yeast and primary neuronal models. We have identified functional mutations in the GTPase domain of LRRK2 that can modulate GTP binding and hydrolysis. We will explore how the introduction of these functional mutations into G2019S LRRK2 influences progressive dopaminergic neuronal degeneration in a rat adenoviral-mediated gene transfer model. These experiments will determine if inhibiting GTP binding or enhancing or reducing GTP hydrolysis can attenuate the neurotoxic actions of G2019S LRRK2. Furthermore, these experiments will validate the GTPase domain as a molecular target for interfering with LRRK2-dependent neuronal damage, and will clarify whether activation or inhibition of GTPase activity is the most effective strategy. These studies will provide an important guide for the development of new therapeutic agents that target LRRK2.

In Aim 2 we will examine the requirement of endogenous a-synuclein for LRRK2-induced neuronal toxicity. PD brains with LRRK2 mutations predominantly exhibit a-synuclein-positive Lewy pathology but the significance of this pathology for neurodegeneration is not known.

LRRK2 animal models fail to develop a-synuclein aggregates but whether a-synuclein is required for neuronal degeneration through the development of soluble oligomeric species or submicroscopic aggregates remains a possibility. We have recently shown that A53T a-synuclein-induced pathology that develops in transgenic mice is subtly regulated by LRRK2 expression. To further explore the relationship of LRRK2 and a-synuclein we will determine whether gene silencing or genetic deletion of a-synuclein in cultured primary neurons influences LRRK2-induced neuronal toxicity through measurements of neurite outgrowth and cell death. Furthermore, we will determine whether genetic deletion of a-synuclein in mice influences dopaminergic neuronal degeneration induced by the adenoviral-mediated expression of human G2019S LRRK2 in the nigrostriatal pathway. Therefore, we hope to clarify whether a-synuclein is critically required for mediating the neurotoxic effects of mutant LRRK2 in primary neuronal and animal models.

In Aim 3 we will validate the LRRK2-dependent phosphorylation of two putative substrates and explore the contribution of substrate phosphorylation and expression for mediating LRRK2-induced neuronal toxicity. LRRK2 can function as a kinase in vitro but substrate phosphorylation in mammalian cells or tissues has not been clearly demonstrated. LRRK2-induced neuronal toxicity that occurs in primary cultures and in mice is dependent on kinase activity but whether abnormal substrate phosphorylation mediates this process is not known. We recently identified ArfGAP1 as a novel substrate of LRRK2 in vitro and demonstrated that ArfGAP1 expression is critically required for LRRK2-induced neuronal toxicity. Furthermore, a quantitative proteome-wide screen for phospho-peptides that are modulated by LRRK2 kinase activity in human neural cells identified a number of putative LRRK2 substrates, including GOLGA4. To validate new robust substrates for LRRK2 we will characterize the LRRK2-dendent phosphorylation of ArfGAP1 and GOLGA4 in vitro, in mammalian cells and in brain tissue from LRRK2 animal models. We will also identify and confirm the sites of phosphorylation and develop phospho-specific antibodies to these substrates. To functionally validate the contribution of substrate phosphorylation to LRRK2-induced neuronal toxicity, we will explore how the overexpression and silencing of each substrate influences the LRRK2-dependent inhibition of neurite outgrowth in primary cultures. Finally, we will explore how the genetic deletion of ArfGAP1 and GOLGA4 in mice influences dopaminergic neuronal degeneration induced by the adenoviral-mediated expression of human G2019S LRRK2 in the nigrostriatal pathway. We hope to identify robust substrates of LRRK2 kinase activity and explore their contribution to LRRK2-dependent neurotoxicity. Furthermore, these studies may identify new targets or signaling pathways for the development of therapeutic strategies to ameliorate the toxic actions of disease-associated LRRK2 variants

Ultimately, by exploring novel aspects of the cell biology and pathophysiology of LRRK2 we hope to reveal potential new molecular targets or pathways for interfering with LRRK2-dependent neurodegeneration in PD.

Lay Summary
Further information available at:

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