Mini-Symposium

The Roles of Kinases in Familial Parkinson's Disease

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The purpose of this mini-symposium is to discuss some of the inherited forms of Parkinson's disease (PD) in view of recent data suggesting that some of the proteins affect cellular signaling pathways. As an illustration, we shall focus on two different kinases associated with recessive and dominant forms of PD. Mutations in the mitochondrial kinase PTEN (phosphatase and tensin homolog)-induced kinase 1 (PINK1) are loss-of-function mutations in a normally neuroprotective protein. Loss-of-function mutations in model organisms have variable effects, from dramatic muscle and spermatid defects in *Drosophila* to more subtle neurophysiological abnormalities in mice. Several lines of evidence relate these to the action of a second gene for familial PD, parkin, an E3 ubiquitin ligase shown recently to have effects on Akt signaling. Mutations in leucine-rich repeat kinase 2 (LRRK2), a cytosolic kinase, are dominant and have the opposite effect of causing neuronal damage. The mechanism(s) involved are uncertain at this time because LRRK2 is a large and complex molecule with several domains. Increased kinase activity accounts for the action of at least some of the mutations, suggesting that hyperactive or misregulated kinase activity may lead to the damaging effects of LRRK2 in neurons. For both PINK1 and LRRK2, the following key question that needs to be answered: what are the physiological substrates that mediate effects in cells? Here, we will discuss some of the recent thinking about physiological and pathological roles for signaling in PD and how these may have therapeutic implications for the future.

Key words: **Parkinson's disease; dopaminergic; genetics; human; knock-out mice; mitochondria; neuron death; phosphorylation; protein kinases; signal transduction**

Introduction

Parkinson's disease (PD) affects >1 million people in North America alone. PD is characterized by motor and cognitive dysfunction reflecting widespread neurodegeneration, especially of midbrain dopaminergic neurons. Although PD is typically a sporadic illness, there is growing recognition that genetic susceptibility plays an important role. Indeed, the discovery of mutations underlying rare inherited forms of PD has shed light onto the molecular mechanisms that contribute to the sporadic disease.

The purpose of this article, linked to a Society for Neuroscience mini-symposium, is to review recent insights into the function of two PD genes, PTEN (phosphatase and tensin homolog)-induced kinase 1 (PINK1) and leucine-rich repeat kinase 2 (LRRK2), and to illustrate how the study of these genes is beginning to uncover signaling events underlying PD-related neurodegeneration.

Pink1, parkin, and pathways to recessive parkinsonism

Recessively inherited *PINK1* mutations are a relatively frequent cause of parkinsonism (Valente et al., 2004). *PINK1*-related disease is clinically similar to idiopathic PD and responds well to dopamine replacement therapies. PINK1 contains a serine/threonine protein kinase domain (Valente et al., 2004), preceded by an N-terminal mitochondrial targeting sequence that localizes the protein to mitochondria. Mutations are found throughout the protein, although missense mutations are commonly found in the kinase region. Both truncating and destabilizing mutations are also found, which support the idea that pathogenic mutations cause disease through a loss of function. In addition, PINK1 overexpression protects against oxidative and apoptotic stressors in a kinase-dependent manner (Petit et al., 2005). Thus, PINK1 loss-of-function appears to promote PD-related neurodegeneration.

One of the problems with testing whether PINK1 mutations are simple loss-of-function mutations is that knowledge of direct substrates is limited. Therefore, most reports to date have used autophosphorylation or generic substrates to measure enzyme activity. A limitation of these assays is that they may not fully capture all the ways in which mutations work; for example, if a mutation on the protein surface disrupted docking of the enzyme to a pathological substrate, then this might not be captured in an autophosphorylation mode. In part because of this limitation, but more importantly because substrates are probably key to understanding pathogenesis, the search for PINK1 substrates is a critical step for the field. Recently, a mitochondrial chaperone, tumor necrosis factor receptor-associated protein 1, has been

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shown to be phosphorylated by PINK1 *in vitro* and in cell models (Pridgeon et al., 2007). Additional confirmation that this substrate contributes to PINK1 pathogenesis *in vivo* is awaited, perhaps in some of the models recently described for PINK1 deficiency.

Studies of PINK1 *in vivo* support the notion that it functions at mitochondria. Loss-of-function of the *Drosophila* PINK1 homolog (Clark et al., 2006; Park et al., 2006; Yang et al., 2006) leads to morphological abnormalities of mitochondria and apoptosis in testes and flight muscles. Some, but not all, of these studies also report dopaminergic cell loss. These findings were similar to loss of function of the *Drosophila* parkin homolog, leading investigators to explore a potential link between these two PD genes. Indeed, parkin overexpression rescues the PINK1 phenotype, but PINK1 overexpression does not rescue parkin loss of function. These findings suggest a functional relationship between PINK1 and parkin and indicate that PINK1 functions upstream of parkin. A similar relationship awaits demonstration in mammalian systems. *PINK1* knock-out (KO) (Kitada et al., 2007) mice exhibit abnormalities of evoked dopamine release but show no evidence of neurodegeneration. Electron microscopy analysis revealed that the density and morphology of mitochondria in the striatum of *PINK1*$^{-/-}$ and control mice is similar (T. Kitada and J. Shen, unpublished results). These results indicate that, in contrast to *Drosophila* mutants, loss of PINK1 function in the mouse brain does not cause severe mitochondrial morphological defects. The reason for this apparent discrepancy is not clear and is the subject of active discussion.

Like PINK1, parkin mutations cause autosomal recessive parkinsonism (Kitada et al., 1998) and account for a large fraction of familial early onset cases (Lucking et al., 2000). Parkin is an E3 ubiquitin (Ub) ligase, and many PD-associated mutations likely lead to a loss of parkin Ub-ligase activity. This would be expected to cause target substrate accumulation, because E3 ubiquitination (via lysine 48, or "K48" of ubiquitin) is required for proteasomal degradation. However, not all mutations inactivate the ubiquitination activity of parkin (Hampe et al., 2006; Matsuda et al., 2006), and most candidate parkin substrates do not accumulate in the brains of parkin KO mice (Goldberg et al., 2003; Itier et al., 2003; Von Coelln et al., 2004; Ko et al., 2005; Perez and Palmiter, 2005; Periquet et al., 2005). Thus, a number of key questions remain about the pathogenic mechanisms related to parkin and whether proteasomal degradation is indeed the critical function.

Ubiquitination has been implicated in other cellular functions (Hicke and Dunn, 2003; Mukhopadhyay and Riezman, 2007), including signaling and protein trafficking. This typically involves mono-, multi-mono, or K63-linked-Ub conjugation (Hampe et al., 2006; Matsuda et al., 2006), and parkin is capable of catalyzing such linkages. For example, parkin monoubiquitinates epidermal growth factor (EGF) receptor pathway substrate 15 (Eps15), a Ub-binding adaptor protein involved in the endocytosis and trafficking of the EGF receptor (EGFR), a receptor tyrosine kinase. Ubiquitination of Eps15 may interfere with the ability of the Eps15 to bind ubiquitinated EGFR, thereby delaying EGFR internalization and degradation and promoting prosurvival phosphatidylinositol 3 kinase-Akt signaling. Parkin also monoubiquitinates protein interacting with C-kinase 1 (PICK1) (Joch et al., 2007), an adaptor protein involved in the trafficking of neurotransmitter receptors, transporters, and ion channels (Madsen et al., 2005). In contrast to other E3 Ub-ligases, which ubiquitinate cell surface receptors and channels, the action of parkin on Eps15 and PICK1 indicate that it affects kinase signaling pathways by ubiquitinating downstream adaptor proteins involved in endocytosis and trafficking. Such a role for parkin may explain the lack of accumulation of most known parkin substrates in parkin null mice and the potential functional link between parkin and PINK1.

LRRK2, a kinase for dominant PD

Dominant mutations of LRRK2 are the most common cause of inherited PD (Paisan-Ruiz et al., 2004; Zimprich et al., 2004; Bonifati, 2007), and most cases of LRRK2-related PD are clinically and pathologically indistinguishable from the idiopathic disease. LRRK2 contains both GTPase and kinase domains, as well as two protein–protein interactions domains (leucine-rich and WD40 repeats). Definitively pathogenic mutations have been identified in the GTPase and kinase domains, as well as the region between these domains.

Significant efforts have been made to determine whether PD mutations alter LRRK2 kinase activity. There is consensus that G2019S significantly increases LRRK2 kinase function in assays of either autophosphorylation or phosphorylation of generic substrates (West et al., 2005; Greggio et al., 2006; MacLeod et al., 2006; Hatano et al., 2007; Jaleel et al., 2007; Luzon-Toro et al., 2007). However, controversy exists regarding whether the other PD mutations alter LRRK2 kinase function (Gloeckner et al., 2006; Jaleel et al., 2007). Mutations in the GTP-binding domain diminish the (admittedly low) rate of GTP hydrolysis seen with wild-type LRRK2 (Lewis et al., 2007; Li et al., 2007), suggesting that these mutations may not affect kinase activity per se but how kinase activity is regulated by GTP binding. Clarifying the effects of PD mutation on kinase function awaits the identification of true LRRK2 substrates, because autophosphorylation or model substrate assays may not accurately reflect LRRK2 function.

Multiple LRRK2 PD mutants show enhanced toxicity, causing significantly greater cell death than the wild-type protein in cell lines and primary neurons. Notably, abolishing LRRK2 kinase function diminishes the toxicity of all PD mutants (Greggio et al., 2006; Smith et al., 2006) although most of these mutations do not appear to enhance kinase function. Thus, most PD mutations appear to cause cell death by altering some other feature of LRRK2 biology but that nonetheless requires intact (basal) kinase function. The logical connection is that LRRK2 is a signaling molecule and that kinase activity is one key part of the signaling process. Presumably, LRRK2 becomes pathogenic when the kinase is hyperactive or misregulated, and this may involve signaling pathways. Some recent evidence suggests that LRRK2 or homologs in other species have roles in neurite outgrowth and sorting of molecules along axons (MacLeod et al., 2006; Sakaguchi-Nakashima et al., 2007). Therefore, LRRK2 probably has activities that are important (perhaps even required) for normal neuronal function. Understanding the relationship between pathological and normal signaling is clearly the key step for the field: is it the same set of signals but misregulated or novel pathways only accessed by the mutant proteins?

As an example of a novel property of mutant LRRK2, some mutations dramatically alter the subcellular distribution of the protein, causing it to concentrate in string-like filamentous structures rather than its normal diffuse cytosolic pattern (C. C.-Y. Ho and H. Rideout, unpublished observations). Notably, blocking kinase activity virtually abolishes filament formation, linking LRRK2 filament formation to neurotoxicity. In previously identified cases, filament formation reflects a homotypic protein–protein interaction that mediates signaling, and oligomerization promotes the recruitment of signaling molecules into spatially

defined complexes that facilitates their interaction and activation. LRRK2 oligomerizes and filament-forming mutations enhance its oligomeric state (Dauer, Ho, and Rideout, unpublished observations). LRRK2 oligomerization appears to generate a protein scaffold that recruits other signaling molecules. Filament-forming mutations may act by leading to a structural change in LRRK2 that exposes an autophosphorylation site and that blocking kinase function prevents filament formation (and therefore substrate recruitment and toxicity) by preventing the formation of the phosphomotif required for oligomerization. It is of interest that a kinase-dependent accumulation of LRRK2 has also been proposed for other mutations both *in vitro* (Greggio et al., 2006) and *in vivo* (MacLeod et al., 2006), although not all studies have reported increased inclusion bodies (West et al., 2005), suggesting that experimental details such as choice of mutation and expression levels may be important. These data suggest a novel signaling mechanism for LRRK2 and highlight the notion that blocking LRRK2 self-association may be a novel therapeutic strategy for PD.

Summary

The identification PD genes have provided an array of new tools with which to unravel PD pathogenesis. PINK1 and parkin appear functionally related and to be involved in neuroprotective signaling, although the mechanistic aspects are poorly understood. LRRK2 mutations appear to cause a toxic gain of function that requires intact kinase function. The fact that all of these proteins contain well understood catalytic motifs is enabling mechanistic studies, and the identification of bona fide substrates should greatly enhance our understanding of the detailed relationship between genes and neuronal survival.

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