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Review

Novel insights into the neurobiology underlying LRRK2-linked Parkinson's disease

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ABSTRACT

Mutations in LRRK2 (leucine-rich repeat kinase 2) are found associated with both sporadic and familial Parkinson's disease (PD). Pathogenic mutations are localized to the catalytic domains of LRRK2, including kinase and GTPase domains. Altered catalytic activity correlates with neurotoxicity, indicating that targeting those activities may provide clues as to novel therapeutic strategies for LRRK2-linked PD. However, the cellular readout of such altered catalytic activities remains largely unknown. Recent cell biological studies have started to highlight possible early cellular events which are altered in the presence of pathogenic LRRK2 and may ultimately lead to neuronal demise, and these studies link altered LRRK2 function to various abnormal endolysosomal vesicular trafficking events. This review examines our current knowledge of LRRK2 neurobiology and how pathogenic mutations may lead to neurodegeneration in PD.

1. Introduction

Parkinson's disease (PD) is an age-related neurodegenerative movement disorder, characterized by resting tremor, slowness of movement, muscular rigidity and postural instability. The neuropathological hallmarks of PD include progressive degeneration of dopaminergic neurons in the substantia nigra pars compacta, and the presence of intraneuronal cytoplasmic inclusions called Lewy bodies in surviving neurons, which are rich in α-synuclein (reviewed in Halliday et al., 2011). The clinical motor symptoms of PD result from reduced dopamine input to the striatum due to the severe loss of dopaminergic neurons.

There is currently no cure for PD, and its prevalence will increase as our life expectancy continues to rise. Despite decades of research, the molecular and cellular mechanisms underlying the pathogenesis of Parkinson's disease (PD) have largely remained unknown. This is in part due to the fact that the majority of PD cases appear to be idiopathic and probably reflect a complex interaction between age, genetic predisposition and environmental factors. This has made the establishment of PD disease models very difficult. However, over the past years, mutations in several genes with both recessive and dominant modes of inheritance have been clearly linked to familial forms of PD, including mutations in *SNCA* (α-synuclein), *PARK2* (parkin), *PINK1* (phosphatase and tensin homologue deleted on chromosome 10-induced putative kinase 1), *PARK7* (DJ-1), *GBA* (acid β-glucosidase) and *LRRK2* (leucine-rich

Abbreviations: AKAP, A-kinase anchoring protein; ANK, ankyrin repeat; AP, adaptor protein; ARM, armadillo repeat; CHIP, carboxyl terminus of hsp70-interacting protein; CMA, chaperone-mediated autophagy; COR, C-terminal of ROC; ERM, ezrin/radixin/moesin protein family; GAD, GTPase activated by nucleotide-dependent dimerization; GAP, GTPase activating protein; GEF, guanine nucleotide exchange factor; LAMP, lysosome-associated membrane glycoprotein; LRRK2, leucine-rich repeat kinase 2; M6PR, mannose 6-phosphate receptor; NEM, N-ethylmaleimide; NSF, N-ethylmaleimide sensitive fusion protein; PD, Parkinson's disease; PKA, protein kinase A; ROC, Ras of complex proteins; SNARE, soluble NSF attachment protein receptor.

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repeat kinase 2) (reviewed in Singleton et al., 2013). These findings allow for the generation of well-defined cellular and animal models carrying the mutant genes to define disease mechanisms, and this fact has opened up a new area in PD research, allowing us to more precisely identify the molecular mechanisms underlying this disease.

2. LRRK2-linked PD

Autosomal-dominant missense mutations in LRRK2 comprise the most common monogenic form of PD, which clinically and pathologically resembles the sporadic disease (Haugarvoll and Wszolek, 2009). In addition, genome-wide association studies have identified common variations in the LRRK2 gene as risk factors for sporadic PD (Satake et al., 2009; Simón-Sánchez et al., 2009; Nalls et al., 2011), thus implicating LRRK2 in both sporadic and familial forms of the disease. Amongst several putative variants, at least seven point mutations clearly segregate with disease in familial forms of PD, and thus are truly pathogenic (G2019S, R1441C, R1441G, R1441H, N1437H, Y1699C and I2020T) (Fig. 1) (Cookson, 2010; Aasly et al., 2010).

LRRK2 belongs to the ROCO family of proteins, characterized by the presence of a conserved ROC (Ras of complex proteins) and COR (C-terminal of ROC) bi-domain (Marin, 2006). The ROC domain of LRRK2 can bind and hydrolyse GTP, and thus displays true GTPase activity. LRRK2 also contains a kinase and various protein–protein interaction domains such as armadillo (ARM), ankyrin (ANK), leucine-rich and WD40 repeats (Fig. 1). There seems to exist a link between the enzymatic function(s) of LRRK2 and neuronal cell death in the presence of pathogenic mutations (reviewed in Cookson, 2010). Thus, LRRK2 has emerged as prime drug target for novel approaches to PD therapeutics.

3. LRRK2 kinase activity

Even though from a pharmacological standpoint, a kinase generally makes for a great drug target, studies into LRRK2 kinase activity and identification of substrate(s) have been difficult. Early studies focused on LRRK2 autophosphorylation, an inherent feature of many protein kinases (reviewed in Nolen et al., 2004). Data of this type consistently indicate that the common G2019S mutation in the activation loop of the kinase domain increases catalytic activity, even though the effects of other pathological mutations on kinase activity have remained controversial (Fig. 1) (reviewed in Greggio and Cookson, 2009). In contrast, a recent study indicates that most pathogenic mutants display increased autophosphorylation at one particular site (S1292) *in vitro* and *in vivo* (Sheng et al., 2012), and further studies will be required to determine whether altered kinase activity is a common readout of all pathogenic LRRK2 mutations, or rather specific to the G2019S mutation.

Active LRRK2 seems to be a dimeric protein (Greggio et al., 2008; Sen et al., 2009; Berger et al., 2010), with autophosphorylation of the full-length protein occurring through an intramolecular (cis) event (Greggio et al., 2008; Sheng et al., 2012), and further regulated by protein interactions. Thus, in intact cells, activity may be subjected to regulation by dimerization and modulated by the recruitment of LRRK2 to protein complexes and/or to membranes (see below). Whilst a reliable readout for catalytic activity, the physiological relevance of LRRK2 autophosphorylation remain unclear. Autophosphorylation sites have been mapped mainly to the ROC domain (Greggio et al., 2009; Kamikawaji et al., 2009; Pungaliya et al., 2010; Gloeckner et al., 2010; Webber et al., 2011). Since the sites within the ROC domain are very close to the motifs which mediate GTP binding, autophosphorylation of ROC may alter this function (see below) (reviewed in Taymans, 2012).

Apart from LRRK2 itself, few substrates for its kinase activity have been described thus far. Interestingly, the ERM (ezrin/radixin/moesin) family of proteins, which function to anchor the actin cytoskeleton to the plasma membrane and play important roles in regulating membrane structure and organization, has been identified as LRRK2 kinase substrates. Indeed, phospho-ERM levels are altered in cultured neurons from G2019S LRRK2 transgenic mice, concomitant with alterations in actin cytoskeleton dynamics (Parisiadou et al., 2009), suggesting that LRRK2 may directly or indirectly regulate ERM phosphorylation. Studies of this type have also allowed development of efficient model substrate peptides such as LRRKtide and Nictide, derived from ERM sequences, which has significantly improved analysis of LRRK2 kinase activity *in vitro* (Jaleel et al., 2007; Nichols et al., 2009). Specific LRRK2 kinase inhibitors, some of them brain permeant, have been described (Liu

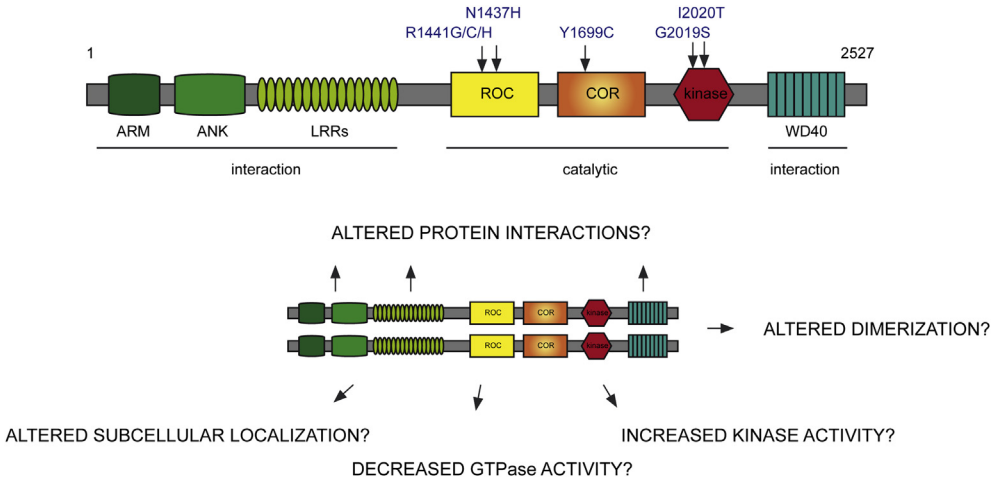

Fig. 1. Top: Schematic representation of LRRK2. Full-length LRRK2 is indicated with its distinct domains, as well as pathogenic mutants indicated in blue. ARM, armadillo repeats; ANK, ankyrin repeats; LRRs, leucine-rich repeats, WD40, WD40 repeats. Bottom: proposed altered functional readouts for pathogenic LRRK2. Pathogenic mutations may alter kinase output towards substrates or towards itself. The latter may inhibit the GTPase activity, thus prolonging effector interactions, and this may be the relevant physiological readout of LRRK2. Alternative scenarios include a change in the dimeric status of LRRK2, which may modulate activity and/or subcellular localization, or a change in distinct protein interactions of LRRK2, which may serve a scaffolding role for the formation of distinct protein complexes.