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Review

ERKed by LRRK2: A cell biological perspective on hereditary and sporadic Parkinson's disease☆,☆☆

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ABSTRACT

The leucine rich repeat kinase 2 (LRRK2/dardarin) is implicated in autosomal dominant familial and sporadic Parkinson's disease (PD); mutations in *LRRK2* account for up to 40% of PD cases in some populations. LRRK2 is a large protein with a kinase domain, a GTPase domain, and multiple potential protein interaction domains. As such, delineating the functional pathways for LRRK2 and mechanisms by which PD-linked variants contribute to age-related neurodegeneration could result in pharmaceutically tractable therapies. A growing number of recent studies implicate dysregulation of mitogen activated protein kinases 3 and 1 (also known as ERK1/2) as possible downstream mediators of mutant LRRK2 effects. As these master regulators of growth, differentiation, neuronal plasticity and cell survival have also been implicated in other PD models, a set of common cell biological pathways may contribute to neuronal susceptibility in PD. Here, we review the literature on several major cellular pathways impacted by LRRK2 mutations – autophagy, microtubule/cytoskeletal dynamics, and protein synthesis – in context of potential signaling crosstalk involving the ERK1/2 and Wnt signaling pathways. Emerging implications for calcium homeostasis, mitochondrial biology and synaptic dysregulation are discussed in relation to LRRK2 interactions with other PD gene products. It has been shown that substantia nigra neurons in human PD and Lewy body dementia patients exhibit cytoplasmic accumulations of ERK1/2 in mitochondria, autophagosomes and bundles of intracellular fibrils. Both experimental and human tissue data implicate pathogenic changes in ERK1/2 signaling in sporadic, toxin-based and mutant LRRK2 settings, suggesting engagement of common cell biological pathways by divergent PD etiologies. This article is part of a Special Issue entitled: Misfolded Proteins, Mitochondrial Dysfunction, and Neurodegenerative Diseases.

1. Introduction

Parkinson's disease (PD) is the second most common neurodegenerative disorder, affecting approximately 1–2% of the population over the age of 65 [1]. Clinically, PD is characterized by resting tremor, rigidity, bradykinesia, and stooped posture. The majority of these motor impairments arise from the loss of dopaminergic neurons in the substantia nigra pars compacta (SNpc), which results in depletion of dopamine from the nigro-striatal system. Formation of Lewy bodies (LB), intracytoplasmic inclusion bodies that contain α-synuclein, often accompanies this selective neurodegeneration [2]. Though the underlying cause of sporadic PD remains unidentified, exposure to environmental toxins and genetic factors have been implicated in PD pathogenesis.

Higher incidences of sporadic PD have been reported in rural populations associated with agricultural work. This observation suggests that pesticides and herbicides may contribute to PD [3,4]. Rotenone, a mitochondrial complex I inhibitor, is one of the pesticides that has been linked to PD. It was shown to cause selective degeneration of dopaminergic neurons after systemic administration to rats, and is currently used as a toxin model to study sporadic PD [5]. A toxin previously shown to induce human parkinsonism is MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine). The oxidized product of MPTP, MPP+ is selectively taken up by dopaminergic neurons, accumulates in the mitochondria and inhibits complex I [6]. Both toxins induce mitochondrial damage, implicating a key role for dysregulated mitochondrial homeostasis in sporadic PD.

Though comprising only a fraction of total PD cases, a genetic component to parkinsonian neurodegeneration has become well established (reviewed in [7]). There are at least 16 *PARK* loci that have been identified and are associated with either autosomal recessive or autosomal dominant PD [8]. Causal mutations have been identified in a number of these loci, and the functional consequence of these mutations on the encoded proteins is an area of intense study. Mutations in the leucine-

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rich repeat kinase 2 gene (*LRRK2*) are the most common known cause of familial PD [9–12]. The biochemistry of LRRK2 has been reviewed elsewhere [13,14]. In this review we discuss major cell biologic processes and signaling pathways that may be regulated by LRRK2, and how mutations in *LRRK2* may serve to promote neurodegeneration.

2. *LRRK2* and Parkinson's disease

The *LRRK2* locus was first identified in a Japanese family with autosomal dominant parkinsonism by linkage analysis, involving a novel PARK locus on chromosome 12q12 that was named *PARK8* [15]. In 2004, two independent groups cloned the responsible gene and identified pathogenic mutations in the *PARK8* locus [11,16].

LRRK2 is a multidomain, 2527 amino acid (~280 kDa) protein. The N-terminus consists of an ankyrin-like domain and leucine-rich repeats (LRR). LRRK2 also contains a Ras of complex (ROC) GTPase domain adjacent to a C-terminal of ROC (COR) domain that functions as a linker between the N- and C-terminus of the protein. The kinase domain and a WD40 domain are present in the C-terminal portion of the protein [17]. The LRRK2 kinase domain shares a close homology with mixed linkage kinases (MLKs), which comprise a sub-class of the mitogen activated protein kinase kinase kinase (MAPKKK) family.

Expression analysis by RT-PCR [11] and Northern Blot [16] revealed that LRRK2 is widely expressed in various tissues and cell types, including the brain, lungs, liver, skeletal muscle and kidneys. Within the brain, it is expressed in multiple regions, with a high level of expression in the putamen, where terminals of the substantia nigra region that are vulnerable to neurodegeneration in PD project. LRRK2 is predominately localized in the cytoplasm, where it associates with mitochondria [18], endoplasmic reticulum, Golgi, microtubules [10] and vesicular structures such as lysosomes and endosomes [19].

Multiple pathogenic mutations in *LRRK2* have been linked to familial parkinsonism. These include I2020T [12], G2019S [20–22], R1441G [9], R1441C [11] and Y1699C [16]. The most prevalent mutation is G2019S in the kinase domain which increases LRRK2 kinase activity and is estimated to occur in approximately 6–8% of familial and 1–2% of sporadic PD cases [23]. LRRK2-G2019S mutants can form inclusion bodies when expressed in cultured cells or neurons, associated with neuronal toxicity [24]. The toxic effects of this mutation can be ameliorated using the selective LRRK2 inhibitor, LRRK2-IN-1 [25].

Most LRRK2 kinase activity studies have been done *in vitro* using generic substrates, such as myelin basic protein (MBP); identifying *in vivo* targets of LRRK2 remains an area of active investigation. A number of proteins represent potential substrates, including: Ezrin/Radixin/Moesin (ERM) [26], beta-tubulin [27], 4E-BP1 [28] and alpha-synuclein [29]. In addition to the *in vitro* analyses of LRRK2 targets, a variety of cell culture and animal studies indicate that LRRK2 may play a role in the regulation of cell death, autophagy, the cytoskeleton, protein translation and cell signaling pathways.

3. The cell biology of *LRRK2* and its mutations

3.1. LRRK2 and autophagy

Autophagy is an important catabolic process in which cytoplasmic components are engulfed into a double membrane vesicle called an autophagosome, after which they are delivered by fusion events to the lysosome for hydrolytic degradation [30]. Cells use autophagy to maintain protein and organellar integrity on an ongoing basis. Autophagy is induced in response to starvation [31,32] and other cellular stresses implicated in a number of neurodegenerative diseases (reviewed in [33]). In neurons, suppression of basal autophagy results in the accumulation of misfolded proteins and neurodegeneration [34,35]. Autophagy is a common response to injury, but depending on the context, autophagy induction can be adaptive or maladaptive [36].

A number of studies have emerged that implicate a link between LRRK2 and autophagy regulation, but the exact mechanisms are still poorly understood. Overexpression of pathogenic LRRK2 mutants induces autophagosome accumulation in multiple cell types, including neurons [37,38], neuronal cell lines [39] and kidney cells [40].

The first study implicating LRRK2 in the (mis)regulation of autophagy utilized retinoic acid differentiated SH-SY5Y cells to show that the G2019S mutant of LRRK2, but not the kinase-deficient mutant, increased autophagosomes in neurites. More mature autophagic vacuoles (multivesicular-like bodies and lysosomes) were observed in the soma [39] suggestive of retrograde trafficking and maturation. RNAi knockdown of components of the autophagic machinery, LC3 and Atg7, indicated that the LRRK2-induced neurite retraction is mediated by autophagy [39], confirming that mutations in LRRK2 regulate the morphology of neuronal processes in PD [41]. Subsequent studies in primary neurons demonstrated that Protein kinase A protects against G2019S- or R1441C-LRRK2-mediated dendrite shortening by phosphorylating and inactivating LC3, the mammalian homolog to the yeast Atg8 autophagy protein [37].

Perturbed calcium homeostasis has been shown to play a role in LRRK2-induced autophagy. Autophagy induced by overexpression of wild type or G2019S LRRK2 has been reported to occur through activation of an NAADP related Ca^{2+}/CaMKK/AMPK pathway [42]. Treatment with inhibitors of this pathway, such as compound c (AMPK inhibitor), STO-609 (CaMKK-a/b inhibitor), or chelation of intracellular calcium with BAPTA-AM abrogates the increase in autophagosome number induced by LRRK2 overexpression [42]. Additionally, deficiencies in calcium handling lie upstream of dendritic mitophagy, which contributes to dendrite retraction in primary neurons overexpressing G2019S- or R1441C-LRRK2 [38]. Inhibition of calcium influx through L-type voltage gated calcium channels prevents mitochondrial depolarization, mitophagy and dendrite shortening [38].

It remains controversial whether the autophagosome accumulation induced by LRRK2 is due to an increase in autophagy induction, inhibition of autophagic flux, or both. Overexpression of the LRRK2-R1441G in HEK293 cells caused accumulation of p62 and large autolysosomes, consistent with decreased flux [40]. However, a subsequent study showed that the LRRK2-mediated increase in p62 could be blocked by a translation inhibitor [42]. The earlier study also reported that knockdown of GFP-tagged LRRK2 prevented cell death caused by bafilomycin under starvation conditions. Since bafilomycin inhibits late stages of lysosomal degradation [43], it is unclear how LRRK2 siRNA would be able to restore flux through the system, suggesting additional mechanisms of cytoprotection. In a recent study involving a LRRK2 kinase inhibitor (LRRK2-IN-1), LRRK2 activity was placed upstream of autophagy initiation. As this highlights a possible role of endogenous LRRK2 in suppressing autophagy initiation, it is possible that either too little or too much LRRK2 kinase activity may induce autophagy [44].

In vivo studies also suggest that altered LRRK2 expression leads to dysregulation of autophagy. *Lrrk2*$^{-/-}$ mice are viable, physically normal, and have an intact dopaminergic system [45]. However, alpha-synuclein and ubiquitinated protein accumulation, accompanied by dysregulation of the autophagy-lysosomal pathway, has been observed in the kidneys. The observed alterations of autophagic activity are age-dependent and bi-phasic. At 1 month of age there are no observable differences, but autophagic activity is enhanced at 7 months, and then reduced at 20 months [46]. The authors suggest that impaired clearance of proteins observed in aged mice was due to decreased recycling of induced autolysosomes and increased accumulation of autophagic vacuoles.

Transgenic mice expressing LRRK2-G2019S show progressive degeneration of dopaminergic neurons, as demonstrated by shorter neurite length and branching, leading to an autophagy-induced reduction in neurite complexity. This loss of DA neurons, is more pronounced in older G2019S-LRRK2 transgenic mice than younger transgenic or wild type mice, and requires a certain level of LRRK2 overexpression

[47]. In *Drosophila*, overexpression of LRRK2-G2019S mutation causes increased loss of photoreceptors in the retina due to elevated autophagy caused by upregulation of key autophagy gene, Atg5, compared to wild type LRRK2 overexpression [48].

LRRK2 may also be a victim of dysregulated autophagy rather than the perpetrator. Conditional deletion of the essential autophagic gene, Atg7, in DA neurons caused a presynaptic accumulation of alpha-synuclein, p62 and LRRK2 proteins in large intracellular inclusions in the brain [49]. Similarly, there was almost a 4-fold increase in LRRK2 mRNA in Atg5$^{-/-}$ MEFs compared to control cells. These results showed that dysregulation of autophagy could lead to an abnormal elevation in expression of LRRK2 [49].

The role that LRRK2 plays in autophagy regulation remains unclear. However, a large body of evidence suggests that autophagosome accumulation induced by expression of PD-associated mutants contributes to injury in LRRK2 models of PD. Alterations in calcium handling and LRRK2 kinase activity have been shown to contribute to this phenotype, but further investigation is required to unravel the mechanism of autophagy dysregulation in these models.

3.2. LRRK2 and microtubule dynamics

Cytoskeletal components are important for maintaining the structural support for neurons, in vesicular biogenesis, organelle or vesicle transport, and synaptic signaling [50]. Dysfunction in cytoskeleton dynamics is often associated with neurodegenerative diseases [51,52]. Rotenone, a mitochondrial complex I inhibitor, was shown to induce depolymerization of microtubules leading to disruption in the transport of dopamine vesicles and their accumulation in the soma and eventually, oxidative stress due to the leakage of dopamine from these vesicles [53].

LRRK2 has been shown to co-localize with β-tubulin [10,19] and to interact directly with α/β tubulin through the ROC GTPase domain [19,54]. Wild-type and R1441C LRRK2 had similar affinities for α/β tubulin [19]. Human LRRK2 interacted with β tubulin and preferentially phosphorylated tubulin from bovine brain, which was enhanced three-fold by the G2019S mutation [27]. Interestingly, phosphorylated β-tubulin did not immunoprecipitate with LRRK2 in HEK293 cells despite this interaction. When incubated with microtubule associated protein-rich medium, G2019S-LRRK2 significantly enhanced microtubule assembly/stability. These results suggest that increased phosphorylation of β tubulin by G2019S-LRRK2 mutant results in decreased microtubule dynamics, affecting the structure of the neurons [27]. Recently, multiple PD associated LRRK2 mutants (R1441C, R1441G, Y1699C and I2020T) have been shown to form filamentous structures that associate with microtubules [55]. This association requires kinase function and the WD40 domain. Interestingly, the G2019S mutation did not show any filament formation [55], raising the possibility of different pathogenic mechanisms elicited by the mutation that clearly elevates kinase activity versus the mutations that do not.

The interaction of LRRK2 and tau (a microtubule-associated protein) leads to increased phosphorylation of tau and shorter neurite processes [41]. This phenotype was prevented with the expression of kinase dead (K1906M) fragment of LRRK2, although caution should be taken due to the need for further characterization of this fragment. Interestingly, this interaction and the *in vitro* phosphorylation of tau by LRRK2 are dependent on tubulin [51]. G2019S- [56] and I2020T- [57], but not R1441C-, LRRK2 mutants phosphorylated more tau than wild-type LRRK2 [58]. Overexpression of the G2019S PD-associated mutant of LRRK2 led to tau-positive inclusions in neurons and LRRK2 co-localized with tau in these inclusions. In *Drosophila*, expression of G2019S-LRRK2 induced mislocalization of tau in the dendrites that leads to dendrite degeneration [59]. Tau phosphorylation was dependent on the expression of LRRK2, as increased LRRK2 expression increased tau phosphorylation, which was conversely reduced by RNAi knockdown of LRRK2. The expression level of LRRK2 was inversely correlated with neurite length [57,58]. Taken together with the autophagy and mitophagy data discussed above, it appears that multiple LRRK2-associated mechanisms may contribute to LRRK2-mediated neurite shortening.

Elongation factor 1A (EF1A), a GTPase that transports aminoacyl-tRNA to the ribosomes during protein translation and is essential for cytoskeletal organization [60,61], has also shown to interact with LRRK2 [62]. Binding of EF1A significantly reduced the autophosphorylation activity of wild type-, G2019S- and R1441C-LRRK2. The microtubule assembly activity of EF1A was shown to be impaired in the presence of wild-type and G2019S-LRRK2 in an *in vitro* microtubule polymerization assay. Phospho-EF1A could not be detected in this study suggesting that EF1A is not a direct target of LRRK2 [62].

In dopaminergic neurons, either knockdown of LRRK2 [63] or transgenic mice expressing LRRK2-G2019S mutations [64] led to shortening of neurite extensions. Using the KinasE Substrate Tracking and ELucidation screening (KESTREL) approach, moesin, a protein that anchors the actin cytoskeleton to the plasma membrane, as well as ezrin and radixin were proposed as G2019S-LRRK2 targets [26]. A follow-up study from the same group, however, failed to detect phosphorylation of ERM proteins in HEK293 cells, even after overexpression of LRRK2-wt or LRRK2-G2019S [65]. On the other hand, mutant LRRK2-G2019S did enhance phosphorylation of ERM proteins in another study, which was correlated with retardation of neurite outgrowth, and inhibition of ERM phosphorylation was able to rescue the G2019S-phenotype [64]. Data from NIH3T3 cells suggest that LRRK2 can directly bind F-actin, affecting its polymerization and depolymerization *in vitro*. These discrepancies suggest that either LRRK2 status modulates ERM phosphorylation indirectly, or that detection of phosphorylated ERM proteins might be dependent on experimental conditions. It will be interesting to see if other groups can detect phospho-ERMs in mammalian neurons.

In summary, current evidence suggests that either too much or too little LRRK2 activity may contribute to neurite shortening, although initial studies indicated that LRRK2 knockdown caused elongated neuronal processes [41]. LRRK2 may interact with and/or phosphorylate or regulate the phosphorylation of several structural and regulatory components of the actin cytoskeleton and microtubule network. Given that neurite shortening is a commonly observed phenotype in neuronal cells expressing pathogenic LRRK2 mutants, understanding these cytoskeletal associations and their possible relationships with autophagy or mitochondrial dynamics may provide insight into the PD pathogenesis.

3.3. LRRK2 and protein translation control

LRRK2 was shown to interact with genes in the TOR/4E-BP pathway in *Drosophila* [28]. In the same study, human wild type LRRK2 and an I2020T mutant were able to phosphorylate 4E-BP1. Eukaryotic 4E-BP1 binds to initiation factor 4E (eIF4E) and inhibits its function, while phosphorylation alleviates this inhibition. Phosphorylation of 4E-BP1 by LRRK2 decreases survival of *Drosophila* neurons. In contrast, loss of LRRK2 or gain of 4E-BP1 function rescues pathology in Drosophila *Parkin*/*PINK1* models [66]. Hence, it has been hypothesized that increased LRRK2 expression or kinase activity increases protein translation to a level that overwhelms the cellular degradation machinery, resulting in accumulation of unwanted proteins [28,66].

However, this interpretation has been challenged by other studies. While phosphorylation of 4E-BP by LRRK2 can be demonstrated *in vitro*, it is a relatively poor substrate [67]. Moreover, increased phosphorylation was not observed when LRRK2 was overexpressed in HEK cells. This study suggested that 4E-BP phosphorylation may be an indirect phenomenon related to p38-mediated cell stress [67]. Similar results were obtained in another study in which the phosphorylation status of endogenous 4E-BP1 was not altered in the brains of LRRK2 knockout or mutant LRRK2 transgenic mice, nor were there changes in idiopathic or G2019S PD patient brains, suggesting that 4E-BP1 is not a direct substrate for LRRK2 in mammalian systems [68].

3.4. Mitochondrial pathology in LRRK2 pathogenesis

Mitochondrial dysfunction is widely recognized as a contributor to parkinsonian injury. Common themes observed in multiple genetic or toxin models include increased reactive oxygen species production, altered mitochondrial dynamics, decreased mitochondrial membrane potential, and impaired calcium homeostasis. The recessive parkinsonian genes PINK1, Parkin and DJ-1 have been particularly implicated in mitochondrial homeostasis, and LRRK2 interacts with these recessive PD genes in *Drosophila* [69]. Interestingly, recent studies also implicate mitochondrial dysfunction in LRRK2 pathogenic mechanisms.

A loss of mitochondrial membrane potential has been reported in primary mouse cortical neurons, immortalized cell lines, and PD patient fibroblasts expressing mutant LRRK2 [38,70]. In addition, elevated ROS and diminished ATP levels have been reported in human fibroblasts derived from patients with the G2019S pathogenic mutation [71]. Decreased $\Delta\Psi_m$ may initiate mitochondrial fission, and knockdown of *PINK1* limits phosphorylation of Drp1 at Ser637, enabling it to be recruited to mitochondria to facilitate fission [72,73]. Two recent studies suggest that overexpression of WT or mutant LRRK2 also leads to mitochondrial fragmentation in mouse primary cortical neurons, SH-SY5Y, and HeLa cells [74,75]. Further, a direct interaction between LRRK2 and Drp1 was described in these reports.

In order to maintain healthy mitochondrial networks, damaged mitochondria need to be recycled by mitophagy. Parkin and PINK1 are integrally involved in regulating the selective, autophagic clearance of mitochondria known as mitophagy [76,77]. We have recently reported that loss of dendritic mitochondria in G2019S or R1441C LRRK2 neurons can be reversed by inhibition of autophagy [38]. Interestingly, perturbed calcium homeostasis was the underlying cause of dendritic mitophagy induced by mutant LRRK2 expression. Consistent with our findings, another group has reported that elevated intracellular calcium levels are necessary for LRRK2 induced autophagy [42]. Delayed calcium clearance has also been observed following PINK1 knockdown and in cells expressing mutant PINK1; thus, impaired calcium buffering is a shared feature of mutant LRRK2 and PINK1-related pathogenic mechanisms [78–80].

Overexpression of PKA protects against injuries induced by either PINK1 knockdown [72] or G2019S-LRRK2 expression [37], providing further evidence that converging signaling cascades contribute to pathogenesis in PINK1 and LRRK2 models. Phosphorylation of Drp1 by PKA appears to be the primary mechanism of protection in PINK1 deficient cells [72], whereas of phosphorylation of LC3 is involved in PKA-mediated protection against injury in cells expressing mutant LRRK2 [37]. PINK also regulates phosphorylation of LC3, albeit through indirect mechanisms, and overexpression of WT-PINK1 prevents autophagy and neurite retraction in cells expressing G2019S-LRRK2 (SJ Cherra & CT Chu, unpublished data).

As with other genetic models of PD, altered mitochondrial dynamics, impaired mitochondrial calcium buffering, elevations in ROS levels, and decreased $\Delta\Psi_m$ have been reported in cells expressing PD-associated LRRK2 mutants. The parallels that can be drawn between pathogenic mechanisms in genetic models of PD provide compelling evidence that aberrant regulation of convergent signaling pathways contributes to mitochondrial dysfunction.

3.5. Endosomal/synaptic dysregulation in LRRK2 pathogenesis

The cytoskeletal, autolysosomal and protein synthesis effects of LRRK2 have been shown to affect synaptic vesicle function/dysfunction and the dynamics of recycling endosomes [81]. In *Drosophila*, loss of LRRK2 causes synaptic overgrowth, while its overexpression has opposite effects [82]. LRRK2 protein targets have been proposed in both the pre- and post-synaptic compartments, namely the microtubule-binding protein Futsch and the protein translation inhibitor 4E-BP, respectively. Presynaptic silencing of LRRK2 causes an increase in vesicular dynamics within the recycling pool [83]. In the postsynaptic compartment, mutant LRRK2 promotes calcium overload through engagement of L-type calcium channels [38] and glutamate receptors (E Plowey & CT Chu, unpublished data). While the molecular mechanisms underlying these synaptic alterations remain unknown, other studies indicate that they may reflect more general problems with RAB7L1-related vesicular protein transport and endosomal, lysosomal and Golgi trafficking [84]. LRRK2 was shown to interact and partially co-localize with Rab5b. Additionally, altering the levels of LRRK2 expression can modulate endocytosis of synaptic vesicles [85]. Interestingly, either overexpression of wild type or PD associated mutants, or knockdown of LRRK2 was shown to slow down the rate of endocytosis. Using yeast as a model system, the defect in endocytosis by LRRK2 was found to be dependent on its GTPase activity [86]. A recent study identified EndoA as a direct substrate for LRRK2, and phosphorylation of EndoA in a *Drosophila* model system affects the endocytotic process [87]. The increased demands for autophagy generated by protein aggregation and mitochondrial dysfunction, combined with defects in endosomal–lysosomal processes, create an imbalance in cellular quality control mechanisms and promotes autophagic stress [88].

In summary, LRRK2 has been shown to play a role in maintaining homeostasis at the synapse through multiple mechanisms. Interactions with known regulators of protein translation, vesicular transport, and autophagy and endocytosis are important for proper synaptic function. As many of the studies have been performed in *Drosophila*, further investigation is needed to determine the role of these mechanisms in mammalian LRRK2 models of PD.

4. Interactions of LRRK2 with the Wnt signaling pathway

4.1. The Wnt pathway and PD

Wnt (Wingless/Int) signaling pathways have been implicated in the regulation of neurogenesis and function of mature and post mitotic neurons [89]. Wnt ligands are extracellular factors that regulate the differentiation of neuronal stem cells into mature neurons [90]. The Wnt signaling cascade has three major branches; canonical, non-canonical/planar cell polarity and Wnt-Ca^{2+}, of which the canonical pathway has been best described [91]. Activation of this pathway results in stabilization and nuclear recruitment of beta-catenin, which regulates gene expression [92,93].

The Wnt signaling pathway is important to the development of DA neurons in the midbrain. Wnt1 and Wnt3a were shown to induce specification of committed dopaminergic precursors, whereas, Wnt5a induced their differentiation by promoting the maturation of $Nurr^{+}$, a nuclear receptor essential for the development and maintenance of DA precursors into DA neurons [94]. Induced expression of Wnt5a in ventral midbrain neural stem cells generated 10 fold more DA neurons, and transplantation of these cells in Parkinson's mice model resulted in functional recovery [95]. Moreover, compared to single KO mice, $Wnt1^{-/-}$ and $Wnt5^{-/-}$ double knockout mice showed greater loss of DA neurons and $Nurr1^{+}$ cells [96]. In the absence of Wnt ligand, Nurr1 associates with LEF1 and functions as a co-repressor. The Nurr1 and beta catenin interaction is increased upon Wnt-mediated stabilization of beta-catenin. This disrupts the Nurr1-LEF1 co-repressor complex and induces the expression of beta-catenin responsive genes [97].

Several key components of Wnt-beta-catenin pathway are down-regulated in monkeys treated with MPTP [98]. In mice, MPTP treatment of the ventral midbrain increases Wnt1 expression as well as deregulation of FZD-1 and beta-catenin expression, suggesting that the Wnt signaling pathway may contribute to neuroprotection against MPTP toxicity. Aged mice have decreased Wnt1 and loss of beta-catenin, which is correlated with failure to recover from acute MPTP toxicity [99]. Pesticide treatment, which induces sporadic PD, alters expression of Wnt signaling pathway components [100], and inhibits Wnt signaling through Dkk1 to promote neurodegeneration in mice treated with

6-OHDA [101]. Likewise, canonical Wnt signaling has been shown to be protective in the human neuroblastoma cell line SH-SY5Y when treated with 6-OHDA [102].

4.2. LRRK2-Wnt interactions

LRRK2 and Wnt signaling were first linked, when mRNA species involved in the Wnt signaling pathway cascade was shown to be deregulated by knockdown of LRRK2 in SH-SY5Y cells [103]. Similarly, a systems biology approach revealed that many transcripts in the Wnt signaling cascade are co-regulated with LRRK2 [104].

The LRRK2 ROC-COR tandem domain was reported to interact with Dvl-1, an important mediator in Wnt signaling [105]. This interaction is disrupted by the Y1699C mutation and strengthened by R1441C and R1728 mutations in LRRK2 [105]. Additionally, LRRK2 functions as a scaffold where it interacts with components of the beta-catenin destruction complex. Once recruited to the plasma membrane, it has been shown to bind the Wnt-corepressor LRP6 upon stimulation. However, overexpression of LRRK2 itself does not stimulate the Wnt pathway. Instead, it requires co-expression of Dvl proteins to be stimulated by Wnt3a. This induction of Wnt signaling is dependent on the kinase and GTPase activity of LRRK2, as inhibition of kinase activity by LRRK2-IN-1 or the inactivating K1347A GTPase mutation prevents Wnt activation [106]. An interaction between LRRK2 and Sgg (the *Drosophila* homolog of GSK3β), a component of the beta-catenin destruction complex, has been reported in *Drosophila* [59]. This would imply a role for LRRK2, however, in downregulating canonical Wnt signaling, in contrast to the prior study.

As differential effects among *Drosophila* and mammalian systems have been observed with other PD-linked genes, it would be interesting to determine if LRRK2 promotes beta-catenin degradation in mammalian cells. As the Wnt signaling pathway is important for neurogenesis, the possible role of LRRK2 in regulation of this pathway, and how it is affected in PD, still needs to be determined. As chemical (kinase specific inhibitors) and molecular tools (knock-out, transgenic animals) become increasingly available, future research linking Wnt signaling and LRRK2 would be interesting.

5. Interactions of LRRK2 with MAPK/ERK1/2 signaling pathways

5.1. ERK1/2 signaling in parkinsonian toxin models

Extracellular-regulated kinase 1 and 2 (ERK1/2) belongs to the mitogen activated protein kinase (MAPK) family, which also includes c-Jun NH2-terminal kinase (JNK) and p38 kinase. These MAPKs are serine/threonine protein kinases that regulate diverse cellular functions such as cell growth, division, differentiation and cell death [107,108]. LRRK2 has been implicated in regulating multiple branches of the MAPK superfamily, including the dual specificity kinases for the p38 MAPK and the JNK pathways [109,110].

Activation of ERK1/2 has been shown to be protective as well as cytotoxic in different cell models. Transient, early activation of ERK1/2 is typically neuroprotective [111], whereas delayed and sustained ERK1/2 activation promotes neuronal cell death [111–114]. In the 6-hydroxydopamine (6-OHDA) model, it was found that there is a delayed phase of ERK1/2 activation that is attributable to mitochondrial ROS [115]. Interestingly, activated ERK1/2 is observed in midbrain neurons of human PD and diffuse Lewy body disease patients [116]. A similar increase in activated ERK1/2 was observed in tau associated neuropathology [117], which can be seen in some families with mutant LRRK2 [11]. Ultrastructural and dual immunofluorescence studies have demonstrated that activated ERK1/2 is associated with abnormal mitochondria, autophagosomes, and intracellular filaments [118]. Sustained ERK1/2 activation has also been shown to promote the autophagic clearance of mitochondria in neuronal cells [119], and suppress mitochondrial

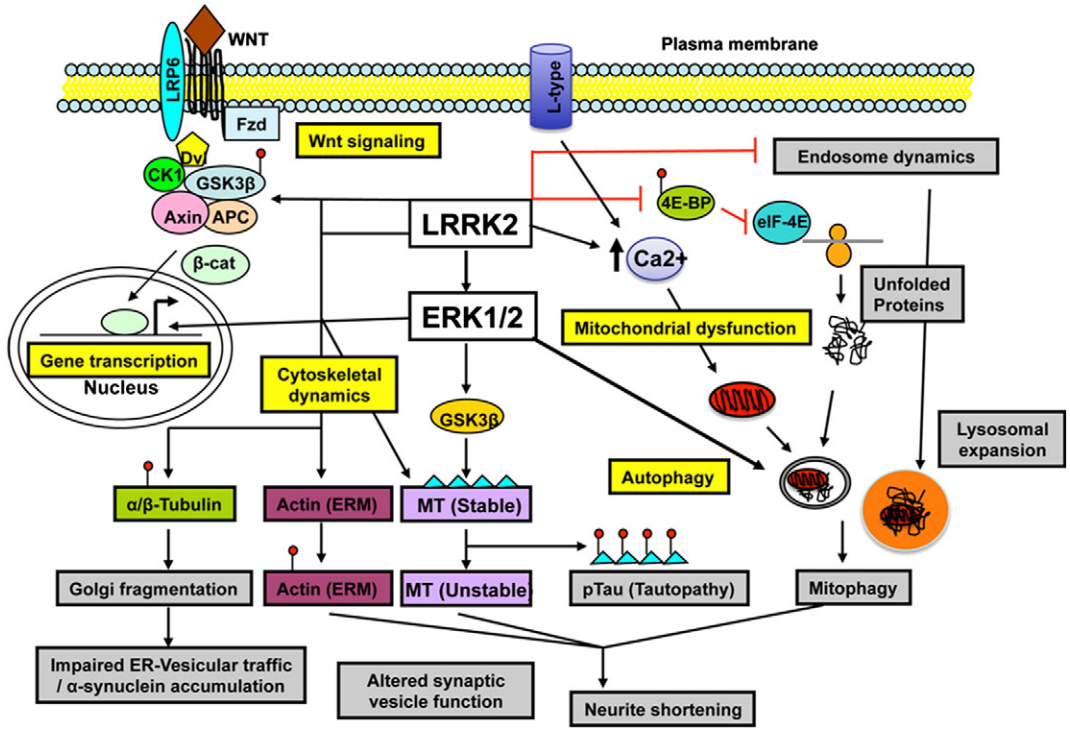

Fig. 1. Cell biological pathways implicated in LRRK2-associated pathobiology: intersection with ERK1/2 and Wnt signaling pathways. Altered endosomal dynamics, increased protein translation by inhibition of repressor protein 4E-BP1, and increased mitophagy contribute to a state of autophagic/lysosomal stress. LRRK2 promotes increased intracellular calcium and activates ERK1/2-dependent autophagy and downregulation of dendritic mitochondria. LRRK2 and ERK1/2 also regulate microtubule dynamics by phosphorylation of tau, actin and α/β-tubulin. LRRK2 also triggers Wnt-β-catenin and ERK1/2-dependent changes in gene transcription. The autophagic, transcriptional and cytoskeletal effects of mutant LRRK2 expression contribute to ERK1/2-dependent neurite shortening, while altered endosomal/vesicular dynamics affect synaptic function. 4E-BP1: translation repressor protein; APC: Adenomatous polyposis coli; CK1: Casein kinase 1; Dvl: Dishevelled; eIF-4E: eukaryotic translation inition factor 4E; ERK1/2: Extracellular-signal regulated kinase 1/2; ERM: Ezrin-Radixin-Moesin; GSK3β: Glycogen synthase kinase 2beta; LRP6: low density lipoprotein receptor-related protein 6; LRRK2: Leucine-rich repeat kinase 2; MT: Microtubule; β-cat: beta catenin.

biogenesis in response to chronic MPP+ toxicity [120]. In addition, ERK1/2 has also been shown to modulate reactive oxygen species (ROS) [121–123], nitric oxide [124] and calcium (reviewed in [125]). Thus, ERK1/2 may be involved in several cellular pathways implicated in LRRK2 pathobiology: autophagy, microtubule dynamics, neurite shortening, altered mitochondrial calcium regulation and mitophagy.

5.2. LRRK2: the ERK1/2 link

The most common pathogenic mutation associated with LRRK2 is G2019S, which occurs in the MAPKKK domain of the protein. In the search for possible LRRK2 substrates, leukocytes from patients carrying the G2019S mutation were compared to healthy counterparts. Src, a non-receptor tyrosine kinase that activates both ERK1/2 and p38, was less phosphorylated in G2019S mutants. Similarly, HSP27, a downstream target of p38 was also less phosphorylated. However, there was an increase in phosphorylated ERK1/2 in G2019S leucocytes, suggesting that ERK1/2 could be activated by a different mechanism in these patients [126].

Neurite retraction, a consistent phenotype caused by expression of pathogenic LRRK2 mutations, is driven by ERK1/2-dependent mechanisms, including autophagy [39]. This suggests that ERK1/2 activation may contribute to mutant LRRK2 pathogenicity. In contrast, neuroprotective effects of wild type LRRK2 against hydrogen peroxide stress also seemed to be mediated through ERK1/2 signaling in HEK293 and SH-SY5Y cells [127]. While the G2019S mutant seemed to act through ERK1/2-dependent mechanisms, other studies reveal that the pathogenic Y1699C LRRK2 mutant failed to activate ERK1/2 in SH-SY5Y [127] or SN4741 cell lines [128].

A study using an inducible system confirmed a role for ERK1/2 in LRRK2-associated pathology. Overexpression of wild type, G2019S and R1441C LRRK2 increases phosphorylated ERK1/2 in a time dependent manner, with delayed ERK1/2 activation compared to the wild type in HEK293 cells. Increased phospho-ERK1/2 levels correlate with induction of *SNCA*, which encodes for α-synuclein. LRRK2 also promoted increased phosphorylation of MEK2, an upstream kinase of ERK1/2 [129]. Indeed, either inhibitors of MEK or dominant negative ERK2 prevents G2019S-mediated autophagic neurite shortening [39], and involvement of ERK1/2 was subsequently confirmed in relation to G2019S LRRK2-induced increases in basal autophagy [130]. In a recent study using patient derived iPS cells, genetic correction of LRRK2 G2019S mutation significantly ameliorated PD associated phenotypes, which could also be achieved by inhibition of ERK1/2 [131].

6. Conclusions and future direction

Alterations in autophagy, mitophagy, cytoskeletal dynamics, mitochondrial function and the balance of protein synthesis and degradation have been implicated in relation to the normal or pathological function(s) of LRRK2 (Fig. 1), as well as in sporadic or toxin-based parkinsonism. Interestingly, LRRK2 interacts genetically with α-synuclein and with recessive PD genes. In contrast to α-synuclein, whose normal expression is predominantly in axon terminals, it is clear that LRRK2 shows both pre-synaptic and post-synaptic effects in neurons. Moreover, dendrite shortening is a prominent phenotype of neurons expressing the mutant *LRRK2* gene. Future studies are needed to address the role of the various cell biological processes impacted by LRRK2 expression, including Wnt-related differentiation, in the context of human neurons affected in PD.

ERK1/2-related cellular pathways may represent a point of mechanistic convergence between toxin-based models of sporadic PD, and newer studies of pathogenic mutations in LRRK2, which comprise the most common familial forms of PD (Fig. 1). Various studies have emerged linking LRRK2 to the ERK1/2 signaling pathway [39,129]. Increased mitophagy observed with LRRK2 and associated pathogenic mutations [38,132] has also been observed when ERK1/2 activity is modulated [114]. Cytoplasmic granules of phosphorylated ERK1/2 are observed in the context of both sporadic and G2019S LRRK2-associated PD patient substantia nigra neurons (Fig. 2) [116,133]. Most studies have implicated increased kinase activity of LRRK2 in activation of ERK1/2, which could be prevented by MEK inhibitors. These studies would place LRRK2 upstream of ERK1/2. However, as ERK1/2 activation is not universally observed in cells expressing pathogenic LRRK2 mutants, more studies will be required to confirm how LRRK2 interacts with this pathway. A better understanding of mechanisms by which ERK1/2 regulates mitochondrial turnover, protein expression and cytoskeletal dynamics may add insights for sporadic and familial forms of PD linked to LRRK2 as well as several recessive PD genes.

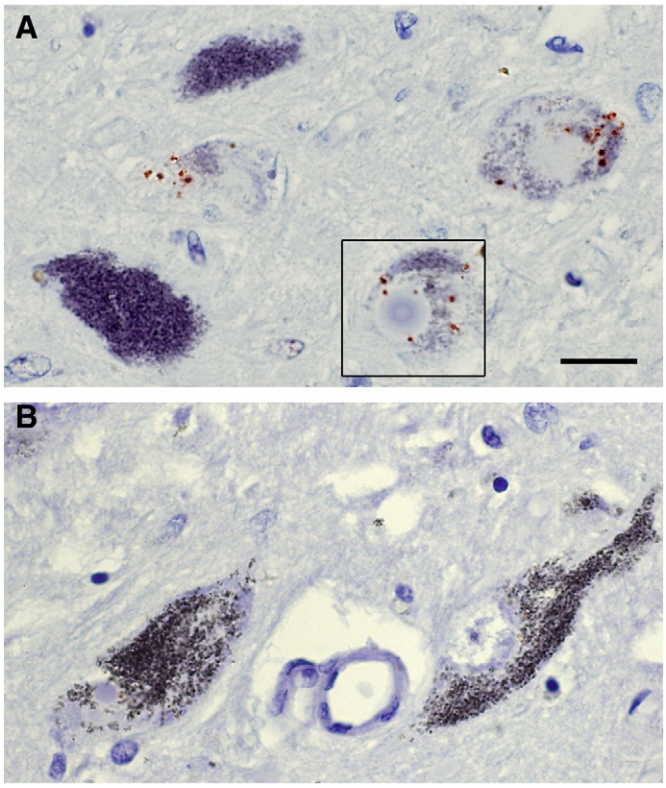

Fig. 2. Post-mortem analysis of phosphorylated ERK1/2 in a LRRK2 G2019S PD/transitional Lewy body disease (LBD) patient. Note the punctate, cytoplasmic staining of phosphorylated ERK1/2 (red chromagen) in pigmented substantia nigra neurons from the G2019S patient (A). These are similar in appearance and numbers to those previously reported in a series of sporadic PD/LBD cases [102], whereas this staining pattern was not seen in the midbrains of control subjects (B). Previous ultrastructural studies revealed phosphorylated ERK1/2 in association with abnormal mitochondria, autophagosomes, and bundles of intracellular filaments in PD/LBD substantia nigra neurons [104]. As reviewed in the text, alterations in mitochondrial homeostasis, autophagy, microtubule dynamics and ERK1/2-dependent protein expression are observed in models of LRRK2-associated pathobiology, suggesting the involvement of common cell biological pathways among sporadic and mutant LRRK2-triggered neurodegeneration. Scale = 20 μm.

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