Insights into LRRK2 function and dysfunction from transgenic and knockout rodent models

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Abstract
Mutations in the LRRK2 (leucine-rich repeat kinase 2) gene on chromosome 12 cause autosomal dominant PD (Parkinson’s disease), which is indistinguishable from sporadic forms of the disease. Numerous attempts have therefore been made to model PD in rodents via the transgenic expression of LRRK2 and its mutant variants and to elucidate the function of LRRK2 by knocking out rodent Lrrk2. Although these models often only partially recapitulate PD pathology, they have helped to elucidate both the normal and pathological function of LRRK2. In particular, LRRK2 has been suggested to play roles in cytoskeletal dynamics, synaptic machinery, dopamine homoeostasis and autophagic processes. Our understanding of how these pathways are affected, their contribution towards PD development and their interaction with one another is still incomplete, however. The present review summarizes the findings from LRRK2 rodent models and draws potential connections between the apparently disparate cellular processes altered, in order to better understand the underlying mechanisms of LRRK2 dysfunction and illuminate future therapeutic interventions.

Introduction
Mutations in the LRRK2 (leucine-rich repeat kinase 2) gene at the PARK8 locus cause incompletely penetrant autosomal dominant PD (Parkinson’s disease), which is considered clinically and neuropathologically indistinguishable from sporadic PD. LRRK2 mutations account for 5–13% of familial PD cases and 1–5% of those previously considered to be sporadic [1]. The LRRK2 gene encodes a protein incorporating several repeat regions followed by ROC (Ras of complex proteins–COR (C-terminal of ROC) GTPase, kinase and WD40 domains [2]. LRRK2 can also potentially self-interact and dimerize, which has been proposed as critical to its function [3]. Mutations at several sites within either the GTPase (N1437H, R1441G/C/H and Y1699C) or kinase (G2019S and I2020T) domains can lead to PD [2]. The normal function of LRRK2 remains unclear, although it has been implicated in numerous cellular processes, including cytoskeletal dynamics [4–7], vesicle trafficking [8,9] and autophagy [10–12] (Figure 1). The effects of mutations on the function of the LRRK2 protein also remain controversial, with evidence of increased kinase activity for some mutations, but not all, and both gain- and loss-of-function effects reported [13].

Expression of wild-type or mutant forms of Parkinson’s disease-associated genes in rodents has been widely used to attempt to recapitulate the pathology of PD [14]. Consequently, efforts have been made to develop rodent models of LRRK2 PD. Although such models do not model all aspects of PD, they have provided insight into systems affected by LRRK2 function and dysfunction. The present review evaluates the insights gained from transgenic rodent models, focusing on the proposed roles of LRRK2 in neurite morphology, cytoskeletal processes, dopaminergic homoeostasis and vesicle trafficking, autophagy and interactions with α-synuclein.

Neurite morphology
Neurons overexpressing mutant LRRK2 show impaired neurite outgrowth both in vivo [15,16] and in primary culture [7,16–19]. This phenotype can be rescued using inhibitors of LRRK2 kinase activity [17] or conditional suppression of mutant transgene expression [20]. Lrrk2−/− in mice, or knockdown in primary neurons [5,7,16,17,21], also alters neurite morphology, although whether it increases or attenuates outgrowth is disputed. Wild-type LRRK2 overexpression has been found to cause a phenotype similar to mutant overexpression [22], but often no effect is reported [7,16]. Mutant LRRK2 phenotypes seem to be kinase-dependent as no morphological abnormalities result from expression of kinase-dead LRRK2 variants [22]. Furthermore, neurite outgrowth caused by LRRK2 knockdown can be attenuated by transfection of either wild-type or mutant kinase domain alone [16]. Interestingly,
kinase-dead LRRK2 expression produces a phenotype resembling LRRK2 knockdown [16]. This may reflect interference with endogenous LRRK2, potentially through disruption of dimer function.

Whether the changes to neurite morphology reflect elevated cellular toxicity is unclear. However, altered neurite morphology is not exclusive to LRRK2 overexpression. For example, up-regulation of TNF (tumour necrosis factor), a cytokine involved in apoptotic and inflammatory processes, both of which have been linked to mutant LRRK2 function [16,19,23], causes a similar morphological phenotype [24]. Interestingly, both TNF and LRRK2 have been linked to inflammatory bowel disease [25]. It therefore seems possible that the morphological phenotype actually reflects upstream cellular toxicity processes. Alternatively, LRRK2 is reported to have modulatory effects upon the cytoskeleton and autophagy, which can also modulate neurite morphology. Therefore whether the alterations in neurite outgrowth are simply secondary events is still unclear.

Cytoskeletal processes
LRRK2 has been repeatedly implicated as a modulator of cytoskeletal dynamics [26]. Specifically, microtubules, formed from polymerized α/β-tubulin heterodimers, and actin filaments are reportedly altered by LRRK2 mutant expression or Lrrk2−/−. In vivo, LRRK2 is recruited to microtubules in a kinase-dependent fashion [6]. LRRK2 has also been shown to phosphorylate β-tubulin [5]. In both cases, LRRK2 mutations reportedly enhance these effects. Although the role of β-tubulin phosphorylation is not completely clear, it has been linked to microtubule assembly during neurite outgrowth [27].

Mice overexpressing either mutant or wild-type LRRK2 show elevated brain α/β-tubulin polymerization [4,19]. Conversely, brains of Lrrk2−/− mice demonstrate reduced tubulin polymerization [5]. Overstabilization of microtubules by LRRK2 may impair cellular function. In support of this, Golgi apparatus fragmentation, which has been linked to altered microtubule stability, has been reported in neurons expressing mutant LRRK2 in vivo [4,19]. In particular, dopaminergic neurons, which are selectively lost in the SNpc (substantia nigra pars compacta) in PD, show elevated Golgi fragmentation [19]. In line with this, dopaminergic neurons have shown specific susceptibility to perturbations in microtubule dynamics [28]. The process by which LRRK2 alters microtubule stability is not well established, however.

Although it is possible that LRRK2 stabilizes microtubules directly, the interaction of LRRK2 with microtubule-associated proteins such as tau, which play critical roles in microtubule stability, may also modulate microtubule dynamics. LRRK2 PD patients often present with tauopathy [29] and genome-wide association studies have implicated microtubule-associated protein tau (MAPT or tau) as playing a key role in PD [30]. Tau is also reportedly involved in neurite extension [31]. In agreement with this, both wild-type and, to a greater extent, mutant LRRK2 overexpression leads to tau accumulation [16,32–34] and inclusion formation [16] in rodent models. The deposited tau is phosphorylated at multiple residues, such as at those detected by the AT8 (Ser205/Thr205) [32,33], 12E8 (Ser356), sc-12952 (Ser404) and CP-13 (Ser202) antibodies [16,34]. Conversely, reductions in AT8-detectable tau have been reported in Lrrk2−/− mice [5]. LRRK2 therefore appears to have a role in tau phosphorylation and accumulation, with mutations in LRRK2 augmenting this effect. In support of this, biochemical studies have shown that LRRK2 phosphorylates tubulin-associated tau directly [35]. Tau phosphorylation typically reduces the affinity of tau for microtubules, potentially enhancing the tendency of unbound tau to aggregate. However, as a reduction in tau binding usually leads to reduced microtubule stability [36], it is unclear how these findings align with increased tubulin polymerization in LRRK2 overexpression.

LRRK2 has also been implicated in actin dynamics. Actin cytoskeleton-related genes are reportedly dysregulated in blood mononuclear cells from patients carrying LRRK2 mutations [37]. The accumulation at the filopodia of cultured hippocampal neurons of polymerized actin and phosphorylated ERM (ezrin, radixin and moesin) proteins, which cross-link the actin cytoskeleton, is enhanced by mutant LRRK2 [7]. Reduction of LRRK2 protein levels or Lrrk2 knockout reverses this phenotype. Furthermore, F-actin (filamentous actin) depolymerization or suppression of ERM phosphorylation rescued impaired neurite outgrowth in neurons expressing mutant LRRK2. Although interactions between the actin and microtubule cytoskeleton, as well as with tau, are well documented and both are required for axonal branching, it is unclear how their interaction may be altered by LRRK2 mutations [38]. Future investigation into the effect that LRRK2 has upon the interplay of various aspects of the cytoskeleton may reveal further underlying mechanisms of LRRK2 pathology.
Dopaminergic homoeostasis and vesicle trafficking

Deregulation of dopamine neurotransmission is characteristic of PD and hence it has been investigated in rodent models. Studies have reported L-dopa-sensitive motor deficits in Lrrk2 transgenic mice, suggesting impaired dopamine homoeostasis [33,39]. Furthermore, mice expressing mutant LRRK2 reportedly display reductions in baseline extracellular striatal dopamine and its metabolites [19,33,34]. Other studies have shown reduced dopamine levels outside of the striatum, possibly due to the anatomical pattern of transgene expression [18]. In contrast, the temporal expression of mutant LRRK2 in adult rats [40] caused elevated extracellular dopamine levels leading to hyperactivity, which may reflect different effects of mutant LRRK2 expression depending on the disease progression stage. The effects of overexpressing wild-type LRRK2 are much less clear, with some studies reporting no effect [18,33] and some a reduction [34] of extracellular dopamine levels.

Extracellular dopamine levels are linked to dopamine release, re-uptake and dopamine receptor function, all of which have been investigated. Mice expressing mutant LRRK2 display impaired dopamine release [33,41] and chromaffin cells from Lrrk2 mutant knockin mice also show impaired catecholamine release [42]. Conversely, mice overexpressing wild-type LRRK2 demonstrate enhanced dopamine release [41]. Mutant LRRK2 may affect dopamine release by altering vesicle recycling mechanisms. Previously, Lrrk2 has been found to localize to vesicles [43], and studies have shown interactions between Lrrk2 and other vesicle proteins [8]. Lrrk2 knockdown in cortical neurons reportedly caused increased ePSC (excitatory postsynaptic current) probability and amplitude followed by an attenuation of ePSC after a second pulse [8]. This suggests elevated levels of initial neurotransmitter release followed by an attenuation of subsequent release levels. Whereas Lrrk2−/− neurons were not found to have altered vesicle numbers, electron microscopy revealed fewer docked vesicles at the presynaptic membrane. It is therefore possible that transmitter release, and, potentially, extracellular dopamine levels, are affected by alterations in vesicle exocytosis. This is supported by the fact that, whereas levels of dopamine release were reduced in mutant and wild-type lines overexpressing LRRK2, an amphetamine challenge which should cause total release of dopamine into the synaptic cleft eradicated differences between genotypes [34]. What caused the alterations in vesicle localization is unclear. However, perturbations in vesicle endocytosis have been reported in LRRK2-overexpressing neurons and this may lead to the abnormal presynaptic vesicular localization [9]. Alternatively, disrupted vesicle trafficking due to cytoskeletal abnormalities may account for altered vesicle localization.

In addition to altered dopamine release, impairments in dopamine re-uptake have also been reported. Specifically, differences in extracellular dopamine between genotypes were abolished by DAT (dopamine active transporter) inhibition [40]. This suggests that changes in dopamine re-uptake are responsible for the altered extracellular dopamine levels seen in mice expressing mutant LRRK2. Furthermore, reduced DAT levels in the striatum of mice expressing mutant LRRK2 have also been reported, potentially reflecting the death of dopaminergic neuron terminals [39].

Whereas altered release/reuptake upon mutant LRRK2 expression may explain the altered extracellular dopamine levels found, this may in turn lead to a change in dopamine receptor function, something seen in PD patients [44]. Such changes in receptors may reflect cellular compensatory mechanisms responding to reduced dopamine levels. Consistent with this, attenuated locomotor effects of quinpirole, a D2 receptor agonist, were seen in mice expressing mutant LRRK2 [42]. Additionally, SNpc neurons showed reduced hyperpolarization responses to amphetamine or dopamine challenges, compatible with impaired autoreceptor function. These changes may occur as a result of a reduction in receptor density which may, in turn, reflect a reduction in receptor expression. Alternatively, it may reflect impaired receptor trafficking to the membrane which may be disrupted by impairments to the cytoskeleton.

Autophagy

LRRK2 puncta have been found to co-localize with AVs (autophagic vacuoles) and MVBs (multivesicular bodies) in cultured cells with a pattern similar to that found in human brains [45]. In agreement with this, punctate Lrrk2 co-localizes with lysosomal and endosomal vesicles in rat brains [43]. In mice expressing mutant LRRK2, striatal and cortical AVs were noticeably enlarged [18]. Furthermore, MVBs and altered lysosomal markers were observed in primary cultures expressing mutant LRRK2 [16]. Finally, modulation of mTOR (mammalian target of rapamycin), a regulator of autophagy, has been reported in both Lrrk2 mutant and Lrrk2−/− mice [11].

A number of studies also report altered autophagic and lysosomal markers in the kidneys of Lrrk2−/− mice [10–12]. Interestingly, many of these changes in the kidney appear biphasic in nature, often involving an initial up-regulation, followed by a subsequent depletion of autophagic activity [10]. Such initial changes could reflect either a primary up-regulation of autophagy upon LRRK2 depletion brought about by modifications to autophagic machinery or a compensatory mechanism for changes in other processes such as altered lysosomal pH [46] which falls over time. No alterations in brain autophagy have been reported so far in Lrrk2−/− mice, potentially due to the kidneys expressing higher comparative levels of LRRK2, or because of the low level of expression in the kidneys of LRRK1 (leucine-rich repeat kinase 1), which has been theorized to compensate for the absence of LRRK2. In addition to the potential compensatory role of LRRK1, future studies should investigate the effect of the expression of LRRK2 mutations on autophagy function.
It is possible that alterations in the endocytic pathway, an important process in the formation of vesicles, are responsible for the dysregulation in autophagic processes. LRRK2 overexpression has been suggested to slow endocytosis in a mutant- and kinase-independent fashion [9]. Interestingly, Lrrk2 knockdown had a similar effect, perhaps implying a dominant-negative effect. Overexpression of Rab5, a GTPase involved in endocytosis and implicated in autophagic degradation pathways, was able to rescue the slowed endocytosis. As such, both impairments in autophagy and synaptic vesicle release related to LRRK2 dysfunction could rely to an extent upon the endocytotic pathway.

Potential effects of mutant LRRK2 on autophagic degradation may be partially responsible for the accumulation of tau as impaired lysosomal and autophagic activity has been shown previously to promote tau aggregation [47]. How impaired autophagy links with the other cytoskeletal abnormalities observed in LRRK2 mutants remains uncertain.

LRRK2 and α-synuclein

Mutations or duplications in α-synuclein cause early-onset familial PD. Attempts have therefore been made to investigate the interplay between LRRK2 and α-synuclein via the generation of LRRK2/SNCA (α-synuclein) double transgenic mice which show augmented effects of LRRK2 overexpression upon the progression of mutant α-synuclein-induced pathology [4,48]. Conversely, expressing the A53T-SNCA transgene on a Lrrk2+/− background attenuated the A53T-SNCA phenotypes [4], suggesting a convergence of LRRK2 and α-synuclein pathogenic pathways. Interestingly, Lrrk2−/− mice demonstrated decreased α-synuclein levels in kidneys at 7 months of age together with increased autophagy, which reversed with aging to elevated α-synuclein levels and decreased autophagy by 20 months [12]. It has been shown previously that α-synuclein is degraded partially via autophagic pathways [49]. Perturbations in autophagic processes brought about by LRRK2 mutations may therefore cause enhanced α-synuclein accumulation, although rodent models expressing mutant LRRK2 alone have failed so far to produce dramatic α-synuclein pathology. Alternatively, depolymerization of microtubules, which may occur via tau phosphorylation, has been shown to promote α-synuclein oligomerization [50]. Given that LRRK2 variably affects α-synuclein pathology both in different rodent models and in patients harbouring LRRK2 mutations [29], an alternative hypothesis would be that LRRK2 and mutant α-synuclein act upon parallel pathways rather than in concert.

The limitations of LRRK2 rodent models

Whereas some papers report SNpc neurodegeneration in Lrrk2 transgenic rodents, they represent a minority [18,32,33,39]. Furthermore, Lewy bodies, the hallmark of PD, have never been described. This may reflect the incomplete penetrance and comparatively mildness of LRRK2 mutations compared with other mutations associated with PD. It is also possible that a level of developmental compensation occurs which may attenuate subsequent mutant phenotypes. In support of this, studies which involve temporal restriction of Lrrk2 transgene expression either using viral transduction or conditional gene expression often display more dramatic phenotypes [32,40]. Differences in the ability of different tissues to compensate during development may also explain why Lrrk2−/− phenotypes are seen in the kidneys and lungs which show higher than normal levels of LRRK2 [11].

Variation in the use of promoters and the inconsistent use of human or murine genes makes comparison between studies difficult. Heterologous promoters result in inappropriate spatiotemporal expression and splicing, both of which are likely to be crucial for accurate disease recapitulation when working with a gene as complex as LRRK2. Another issue is that, whereas SCNA duplications and triplications lead to exacerbated pathology in patients, this has not been shown for LRRK2, making it difficult to determine which expression levels of the Lrrk2 transgene in rodents are relevant to human disease. Finally, rather than trying to model late-stage human PD comprising a fully developed pathology of Lewy bodies and protein aggregation, it would perhaps be more useful to concentrate on reproducing earlier pathogenic pathways in which to investigate neuroprotective therapies.

Conclusions

Both the normal and pathological functions of LRRK2 remain to be established. It will be particularly informative to determine more specifically the functional impact of LRRK2 mutations on protein function and whether mutations in different domains produce different effects. More extensive phenotype characterization of transgenic mouse and rat lines expressing wild-type or mutant LRRK2 protein will probably provide useful insight into these issues, and hopefully model early-stage PD within the therapeutically tractable window.

Funding

J.A.-A. is funded by Parkinson’s UK.

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Received 25 June 2012
doi:10.1042/BST20120151