LRRK2 and autophagy: a common pathway for disease

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Abstract
LRRK2 (leucine-rich repeat kinase 2) is an enzyme implicated in human disease, containing kinase and GTPase functions within the same multidomain open reading frame. Dominant mutations in the LRRK2 gene are the most common cause of familial PD (Parkinson’s disease). Additionally, in genome-wide association studies, the LRRK2 locus has been linked to risk of PD, Crohn’s disease and leprosy, and LRRK2 has also been linked with cancer. Despite its association with human disease, very little is known about its pathophysiology. Recent reports suggest a functional association between LRRK2 and autophagy. Implications of this set of data for our understanding of LRRK2’s role in physiology and disease are discussed in the present paper.

LRRK2 (leucine-rich repeat kinase 2) in PD (Parkinson’s disease)
LRRK2 is a ubiquitously expressed [1] member of the ROCO protein family [2]. It is composed of an active serine/threonine kinase domain with sequence similarities to the RIPKs (receptor-interacting protein kinases) [3] and of an active Ras-like GTPase ROC (Ras of complex proteins) domain separated by a COR (C-terminal of ROC) domain [4]. This enzymatic core is flanked by protein–protein interaction motifs. Single nucleotide mutations located in the enzymatic core of LRRK2 are the most frequent genetic cause of PD, although they display an incomplete and age-dependent penetrance [1,5,6]. Genome-wide association studies have identified variations at the LRRK2 locus increasing the risk of developing sporadic PD [7]. The strong links between LRRK2 and PD have driven an extensive research effort to improve our understanding of its physiological function and dysfunction in disease.

LRRK2: an enigma of function(s)
The presence of an active kinase domain encoded in the LRRK2 sequence strongly suggests that LRRK2 can be involved in signalling cascades, and the fact that LRRK2 itself can be phosphorylated on different sites [8] strengthens this hypothesis. As a consequence, many studies have aimed to identify proteins phosphorylated by LRRK2 and the kinase signalling pathways associated with them. More than ten potential LRRK2 substrates have been identified, with a number of other proteins suggested to interact with LRRK2 [9,10]. For the majority of these proteins, however, their physiological relevance to LRRK2 needs to be confirmed, and a role for LRRK2 in related signalling cascade(s) still needs to be established. Many functional roles for LRRK2 have been suggested, including vesicular trafficking and endocytosis [11,12], protein synthesis [13], immune response regulation [14], inflammation [15] and cytoskeleton homoeostasis [16]. Finally, LRRK2 has been associated with TNFα (tumour necrosis factor α) [17], Met [18], interferon γ [19] and MAPK (mitogen-activated protein kinase) [20] signalling pathways. However, there is still no consensus on LRRK2 function, and its physiological role(s) remain to be elucidated.

The presence of two different enzymatic functions within the same primary structure complicates functional studies, owing to the experimental difficulty in dissecting its kinase and its GTPase activities. However, this peculiarity increases the interest in (and importance of) the biochemical characterization of LRRK2. To date, very little is known about the role of the LRRK2 GTPase domain. It has been suggested that the GTPase activity of LRRK2 controls and modulates its kinase function [21]. Vice versa, LRRK2 autophosphorylation was suggested to play a role in GTP binding [22]. On the basis of our extant knowledge, several hypotheses can be considered. First, both LRRK2 enzymatic functions could be implicated in different molecular processes so that LRRK2 could be concurrently responsible for different cellular functions (Figure 1A) or LRRK2 kinase and GTPase activities could be involved in the control of the same pathway. If the last scenario holds true, the two enzymatic functions could be orchestrating the same step within the same pathway (Figure 1B), otherwise the kinase and the GTPase functions could be controlling different molecular events within the same pathway so that, from a mechanistic point of view, LRRK2 would be responsible for the control of two different molecular activities, but, in toto, it would be implicated in only one singular cellular function (Figure 1C).
**Figure 1** Hypothetical implication of LRRK2 kinase (K) and GTPase (G) activities in cellular signalling pathways

The two enzymatic functions could be working to: (A) control different effectors implicated in divergent pathways (*in toto* two different LRRK2 functions); (B) orchestrate the same step in the same pathway (one LRRK2 function); (C) modulate different effectors responsible for different steps in the same pathway (two different LRRK2 functions).

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**LRRK2 localizes to lipid membranes and vesicles**

A number of studies have described an association of LRRK2 with membranes in various mammalian cell models, in rat and mouse brains. LRRK2 was shown to co-localize with endosomes, lysosomes, mitochondrial membranes, Golgi transport vesicles, plasma membrane and synaptosomes. The use of cell lines allowed the ultrastructural localization of LRRK2 in microvilli/filopodia, neck of caveolae, multivesicular bodies and autophagosomes [23–25]. This subcellular localization has been reported to be a weak interaction occurring on lipidic surfaces and several pieces of evidence indicate that LRRK2 is not homogeneously distributed; it is likely to be partially located to specific microdomains within membranous structures [25]. The membrane association of LRRK2 has been shown to be crucial for the control of its enzymatic activity. It has been hypothesized that membrane association recreates the optimum microenvironment for LRRK2 dimerization, thus improving GTP binding and increasing its kinase activity. The membrane-associated LRRK2 dimer has been suggested to be the only physiologically active form of the protein [26]. Overexpression of LRRK2 in cultured cells revealed that its distribution within membranes was not altered when mutant LRRK2 proteins (carrying PD-associated dominant mutations) were compared with the wild-type sequence. This suggests that the LRRK2 pathology is not triggered by a change in its subcellular localization, but rather by either an alteration of its physiological function or a gain of a new toxic and membrane-associated activity [25]. These data steer research into pathophysiological LRRK2 function towards membrane-associated processes.

**LRRK2 is implicated in autophagy**

Reports from post mortem investigations of PD human brains carrying mutations in *LRRK2* reveal dystrophic neurites and the accumulation of Lewy bodies in most cases, with a minority characterized by the presence of neurofibrillary tangles, TDP-43 (TAR DNA-binding protein 43) inclusions or ubiquitin-positive pathology [27,28]. Transgenic mice expressing the dominant R1441C mutation in LRRK2 showed increased density of autophagic vacuoles in the brain cortex. An even stronger increase was described in the cortex and in the striatum of LRRK2 transgenic mice expressing the dominant G2019S mutation in comparison with non-transgenic littermates [29]. These reports suggest that LRRK2 could be associated with autophagy- and lysosome-related pathways implicated in the control of protein turnover and in clearance mechanisms. A study in *Caenorhabditis elegans* transgenic lines led to the same conclusion, showing that genes associated with autophagy are the most altered in relation to LRRK2 activity [30]. If the correlation between LRRK2 and autophagy holds true, the first question is whether altered autophagy is the primary causative insult in LRRK2-associated PD or whether it is a consequence of the disease status, which is triggered by an upstream LRRK2-dependent molecular event. If the first hypothesis is true, another issue is whether LRRK2 is physiologically implicated in the regulation of autophagy, and this function is altered...
in PD, or whether the correlation between LRRK2 and autophagy only happens in the disease status as a consequence of a toxic gain-of-function. One way to address these questions would be to determine the molecular mechanism linking LRRK2 to autophagy in both physiological and pathological conditions. A number of studies are currently trying to answer these still debated questions.

The overexpression of G2019S-LRRK2 in SHSY5Y neuroblastoma cells resulted in a dramatic change in cell morphology, a reduction of branching and an increase in the content of autophagosomes. In this cell model, LRRK2 toxicity was enhanced by exogenous stimulation of autophagy and was reduced by the molecular ablation of the autophagic machinery, thus suggesting that autophagy plays an active role in cell toxicity induced by the overexpression of G2019S-LRRK2 [31]. Another study described an association between the expression of R1441C-LRRK2 and an abnormal increase in the number of autophagosomes within the cells. The autophagic phenotype was reported to be a consequence of a reduction rather than an increase in autophagy. This hypothesis was supported further by the fact that the siRNA (small interfering RNA) knockdown of LRRK2 resulted in an increase of the autophagic flux [23]. Finally, a recent report showed accumulation of autophagosomes after overexpression of wild-type or G2019S-LRRK2 in various cell lines [32]. This was shown to be due to an LRRK2-dependent alteration of Ca\(^{2+}\) homoeostasis with the consequent activation of the CaMKK\(\beta\) (Ca\(^{2+}\)-/calmodulin-dependent protein kinase kinase \(\beta\))/AMPK (AMP-activated protein kinase) pathway causing induction of autophagy [32]. However, a decrease in the acidification of lysosomes was detected as well, and this can lead to a concomitant reduction of autophagy by blocking autophagosome turnover [32].

These reports suggest a strong association between LRRK2 and autophagy in the disease context, but they also indicate that the role of LRRK2 in the control of the autophagy–lysosome pathway is likely to be complicated. At the moment, it is still not clear whether the PD-associated mutations are able to induce an increase or a decrease in the autophagic flux; consequently, it is not clear whether there is a positive or a negative contribution of LRRK2 in the control of autophagy. It can be hypothesized that, in PD brains, autophagy could arise from a cellular response to the accumulation of misfolded proteins, and that activating mutations in LRRK2 would amplify this process. The consequent hyperactivation of autophagy could be efficient and beneficial in the short term, but deleterious, and in the end insufficient, when chronically applied, thus leading to disease [33]. Excessive induction of autophagy can, in fact, be coupled to an excessive demand of energy to sustain the process and this can result, especially in aged brains, in autophagic stress, neuronal atrophy, degeneration and finally cell death [34,35] (Figure 2A). This scenario is supported by studies investigating mechanisms of cell death in PD. The overexpression of the pathogenic mutant A53T synuclein was suggested to induce autophagic cell death [36]; and cell death induced by MPP\(^{+}\) (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) has been linked to increased autophagy [37]. In a converse scenario, pathogenic mutations in LRRK2 could act by reducing autophagy; this would cause misfolded proteins to accumulate instead of being disposed of, leading, eventually, to neuronal degeneration and cell death (Figure 2B). A number of studies on other neurodegenerative disorders strengthen this hypothesis. The pharmacological enhancement of autophagy, leading to an increase in the removal of aggregated proteins, was shown to be protective in Huntington’s disease models and in other models of protein aggregate toxicity [38].

Knockout mice have been developed to gain insights into the function of LRRK2. Surprisingly, no major alterations were observed in the brains of these animals; however, they develop a marked kidney phenotype. If there were a hope that these mice could end the debate as to whether LRRK2 is a positive or negative regulator of autophagy, it was a forlorn one, and instead the data from these models have complicated this biological puzzle even more. Kidneys from two independently generated LRRK2-deficient mice showed an age-dependent accumulation of autophagosomes, p62, lipofuscin granules, ubiquitinated proteins and \(\alpha\)-synuclein-positive inclusions, thus indicating an impairment of the autophagy–lysosome pathway [39]. In a more detailed analysis, these mice displayed a biphasic alteration in autophagy that first increased in young mice and then decreased with age, as verified in 20-month-old mice [40]. Another independent LRRK2-knockout mouse showed an accumulation of secondary lysosomes in the kidney, but, in this case, autophagy was reported not to be involved since no changes in the marker LC3 (light chain 3) were detected [41].

In conclusion, there is strong evidence that the autophagy process is altered in LRRK2 PD models, but, at the moment,
we do not know in which direction this occurs, let alone the specific mechanism. Whether this alteration in autophagy is a consequence of the pathological process or whether it is the insult triggering PD in patients with LRRK2 mutations still remains to be determined.

Considerations

From both a pathological and an enzymatic point of view, the hypothesis that LRRK2 could have a role in the autophagy–lysosome pathway is intriguing.

From the pathological side, the involvement of LRRK2 in autophagy is consistent with the degeneration described in LRRK2-PD brains; the fact that other PD-related genes, such as GBA (glucosidase β acid) [42], SCNA (α-synuclein) [42], VPS35 (vacuolar protein sorting 35) [43] and ATP13A2 (ATPase type 13A2) [44], have been implicated in autophagy and lysosomal pathways as well begins to draw a picture of altered autophagy–lysosome degradation as a common feature of a number of genetic forms of PD. The LRRK2 locus has also been associated with a number of other human pathologies including Crohn’s disease [45], cancer [46] and leprosy [47]. Since we do not know the physiological role of LRRK2, we are currently not in a position to understand the association with such a heterogenous collection of diseases. LRRK2 could be involved in different physiological pathways in different cell types. Alternatively, it could control one single pathway, exerting its effects through tissue-specific effectors and modulators in different cell populations [48]. However, alterations in the autophagy–lysosome pathway have also been genetically implicated in Crohn’s disease [49] and leprosy [50]. Therefore autophagy and lysosomal metabolism can be at the intersection of different pathological pathways with no apparent commonalities, all of them showing a relationship with LRRK2.

From a biochemical point of view, kinase and GTPase activities in different proteins are required for the autophagic process to occur properly, and LRRK2 is a protein that combines these enzymatic activities in one open reading frame so that it can be implicated in one or both of these functions in autophagy; the presence of protein–protein interaction domains in the LRRK2 sequence may suggest that it could act as a scaffolding protein for fusion of vesicles as well. With the experimental models currently available, we are limited in our ability to examine separately each enzymatic activity of LRRK2. By focusing on either one or the other of its enzymatic functions, the data generated vary from one study to another; these discrepancies may suggest that LRRK2 could be implicated in autophagy by being concurrently both a positive and a negative regulator of autophagy.

Further work will need to be done to understand the role of LRRK2 in autophagy. The elucidation of the fine interplay between LRRK2 and the autophagy–lysosome pathway is likely to be essential for understanding the fundamental biology underlying autophagy, and for the successful development of therapeutic strategies for PD and other LRRK2-related disorders.

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References


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