Possible involvement of the relationship of LRRK2 and autophagy in Parkinson’s disease

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Abstract
PD (Parkinson’s disease) is a neurodegenerative disorder caused by loss of dopamine-generating cells in the substantia nigra. The implication of genetic factors in the aetiology of PD has an essential importance in our understanding of the development of the disease. Mutations in the LRRK2 (leucine-rich repeat kinase 2) gene cause late-onset PD with a clinical appearance indistinguishable from idiopathic PD. Moreover, LRRK2 has been associated with the process of autophagy regulation. Autophagy is an intracellular catabolic mechanism whereby a cell recycles or degrades damaged proteins and cytoplasmic organelles. In the present paper, we discuss the role of LRRK2 in autophagy, and the importance of this relationship in the development of nigral degeneration in PD.

Parkinson’s disease
PD (Parkinson’s disease) was first described in 1817 by James Parkinson (1755–1824) in An Essay on the Shaking Palsy. The study described a series of symptoms that Parkinson observed in six patients who attended his medical clinic. Initially, he named the illness of the six patients “paralysis agitans”. He observed that the illness was characterized by “Involuntary tremulous motion, with lessened muscular power, in parts not in action and even when supported; a propensity to bend the trunk forward, and to pass from a walking to a running pace affecting the parts that are at rest and that also provokes a tendency of the body to forward inclination and a manner of walking with short, quick steps. The senses and intellects being uninjured”. The initial description provided by Parkinson was comprehensive and detailed, but he did not mention two fundamental symptoms of the illness: rigidity and cognitive disorders. Years later, Charcot and Vulpian described in their work De la Paralysie Agitante the condition of the mental faculties in individuals presenting with the disease. Charcot began to refer to the paralysis agitans illness as PD in honour of the English physician who provided the first detailed description of the illness.

In general, neurodegenerative illnesses are chronic and progressive processes that are characterized by a loss of neurons in different regions of the nervous system. The most well-known examples of these pathologies are AD (Alzheimer’s disease) and PD, although other important conditions include Huntington’s disease, the ataxias and amyotrophic lateral sclerosis. PD is the second common neurodegenerative disorder, after AD, with a prevalence rate of approximately 3 in every 1000 people, and incidence and prevalence rates rise with advancing age [1]. Loss of at least 50% of dopaminergic neurons in the substantia nigra pars compacta is responsible for the origin of this disorder. The death of these neurons in the substantia nigra leads to a depletion of dopamine in the corpus striatum [2], which is responsible for the patients’ motor symptoms, especially akinesia [3].

The aetiology of PD is multifactorial, in which both genetic and environmental factors are included [4]. At the end of the 19th Century, Gowers (1886) noted, with regard to PD, that “…in any occasions the influence of the inheritance could be important…”. This remained the general opinion among researchers until 1980, when the toxin MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) was discovered [5]. This drug causes the selective death of substantia nigra neurons in humans and experimental models [6] and induces motor symptoms similar to those observed in PD. This discovery opened the door to more extensive research on the possible environmental factors that could be related to the illness. Today, many pesticides, herbicides and industrial chemicals are known to be involved in the development of PD. The most studied compounds include rotenone, which is a substance of plant origin that is used as an insecticide and acts as a potent complex I inhibitor in the mitochondrial respiratory chain, 6-hydroxydopamine, which is a neurotoxin that induces oxidative stress [7], bipyrindinic compounds, such as the MPP+ (1-methyl-4-phenylpyridinium ion), and the herbicide...
Table 1 | Principal genes related to PD

<table>
<thead>
<tr>
<th>Gene</th>
<th>Locus</th>
<th>Protein name</th>
<th>Inheritance pattern</th>
</tr>
</thead>
<tbody>
<tr>
<td>PARK1/4</td>
<td>4q21.3-q22</td>
<td>α-Synuclein</td>
<td>Autosomal dominant</td>
</tr>
<tr>
<td>PARK2</td>
<td>6q25.2-27</td>
<td>Parkin</td>
<td>Autosomal recessive</td>
</tr>
<tr>
<td>PARK3</td>
<td>2p13</td>
<td>?</td>
<td>Autosomal dominant</td>
</tr>
<tr>
<td>PARK5</td>
<td>4p14</td>
<td>UCH-L1</td>
<td>Autosomal dominant</td>
</tr>
<tr>
<td>PARK6</td>
<td>1p35-36</td>
<td>PINK1</td>
<td>Autosomal recessive</td>
</tr>
<tr>
<td>PARK7</td>
<td>1p36</td>
<td>Dj-1</td>
<td>Autosomal recessive</td>
</tr>
<tr>
<td>PARK8</td>
<td>12q12</td>
<td>LRRK2</td>
<td>Autosomal dominant</td>
</tr>
<tr>
<td>PARK9</td>
<td>1p36</td>
<td>ATP13A2</td>
<td>Autosomal recessive</td>
</tr>
<tr>
<td>PARK10</td>
<td>1p32</td>
<td>?</td>
<td>Autosomal dominant</td>
</tr>
<tr>
<td>PARK11</td>
<td>2q36-q37</td>
<td>GIGYF2</td>
<td>Autosomal dominant</td>
</tr>
<tr>
<td>PARK12</td>
<td>1q21-q25</td>
<td>?</td>
<td>X-linked</td>
</tr>
<tr>
<td>PARK13</td>
<td>2p13</td>
<td>HTRA2/OMI</td>
<td>Autosomal dominant</td>
</tr>
<tr>
<td>PARK14</td>
<td>22q13.1</td>
<td>PLA2G6</td>
<td>Autosomal recessive</td>
</tr>
<tr>
<td>PARK15</td>
<td>22q11.2</td>
<td>FBX07</td>
<td>Autosomal recessive</td>
</tr>
<tr>
<td>PARK16</td>
<td>1q32</td>
<td>?</td>
<td>?</td>
</tr>
</tbody>
</table>

paraquat, which has been studied at great length for its effect on neurodegenerative processes [8].

On the other hand, knowledge about the genetic factors involved in the disease is essential when clarifying the possible causes and mechanisms underlying its development. A family history of PD constitutes a risk factor at the time of PD development [9]. Since 1997, many PD-related genes have been studied, grouped under the name of PARK genes (Table 1). In the following years, a lot of number of genes was discovered implicated in PD. However, in recent years, has acquired special relevance the studies of PARK8 gene, which codes for the LRRK2 (leucine-rich repeat kinase 2) protein and could be directly associated with the development of PD.

PD and PARK8

PARK8 gene mutations were described as one of the major genetic causes associated with hereditary Parkinsonism [10]. The protein LRRK2 belongs to the family of ROCO proteins, which includes at least 40 proteins of various species that are characterized by the presence of a ROC (Ras of complex proteins) domain in their structure, followed by another domain called COR (C-terminal of ROC). In general, these domains are followed by a kinase domain. Moreover, LRRK2 has a domain rich in leucine and ankyrin repeats as well as a WD40 domain, which enables LRRK2 to interact with other proteins [11].

LRRK2 is a protein that it has a homodimeric structure [12], which suggests that it could have the capacity to self-regulate its kinase activity and GTPase activity [13]. It was first thought that the GTPase domain was responsible for regulating the kinase activity of LRRK2 [14], although some studies indicated that this view was not entirely correct [15]. Furthermore, studies have demonstrated that the LRRK2 protein, in addition to phosphorylating external substrates, has the capacity for autophosphorylation because it has various residues that can be phosphorylated by the kinase domain of the molecule [16]. Another hypothesis proposes that the union of the GTP molecule and the GTPase domain transforms LRRK2 into its active state, which would explain how mutations that modify the interaction of the GTP-ROC domain interrupt the kinase activity of the protein [17], which is essential for maintaining the dimeric structure [18].

A number of mutations are known in the LRRK2 structure [19]. However, the G2019S mutation in the kinase domain shows a higher prevalence in familial PD in the Caucasus [20], where it is responsible for 0.5–2% of the sporadic cases of PD and more than 10% of familial PD cases [21]. However, in Northern Africa, studies have related this mutation to 37% of familial PD cases and 30% of sporadic cases [22].

LRRK2 domain structure

The LRRK2 protein has six essential domains in its structure to interact with other proteins and regulate the different activities of protein (Figure 1).

Ankyrin repeat domain

There are seven ankyrin repeats located at the extreme N-terminus with a slightly curved configuration. These repeats are found on many proteins, including cytoskeletal proteins, transcription factors and proteins that regulate the cell cycle.

LRR (leucine-rich repeat) domain

The LRR domain of dardarin is composed of 13 repeats, each with a β-loop, followed by an α-helix that forms an
arch-shaped structure. The domain also participates in protein–protein interactions.

**WD40 domain**

The WD40 domain is formed by 40 repeats, each one with four antiparallel β-sheets forming a circular structure. The WD40 domain can interact with other proteins that contain WD40 domains.

**Kinase domain**

The catalytic site of the tyrosine kinase domain has a small nucleus in the N-terminus and a large lobe in the C-terminus that are connected by a hinge region. The hinge forms a fissure in which Mg²⁺-ATP and the protein substrate unite. The activation loop has a length of 20–35 amino acids and is flanked by two well-preserved sequences: DYG (Asp-Tyr-Gly) and APE (Ala-Pro-Glu) [11]. Most protein kinases need to be phosphorylated to be activated, after which the activation segment adopts an active configuration that allows the catalysis of the substrate. The phosphorylation of LRRK2 can be performed by another protein kinase or by autophosphorylation [23]. The kinase domain of LRRK2 is highly homologous with other MAPKKKs (mitogen-activated protein kinase kinase kinases) of the tyrosine kinase group [24], in which various mutations have been detected. These mutations have been mostly found in the preserved DF/YG (Asp-Phe/Tyr-Gly) sequence, which has been linked to PD.

The G2019S mutation is found in a preserved region of the activation segment (ADYGIAQYCC). Specifically, it is found in the Mg²⁺ union site of the kinase domain. The exchange of glycine for serine facilitates the access of the kinase domain to its substrates, thereby increasing its capacity for autophosphorylation 2.5-fold and its capacity to phosphorylate other substrates 3-fold. For example, the phosphorylation of MBP (myelin basic protein) by LRRK2 is increased by the mutation [25,26], which results in increased toxicity of MBP [27]. The I2020T mutation is found in the zone adjacent to the residue at position 2019, and it influences the activation site of the kinase domain. The exchange of isoleucine for tyrosine next to the DYG activation site increases the autophosphorylation capacity of LRRK2 by 40%. Such a mutation can also modify the specificity for substrates and result in an increase in toxicity [23].

Various studies have associated changes in LRRK2 kinase activity with cellular death processes; thus the search for physiological substrates is of great importance. The first protein described as a substrate for LRRK2 was moesin [26], which is involved in the cytoskeleton and the cytoplasmic membranes [28]. β-Tubulin has also been described as a LRRK2 substrate, and it has been suggested to be a LRRK2 regulator in the regulation of cytoskeletal stability [29]. 4E-BP (eukaryotic initiation factor 4E-binding protein) has also been postulated as a LRRK2 substrate [30], in addition to certain members of the MAPKKK and MKK (mitogen-activated protein kinase kinase) 3, 4, 6 and 7 families [31].

In addition, LRRK2 has a high autophosphorylation capacity. Depending on the domain in which these autophosphorylations occur, variations in the activity of GTPase and kinase activity can occur [32]. MS has revealed that Ser303, Thr1410 and Thr1401 in the Roc domain, like Thr1467 and Thr1465 in the kinase domain, can be phosphorylated by the LRRK2 molecule [33]. Ser305 and Ser355 can also be autophosphorylated and are essential for maintaining the molecule's stability [34].

**Roc domain**

GTPases are molecules with the capacity to regulate various kinases. MAPKs (mitogen-activated protein kinases) are examples of signalling molecules that are regulated by GTPases. GTPase activity requires the molecules GEF (guanine-nucleotide-exchange factor) and GAP (GTPase-activating protein), which regulate the exchange and binding of GTPase to GTP (active) or GDP (inactive) [35].

The Roc domain of LRRK2 has GTPase activity and, like many other GTPases, its function is to regulate cellular processes. The Roc domain is responsible for increases in kinase activity [36], although little is known about how this regulation occurs. Three mutations have been found in the Roc domain that affect the residue at position 1441 of the domain, which corresponds to arginine. These mutations do not increase GTP's binding capacity, but they do reduce its hydrolysis capacity, thus increasing the duration of the active state of the molecule [37]. Studies have indicated that a loss of GTP-binding capacity (mutation K1347A) produces an increase in kinase activity [25]. However, other studies have indicated that the loss of GTP, GDP or GMP binding does not influence the kinase activity of LRRK2 [15]. Furthermore, the influence of the R1441C mutation on kinase activity remains controversial. In some studies, an increase is observed [36], whereas in others, no changes are observed [26].

**LRRK2 functions**

Functions of LRRK2 are not yet completely known. In fact, the number of domains in its structure indicates a large number of proteins that may interact with LRRK2. Previous studies have demonstrated multiple proteins that interact with LRRK2 such as cytoskeleton protein (β-tubulin or actin) [29], Hsp90 (heat-shock protein 90), CHIP (C-terminus of the Hsc (heat-shock cognate) 70-interacting protein), FADD (Fas-associated death domain), Rab5b and other proteins related to PD, such as parkin [38], PINK-1 [PTEN (phosphatase and tensin homologue deleted on chromosome 10)-induced putative kinase 1], DJ-1 [39] and α-synuclein [40]. Moreover, LRRK2 interacts with MAPKs such as ERK (extracellular-signal-regulated kinase), JNK (c-Jun N-terminal kinase) and p38 [41]. All of these interactions suggest that LRRK2 may participate in different processes, especially with regard to the transport of proteins through synaptic vesicles and the process of ubiquitination. Some studies have also associated LRRK2 with autophagy [42] and apoptosis [43].
Link between LRRK2 and autophagy

Autophagy is a catabolic cellular mechanism that has been highly preserved throughout evolution; it is the process by which the cell recycles or degrades proteins or damaged cytoplasmic organelles [44]. Autophagy is a tightly regulated process that plays a normal part in neonatal development [45] and in illnesses such as cancer, cardiomyopathies or neurodegenerative processes [8]. It is a degradative process with a complex regulation (Figure 2). There are several negative regulators such as mTOR (mammalian target of rapamycin), the PI3K (phosphoinositide 3-kinase) class I route, NF-κB (nuclear factor κB), p38, caspase 8 or Bel-2. However, many pathways are capable of positively regulating autophagy. The most well known pathway is the PI3K class III Beclin-1-dependent route [46]. However, it is not the only positive regulator, since it is known that the MEK (MAPK/ERK kinase)/ERK pathway, AMPK (AMP-activated protein kinase), JNK or the presence of ROS (reactive oxygen species) has been involved in the regulation of autophagy.

There are several indications of the involvement of LRRK2 in regulating autophagy starting with the fact that an endogenous part of LRRK2 is localized to membranous structures of the cell, including the endoplasmic reticulum and endosomes [47]. Moreover, LRRK2 interacts with various proteins that are implicated in the regulation of autophagy. However, the exact mechanism of interaction between LRRK2 and autophagy remains unknown.

Conclusions

There is evidence of the relationship between autophagy and LRRK2, and the deregulation could be involved, at least in part, in the aetiology of PD. In fact, an alteration in kinase activity of LRRK2 has been associated with death process in different models. Therefore it is very important to understand the complete link between LRRK2 and autophagy, to elucidate whether this degradative process was implicated in cellular death that has been identified in studies of PARK8 mutations. This information is essential for the development of strategies for reducing the cellular sensitivity and cell death that could trigger the development of PD.

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