LRRK2 and vesicle trafficking

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Abstract

Mutations in LRRK2 (leucine-rich repeat kinase 2) (also known as PARK8 or dardarin) are responsible for the autosomal-dominant form of PD (Parkinson’s disease). LRRK2 mutations were found in approximately 3–5% of familial and 1–3% of sporadic PD cases with the highest prevalence (up to 40%) in North Africans and Ashkenazi Jews. To date, mutations in LRRK2 are a major genetic risk factor for familial and sporadic PD. Despite the fact that 8 years have passed from the establishment of the first link between PD and dardarin in 2004, the pathophysiological role of LRRK2 in PD onset and progression is far from clearly defined. Also the generation of different LRRK2 transgenic or knockout animals has not provided new hints on the function of LRRK2 in the brain. The present paper reviews recent evidence regarding a potential role of LRRK2 in the regulation of membrane trafficking from vesicle generation to the movement along cytoskeleton and finally to vesicle fusion with cell membrane.

Introduction

PD (Parkinson’s disease) is the second most common neurodegenerative disorder after Alzheimer’s disease, affecting more than 1 million people in North America and more than 4 million people worldwide. Characterized by a core phenotype of motor deficits, akinnesia, rigidity, postural disturbance and tremor, PD is complicated by other neurological deficits during its long progression [1]. The most characteristic features of this neuropathology are the loss of pigmented neurons in the SNpc (substantia nigra pars compacta) and presence of eosinophilic cytoplasmic inclusion bodies (called Lewy bodies). In particular, the loss of dopaminergic neurons is responsible for both depletion of dopamine in the caudate putamen and motor dysfunction.

Although the pathogenesis of PD remains incompletely understood, both genetic susceptibility and environmental factors appear to be involved [2]. The identification of rare familial forms of parkinsonism and the subsequent cloning of causal genetic mutations has had a significant impact on our understanding of the molecular mechanisms underlying idiopathic PD. Genes whose mutations have been associated with parkinsonism include SNCA (α-synuclein), PARK2 (parkin), PARK7 (DJ-1), PINK1 (phosphatase and tensin homologue deleted on chromosome 10-induced putative kinase 1), ATP13A2 (ATPase type 13A2) and LRRK2 (leucine-rich repeat kinase 2) (also known as PARK8 or dardarin) [3].

Specific mutations in the LRRK2 gene have been identified as being responsible for autosomal-dominant familial PD variants, and most LRRK2-linked families show a clinical and in vivo neurochemical phenotype that is indistinguishable from idiopathic forms of PD [4,5]. Clinicogenetic studies from several independent groups have evaluated the frequency of LRRK2 mutations in many different populations, and such mutations have been found not only in 3–5% of familial PD, but also in approximately 1–3% of idiopathic PD cases [6]. The pattern of dominant inheritance is consistent with a gain-of-function mechanism, and the most frequent pathological mutations appear to increase the protein kinase activity [7,8]. Despite extensive studies using both animal and cellular models, the pathophysiological role of LRRK2 in PD onset and progression is still largely unclear, and LRRK2 substrate(s) remain pretty elusive. Different hypotheses on LRRK2 function have been formulated ranging from miRNA (microRNA) processing [9], translation regulation [10], cytoskeletal organization [11–13] and autophagy–lysosome pathways [14,15] to immunoregulatory function [16]. The present review focuses on recent evidence for a potential role of LRRK2 in the regulation of vesicle trafficking.

Key words: cytoskeleton, endocytosis, exocytosis, leucine-rich repeat kinase 2 (LRRK2).

Abbreviations used: A53T, amyotrophic lateral sclerosis; Arf, ADP-ribosylation factor; ArfGAP1, Arf GTPase-activating protein 1; COP, coatomer protein; EPSC, excitatory postsynaptic current; ER, endoplasmic reticulum; ERK, extracellular signal-regulated kinase; LRRK2, leucine-rich repeat kinase 2; PD, Parkinson’s disease; PINK1, phosphatase and tensin homologue deleted on chromosome 10-induced putative kinase 1; siLRRK2, LRRK2 short interfering RNA; SNpc, substantia nigra pars compacta; SOD, superoxide dismutase.

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LRRK2 localization in dopaminergic areas

The LRRK2 expression pattern in different brain regions appears to be quite different from the expression profile of all other genes linked to PD: SCNA, PARK2, UCHL1 (ubiquitin C-terminal hydrolase isoenzyme L1), PARK7 and PINK1. mRNA are all expressed in many, if not all, neurons of the brain, showing very high levels in dopamine neurons of the midbrain [3].

Initial Northern blot analysis showed that LRRK2 is expressed in diverse brain areas such as the cerebellum, cerebral cortex, medulla, spinal cord and putamen [5]. Before the development of specific antibodies against LRRK2, an in situ hybridization approach was used, showing a high level of LRRK2 mRNA in different brain areas,
but low/moderate [17–19] or undetectable levels [20,21]
in dopaminergic neurons. The development of specific antibodies for multiple LRRK2 epitopes has allowed a more detailed analysis of LRRK2 protein expression in the different brain areas and in particular in dopaminergic neurons. Overall, the regional distribution of LRRK2 immunoreactivity in rodents, monkeys and humans is quite similar, whereas some discrepancies have been raised regarding the expression of LRRK2 in Lewy bodies or Lewy neurites, the pathological hallmark of PD [17,18,22]. The analysis clearly demonstrates the presence of LRRK2 protein in neurons, but not in glial cells in the SNpc and VTA (ventral terminal area), although at a lower level compared with dopaminergic target areas. Notably, not all tyrosine hydroxylase-positive cells were positive for LRRK2 staining [23,24]. Thus, despite the development of new tools, the first hypothesis that LRRK2 dysfunction may affect mainly the dopaminergic target areas or surrounding cells, which in turn influence the dopaminergic neurons, is still under debate. For instance, the expression of LRRK2 mutant R1441G increases pro-inflammatory cytokine release from activated primary microglial cells and resultant neurotoxicity [25]. On the other hand, the expression of LRRK2 pathological mutants is toxic in neuronal cell lines [7,26], suggesting a death mechanism independent of cell–cell communication. However, this phenomenon is also observed for other genes linked to degeneration of specific sets of neurons; for instance, the expression of SOD (superoxide dismutase) 1 pathological mutants, responsible for familial ALS (amyotrophic lateral sclerosis), is toxic in neuronal cells, although, to date, it is largely accepted that SOD1-ALS is not a cell-autonomous disease [27]. In this respect, cell–cell communication between dopaminergic and non-dopaminergic cells could play an important role in the neuronal toxicity induced by LRRK2 pathological mutant expression. Vascular trafficking plays a major role in cell–cell interactions, and underlies virtually all functions of the nervous system: from neuronal sustenance through continuous new cell membrane supply and trophic factor release to neuronal communication through the control of protein or neurotransmitter release.

LRRK2 intracellular localization

More unequivocal are the experimental results on the LRRK2 intracellular localization. Different experimental approaches have demonstrated that LRRK2 is localized throughout the cytoplasm of neuronal perikarya and dendritic processes, where it is associated with vesicular and membranous structures, the microtubule network, mitochondria and other membrane-bound organelles [24,28]. Moreover, LRRK2 interacts with membrane regions that resist solubilization by ice-cold 1% Triton X-100, indicating its association through lipid rafts [28]. Although the pathological mutations do not affect the LRRK2 membrane association, this localization is of particular interest since the lipid rafts play important roles in cellular functions such as signal transduction, membrane trafficking and cytoskeletal organization [29]. Moreover, lipid rafts associate with SNARE (soluble N-ethylmaleimide-sensitive fusion protein-attachment protein receptor) proteins and regulate endocytosis and exocytosis [30,31]. Notably, the membrane-associated fraction of LRRK2 possesses greater kinase activity than cytosolic LRRK2. In addition, membrane-associated LRRK2 binds GTP more efficiently than cytosolic LRRK2, but demonstrates a lower degree of phosphorylation [32]. The membrane association also increases the LRRK2 dimer formation [32], although this LRRK2 status is still debated [33,34].

The most extensive analysis regarding the LRRK2 intracellular localization was performed by Biskup et al. in 2006 [24]. Using a large panel of antibodies, they showed, both in rat primary cortical neuronal cultures and in rodent brains, that LRRK2 co-localizes consistently to the Golgi apparatus and Golgi-associated vesicles, ER (endoplasmic reticulum), lysosomes and mitochondria, and, to a significantly lesser degree, to vesicle markers such as synaptotagmin. The LRRK2 association with microtubule networks is significant in rodent brain slices, but less prominent in rat primary cortical neuronal cultures.

The mitochondrial localization is of particular interest since many PD-related genes are associated with or localized inside the mitochondria, including PARK2, PARK7 and PINK1 [35]. Moreover, mitochondrial involvement in LRRK2 neuronal toxicity has been observed under different experimental conditions [26,36]. LRRK2 is thought to be tightly associated rather than membrane-integrated with mitochondria, although LRRK2 does not contain any hydrophobic transmembrane domains or obvious mitochondrial sequence. To exclude the presence of LRRK2 inside the mitochondria, we used two different approaches: the insertion of a mitochondrial localization signal in LRRK2 cDNA and the trypsin treatment of purified mitochondria. We fused two mitochondrial signal peptides, one for the matrix (mouse cytochrome c oxidase) or one for the intermembrane space localization (mouse cytochrome c), to the N-terminal position of LRRK2. We transiently transfected neuronal cell lines (SH-SY5Y) to evaluate the mitochondrial localization of the different proteins. As shown in Figure 1, the presence of both mitochondrial localization signals increases only slightly the LRRK2 protein level associated with mitochondria. However, a mild trypsin treatment of purified mitochondria from the different samples leads to the complete disappearance of LRRK2, whereas mitochondrial MnSOD protein is unaffected. This last result is comparable with the one obtained by West et al. [7].

LRRK2 and membrane trafficking

To elucidate the physiological role of LRRK2, Piccoli et al. [37] analysed the presynaptic and postsynaptic properties of cortical neurons in which LRRK2 had been silenced by short hairpin-mediated RNA interference. Electrophysiological analysis of these neurons revealed that LRRK2 silencing alters synaptic transmission. In particular, EPSC (excitatory
postsynaptic current) amplitude in postsynaptic neurons connected to siLRRK2 (LRRK2 short interfering RNA)-silenced presynaptic neurons increased more than 2-fold compared with that measured in control pairs. Furthermore, siLRRK2 pairs showed a higher probability of generating an EPSC over the baseline after a presynaptic trigger. LRRK2 silencing perturbs vesicle dynamics and distribution within the recycling pool, determining a significant decrease in docked vesicles, but an increase in the amount of vesicle recycling. At the molecular level, LRRK2 interacts, mainly through the WD40 domain, with a number of proteins involved both in endo- and exo-cytosis of synaptic vesicles. Unfortunately, under the same experimental conditions, the overexpression of LRRK2 is toxic, therefore not allowing a comparative analysis between protein absence and overexpression. In Caenorhabditis elegans, the absence of LRK-1 (the only LRRK-like protein present in this organism) alters the axonal–dendritic polarity of synaptic vesicle localization [38]. The synaptic vesicles, strictly localized to the axonal regions, in the absence of LRK-1 are localized also in the dendritic processes [38]. In this animal model, LRK-1 protein kinase functions on the TGN (trans-Golgi network) to exclude synaptic vesicle proteins from dendrite-specific transport. In C. elegans, the overexpression of human LRRK2 proteins causes age-dependent dopaminergic neurodegeneration, behavioural deficits and locomotor dysfunctions that are accompanied by a reduction in dopamine levels in vivo. In comparison, R1441C and G2019S mutants cause more severe phenotypes than the wild-type protein [39].

Different LRRK2 rodent transgenic animals have been generated by independent groups. The comparison between different animal models is quite difficult because of the use of different pathological mutants, human or mouse cDNAs, different promoters or BAC (bacterial artificial chromosome) transgenes. In a few cases, the authors describe dopaminergic degeneration in animals expressing pathological mutants [14,40]; some alterations in dopamine neurotransmission are observed more consistently, although not in all transgenic animals [14]. The prevalence is a reduction in dopamine extracellular content without [13,41] or with [40,41] pharmacological manipulation. In a few cases, the authors describe an increase in dopamine extracellular content in wild-type Lrrk mouse transgenes [41] or rats expressing the inducible LRRK2 G2019S pathological mutant [42]. Moreover, the dopaminergic system of LRRK2-knockout mice appears normal, and the number of dopaminergic neurons and levels of striatal dopamine are unchanged [15]. Some of the results discussed above may be due to an alteration in endocytosis mechanisms rather than exocytosis. Indeed, at least two independent studies have highlighted a role for LRRK2 in the control of cellular mechanisms of endocytosis [37,43]. In particular, alteration of LRRK2 expression by either overexpression or knockdown of endogenous LRRK2 in primary neuronal cell lines significantly impairs synaptic vesicle endocytosis [43], whereas Piccoli et al. [37] obtained a similar phenotype by LRRK2 silencing. At the molecular level, the alteration of synaptic vesicle endocytosis may be mediated by the interaction between LRRK2 and Rab5 [43]. Recently, ArfGAP1 (ADP-ribosylation factor GTPase-activating protein 1) has been identified as an LRRK2 interactor both in vitro and in vivo [44,45]. Interestingly, ArfGAP1 promotes the GTP hydrolysis of Arf1 (ADP-ribosylation factor 1), a small GTPase that is critical for maintaining normal Golgi morphology, and Arf1 GTP hydrolysis is also required for the dissociation of coat proteins from Golgi-derived membranes and vesicles [46]. Although some results are somewhat contradictory or
LRRK2 (indicated by asterisks) largely co-localizes with the ER, Golgi apparatus and Golgi-associated vesicles, cytoskeleton, synaptic vesicles and lipid rafts. All of these structures are involved to a different extent in both anterograde and retrograde transport regulating vesicle generation, motility, secretion and endocytosis. Moreover, LRRK2 also co-localizes with lysosomes that are generated by the addition of hydrolytic enzymes to early endosomes from the Golgi apparatus. Thus membrane trafficking regulates different aspects of neuronal physiology ranging from neurotransmitter or neurotrophic factor release, insertion of cell membrane components (including both membrane proteins and lipids) and, not least, organelle biogenesis.

difficult to interpret, it is evident that LRRK2 participates in a protein network regulating synaptic vesicle trafficking.

An alternative explanation for the dysregulation of vesicle trafficking could arise from the described role of LRRK2 in the modulation of cytoskeleton dynamics. Both the microtubule and actin cytoskeletons make essential contributions to intracellular vesicle and organelle motility [47]. There are two mechanisms for actin-based motility [29]. First, actin polymerization itself can propel vesicles, and, secondly, actin serves as a track for the motor protein myosin belonging to the ERM (ezrin/radixin/moesin) family. In fact, LRRK2 modulates both ERM protein phosphorylation and actin polymerization mainly in filopodia structures [11]. The growth cone filopodia contain vesicles that transport synaptic vesicle proteins bidirectionally along filopodia and fuse with the filopodia surface in response to focal stimulation, allowing for both local secretion of vesicular contents and rapid changes in plasma membrane composition [48]. On the other hand, microtubules serve as tracks for vesicle transport via the motor proteins dynein and kinesin. Notably, human LRRK2 appears to interact [49] and preferentially phosphorylates β-tubulin at Thr107 in mouse brain, and the phosphorylation is significantly enhanced by G2019S mutation [12]. Moreover, LRRK2 directly phosphorylates tau in the presence of tubulin and facilitates dissociation of tau from tubulin, thus suggesting an important physiological role for LRRK2 in microtubule dynamics [50]. Each of the three major classes of transport vesicle coats COP (coatamer protein) II, COPI, and clathrin [47] form binding interactions with regulatory or structural components of the cytoskeleton-based motility machinery. Multiple types of coat-bound accessory proteins participate at the interface between vesicle formation and the cytoskeleton. Cytoskeletal dynamics, motor protein function and vesicle trafficking are all regulated through the Rab, Arf and Rho families of GTP-binding proteins. Notably, LRRK2 contains a GTP-binding domain and interacts with ArfGAP1, a protein regulating the GTP hydrolysis of Arf1 [46], leading to the interesting possibility that LRRK2 may affect, directly or indirectly, the secretory and endocytic pathways modulating cytoskeleton dynamics and vesicle motility. Finally, lysosomes are created by the addition of COPI vesicle coats containing hydrolytic enzymes to early endosomes from the Golgi apparatus. Thus the dysregulation of vesicle trafficking may also affect the autophagy–lysosome pathway.

Future prospects

LRRK2 appears to be localized in different intracellular districts that play a critical role in the control of vesicular trafficking: ER, Golgi apparatus and associated vesicles, cytoskeleton, lipid raft and finally lysosomes (Figure 2). Although there are some discrepancies between different experimental approaches and animal or cellular models, the involvement of LRRK2 in the regulation of vesicle trafficking appears to be quite consistent. Most of the functional studies on LRRK2 have focused on the uptake/release of vesicles around the plasma membrane and mainly related to neurotransmitters on the basis of the fact that PD is a neuronal disease. Vesicle trafficking is a complex process regulating multiple different cellular functions, in addition to neurotransmitter release, such as neurotrophic factor release, localization of membrane receptors, changes in plasma membrane composition at the cell surface and, not least,
organellar biogenesis. Most small-molecule neurotransmitters are stored in small vesicles that range from 40 to 60 nm in diameter and, in electron micrographs, appear to have a clear centre, whereas the vesicles that store neuropeptides are larger, ranging from 90 to 250 nm in diameter. These vesicles appear dark and electron-dense in electron micrographs. The molecular mechanisms regulating the exo/endo-cytosis for proteins or membranes are different from those regulating neurotransmitter release. The LRRK2 cellular localization mainly with the ER and the Golgi apparatus and Golgi-associated vesicles, but to a significantly lesser degree with vesicle markers [24], could suggest an involvement of LRRK2 in anterograde or retrograde vesicle trafficking between the ER and Golgi apparatus or Golgi and cell membrane or Golgi and lysosome, rather than a simple control of neurotransmitter release at the synapse. These last aspects have not been investigated in depth yet in the different cellular or animal models lacking LRRK2 or overexpressing the pathological mutants.

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