Published in final edited form as:

Brain Res Bull. 2017 July ; 133: 51–59. doi:10.1016/j.brainresbull.2016.12.004.

Parkin and PINK1 functions in oxidative stress and neurodegeneration

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Abstract

Loss-of-function mutations in the genes encoding Parkin and PINK1 are causally linked to autosomal recessive Parkinson's disease (PD). Parkin, an E3 ubiquitin ligase, and PINK1, a mitochondrial-targeted kinase, function together in a common pathway to remove dysfunctional mitochondria by autophagy. Presumably, deficiency for Parkin or PINK1 impairs mitochondrial autophagy and thereby increases oxidative stress due to the accumulation of dysfunctional mitochondria that release reactive oxygen species. Parkin and PINK1 likely have additional functions that may be relevant to the mechanisms by which mutations in these genes cause neurodegeneration, such as regulating inflammation, apoptosis, or dendritic morphogenesis. Here we briefly review what is known about functions of Parkin and PINK1 related to oxidative stress and neurodegeneration.

Keywords

PINK1; Parkin; Ubiquitin; Neurodegeneration; Oxidative stress; Mitophagy

1. Introduction

Oxidative stress has been implicated as a likely cause of many neurodegenerative diseases including Parkinson's disease (PD). PD is the most common neurodegenerative movement disorder and affects millions of people worldwide. PD is defined clinically by bradykinesia, resting tremor, rigidity and abnormal gait. It is diagnosed neuropathologically by relatively selective loss of dopaminergic neurons in the substantia nigra and the presence of Lewy body intraneuronal inclusions containing α -synuclein. Current pharmacological therapies treat the symptoms, mostly by enhancing dopaminergic signaling, which is required for

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Conflict of interest

The authors declare that there are no conflicts of interest.

normal movement. There are currently no therapies proven to slow down disease progression or to protect against neurodegeneration. Most PD cases are sporadic, but the recent identification of genes with mutations linked to familial forms of PD provides important clues that could help determine the causes of both familial and idiopathic PD, and could lead to the development of more effective therapies (Kumaran and Cookson 2015). Even prior to the identification of the first genetic mutations linked to PD, mitochondrial dysfunction and oxidative stress were implicated in PD pathogenesis because neurotoxins, such as 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), can induce parkinsonism in humans and animal models by inhibiting mitochondrial respiration and increasing production of reactive oxygen species (ROS) (Langston 1987; Mizuno et al. 1995; Jenner and Olanow 1996; Fukae et al. 2007; Zhou et al. 2008; Camilleri and Vassallo 2014; Gautier et al. 2014; Moon and Paek 2015). Even under normal physiological conditions, electron leakage from the mitochondrial electron transport chain is a major cellular source of ROS that damage proteins, lipids and DNA (Beal 2005). Because this damage likely accumulates with age and because age is the greatest PD risk factor, mitochondrial dysfunction and oxidative stress are likely causes of idiopathic PD initiation and progression (Beal 2003; Shults 2004). Consistent with this, oxidatively damaged subunits of mitochondrial complex I are increased in PD brains (Keeney et al. 2006) and impaired complex I activity has been observed in multiple tissues and peripheral blood leukocytes from PD patients (Mann et al. 1992; Albers and Beal 2000; Muftuoglu et al. 2004; Schapira 2008). Perhaps the most compelling evidence for mitochondrial dysfunction as a direct cause of parkinsonism (rather than a consequence or an age-coupled epiphenomenon) comes from the genetic linkage of loss-of-function mutations in the mitochondrial kinase Phosphatase and tensin homolog (PTEN)-induced kinase 1 (PINK1) to early onset recessive parkinsonism (Valente et al. 2004). PINK1 is a mitochondrial kinase that accumulates on the surface of defective mitochondria and recruits Parkin to promote selective degradation of dysfunctional mitochondria (Narendra et al. 2008; Matsuda et al. 2010). Loss-of-function mutations in Parkin account for about 50% of all cases of early onset PD (Kitada et al. 1998; Lucking et al. 2000). Here, we briefly review the known functions of PINK1 and Parkin with respect to potential mechanisms of PD pathogenesis.

2. Mitochondrial dysfunction and oxidative stress in PD

The single greatest risk factor for PD is age, which strongly implicates cumulative oxidative damage as a causative mechanism. There is now overwhelming evidence that oxidative damage plays a key role in idiopathic PD and inherited parkinsonism, as well as neurotoxin-induced PD animal models (Fahn and Cohen 1992; Cassarino et al. 1997; Beal 2005). Low-level oxidative stress may promote mitochondrial biogenesis and elimination or repair of damaged mitochondria while high-level oxidative stress beyond the cellular capacity to repair or remove oxidative damage may lead to the accumulation of damaged mitochondria (Lee and Wei 2005). Of all the proteins with mutations so far linked to parkinsonism, Parkin is one of the most sensitive to oxidation and mounting evidence suggests Parkin is important for protecting cells from oxidative stress (Hyun et al. 2002; Palacino et al. 2004; Pesah et al. 2004; Greene et al. 2005). Observations that Parkin is sensitive to oxidative damage by nitrosylation supports the idea that oxidative damage to endogenous Parkin may contribute

to idiopathic PD (Yao et al. 2004). Dopamine readily oxidizes to form reactive oxygen species and dopamine quinone, which can covalently modify and inactivate Parkin, providing further evidence that progressive loss of Parkin function in dopamine neurons in combination with oxidative stress may contribute to onset or progression of idiopathic PD (LaVoie et al. 2005). Although inherited mutations in Parkin cause only a small fraction of all clinical parkinsonism cases, oxidative damage to Parkin protein is observed in the brains of sporadic PD patients, which supports the hypothesis that inactivation of Parkin in conjunction with oxidative damage could cause idiopathic PD (Chung et al. 2004; Yao et al. 2004).

3. Mutations in Parkin and PINK1 causally linked to PD

Mutations in five genes have so far been definitively linked to familial PD. Gain-of-function mutations in *α-synuclein* and *LRRK2* have been linked to dominantly inherited parkinsonism (Polymeropoulos et al. 1997; Paisan-Ruiz et al. 2004; Zimprich et al. 2004), and loss-of-function mutations in *parkin*, *DJ-1*, and *PINK1* have been linked to recessively inherited parkinsonism (Kitada et al. 1998; Bonifati et al. 2003; Valente et al. 2004).

Parkin was the first gene to be identified with mutations linked to recessive parkinsonism (Kitada et al. 1998). Over 100 different *parkin* mutations affecting each of *parkin*'s 12 exons have since been identified in parkinsonian patients, including missense point mutations, truncation mutations, large chromosomal deletions and duplications spanning one or more exons, as well as promoter mutations (Hedrich et al. 2004; Lesage et al. 2007). The recessive mode of inheritance and the absence of Parkin protein (or radically truncated protein in some patients) are consistent with a loss-of-function mechanism by which *parkin* gene mutations cause disease (Shimura et al. 1999). Parkin is expressed widely throughout the brain and other tissues, with the highest mRNA abundance in brain, heart and skeletal muscle (Kitada et al. 1998).

Loss-of-function mutations in *parkin* are found in nearly 50% of parkinsonism cases with onset of symptoms before age 45 (Lucking et al. 2000). Other than an earlier average age at onset, the clinical symptoms of patients bearing *parkin* mutations resembles that of typical late-onset idiopathic PD, with good therapeutic response to L-DOPA and slightly slower disease progression (Lucking et al. 2000). There is so much overlap in clinical symptoms between idiopathic PD and *parkin*-linked disease that it is not possible to distinguish patients bearing *parkin* mutations from idiopathic PD based on clinical criteria alone (Lucking et al. 2000). Although *parkin* mutations were first identified in patients with very young age at onset, *parkin* mutations have since been identified in typical late-onset PD patients and *parkin* polymorphisms or heterozygous mutations are suspected of increasing susceptibility to typical late-onset PD (Klein et al. 2000; Schlitter et al. 2006). *Parkin* polymorphisms, in combination with increased environmental exposures to substances suspected of causing idiopathic PD, are associated with earlier onset of symptoms more than either factor alone (Ghione et al. 2007).

The initial neuropathological examinations reported for cases bearing *parkin* mutations led to the assumption that Parkin is required for Lewy body formation because no Lewy bodies

were observed in the initial autopsies, however, Lewy bodies have since been observed in cases from 2 independent families bearing *parkin* mutations (Farrer et al. 2001; Pramstaller et al. 2005). All autopsies of *parkin*-linked PD showed profound loss of neuromelanin-containing neurons in the substantia nigra pars compacta as well as prominent loss of locus coeruleus neurons, which is also observed in idiopathic PD. Given that cortical Lewy bodies are commonly observed in neuropathological examinations of aged brains, it is likely that the absence of Lewy bodies in some cases of *parkin*-linked PD reflects the earlier age at onset of these cases and the many years or decades that may be required for insoluble protein aggregates to accumulate to the extent that they can be detected by visible light microscopy in the form of Lewy bodies. We and others have put forward the hypothesis that small protein aggregates are more likely to be mechanistically involved in PD pathogenesis than Lewy bodies or large fibrillar aggregates that can be detected by microscopy (Goldberg and Lansbury 2000). We speculate that parkin and PINK1 function, at least in part, to prevent the accumulation of small protein aggregates or oxidized proteins that could be neurotoxic.

Loss-of-function mutations in *PINK1* cause clinical symptoms and neuropathology indistinguishable from PD with young onset (Valente et al. 2004; Samaranch et al. 2010). PINK1 was first cloned in the course of a search for genes upregulated by the tumor suppressor gene PTEN (Unoki and Nakamura 2001). Loss-of-function mutations in *PINK1* were subsequently identified as the cause of recessive parkinsonism linked to the PARK6 locus on chromosome 1 (Valente et al. 2004). The *PINK1* gene contains 8 exons encoding a 581 amino acid protein with an N-terminal mitochondrial targeting motif, a transmembrane domain (amino acids 94–110) and a highly conserved kinase domain (amino acids 156–509) with sequence homology to the serine/threonine kinases of the calcium/calmodulin family (Valente et al. 2004). PINK1 is ubiquitously expressed throughout the human and rodent brain (including in the substantia nigra) (Taymans et al. 2006; Blackinton et al. 2007) and in most adult human tissues, but at higher levels in skeletal muscle and heart (Unoki and Nakamura 2001).

Over 50 mutations causally linked to recessive parkinsonism have been identified throughout the length of the *PINK1* gene (Kawajiri et al. 2011) (Figure 1). These include missense point mutations, truncating mutations, genomic rearrangements and whole gene deletions. The penetrance of homozygous mutations is very high and heterozygous mutations may increase susceptibility for PD (Kawajiri et al. 2011). The clinical features of PINK1-linked parkinsonism are indistinguishable from sporadic PD with the exception of an earlier age of onset and slower progression (Kawajiri et al. 2011). Postmortem examination of PINK1-linked PD shows neuropathology similar to idiopathic PD with Lewy bodies and neuronal loss in the substantia nigra accompanied by microgliosis and astrocytic gliosis (Samaranch et al. 2010; Steele et al. 2015), although Lewy bodies were not observed in one of the three autopsies reported to date (Takanashi et al. 2016).

4. Domain structures and functions of Parkin and PINK1

PINK1 encodes a kinase with a mitochondrial targeting sequence at the N-terminus. PINK1 is normally rapidly degraded following mitochondrial import, however, low mitochondrial

membrane potential induced by treating cells with ionophores, such as CCCP or valinomycin, causes PINK1 to accumulate on the outer mitochondrial membrane and to recruit Parkin to selectively target dysfunctional mitochondria for degradation by autophagy (Narendra et al. 2008; Matsuda et al. 2010; Narendra et al. 2010). Autophagy is a highly regulated and conserved process of lysosomal-mediated protein degradation and recycling of organelles. Mitochondria can be degraded by autophagy either non-selectively or selectively in a process termed mitophagy in which defective mitochondria (*e.g.* with low membrane potential) are selectively targeted for degradation (Figure 2). This can promote cell survival by removing dysfunctional mitochondria that produce excess reactive oxygen species via leakage from the electron transport chain and by removing mitochondria that might otherwise signal apoptosis. The prevailing hypothesis regarding the mechanisms by which loss-of-function mutations in PINK1 and Parkin cause PD is that deficiency for either Parkin or PINK1 diminishes mitophagy and causes an age-dependent accumulation of dysfunctional mitochondria that would otherwise be removed, leading to increased ROS and to eventual neurodegeneration of susceptible cells (Figure 2).

PINK1 null *Drosophila* exhibit striking mitochondrial morphology defects, flight muscle degeneration, male sterility and mitochondrial respiration defects that precede the prominent visible flight muscle pathology, suggesting that the mitochondrial defects constitute an early pathogenic mechanism (Clark et al. 2006; Park et al. 2006; Yang et al. 2006). Some, but not all, groups have observed degeneration of dopamine neurons in PINK1 null flies (Clark et al. 2006; Park et al. 2006; Yang et al. 2006). Notably, Parkin null *Drosophila* have the same phenotype and the muscle degeneration and mitochondrial morphology defects in PINK1 null *Drosophila* can be rescued by overexpression of Parkin, consistent with Parkin functioning downstream of PINK1 in the same pathway (Clark et al. 2006; Park et al. 2006; Yang et al. 2006). Similar to Parkin knockout mice (Goldberg et al. 2003), PINK1 knockout mice do not develop nigral dopamine neuron loss but exhibit mitochondrial respiration defects in the striatum but not in the cortex at 3–4 months (Kitada et al. 2007; Zhou et al. 2007; Gispert et al. 2009; Akundi et al. 2011). Multiple groups have reported significant nigral cell loss in 8–9 month old PINK1 knockout rats using rigorous stereology (Dave et al. 2014; Villeneuve et al. 2014).

The *parkin* gene encodes a cytosolic 465 amino-acid protein with a ubiquitin-like (Ubl) domain at the N-terminus and an RBR (RING-between-RING) domain toward the C-terminus (Shimura et al. 2000) (Figure 3). Parkin functions as an E3 ubiquitin ligase (Shimura et al. 2000) and it has been shown to be capable of inducing monoubiquitination (Hampe et al. 2006; Moore et al. 2008), multiple monoubiquitination (Matsuda et al. 2006), as well as K48-linked and K63-linked polyubiquitination (Doss-Pepe et al. 2005; Lim et al. 2005). Parkin has been demonstrated to bind to several E2 ubiquitin conjugating proteins including UbcH7, UbcH8, and a UbcH13/Uev1a heterodimer that is thought to be responsible for the catalysis of K63-linked ubiquitin chains (Shimura et al. 2000; Zhang et al. 2000; Olzmann et al. 2007). In addition to the more common K48-linked and K63-linked ubiquitin chains, Parkin can also form K11 and K6 linked chains (Ordureau et al. 2014). Parkin itself becomes ubiquitinated by the attachment of K6 ubiquitin chains, which may play a role in its own degradation (Durcan et al. 2014).

Parkin has been shown to translocate from the cytosol to mitochondria upon mitochondrial depolarization (Narendra et al. 2008) and to ubiquitinate various proteins including mitochondrial outer membrane proteins such as VDAC1, Mfn1/2, Miro, Hexokinase I, CISD1, and TOMM20 (Weihofen et al. 2009; Gegg et al. 2010; Geisler et al. 2010; Poole et al. 2010; Ziviani et al. 2010; Chan et al. 2011; Glauser et al. 2011; Kane and Youle 2011; Rakovic et al. 2011; Wang et al. 2011; Okatsu et al. 2012; Koyano et al. 2013; Sarraf et al. 2013; Ordureau et al. 2014). Parkin translocation to depolarized mitochondria and Parkin-mediated ubiquitination of mitochondrial proteins correlates with induction of mitochondrial autophagy even in cells lacking PINK1 (Kubli et al. 2015). The Ubl domain is not necessary for the E3-ligase activity of Parkin *in vitro* because a C-terminal fragment of Parkin is capable of auto-ubiquitination (Matsuda et al. 2006). Furthermore, deletion of the Ubl domain actually increases Parkin auto-ubiquitination, consistent with the Ubl domain functioning as a constitutive inhibitor of Parkin's E3 ligase activity (Chaugule et al. 2011).

Crystal and NMR structures of Parkin show that Parkin protein exists in an auto-inhibited state (Riley et al. 2013; Spratt et al. 2013; Trempe et al. 2013; Wauer and Komander 2013). This detailed structural knowledge has enabled the generation of Parkin variants, such as W403A, that alleviate the auto-inhibition of Parkin and promote Parkin E3 ubiquitin ligase activity in cell-based and cell-free *in vitro* assays (Riley et al. 2013; Trempe et al. 2013; Koyano et al. 2014; Zhang et al. 2014; Fiesel et al. 2015b; Koyano and Matsuda 2015). Parkin has been found to be activated by PINK1-mediated phosphorylation at serine 65 (S65) within the Ubl domain (Kondapalli et al. 2012; Shiba-Fukushima et al. 2012) and by binding to serine 65-phosphorylated ubiquitin (Kane et al. 2014; Kazlauskaitė et al. 2014b; Koyano et al. 2014). More recent crystal and NMR structures have revealed mechanisms by which phosphorylation of Parkin at S65 and binding to S65-phosphorylated ubiquitin induce Parkin activation by decreasing the interactions that mediate Parkin auto-inhibition (Caulfield et al. 2014; Caulfield et al. 2015; Fiesel et al. 2015b; Koyano and Matsuda 2015; Kumar et al. 2015; Sauve et al. 2015; Wauer et al. 2015a) (Figure 4). This knowledge will hopefully expedite the discovery and development of Parkin-activating drugs as potential PD therapeutics that could reduce ROS by enhancing Parkin-mediated autophagy of dysfunctional mitochondria. Additional potential therapeutic mechanisms are discussed below.

5. Alternative functions and potential pathogenic mechanisms

There is compelling evidence that PINK1 and Parkin can function in a common pathway because Parkin overexpression can rescue the mitochondrial dysfunction and flight muscle degeneration phenotypes of PINK1 null *Drosophila* (Clark et al. 2006; Park et al. 2006; Yang et al. 2006) and because PINK1-mediated phosphorylation of Parkin at serine 65 activates Parkin *in vitro* and PINK1-phosphorylated ubiquitin chains function both as receptors for recruiting Parkin from the cytosol to the mitochondrial outer membrane and as enhancers of Parkin's E3 ligase activity (Kondapalli et al. 2012; Shiba-Fukushima et al. 2012; Caulfield et al. 2014; Kane et al. 2014; Kazlauskaitė et al. 2014a; Kazlauskaitė et al. 2014b; Koyano et al. 2014; Ordureau et al. 2014; Sauve and Gehring 2014; Shiba-Fukushima et al. 2014; Caulfield et al. 2015; Fiesel et al. 2015a; Kazlauskaitė et al. 2015; Okatsu et al. 2015a; Okatsu et al. 2015b; Ordureau et al. 2015a; Ordureau et al. 2015b;

Wauer et al. 2015a; Wauer et al. 2015b; Zheng and Hunter 2015). Nevertheless, multiple groups have published data indicating that PINK1 and Parkin can also function independently and in other pathways. For example, it has been shown that PINK1 can recruit the autophagy receptors NDP52 and optineurin directly to mitochondria to activate mitophagy independent of Parkin (Lazarou et al. 2015).

Although much of the recent research on the cellular functions of Parkin and PINK1 has focused on mitochondrial autophagy (Pickrell and Youle 2015), there is no definitive evidence that the mechanism by which loss-of-function mutations in Parkin and PINK1 cause PD involves defective mitophagy. Many of the same proteins that govern autophagy and mitophagy, including Parkin and PINK1, are also involved in adaptive and innate immunity (Dzambo et al. 2015; Netea-Maier et al. 2015). Mutations in Parkin and PINK1 increase susceptibility to inflammation, which may be the mechanism by which loss-of-function mutations in Parkin and PINK1 cause PD (Frank-Cannon et al. 2008; Akundi et al. 2011) (Table 1). Parkin knockout mice have significantly increased susceptibility to nigral dopamine neuron loss induced by chronic peripheral low dose lipopolysaccharide (LPS) administration, which mimics chronic inflammation (Frank-Cannon et al. 2008). There is a Nuclear Factor-Kappa B (NF- κ B) response element in the *parkin* promoter that represses *parkin* transcription, indicating that chronic inflammation can reduce Parkin levels similar to Parkin mutations that cause PD (Tran et al. 2011). Activated macrophages from Parkin-deficient mice have increased expression of pro-inflammatory cytokines such as Tumor Necrosis Factor (TNF) and IL-1 β as well as iNOS (Tran et al. 2011). Furthermore, Parkin-deficient mice have mitochondrial respiration defects within the nigrostriatal pathway and increased markers of reactive oxygen species, which can also activate inflammatory pathways and increase pro-inflammatory cytokines (Palacino et al. 2004). Similar mitochondrial defects were subsequently reported in cells from patients bearing PD-linked mutations in Parkin, suggesting that this mechanism could be operative in humans (Muftuoglu et al. 2004; Mortiboys et al. 2008). Similar to Parkin, PINK1 likely regulates inflammatory cytokine production (Akundi et al. 2011). Transcriptional profiling of PINK1 knockout mouse striatum showed that the largest number of genes with altered expression were those that regulate innate immune responses (Akundi et al. 2011) (Table 1). Consistent with this, peripheral LPS treatment induced higher brain levels of inflammatory cytokines in PINK1 knockout mice compared to controls (Akundi et al. 2011).

Recently, it has been shown that Parkin and PINK1 repress mitochondrial antigen presentation by actively inhibiting the formation of mitochondrial-derived vesicles (MDVs), which are required for mitochondrial antigen presentation independent of mitochondrial autophagy (Matheoud et al. 2016). This data suggests that Parkin and PINK1 function as suppressors of an inflammation-induced immune response pathway. It is noteworthy that Parkin inhibits the mitochondrial recruitment of Rab9 and Sorting nexin 9, which are required for the formation of MDVs and mitochondrial autophagy (Matheoud et al. 2016).

Phosphoproteomic studies identified Rab proteins as direct and indirect targets of PINK1 kinase activity independent of Parkin (Lai et al. 2015). Specifically, even in cells lacking Parkin (but not in cells lacking PINK1), PINK1 activation by mitochondrial depolarization increased phosphorylation of Rab8A, Rab8B and Rab13 at the highly conserved serine 111

(Lai et al. 2015). This suggests that PINK1 can function independently of Parkin to regulate specific Rab GTPase family members that are known to regulate secretory pathways, such as Rab 8A, 8B and 13. It had previously been shown that Rab7, together with its GTPase activating proteins TBC1D15 and TBC1D17, are required for Parkin-mediated mitophagy downstream of PINK1-induced Parkin translocation to mitochondria (Yamano et al. 2014). Together, this highlights the likely possibility that PINK1 and Parkin have both dependent and independent functions and the possibility that some of these functions are distinct from mitochondrial autophagy. Additional examples of independent and distinct functions include Parkin regulation of mitochondrial cytochrome c release, BAX translocation to mitochondria, and apoptosis (Berger et al. 2009; Johnson et al. 2012a; Johnson et al. 2012b; Charan et al. 2014), and PINK1 regulation of neuronal dendritic morphology (Dagda et al. 2014).

6. PINK1 and Parkin in other neurodegenerative diseases

Mitochondrial dysfunction has been implicated in many neurodegenerative diseases, which has prompted investigations of PINK1 and Parkin beyond Parkinson's research. Analysis of human Alzheimer's disease and multiple sclerosis brains shows increased levels of PINK1 in a cell type specific manner (Wilhelmus et al. 2011). Fibroblasts from AD patients show slower mitochondrial membrane potential recovery post insult, alterations in lysosomal and autophagic pathways, increased reactive oxygen species, and protein aggregation (Martin-Maestro et al. 2016). These were attributed to impairment of PINK1-Parkin mediated mitophagy and could be rescued by Parkin overexpression (Martin-Maestro et al. 2016). PINK1-Parkin pathway alterations have also been found in other neurodegenerative diseases such as Amyotrophic Lateral Sclerosis (ALS) and Huntington's disease (HD). It has been recently reported that ALS patients have lower mRNA levels of PINK1 in affected muscles, which suggest a role for PINK1 in ALS progression (Knippenberg et al. 2013). Mitophagy has been shown to be altered in HD and overexpression of PINK1 is able to rescue HD phenotypes in fly and mouse models of HD (Khalil et al. 2015).

6. Therapeutic implications

Increased expression of Parkin protects against cell death induced by various stress conditions, such as mitochondrial stress, endoplasmic reticulum stress, excitotoxicity, and proteotoxic stress (Imai et al. 2000; Petrucelli et al. 2002; Darios et al. 2003; Staropoli et al. 2003; Higashi et al. 2004; Jiang et al. 2004; Muqit et al. 2004; Yang et al. 2005; Rosen et al. 2006; Fett et al. 2010). Parkin overexpression also has been shown to protect against nigral dopamine neuron loss in animal models of PD (Lo Bianco et al. 2004; Vercammen et al. 2006; Ulusoy and Kirik 2008; Bian et al. 2012). Parkin gene expression is upregulated under cellular stress, and transcription factors such as ATF4 and p53 can increase Parkin expression, whereas c-Jun and N-myc act as transcriptional repressors of Parkin (West et al. 2004; Bouman et al. 2011; Zhang et al. 2011). Parkin also has been found to regulate several cell viability pathways including JNK, PI3K and NF- κ B signaling, p53 transcriptional activity, and Bax activation (Cha et al. 2005; Yang et al. 2005; Henn et al. 2007; Hasegawa et al. 2008; da Costa et al. 2009; Sha et al. 2010; Johnson et al. 2012a)

Parkin displays a low basal E3 ubiquitin ligase activity and a small increase in the activation of Parkin could be sufficient to slow the progression of PD in sporadic forms of the disease in which the wild type protein is present. Small molecules that mimic phospho-ubiquitin or disrupt autoinhibitory interactions might enhance Parkin's neuroprotective action. In cultured cells, mutation of Trp403 or Phe463 speeds recruitment of Parkin to mitochondria in a regulated process that remains dependent on PINK1 and mitochondrial depolarization (Trempe et al. 2013). A small molecule that binds tightly to the pocket occupied by the amino acid side chains would be expected to have the same effect. The deubiquitinating enzymes (DUBs) USP30 and USP15 were recently found to oppose Parkin/PINK1 mediated mitophagy, suggesting that inhibitors of these DUBs would be good candidates for drug design (Bingol et al. 2014; Cornelissen et al. 2014). In contrast, USP8 promotes Parkin mediated mitophagy and agonists to USP8 could be developed as potential therapeutics (Durcan et al. 2014). PINK1 has been shown to activate Parkin both by direct phosphorylation and by phosphorylating ubiquitin (Kondapalli et al. 2012; Shiba-Fukushima et al. 2012; Caulfield et al. 2014; Kane et al. 2014; Kazlauskaitė et al. 2014a; Kazlauskaitė et al. 2014b; Koyano et al. 2014; Ordureau et al. 2014; Sauve and Gehring 2014; Shiba-Fukushima et al. 2014; Caulfield et al. 2015; Fiesel et al. 2015a; Kazlauskaitė et al. 2015; Okatsu et al. 2015a; Okatsu et al. 2015b; Ordureau et al. 2015a; Ordureau et al. 2015b; Wauer et al. 2015a; Wauer et al. 2015b; Zheng and Hunter 2015). This suggests that enhancing PINK1 abundance or PINK1 kinase activity may be another potential option for therapeutic development. In spite of many challenges, PINK1 and Parkin appear to offer multiple promising therapeutic targets for the treatment of PD and relevant diseases caused by mitochondrial dysfunction and oxidative stress.

Acknowledgments

This work was supported by grants from the Michael J. Fox Foundation for Parkinson's Research and by the National Institute of Neurological Disorders and Stroke under NIH award number R01NS082565. The content of this publication is solely the responsibility of the authors and does not necessarily represent the official views of the funding agencies.

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Highlights (for review)

- Mutations in PINK1 and Parkin are linked to Parkinson's disease
- PINK1 and Parkin are believed to function in a common pathway in mitochondrial autophagy
- Additional potential functions of PINK1 and Parkin are reviewed

Figure 1.

Primary sequence domain structure of PINK1 and common point mutations linked to PD. PINK1 domains are annotated as follows: mitochondrial targeting sequence (MTS, blue); transmembrane helix (TM, yellow); N-terminal regulatory region (NT, gray); kinase domain consisting of N- and C-terminal lobes (green and light gray, respectively), and a C-terminal domain (CTD, orange). The amino acid residue numbers at the beginning and end of each domain are shown on the top. Some of the more common missense and nonsense mutations are shown on the bottom.

Figure 2.

Proposed normal functions of PINK1 and Parkin (top) and possible mechanisms of neurodegeneration cause by PINK1-deficiency (PINK1 $-/-$) or Parkin-deficiency (Parkin $-/-$). PINK1 protein is targeted to mitochondria but normally rapidly degraded. PINK1 protein accumulates on the outer membrane of mitochondria with reduced or absent membrane potential. PINK1 recruits Parkin from the cytosol to the mitochondrial outer membrane. PINK1 also activates Parkin by phosphorylation of serine 65 on Parkin and serine 65 on ubiquitin. The E3 ubiquitin ligase activity of Parkin targets mitochondria for degradation by autophagy. The selective autophagy of dysfunctional mitochondria via the combined activities of PINK1 and Parkin may be important for removing a major cellular source of reactive oxygen species (ROS) that can damage proteins, lipids and DNA. In the absence of PINK1 or Parkin, the accumulation of dysfunctional mitochondria over time may lead to increased ROS and eventually cause neurodegeneration, such as loss of dopamine neurons in the substantia nigra or other vulnerable cell populations.

Figure 3.

Primary sequence domain structure of Parkin and common point mutations linked to PD. Parkin domains are annotated as follows: ubiquitin like domain (Ubl, orange); really interesting new gene (RING0, RING1 and RING2, green, blue and brown, respectively); In between RING domain (IBR, gray); repressor domain (REP, pink). The amino acid residue numbers at each domain border are shown on the top. Some of the more common missense and nonsense mutations are shown on the bottom. In addition to point mutations, large deletions and duplications of one or more exons are also common.

Figure 4.

Tertiary protein domain structure of Parkin highlighting interactions involved in regulating E3 ligase activity. PINK1 phosphorylates Parkin at serine 65, which causes a conformational change and activates Parkin by removing auto-inhibition mediated by the Ubl domain and REP domain of Parkin. PINK1 also phosphorylates ubiquitin at serine 65, which binds to Parkin and activates Parkin's E3 ubiquitin ligase activity. The locations of the E2 binding site and the catalytic cysteine (C431) are also shown.

Table 1

Phenotypes of PINK1 and Parkin genetic models.

Gene	Animal	Manipulation	DA neuron loss	DA responsive motor deficits	Immune or inflammatory effects	References
Parkin (<i>PARK2</i>)	<i>C. elegans</i>	Knockout	No	No	ND [/]	Springer et al. 2005
	Drosophila	Knockout	Yes	Yes	ND [/]	Greene et al. 2003, Whitworth et al. 2005
		Transgenic	Yes	Yes	ND [/]	Wang et al. 2007, Sang et al. 2007
	Mouse	Knockout	No	ND [/]	Yes	Goldberg et al. 2003, Ilier et al. 2003, Von Coelln et al. 2004, Perez et al. 2005, Frank Cannon et al. 2008.
		Transgenic	Yes	ND [/]	ND [/]	Lu et al. 2009.
	Rat	Knockout	Yes	Yes	ND [/]	Dave et al. 2014
PINK1 (<i>PARK6</i>)	Drosophila	Knockout	Yes		ND [/]	Park et al. 2006, Clark et al. 2006
	Mouse	Knockout	No	Yes	Yes	Kitada et al. 2007, Gautier et al. 2008, Gispert et al. 2009, Akundi et al. 2011.
	Rat	Knockout	Yes	Yes	ND [/]	Dave et al. 2014

[/]ND: Not determined