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Title: Precise control of mitophagy through ubiquitin proteasome system and deubiquitin proteases and their dysfunction in Parkinson's disease

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ABSTRACT

Parkinson's disease (PD) is one of the most common neurodegenerative diseases in the elderly population and is caused by the loss of dopaminergic neurons. PD has been predominantly attributed to mitochondrial dysfunction. The structural alteration of α-synuclein triggers toxic oligomer formation in the neurons, which greatly contributes to PD. In this article, we discuss the role of several familial PD-related proteins, such as α-synuclein, DJ-1, LRRK2, PINK1, and parkin in mitophagy, which entails a selective degradation of mitochondria via autophagy. Defective changes in mitochondrial dynamics and their biochemical and functional interaction induce the formation of toxic α-synuclein-containing protein aggregates in PD. In addition, these gene products play an essential role in ubiquitin proteasome system (UPS)-mediated proteolysis as well as mitophagy. Interestingly, a few deubiquitinating enzymes (DUBs) additionally modulate these two pathways negatively or positively. Based on these findings, we summarize the close relationship between several DUBs and the precise modulation of mitophagy. For example, the USP8, USP10, and USP15, among many DUBs are reported to specifically regulate the K48- or K63-linked de-ubiquitination reactions of several target proteins associated with the mitophagic process, in turn upregulating the mitophagy and protecting neuronal cells from α-synuclein-derived toxicity. In contrast, USP30 inhibits mitophagy by opposing parkin-mediated ubiquitination of target proteins. Furthermore, the association between these changes and PD pathogenesis will be discussed. Taken together, although the functional roles of several PD-related genes have yet to be fully understood, they are substantially associated with mitochondrial quality control as well as UPS. Therefore, a better understanding of their relationship provides valuable therapeutic clues for appropriate management strategies.

INTRODUCTION

Parkinson's disease (PD) is the second most common neurodegenerative disorder (NDD) in elderly individuals aged above 60. It affects predominately dopaminergic neurons in a specific area of the brain called substantia nigra. PD is characterized by movement disorders, such as resting tremor, rigidity, slow movement, impaired posture and balance, and loss of automatic movements (1). The cause of PD is unknown, but several factors appear to play a role, including genetic and environmental factors. About 10~15% of patients diagnosed with PD are known to carry mutations in one of several specific genes, including *α-synuclein, FBXO7, LRRK2, parkin, PINK1,* and *UCHL1*. Similar to other NDDs, PD patients also display clumps of specific substances within the brain cells called Lewy bodies (LBs), which are the pathological hallmarks of PD and mainly composed of α-synuclein (α-Syn) (2). Mitochondrial dysfunction has also been implicated as the main causative factor in PD. Interestingly,

familial PD-linked gene products and their binding partners, such as LRRK2, BAG5, Miro1, DJ-1, PINK1, and parkin, play a role in the process of mitochondrial quality control. Dysregulation of mitophagy, involving removal of damaged and surplus mitochondria, is also implicated in the pathogenesis of NDDs, including Alzheimer's disease (AD) and PD (3).

Mitophagy is a subtype of macroautophagy, which selectively targets defective mitochondria to the lysosome for degradation. Mitophagy plays a pivotal role in cells, in preserving mitochondrial homeostasis, biogenesis, fission, and fusion for mitochondrial quality control (4). Two PD genes, the mitochondrial kinase *PINK1* and ubiquitin ligase *parkin*, degrade damaged mitochondria via mitophagy. Under resting state, PINK1 is normally transported into the inner mitochondria membrane (IMM) of healthy mitochondria and cleaved by presenilin-associated rhomboid-like protein (PARL). The cleavage of PINK1 results in the formation of a 52-kDa fragment and release into cytosol. PINK1 fragment is then rapidly ubiquitinated and degraded by the ubiquitin proteasome system (UPS). However, various stimuli causing mitochondrial damage interfere with the processing and cytosolic translocation of PINK1, resulting in the accumulation of 63-kD full-length PINK1 on the outer mitochondrial membrane (OMM). PINK1 is then activated by auto-phosphorylation during mitophagy, triggering the recruitment of parkin to the OMM and facilitating its E3 ligase activity (2, 5). Parkin and ubiquitin are then phosphorylated, resulting in the assembly of K6-, K11-, K48-, and K63-linked ubiquitin chains on the OMM target proteins for degradation (6). Finally, parkin builds ubiquitin chains on the damaged mitochondria for lysosomal degradation.

Deubiquitinating enzymes (DUBs) are members of cysteine and metalloprotease family that cleave ubiquitin from the protein substrates. DUB-mediated cleavage of ubiquitin chains from substrate plays a crucial role in various cellular processes by changing its biochemical properties, such as protein stability, function, and localization. DUBs can be classified into five families based on their sequence and structural homology: ubiquitin specific protease (USP), ubiquitin C-terminal hydrolase (UCH), otubain protease (OTU), Machado-Joseph Disease protease (MJD) and JAB1/MPN/Mov34 metalloenzyme (JAMM) (7). The majority of them belong to the ubiquitin-specific proteinase (USP) family. As expected, DUBs play a crucial role in the ubiquitin pathways and are responsible for the recycling of mono- or poly-ubiquitin. DUBs also reverse the ubiquitin-like modification of target proteins (7, 8). As autophagy entails the removal of protein aggregates, the turnover of organelles, as well as the elimination of intracellular pathogens, the potential targets should be selectively marked by the attachment of ubiquitin in order to be recognized by autophagy receptors. Thus, ubiquitination and ubiquitin-dependent autophagy (including mitophagy) are balanced by deubiquitination. In this article, we highlight the functional link between the most well-known degradation pathway of damaged mitochondria and PD. In addition, the role of a single subclass of deubiquitinating enzymes, USP, in

regulating mitophagy and the pathologic features of PD will be discussed.

1. Mitochondrial dysregulation, defective mitophagy, and Parkinson's disease

(1) Mitochondrial dysfunction in neurodegenerative diseases including PD

Mitophagy plays an important role in mitochondrial quality control, and the accurate clearance of the damaged mitochondria is critical for the maintenance of mitochondrial and cellular homeostasis. Therefore, mitochondria dysfunction results in several neurodegenerative diseases, including AD and PD. For example, oxidative stress and mitochondrial dysfunction are connected to genetic mutations in the mitochondrial DNAs, which are involved in AD pathogenesis (9). A defective mitochondrial respiratory chain, especially the reduced activity of complex I, was found in post-mortem brains obtained from sporadic PD patients. In addition, abnormal mitophagy was observed in several PD models, including environmental or genetic forms (10). While α-Syn is not only localized to mitochondria, it also directly regulates the mitochondrial morphology (12) as well as Ca^{2+} signaling (13). Further, two PD-associated proteins, parkin and PINK1, primarily regulate the mitochondrial quality control. These findings suggest the need for prompt and precise mitophagy in mitochondrial homeostasis, and its alteration contributes to PD pathogenesis. When the mitochondria are damaged, PINK1 recruits parkin to the OMM (14). Once parkin is localized to OMM, PINK1 phosphorylates the UBL domain of parkin at S65 residue (14), which then initiates the clearance of damaged mitochondria via autophagy (15). Likewise, the PINK1-mediated mitophagic progression is critical for the regulation of parkin E3 ligase activity via a feed-forward mechanism. Thus, a better understanding of the mitochondrial quality control events is required to develop novel therapeutic interventions for PD.

(2) The role of protein ubiquitination in mitophagy

In eukaryotes, UPS and autophagy are the two major intracellular degradation pathways responsible for eliminating unfolded/misfolded proteins. Ubiquitin (Ub) is a small regulatory protein consists of 76 amino acids, and can be attached to the target substrates (16). This protein is highly conserved among eukaryotes and the process of ubiquitin tagging to substrates is known as ubiquitination. Protein ubiquitination is a type of post-translational modifications (PTMs) in which three enzymes, E1 (ubiquitin activating enzyme), E2 (ubiquitin conjugating enzymes), and E3 (ubiquitin ligase), catalyze the sequential reaction of covalent ubiquitin conjugation (8). With regard to mitophagy, there are two different subtypes, receptor- and ubiquitin-mediated mitophagies. In this article, we focus on the ubiquitin-mediated mitophagy and review its role in controlling the PINK1/parkin-dependent mitophagy to maintain mitochondrial quality control. Besides UPS, parkin

also uses ubiquitin as a key substrate to regulate the degradation of defective organelles. Upon mitochondria depolarization, both parkin and ubiquitin are activated via phosphorylation by PINK1 (17). Subsequently, the activated parkin is attached to target proteins in OMM via an active site of cysteine as an ubiquitin-thioester intermediate (18). The ubiquitinated OMM proteins, such as VDAC1, are promoted for degradation via K27- and K63-linked ubiquitin chains (19). Eventually, the conjugation of ubiquitin to parkin facilitates and completes the degradation of dysfunctional mitochondria in PINK1/parkin-dependent mitophagy.

2. Several PD-linked proteins affect mitophagy

Given their widespread localization and biochemical properties, multiple PD-related genes have been associated with various cellular functions and signaling pathways, including mitophagy (Table 1). First, α-Syn aggregates compromise the autophagic mechanism by impairing the phagocytosis required for protein degradation in neuronal cell lines and α-Syn transgenic mice, which suggests a close association between α-Syn and autophagy (or/and mitophagy) (20, 21). Thus, the defective autophagy induced by α-Syn disrupts the mitochondrial clearance. Other studies have also proposed α-Syn-derived impairment in autophagy. For example, the overexpression of α-Syn in neuroblastoma cell line was shown to enhance the level of autophagic substrate p62, leading to a significant decrease in the levels of autophagy regulator LC3 as well as in the number of LC3-II-positive vesicles (21). Autophagic activity was impaired by the aggregation of α-Syn (22). Interestingly, the effect of A53T mutation of α-Syn on autophagy may differ. An increase in lysosome-mediated mitophagy has been reported in dopaminergic neurons of α-Syn-A53T transgenic mice, which indicate a compensatory response to depletion of defective mitochondria (23). The PD-linked α-Syn mutants, but not wild-type α-Syn, bind to the LAMP2 transporter in the lysosomal membrane and block protein uptake in the chaperone-mediated autophagy pathway, thereby inhibiting self-degradation and that of other substrates (24). Thus, α-Syn overexpression or the physiological effect of α-Syn mutations contributes to impaired autophagy via diverse mechanisms, and in turn compromise the clearance of abnormal mitochondria by mitophagy.

Second, parkin is an E3 ubiquitin ligase, which recruits Ub chains to the target substrate. PINK1 is a Ser/Thr kinase that activates parkin in mitophagy to maintain mitochondrial homeostasis, apoptosis, and oxidative stress (25). Many stressors, such as membrane depolarization, mitochondrial complex dysfunction, mutagenic stress, and proteotoxicity, lead to accumulation of PINK1 on the OMM. Subsequent homodimerization of PINK1 on the OMM leads to auto-phosphorylation, which promotes the kinase activity of PINK1 and facilitates its binding to substrates, parkin and ubiquitin. PINK1 then activates parkin via phosphorylation of ubiquitin on S65 as well as direct phosphorylation of parkin on S65. These results suggest that parkin amplifies a damage detection signal from PINK1 by facilitating

the formation of ubiquitin chains, which recruit additional parkin to the mitochondria (26). Once recruited to the mitochondria, parkin mediates the ubiquitination of multiple targets in OMM, IMM, and mitochondrial matrix. Another report revealed that PINK1 and parkin are associated with the mitochondria-derived vesicles (MDVs), ultimately targeting into the lysosomes for degradation (21). Parkin is ultimately released into the cytosol after carrying the target protein to the lysosome (22).

DJ-1 protein plays an essential role in various cellular pathways, including transcription regulation, mitochondrial homeostasis, and cellular apoptosis. Especially, DJ-1 acts as a redox sensor and regulates autophagy and mitochondrial dynamics during oxidative stress (27). Knockdown of *DJ-1* induces loss of mitochondrial integrity, mitochondrial fragmentation, and polarization; however, antioxidant treatment reverses these effects (28). DJ-1 also regulates mitophagy via association with ER-mitochondria and physically interacts with the IP3R3-Grp75-VDAC1, the mitochondria-associated membrane (MAM)-essential complex. However, the familial PD-associated L166P mutant of DJ-1 displayed a remarkable reduction of the MAM complex. As a result, the knockdown of *DJ-1* induced mitophagy (29), enhancing the mitochondrial mass and activities including the complex I activity (30). In addition, the loss of *DJ-1* increased the parkin recruitment to damaged mitochondria and mitophagy, whereas DJ-1 levels accumulated on the mitochondria under oxidative stress conditions were also dependent on parkin and PINK1. These results suggest a close link between DJ-1 and the PINK1/parkin-mediated pathway (31). This hypothesis was further supported by DJ-1 functions in parallel with the PINK1/parkin pathway to maintain mitochondrial function under an oxidative environment (32). Further, Hao et al have shown the mitochondrial defect in *DJ-1* knockout flies, which is similar to that of *PINK1*- and *parkin*-mutants (33).

Mutations in *leucine-rich repeat kinase 2 (LRRK2)* gene are the most frequent cause of autosomal PD. LRRK2 is a large and multi-domain protein containing two catalytic domains: a Roc GTPase-domain and a kinase domain. Many studies revealed that LRRK2 also plays a key role in regulating mitophagy. For example, patients with familial PD expressing G2019S-LRRK2 mutation, which results in enhanced kinase activity, displayed abnormal mitochondrial function and morphology, and a reduction of mitophagy (34). This LRRK2-2019S mutation also upregulates the intracellular α-Syn level, consequently altering the morphology of lysosomes (35). In addition, LRRK2 activates MAPK/ERK pathway and upregulates transcription of α-Syn in HEK293 cells (36). Further, G2019S-LRRK2 mutant affects the PINK1/parkin-dependent mitophagy pathway that disturbs mitochondrial function, which may lead to accumulation of damaged mitochondria and ultimately increase cellular vulnerability to external stressors (37). In parallel, conflicting findings involving idiopathic and genetic PD suggest that the G2019S mutation of *LRRK2* activates class III HDACs to increase mitophagy in familial PD patients, whereas idiopathic PD cases exhibit considerable downregulation in the clearance

of those defective mitochondria (38).

Molecular chaperones regulate intracellular proteostasis by promoting efficient folding and expediting the refolding or degradation of misfolded proteins under environmental and physiological stress, including heat, oxidative stress, and inflammation. The Bcl-2 associated athanogene (BAG) family of proteins acts co-chaperones in cell survival and cell death pathways. BAG5 enhances dopaminergic neurodegeneration in rodent models of PD (39). While physically interacting with parkin, BAG5 impairs mitophagy by suppressing parkin recruitment to damaged mitochondria and reducing the movement of damaged mitochondria into the lysosomes (40). BAG5 also enhanced parkin-mediated Mcl-1 degradation and cell death following severe mitochondrial insult. Recently, BAG5 was found to interact with PINK1 (41), suggesting a possible role for this co-chaperone in the regulation of the PINK1/parkin-dependent mitophagy. Two other BAG family members, BAG2 and BAG4, have been shown to differentially modulate parkin recruitment to depolarized mitochondria. In addition, BAG3 has been identified as a risk locus for PD (42).

Lastly, mitochondrial Rho GTPase 1 (Miro1) protein is encoded by *RHOT1* gene and regulates mitochondrial homeostasis and apoptosis. Miro1 is localized on the mitochondrial surface and mediates mitochondrial motility. The link between Miro1 dysfunction and PD was established by studies demonstrating Miro1 as a target of mitochondrial quality control via PINK1/parkin-mediated mitophagy and mitochondrial transport. Miro1 is removed from depolarized mitochondria to facilitate their clearance via mitophagy. Miro1 was also identified as an important regulator of mitochondria-ER contact sites (MERCs), where it acts as a sensor of cytosolic calcium levels (43). Recently, there was a report that a group of PD patients expressed *RHOT1* mutations. In addition, the overexpression of Miro1-T351A or -T610A mutants in fibroblasts resulted in calcium dysfunction and reduction in MERCs, both of which play an important role in mitochondrial function. Further, Miro1 interferes with the function of MERCs, indicating that *Miro1* mutants may trigger PD pathology (44). Knockdown of *Miro1* reduces the translocation of parkin to the mitochondria and downregulates mitophagy in a calcium-dependent manner (45). While Miro1 is a component of parkin receptor complex, the interaction of Miro1 with parkin and the following K27-linked ubiquitination of downstream targets are dependent on Ser65 residue of parkin (46). Overall, these results suggest that Miro1 interacts with parkin to regulate mitochondrial dynamics.

3. Dynamic regulation of mitophagy by protein deubiquitination enzymes and its defect in PD

(1) The role of DUBs in mitophagy

Protein ubiquitination is a reversible pathway, and ubiquitin is removed by DUBs upon the

completion of signaling events leading to the covalent conjugation of ubiquitin. In this sense, DUBs act as proteases to cleave ubiquitin or ubiquitin-like proteins from the target proteins (47). As mitophagy is triggered by ubiquitin modification of proteins residing on the surface of mitochondria, it is also subject to the modulation or suppression via deubiquitination and DUBs. Several studies demonstrated that specific DUBs are associated with multiple types of autophagic pathways and NDDs (48) (Fig. 1). For example, USP15, USP30, and USP35 are known to eliminate the ubiquitin- and parkin-mediated signals, consequently delaying or disrupting mitophagy (5). In addition, several E3 ligases, including parkin, undergo autoubiquitination, and DUBs can counteract this activity (18). For instance, USP8 deubiquitinates the K6-linked ubiquitin conjugates from parkin, contributing to the release of parkin autoinhibition to promote CCCP-induced mitochondrial translocation of parkin and parkin-dependent mitophagy (49).

(2) The relationship between DUBs and PD-related Genes

(a) α-Synuclein and DUBs: Patients with sporadic PD generally show extensive mitochondrial dysfunction with toxic accumulation of α-Syn aggregates (12). α-Syn is the main component of LB and its mutation, duplication, or triplication results in autosomal-dominant PD (50). Since ubiquitination plays an essential role in regulating both α-Syn levels and mitochondrial quality control, the degradation of defective mitochondria should be accurately regulated to prevent accumulation of misfolded α-Syn. A few studies reported DUBs targeting to the α-Syn (Table 2). For example, USP8 deubiquitinated K63-linked ubiquitin chains on α-Syn, and knockdown of endogenous *USP8* prevented α-Syn-induced toxicity in a Drosophila model (51). These data demonstrated that altered deubiquitination of α-Syn by USP8 contributes to PD pathogenesis. In addition, USP9X was thought to be a key regulator in altering the mono-ubiquitination level of α-Syn, which also reduces the α-Syn toxicity (52). Based on these findings, the regulation of USP9X deubiquitinase activity might play a role in decreasing the levels of toxic α-Syn aggregates, and their cellular toxicity. Moreover, parkin mediates the clearance of α-Syn (53) and knockdown of *USP13* increases parkin ubiquitination, whereas the clearance of α-Syn is promoted in a parkin-independent manner (54). Although parkin ubiquitination is not directly related to α-Syn degradation, USP13 has a direct effect on the cellular activity of α-Syn and its neuronal death.

Many studies have reported the mitochondrial translocation of α-Syn via its N-terminus, impairing the mitochondrial function (55, 56). Further, α-Syn was also found to impair autophagy, particularly mitophagy. α-Syn impairs mitophagy in numerous ways. In the neurons of PD patients, α-Syn interacts with Miro via its N-terminus and upregulates Miro protein levels, leading to excessive and abnormal accumulation of Miro on the mitochondrial surface and delayed mitophagy. These results suggest that

Miro is a target of α-Syn-associated mitochondrial injury (57). In addition, the overexpression of A53T α-Syn mutant results in p38 MAPK activation, and directly induces the phosphorylation of parkin at serine 131, disturbing the function of parkin and mitophagy (58). In A53T α-Syn-overexpressing mice, α-Syn accumulates on mitochondria, resulting in increased mitophagy and neuronal death, and these mitochondrial deficits can be rescued by silencing parkin and overexpressing Mfn2 or a dominant-negative variant of Drp1 (53, 59). Yeast overexpressing both the human wild-type α-Syn gene and A53T mutant resulted in enhanced mitophagy (60). These studies indicate the role of abnormal mitophagy in α-syn-mediated toxicity.

Another DUB, UCH-L1, which is also associated with familial PD, affects mitophagy. UCH-L1 alters the polyubiquitin chain and increases the availability of free monomeric ubiquitin to the UPS, thus increasing proteasome-dependent proteolysis (61). Interestingly, the I94M mutation in *UCH-L1* has been found in patients carrying autosomal dominant PD (62). Reduced mRNA and protein levels of this DUB were found in samples obtained from frontal cortex and medulla oblongata in patients dying from PD. This UCH-L1 directly interacts with chaperone-mediated autophagy (CMA), by physically binding to LAMP-2A, Hsp70, and Hsp90 (63). It should be noted that a protective S18Y-UCH-L1 variant is negatively correlated with disease onset (64).

(b) PINK1/Parkin and DUBs: Duncan et al. demonstrated that USP8 directly deubiquitinates parkin and abrogates its autoubiquitination effect (49). RNAi-mediated knockdown of *USP8* in various cell lines resulted in delayed parkin recruitment to depolarized mitochondria and in the accumulation of ubiquitinated parkin, which persisted longer in the damaged mitochondria and induced a delay in their clearance by mitophagy. In addition, USP8 was shown to selectively remove K6-linked ubiquitin chains from parkin, and was critical for efficient mitophagy (65). Three additional DUBs were found to be linked with mitophagy (Table 2). USP15 and two mitochondrial DUBs, USP30 and USP35, were shown to oppose parkin-mediated ubiquitination of OMM proteins and attenuate the subsequent clearance of depolarized mitochondria (66, 67). In contrast to USP8, neither USP15 nor USP30 affected the ubiquitination status of parkin or its recruitment to damaged mitochondria. While USP30 delays parkin-mediated mitophagy by interfering with its recruitment to the mitochondria, USP35 does not delay parkin recruitment, suggesting differential regulation of mitophagy via distinct mechanisms (68). Interestingly, USP35 only associates with polarized mitochondria, and rapidly translocates to the cytosol during CCCP-induced mitophagy (Fig. 1).

Recently, another mitochondrial DUB, USP33, present at the OMM was found to deubiquitinate parkin by removing the K6-, K11-, K48- and K63-linked ubiquitin conjugates from parkin (69; Table 2). *USP33* knockdown increased both parkin protein stability and its translocation to depolarized

mitochondria, resulting in enhanced mitophagy and eventual protection of human neuroblastoma cells from the neurotoxin MPTP-induced apoptotic cell death. Interestingly, pharmacological or genetic inhibition of USP14 leads to increased mitochondrial clearance in the absence of PINK1 and parkin (70). Mitochondrial fragmentation and membrane rupture exposes the autophagy receptor prohibitin 2 and the formation of mitophagic vesicles, which are key elements in USP14-induced mitophagy (71). In addition, genetic or pharmacological inhibition of USP14 *in vivo* corrected mitochondrial dysfunction and rectified the impaired locomotion in the established *PINK1*- and *parkin*-mutant *Drosophila* model of PD. Thus, USP14 is closely related to proteasome activity and negatively affects proteasome-mediated proteolysis, thereby inhibiting mitophagy via deubiquitinating activity of USP14 (71).

CONCLUSION

Investigation of cellular mechanisms underpinning mitochondrial quality control in CNS has led to the development of potential therapeutics for neurological disorders. Elucidation of the mitophagy pathway and underlying regulatory mechanisms has revealed the close relationship between the coordinated mitochondrial dynamics and several PD-associated genes. Several genes, such as *LRRK2*, *PINK1*, *parkin*, and *α-synuclein*, are causally linked to idiopathic and familiar PD. These gene products directly or indirectly modulate the mitochondrial quality control system, e.g., mitochondrial fission and fusion, biogenesis, and maintenance of ER/mitochondrial Ca^{2+} balance. Many cases have reported the mitochondrial dysfunction in PD patients. These results consistently support the hypothesis that the balance between mitochondria function and clearance is important for homeostasis in normal cells. Defective mitochondrial function and clearance lead to many NDDs, including PD. Although the development of therapeutic strategies targeting PD are based on diverse molecular mechanisms, selective regulation of autophagy and mitophagy system represents a promising strategy to delay the progression of PD. Moreover, multiple ubiquitin proteases have shown to regulate PINK1/parkin-dependent mitophagy positively or negatively. Several DUBs counteract the auto-ubiquitination pathway of parkin and its target in the sequential reactions of mitophagy. For example, USP8 eliminated K6-linked ubiquitin conjugates from parkin (18), and the overexpression of USP30 and USP35 delayed PINK/parkin-mediated mitophagy (68). In contrast, knockdown of some *DUBs* facilitates mitophagy in a PINK1/parkin-dependent manner, resulting in a neuroprotective effect. Based on the diverse and important roles of DUBs in mitophagy, eliminating abnormal mitochondria via UPS- and DUBs-controlled mitophagy is a key strategy to delay the onset of PD. Collectively, understanding of DUBs functions and their regulation in cellular mitophagic pathway provides a novel but effective therapeutic approach against PD.

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CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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Figure Legends

Figure 1. Many DUBs regulate mitophagy in a positive or negative manner. In healthy mitochondria, PINK1 is constitutively imported into the mitochondria for processing and release into the cytosolic area, followed by rapid degradation. However, when the mitochondrial membrane potential ($\Delta\Psi m$) dissipates, PINK1 is stabilized on the OMM and forms a large complex on the OMM surface where it recruits parkin to the damaged mitochondria. The accumulated PINK1 phosphorylates parkin and attaches ubiquitin chains to several mitochondrial substrates, such as translocase of the outer membrane 20 (Tom20), voltage-dependent anion-selective channel 1 (VDAC1), and mitofusin-2 (MFN2). Such ubiquitinated proteins may act as adaptors for sequestosome-1 (SQSTM1/p62) and promote the translocation of defective mitochondria to the autophagosome followed by sequential steps of mitophagy. Multiple DUBs regulate mitophagy in a positive/negative manner. For example, USP8 and USP13 promote mitophagy by directly detaching ubiquitin from parkin, whereas USP15, USP30, USP33, and USP35 inhibit parkin-mediated ubiquitination of OMM proteins. Accordingly, these DUBs promote or suppress mitophagy during the removal of ubiquitin from the parkin. USP14 negatively regulates proteasome activity, and also acts as a negative regulator of mitophagy.

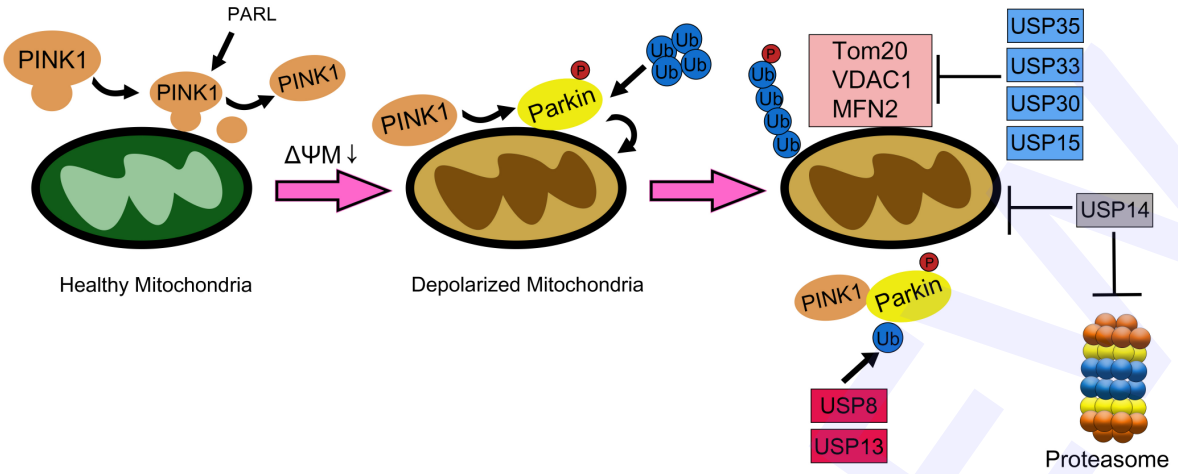

Fig. 1.

Table 1. Diverse functions of many PD-related gene products in a number of cellular pathways, including mitophagy.

Gene	Genetic functions	Regulatory role in the mitophagy and PD	References
α-Synuclein	Accumulated in Lewy bodies and its pathogenic aggregation negatively affects mitophagy	Impairing membrane engulfing process and ultimately leading to the dysfunction in autophagy and mitochondrial clearance	Guhathakurta S et al Sugiura A et al
Parkin	Acts as an E3 ubiquitin ligase which interacts with PINK1 and recruits Ub chains to target substrate to activate mitophagy	Amplifies a damage detection signal from PINK1 by facilitating ubiquitin chain formation	Narendra DP et al McLelland GL et al
DJ-1	Acts as a redox sensor to regulate autophagy as well as mitochondrial dynamics	Knockdown of *DJ-1* recruits the parkin in PINK1/parkin-dependent mitophagy to maintain mitochondrial function	Thomas KJ et al Joselin AP et al
LRRK2	Large multifunctional protein containing the kinase and GTPase domain and regulates mitophagy	G2019S mutant upregulates intracellular α-synuclein level for altering the lysosome morphology and reduces the mitophagy	Walter J et al Obergasteiger J et al
BAG5	Regulates both cell death and survival pathway; BAG5 enhances dopaminergic neurodegeneration and physically interacts with parkin	BAG5 suppresses the parkin recruitment to damaged mitochondria, consequently reducing mitophagy; it also interacts with PINK1	Kalia SK et al De Snoo ML et al Wang X et al
Miro1	Regulates mitochondrial homeostasis, apoptosis, and mediates mitochondrial motility	Knockdown of *Miro1* reduces the translocation of parkin and finally down-regulates the mitophagy	Berenguer-Escuder C et al Safiulina D et al

Table 2. The functional link between DUBs and three PD-related gene products.

PD Genes	DUBs	The role of DUBs in the regulation of PD-related genes	References
α-Synuclein	USP8	Deubiquitinates K63-linked ubiquitin chains of α-synuclein and ameliorates α-synuclein induced toxicity	Alexopoulou Z et al
	USP9X	Regulates mono-ubiquitination of α-synuclein to reduce its aggregation and cellular toxicity	Rott R et al
	USP13	Knockdown shows clearance of α-synuclein in a parkin-independent manner but directly regulates α-synuclein-mediated neuronal death	Liu X et al
PINK1/Parkin	USP15	Attenuates the clearance of dysfunctional mitochondria but doesn't affect the ubiquitination status of parkin	Bingol B et al Cornelissen T et al
	USP30 USP35	Eliminates the parkin-mediated signals and reduces clearance of damaged mitochondria	Wang Y et al
	USP33	Removes several kinds of lysine-linked ubiquitin chains from parkin, whereas its knockdown increases the protein stability of parkin	Niu K et al Chakraborty J et al
	USP14	Negatively regulates proteasome activity, leading to the inhibition of mitochondrial clearance	Chakraborty J et al Wang L et al