PINK1 and Parkin to control mitochondria remodeling

Hyongjong Koh¹, Jongkyeong Chung²
¹Department of Pharmacology, Mitochondria Hub Regulation Center (MHRC), Dong-A University College of Medicine, Busan, ²School of Biological Sciences, Seoul National University, Seoul, Korea

Abstract: Parkinson’s disease (PD), one of the most common neurodegenerative diseases, is characterized by movement disorders and a loss of dopaminergic (DA) neurons. PD mainly occurs sporadically, but may also result from genetic mutations in several PD-linked genes. Recently, genetic studies with Drosophila mutants, parkin and PINK1, two common PD-associated genes, demonstrated that Parkin acts downstream of PINK1 in maintaining mitochondrial function and integrity. Further studies revealed that PINK1 translocates Parkin to mitochondria and regulates critical mitochondrial remodeling processes. These findings, which suggest that mitochondrial dysfunction is a prominent cause of PD pathogenesis, provide valuable insights which may aid in the development of effective treatments for PD.

Key words: PINK1, Parkin, Mitochondria, Parkinson’s disease

Introduction

Parkinson’s disease (PD), the second most common neurodegenerative disease in the world, is characterized by locomotor disorders including rigidity, tremor, bradykinesia, and postural instability (Lang & Lozano, 1998). In addition, massive and selective degeneration of dopaminergic (DA) neurons in the substantia nigra is the neuropathological hallmark of the disease. The majority of PD cases are sporadic; however, familial forms have also been reported. Over the past decade, mutations linked to familial forms of PD have been identified in a number of genes such as alpha-synuclein (also known as SNCA) (Polymeropoulos et al., 1997), leucine-rich repeat kinase 2 (LRRK2) (Paisán-Ruiz et al., 2004), parkin (also known as PARK2) (Kitada et al., 1998), PTEN-induced putative kinase 1 (PINK1) (Valente et al., 2004), DJ-1 (also known as PARK7) (Bonifati et al., 2003), and ATP13A2 (Ramirez et al., 2006). Among these, alpha-synuclein and LRRK2 mediate autosomal dominant forms of PD, and the others mediate autosomal recessive forms. Discovery of these PD-linked genes has enabled an understanding of the molecular mechanisms underlying familial PD pathology, providing valuable insight into the pathological mechanisms involved in sporadic cases.

Mitochondrial dysfunction has been heavily implicated in PD pathogenesis (Henchcliffe & Beal, 2008). The activity of complex I, a major component of the mitochondrial respiratory chain, is decreased in substantia nigra and other tissues in PD patients (Keeny et al., 2006; Parker et al., 2008). Moreover, several complex I inhibitors successfully reproduce key features of PD such as loss of DA neurons and motor deficits. Exposure to 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) causes parkinsonism in humans (Langston et al., 1983). Administration of rotenone or paraquat also induces selective loss of DA neurons and produces locomotor defects in various animal models (Betarbet et al., 2000; Coullom & Birman, 2004; Cicchetti et al., 2005). Recent findings that parkin and PINK1 have...
critical roles in maintaining mitochondrial function and integrity have suggested that mitochondrial dysfunction is the prominent cause of PD pathogenesis, enabling investigation of the pathological mechanisms of PD at the molecular level (Clark et al., 2006; Park et al., 2006; Yang et al., 2006). The following is a brief review of the recent findings related to the roles of parkin and PINK1 in mitochondria.

**Parkin is critical in maintaining mitochondrial integrity**

Parkin is an E3 ubiquitin ligase encoded by parkin, the most commonly affected PD gene conserved in various organisms from Drosophila to humans (Shimura et al., 2000). Parkin is composed of an ubiquitin-like domain in its N-terminus and two RING-finger domains in its C-terminus. In mammalian cell-based studies, Parkin can ubiquitinate and degrade several proteins including CDCrel-1 (Zhang et al., 2000), parkin-associated endothelin receptor-like receptor (Pael-R) (Imai et al., 2001), and cyclin E (Staropoli et al., 2003). From these results, endoplasmic reticulum (ER) stress resulting from accumulated Parkin substrates was proposed as the cause of DA neuronal death by loss of parkin. However, further studies failed to establish a meaningful relationship between these putative Parkin substrates and PD pathogenesis. To overcome the limitations of the cell-based study, several groups generated and characterized parkin null animal models. Although parkin null mice could not reproduce human PD symptoms, Drosophila parkin mutants demonstrated obvious phenotypes including locomotive defects and DA neuron degeneration (Greene et al., 2003; Pesah et al., 2004; Cha et al., 2005). In addition, administration of L-DOPA substantially rectified the behavioral defects of the parkin mutants, further confirming that parkin mutant fly models successfully parallel human PD patients (Cha et al., 2005). These mutants also showed defective wing posture and a crushed thorax. Histological examination of the parkin mutants demonstrated indirect flight muscle degeneration, which probably contributed to the locomotive defects along with DA neuron degeneration. Furthermore, mitochondrial swelling was found in the indirect flight muscles of the parkin mutants, suggesting that mitochondrial dysfunction may be an important cause of PD. However, these data cannot confirm whether mitochondrial swelling is a primary or secondary effect of parkin mutation. Further evidence is needed to confirm the importance of mitochondrial dysfunction in PD pathogenesis.

**PINK1 and Parkin act in a common pathway in mitochondrial protection**

PINK1 is a serine/threonine kinase localized to the mitochondrial membrane via a mitochondrial targeting motif in its N-terminus (Valente et al., 2004). Most of the currently reported mutations are located in its kinase domain, indicating that PINK1 kinase activity is required for its role in PD protection (Klein & Lohmann-Hedrich, 2007). Interestingly, PINK1 fly mutants demonstrated phenotypes remarkably similar to parkin mutants, including flight disability, slow climbing speed, indirect flight muscle degeneration and a reduced number of DA neurons (Clark et al., 2006; Park et al., 2006; Yang et al., 2006). Moreover, mitochondrial swelling was also observed in the indirect flight muscles and DA neurons (Fig. 1). Upon further genetic analysis, over-expression of mitochondrial protein Bcl-2 was found to rescue mitochondrial dysfunction and defective phenotypes in PINK1 mutants, indicating that mitochondrial defects are the main cause of PD-related phenotypes in PINK1 mutants (Park et al., 2006).

Because of the marked phenotypic similarities between parkin and PINK1 mutants, subsequent Drosophila genetic analysis were performed to test whether PINK1 and Parkin act in a common pathway. Transgenic expression of parkin markedly ameliorated the phenotypes of PINK1 mutants; however, parkin mutant phenotypes could not be recovered by over-expression of PINK1 (Clark et al., 2006; Park et al., 2006; Yang et al., 2006; Fig. 2). These data established that PINK1 and Parkin are linked in the same pathway to protect mitochondrial integrity and function with Parkin acting downstream of PINK1. In addition to the Drosophila results, over-expression of parkin successfully rescued the mitochondrial dysfunction induced by PINK1 knockdown in the mammalian system, demonstrating that the PINK1-Parkin pathway is conserved in flies and mammals (Exner et al., 2007).

Following the establishment of the PINK1-Parkin pathway, researchers have been trying to investigate the relationship between these two proteins. Using human DA cells and Drosophila models, Kim et al. demonstrated that PINK1 translocates Parkin to mitochondria in its kinase
activity-dependent manner (Kim et al., 2008). Further analysis suggested that PINK1 phosphorylates Parkin on its linker region and promotes its mitochondrial translocation. Additional studies showed that PINK1 selectively translocates Parkin to impaired mitochondria, confirming the PINK1-dependent Parkin translocation that Kim et al. reported (Matsuda et al., 2010; Narendra et al., 2010).

**PINK1 and Parkin remodel mitochondria**

Since fly genetic analysis clearly demonstrated that the PINK1-Parkin pathway is involved in the protection of mitochondrial integrity and function, various efforts have concentrated on investigating the particular role of this

---

**Fig. 1.** PINK1 mutation induces mitochondrial defects. *Drosophila* PINK1 mutants (B9) demonstrated the crushed-thorax phenotype (top panel, white arrows), an indicator of flight muscle degeneration, and mitochondrial swelling in indirect flight muscles (middle panel) and DA neurons (bottom panel). Toluidine blue staining of longitudinal thorax sections revealed mitochondrial morphology of indirect flight muscles (middle panel). Expression of mitochondria-targeted green fluorescent protein (mito-GFP, green) showed mitochondria shape and size in the DA neurons of adult fly brains (bottom panel). Wild type controls (WT) showed an intact thorax structure and mitochondrial morphology. Scale bar=yellow, 5 μm.

**Fig. 2.** Transgenic expression of parkin ameliorates PINK1 mutant phenotypes, but not vice versa. Over-expression of parkin successfully rescued mitochondrial swelling, mtDNA content, and ATP levels in PINK1 mutants. However, the PINK1 transgene failed to restore these defects in parkin mutants. (A) Mitochondria in indirect flight muscles from PINK1 mutants (B9), Parkin-expressing PINK1 mutants (B9, parkin), parkin mutants (park1), and PINK1-expressing parkin mutants (park1, PINK1). (B) Quantification of mtDNA in fly thoraces. Quantitative real-time PCR was performed using primers from the mtDNA sequences of the following mitochondrial genes: Cox I, cytochrome c oxidase subunit I; Cox III, cytochrome c oxidase subunit III; Cyt B, cytochrome b. Results are expressed as fold change compared relative to WT controls. (C) Comparison of ATP content in fly thoraces. The relative ATP level was calculated by dividing the measured ATP concentration by the total protein concentration. In mtDNA and ATP assays, averages±SDs are from three experiments. Significance was determined by one-way ANOVA (*P<0.01; NS, not significant). Error bars indicate mean±SD. Scale bar=yellow, 5 μm.
pathway in mitochondria. Recent fly genetic studies showed that the PINK1-Parkin pathway regulates the mitochondrial remodeling process including mitochondrial fusion and fission. Mitochondria are dynamic organelles that constantly fuse and divide. Uncontrolled fusion-fission processes lead to severe damage of mitochondria morphology and function, causing the impairment of various cellular process and even cell death (Chan 2006). Surprisingly, in Drosophila, over-expression of Drp1, a guanosine triphosphatase (GTPase) for mitochondrial fission, or down-regulation of Opa1 or Drp1 expression, GTPases for mitochondrial fusion, rescued PINK1 and parkin mutant phenotypes, suggesting that the PINK1-Parkin pathway regulates mitochondria remodeling by promoting mitochondrial fission (Deng et al., 2008; Poole et al., 2008; Yang et al., 2008; Park et al., 2009). Therefore, loss of PINK1 or parkin may induce uncontrolled mitochondrial remodeling leading to severe mitochondrial dysfunction and subsequent DA neuronal degeneration. Results from other human cell-based studies, however, are not in agreement. Co-expression of PINK1 and Parkin, or expression of mitochondria-targeted Parkin induces mitochondrial aggregation in human DA neuroblastoma cells (Kim et al., 2008). Moreover, mitochondrial fragmentation was found in human neuronal cells lacking PINK1 or in the primary cells from human patients with PINK1 mutations (Exner et al., 2007; Kim et al., 2008; Wood-Kaczmar et al., 2008). To account for these different patterns in mitochondrial remodeling, researchers are attempting to dissect the exact mechanism involved in PINK1 and Parkin-mediated mitochondrial remodeling.

In addition to balancing between mitochondrial fusion and fission processes, the PINK1-Parkin pathway may also be involved in another mitochondrial remodeling process: mitophagy, the specific autophagic turnover of mitochondria. Narendra et al. reported that Parkin is selectively translocated to damaged mitochondria upon treatment of mitochondria damaging agents, and induces the turnover of damaged mitochondria through an autophagy-related gene 5 (ATG5)-dependent mechanism (Narendra et al., 2008). This finding elicited further studies to test whether PINK1 is required for the mitophagy-promoting activity of Parkin. In human DA neuroblastoma cells, co-expression of PINK1 and Parkin induces the formation of perinuclear mitochondrial clusters surrounded by autophagic vacuoles (Vives-Bauza et al., 2010). Moreover, knock-down or knock-out of PINK1 successfully inhibits mitochondrial damage-induced mitophagy (Matsuda et al., 2010; Narendra et al., 2010). Further analysis determined that PINK1 is selectively stabilized on impaired mitochondria, and activates the autophagic degradation of these mitochondria by recruiting Parkin (Matsuda et al., 2010; Narendra et al., 2010). These data provide the functional link between PINK1, Parkin, and the selective autophagy of mitochondria, suggesting that PINK1 and Parkin maintain mitochondrial integrity and function by selectively removing impaired mitochondria.

**Mitochondrial targets of the PINK1-Parkin pathway in mitochondrial remodeling**

In biochemical studies using Parkin proteins containing disease causing mutations, the ubiquitin ligase activity of Parkin is critical for normal physiological activities. Moreover, Parkin-mediated mitochondrial ubiquitination was observed in mitochondrial damaging agent-treated cells. Over-expression of dominant negative ubiquitin mutants prevented Parkin-induced mitophagy, demonstrating that ubiquitination links between Parkin and mitophagy (Geisler et al., 2010; Lee et al., 2010; Matsuda et al., 2010). To identify a putative Parkin target on mitochondria, molecular weight changes of various mitochondrial proteins upon mitochondrial damaging-agent treatment were evaluated. Surprisingly, a molecular weight shift of voltage-dependent anion channel 1 (VDAC1) was observed. Further biochemical analysis identified VDAC1 as a target for Parkin-mediated mitochondrial ubiquitination and subsequent mitophagy (Geisler et al., 2010). The ubiquitining binding autophagic component p62 and HDAC6 were also essential for Parkin-dependent mitophagy (Geisler et al., 2010; Lee et al., 2010), suggesting that ubiquitinated VDAC1 may be an important adapter molecule for p62 and HDAC6. In addition, Ziviani et al. reported that the pro-mitochondrial fusion protein Marf is ubiquitinated by Parkin, inferring that Parkin prevents refusion of damaged mitochondria to support proper mitophagic degradation (Ziviani et al., 2010). These mitochondrial Parkin targets further confirmed the role of the PINK1-Parkin pathway in mitochondrial remodeling, suggesting a mitochondrial quality control system driven by PINK1 and Parkin (Whitworth & Pallanck, 2009). Under mitochondrial damaging stress, PINK1 selectively translocates Parkin to impaired mitochondria. In mitochondria, Parkin ubiquitinates its targets, prevents refusion of damaged mitochondria, and finally removes damaged mitochondria.
Roles of PINK1 and Parkin in mitochondria

Concluding remarks

After the cloning of PD-associated genes, their roles in protection against PD were investigated in cell-based PD models. However, pioneering work using a genetic fly model revealed that the most affected PD gene, parkin, acts downstream of another PD gene, PINK1, to protect mitochondria, confirming the link between mitochondrial dysfunction and PD pathogenesis. Additional studies based on this finding revealed the exact role of the PINK1-Parkin pathway in mitochondria: balancing between mitochondria fusion and fission, and selective mitophagy of damaged mitochondria. Further studies investigating the molecular mechanisms of PINK1 and parkin in regulating mitochondrial remodeling will provide an enhanced understanding of PD pathogenesis and the development of an effective treatment strategy for PD.

Acknowledgements

This work was supported by the National Research Foundation of Korea (NRF) grants funded by the Korean government (MEST) (331-2008-1-C00225-2008-0059520, 2009-0075511).

References

Betarbet R, Sherer TB, MacKenzie G, Garcia-Osuna M, Panov AV, Greenamyre JT. (2000). Chronic systemic pesticide exposure reproduces features of Parkinson’s disease. Nat Neurosci 3: 1301-1306
Bonifati V, Rizzu P, van Baren MJ, et al. (2003). Mutations in the DJ-1 gene associated with autosomal recessive early-onset parkinsonism. Science 299: 256-259
Cha GH, Kim S, Park J, et al. (2005). Parkin negatively regulates JNK pathway in the dopaminergic neurons of Drosophila. Proc Natl Acad Sci U S A 102: 10345-10350
Chan DC. (2006). Mitochondrial fusion and fission in mammals. Annu Rev Cell Dev Biol 22: 79-99
Cicchetti F, Lapointe N, Roberge-Tremblay A, et al. (2005). Systemic exposure to paraquat and maneb models early Parkinson’s disease in young adult rats. Neurobiol Dis 20: 360-371
Clark IE, Dodson MW, Jiang C, et al. (2006). Drosophila pink1 is required for mitochondrial function and interacts genetically with parkin. Nature 441: 1162-1166
Coulom H, Birman S. (2004). Chronic exposure to rotenone models sporadic Parkinson’s disease in Drosophila melanogaster. J Neurosci 24: 10993-10998
Deng H, Dodson MW, Huang H, Guo M. (2008). The Parkinson’s disease genes pink1 and parkin promote mitochondrial fission and/or inhibit fusion in Drosophila. Proc Natl Acad Sci U S A 105: 14503-14508
Exner N, Treske B, Paquet D, et al. (2007). Loss-of-function of human PINK1 results in mitochondrial pathology and can be rescued by parkin. J Neurosci 27: 12413-12418
Geisler S, Holmström KM, Skujat D, et al. (2010). PINK1/Parkin-mediated mitophagy is dependent on VDAC1 and p62/SQSTM1. Nat Cell Biol 12: 119-231
Greene JC, Whitworth AJ, Kuo I, Andrews LA, Feany MB, Pallanck LJ. (2003). Mitochondrial pathology and apoptotic muscle degeneration in Drosophila parkin mutants. Proc Natl Acad Sci U S A 100: 4078-4083
Henchcliffe C, Beal MF. (2008). Mitochondrial biology and oxidative stress in Parkinson disease pathogenesis. Nat Clin Pract Neurol 4: 600-609
Imai Y, Soda M, Inoue H, Hattori N, Mizuno Y, Takahashi R. (2001). An unfolded putative transmembrane polypeptide, which can lead to endoplasmic reticulum stress, is a substrate of Parkin. Cell 105: 891-902
Keeney PM, Xie J, Capaldi RA, Bennett JP Jr. (2006). Parkinson’s disease brain mitochondrial complex I has oxidatively damaged subunits and is functionally impaired and misassembled. J Neurosci 26: 5256-5264
Kim Y, Park J, Kim S, et al. (2008). PINK1 controls mitochondrial localization of Parkin through direct phosphorylation. Biochem Biophys Res Commun 377: 975-980
Kitada T, Asakawa S, Hattori N, et al. (1998). Mutations in the parkin gene cause autosomal recessive juvenile parkinsonism. Nature 392: 605-608
Klein C, Lohmann-Hedrich K. (2007). Impact of recent
Genetic findings in Parkinson’s disease. Curr Opin Neurol 20: 453–464
Lang AE, Lozano AM. (1998). Parkinson’s disease. First of two parts. N Engl J Med 339: 1044-1053
Langston JW, Ballard P, Tetrad JW, Irwin I. (1983). Chronic Parkinsonism in humans due to a product of meperidine-analog synthesis. Science 219: 979-980
Lee JY, Nagano Y, Taylor JP, Lim KL, Yao TP. (2010). Disease-causing mutations in parkin impair mitochondrial ubiquitination, aggregation, and HDAC6-dependent mitophagy. J Cell Biol 189: 671-679
Matsuda N, Sato S, Shiba K, et al. (2010). PINK1 stabilized by mitochondrial depolarization recruits Parkin to damaged mitochondria and activates latent Parkin for mitophagy. J Cell Biol 189: 211-221
Narendra D, Tanaka A, Suen DF, Youle RJ. (2008). Parkin is recruited selectively to impaired mitochondria and promotes their autophagy. J Cell Biol 183: 795-803
Narendra DP, Jin SM, Tanaka A, et al. (2010). PINK1 is selectively stabilized on impaired mitochondria to activate Parkin. PLoS Biol 8: e1000298
Paisán-Ruiz C, Jain S, Evans EW, et al. (2004). Cloning of the gene containing mutations that cause PARK8-linked Parkinson’s disease. Neuron 44: 595-600
Park J, Lee G, Chung J. (2009). The PINK1-Parkin pathway is involved in the regulation of mitochondrial remodeling process. Biochem Biophys Res Commun 378: 518-523
Park J, Lee SB, Lee S, et al. (2006). Mitochondrial dysfunction in Drosophila PINK1 mutants is complemented by parkin. Nature 441: 1157-1161
Parker WD Jr, Parks JK, Swerdlow RH. (2008). Complex I deficiency in Parkinson’s disease frontal cortex. Brain Res 1189: 215-218
Pesah Y, Pham T, Burgess H, et al. (2004). Drosophila parkin mutants have decreased mass and cell size and increased sensitivity to oxygen radical stress. Development 131: 2183-2194
Polymeropoulos MH, Lavedan C, Leroy E, et al. (1997). Mutation in the alpha-synuclein gene identified in families with Parkinson’s disease. Science 276: 2045-2047
Poole AC, Thomas RE, Andrews LA, McBride HM, Whitworth AJ, Pallanck LJ. (2008). The PINK1/Parkin pathway regulates mitochondrial morphology. Proc Natl Acad Sci U S A 105: 1638-1643
Ramirez A, Heimbach A, Gründemann J, et al. (2006). Hereditary parkinsonism with dementia is caused by mutations in ATP13A2, encoding a lysosomal type 5 P-type ATPase. Nat Genet 38: 1184-1191
Shimura H, Hattori N, Kubo S, et al. (2000). Familial Parkinson disease gene product, parkin, is a ubiquitin-protein ligase. Nat Genet 25: 302-305
Staropoli JF, McDermott C, Martinat C, Schulman B, Demireva E, Abeliovich A. (2003). Parkin is a component of an SCF-like ubiquitin ligase complex and protects postmitotic neurons from kainate excitotoxicity. Neuron 37: 735-749
Valente EM, Abou-Sleiman PM, Caputo V, et al. (2004). Hereditary early-onset Parkinson’s disease caused by mutations in PINK1. Science 304: 1158-1160
Vives-Bauza C, Zhou C, Huang Y, et al. (2010). PINK1-dependent recruitment of Parkin to mitochondria in mitophagy. Proc Natl Acad Sci U S A 107: 378-383
Whitworth AJ, Pallanck LJ. (2009). The PINK1/Parkin pathway: a mitochondrial quality control system? J Bioenerg Biomembr 41: 499-503
Wood-Kaczmar A, Gandhi S, Yao Z, et al. (2008). PINK1 is necessary for long term survival and mitochondrial function in human dopaminergic neurons. PLoS One 3: e2455
Yang Y, Gehrke S, Imai Y, et al. (2006). Mitochondrial pathology and muscle and dopaminergic neuron degeneration caused by inactivation of Drosophila Pink1 is rescued by Parkin. Proc Natl Acad Sci U S A 103: 10793-10798
Yang Y, Ouyang Y, Yang L, et al. (2008). Pink1 regulates mitochondrial dynamics through interaction with the fission/fusion machinery. Proc Natl Acad Sci U S A 105: 7070-7075
Zhang Y, Gao J, Chung KK, Huang H, Dawson VL, Dawson TM. (2000). Parkin functions as an E2-dependent ubiquitin-protein ligase and promotes the degradation of the synaptic vesicle-associated protein, CDCrel-1. Proc Natl Acad Sci U S A 97: 13354-13359
Ziviani E, Tao RN, Whitworth AJ. (2010). Drosophila parkin requires PINK1 for mitochondrial translocation and ubiquitinates mitofusin. Proc Natl Acad Sci U S A 107: 5018-5023