Parkin Ubiquitinates Drp1 for Proteasome-dependent Degradation

IMPLICATION OF DYSREGULATED MITOCHONDRIAL DYNAMICS IN PARKINSON DISEASE

Hongxia Wang, Pingping Song, Lei Du, Weieli Tian, Wen Yue, Min Liu, Dengwen Li, Bin Wang, Yushan Zhu, Cheng Cao, Jun Zhou, and Quan Chen

From the 4 Department of Genetics and Cell Biology and Tianjin Key Laboratory of Protein Science, College of Life Sciences, Nankai University, Tianjin 300071, China, the 4 National Key Laboratory of Biomembrane and Membrane Biotechnology, Institute of Zoology, Chinese Academy of Sciences, Beijing 100101, China, and the 4 Beijing Institute of Biotechnology, Beijing 100850, China

Mutations in Parkin, an E3 ubiquitin ligase that regulates protein turnover, represent one of the major causes of familial Parkinson disease, a neurodegenerative disorder characterized by the loss of dopaminergic neurons and impaired mitochondrial functions. The underlying mechanism by which pathogenic Parkin mutations induce mitochondrial abnormality is not fully understood. Here, we demonstrate that Parkin interacts with and subsequently ubiquinates dynamin-related protein 1 (Drp1), for promoting its proteasome-dependent degradation. Pathogenic mutation or knockdown of Parkin inhibits the ubiquitination and degradation of Drp1, leading to an increased level of Drp1 for mitochondrial fragmentation. These results identify Drp1 as a novel substrate of Parkin and suggest a potential mechanism linking abnormal Parkin expression to mitochondrial dysfunction in the pathogenesis of Parkinson disease.

Parkinson disease (PD) is one of the most common neurodegenerative diseases affecting over 2% populations over 65 years of age. It is classically characterized by the loss of dopaminergic neurons that project from the midbrain substantia nigra to the striatum (1, 2). Although the loss of dopaminergic neurons is responsible for the symptom of movement disorder in PD, it is now clear that other types of neurons throughout the brain are also affected in the disease (3, 4). The identification of genes linking to PD has greatly advanced our understanding of the molecular pathogenesis of the disease (5–8). Mutations in Parkin represent one of major causes for early onset of familial PD (9–11). Parkin is an E3 ubiquitin ligase that contains two ring finger domains (12–15). A handful of substrates have been identified, including Parkin itself and CDCrel-1, synphilin-1, Pael-R, glycosylated α-synuclein, FBP1 (far upstream element-binding protein 1), and the RNA-processing protein subunit p38/AIMP2 (16–19). A putative mechanism by which mutations of Parkin cause PD would be abnormal accumulation and aggregation of the above substrates due to insufficient E3 ligase activity for ubiquitin-proteasome-dependent protein turnover (18, 20, 21). Surprisingly, only p38/AIMP2 and FBP1 were found to be accumulated in the brain samples of PD patients or in Parkin knock-out mice (16, 17, 19). Even though a number of the putative substrates have been identified, the causative link between these substrates and the PD pathogenesis remains not fully understood.

Over the past few decades, accumulating evidence has suggested that mitochondrial dysfunction and the resulting oxidative damage are associated with PD. This is supported by a large number of reports demonstrating impaired mitochondrial functions in PD patients (22–26). Mitochondria undergo frequent fission, fusion, and redistribution throughout the cytoplasm in response to the energy needs (27, 28). Either disruption of the fusion process or enhancement of the fission process renders the normal, tubular network of mitochondria to fragment into short rods or spheres (29). Abnormal mitochondrial fission or fusion is closely associated with neuronal cell death and a number of neuromuscular diseases (30).

Drp1 is a cytosolic protein responsible for mitochondrial fission (31). It targets to mitochondria to initiate mitochondrial fragmentation (32–34). Enhanced Drp1 expression or a reduced level of mitofusins induces mitochondrial fragmentation, an early event prior to the release of mitochondrial cytochrome c and programmed cell death (20, 35). Strong evidence has shown that Parkin plays a critical role in regulating mitochondrial fission and fusion (36) and mitochondrial quality control (37). Recent studies suggest that Drosophila Parkin genetically interacts with proteins that regulate mitochondrial fission and fusion, although other reports describe inconsistent phenotypes in Parkin- and PINK1-deficient Drosophila cells (38–44). Knockdown of Parkin results in mitochondrial elongation in flies (40). However, studies in mammalian cells suggest that loss of Parkin/PINK1 function may lead to excess mitochondria fragmentation or enhanced mitochondrial biogenesis (45–48). We thus sought to address the molecular
Drp1 Is a Novel Substrate of Parkin

details on how Parkin regulates mitochondrial fission and fusion in mammalian systems. To this end, we have identified Drp1 as a novel substrate of Parkin which effectively promotes the proteasome dependent degradation of Drp1. Our results thus uncover a novel mechanism linking loss of Parkin to mitochondrial dysfunction in the pathogenesis of PD and suggest that Drp1 could be a potential target for fighting against this currently incurable disease.

**EXPERIMENTAL PROCEDURES**

**Plasmids**—The mammalian expression plasmids for Parkin and Drp1 were generated by PCR and cloned into pEGFP-C1 and pRK5-myc vectors. The mammalian expression plasmid for FLAG-ubiquitin was generated by insertion of ubiquitin cDNA in-frame into the pCMV-tag-2B vector. The pCMV-HA-UB and pCMV-HA-UB-K0 plasmids were kindly provided by Dr. Tomohiko Ohta (St. Marianna University, Japan).

**Antibodies and Reagents**—DAPI and antibodies against FLAG, HA, Myc, and β-actin were purchased from Sigma-Aldrich. Antibodies against Parkin (Cell Signaling), Drp1 (BD Biosciences), GFP (Roche Applied Science), ubiquitin (Santa Cruz), rhodamine- and fluorescein-conjugated secondary antibodies (Jackson ImmunoResearch) were from the indicated sources. MG132, PS341, PMSF, and cycloheximide were obtained from Sigma-Aldrich. MitoTracker-Red, CM-H2XRos, and chlororange were from Invitrogen, pepstatin was from BioBasic.

**Cells, siRNAs, shRNAs, and Transfections**—All the cells used in this study were maintained in Dulbecco’s modified Eagle’s medium supplemented with 10% FBS at 37 °C in an atmosphere of humidified air and 5% CO2. Primary neurons were cultured on glass coverslips and maintained in DMEM (Thermo Fisher Scientific) supplemented with 2 mM methionine and cysteine (Sigma). Cells were harvested and then incubated with the chase medium containing 10 μM MG132 for 8 h before harvest. Cell lysate was immunoprecipitated with an antibody against Myc. The precipitates were subjected to Western blotting with an antibody against HA.

**Ubiquitination Assays**—Cells were transfected with GFP-Parkin, Myc-Drp1, and HA-UB or HA-UB-K0 plasmids and incubated with 20 μM MG132 for 8 h before harvest. Cell lysate was immunoprecipitated with an antibody against Myc. The precipitates were subjected to Western blotting with an antibody against HA. In vitro ubiquitination assay was performed in 50 μl of ubiquitination reaction buffer containing 50 mM Tris-HCl, pH 7.5, 5 mM MgCl2, 2 mM DTT, 2 mM ATP, 10 μg of ubiquitin, 100 ng of E1, 200 ng of E2 (UbcH7), 2 μg of purified MBP-Parkin, 2 μg of immunoprecipitated MARCH5, and 2 μg of in vitro translated Drp1. The reaction was performed for 2 h at 30 °C and terminated by addition of the SDS loading buffer. The reaction products were then subjected to Western blotting with anti-ubiquitin and anti-Drp1 antibodies.

**Fluorescence Microscopy**—Cells grown on glass coverslips were transfected with Mito-DsRed together with GFP-Parkin. Cells were fixed with 4% paraformaldehyde for 30 min at room temperature, incubated with primary and secondary antibodies, and then stained with DAPI. Coverslips were mounted with 90% glycerol in PBS and examined with a Zeiss fluorescence microscope.

**[35S]Methionine Pulse-Chase Experiments**—HeLa cells were incubated with the labeling medium containing 10 μCi of Met/Cys Tran35S-label (PerkinElmer Life Sciences) for 3 h. Cells were washed and then incubated with the chase medium containing 2 mM methionine and cysteine (Sigma). Cells were harvested at different time points, and proteins were then immunoprecipitated with anti-Drp1 and anti-β-actin antibodies, separated by SDS-PAGE, and analyzed by autoradiography.

**Statistical Analysis**—Statistical analysis between groups was performed by unpaired two-tailed Student’s t test. Data are presented as means ± S.E.

**RESULTS**

**Alteration of Parkin Expression in Cells Affects Mitochondrial Morphology**—We first examined mitochondrial morphology in cells with altered Parkin expression. Fluorescence microscopy revealed that overexpression of Parkin resulted in perinuclear clustering of mitochondria in both HeLa and SH-SY5Y cells (Fig. 1, A and B), and nearly half of the cells had this phenotype (Fig. 1C). To assess the role of endogenous Parkin in the regulation of mitochondrial morphology, we inhibited the expression of Parkin in SH-SY5Y cells with specific siRNAs (Fig. 1D). Strikingly, although the majority of the cells transfected with control siRNA had a normal reticulum of mitochondria, nearly
FIGURE 1. Alteration of Parkin expression in cells affects mitochondrial morphology. A, HeLa cells were transfected with Mito-DsRed together with GFP-Parkin or GFP vector for 24 h, and cells were then stained with DAPI and analyzed by fluorescence microscopy. Cells with a dense cluster of mitochondria were classified as perinuclear mitochondrial clustering. B, experiments were performed as in A except that SH-SY5Y cells were used. C, experiments were performed as in A and B, and the percentage of cells with mitochondrial clustering was quantified. Cells with an intact network of tubular mitochondria were defined as normal, cells with disrupted and predominantly spherical mitochondria were defined as fragmented mitochondria, and cells with a dense cluster of mitochondria were classified as perinuclear mitochondrial clustering. D, Western blot analysis of the expression of Parkin and β-actin in SH-SY5Y cells transfected with two different Parkin siRNAs or a scramble siRNA control was performed. Parkin protein level was determined by dividing the intensity of Parkin with the intensity of β-actin on the blot. E, SH-SY5Y cells were transfected with control or Parkin siRNAs, stained with Mito-Tracker Red and an anti-Parkin antibody, and then analyzed by immunofluorescence microscopy. F, experiments were performed as in E, and the percentage of cells with mitochondrial truncation or fragmentation was quantified. G, experiments were performed as in E, and the length of mitochondria was measured with ImageJ. All immunofluorescence data shown in the bar graphs represent means ± S.E. (error bars) from three independent experiments, with at least 100 cells counted in a blinded manner. *, p < 0.01 and **, p < 0.001.
Drp1 Is a Novel Substrate of Parkin
half of the cells transfected with Parkin siRNAs had fragmented mitochondria (Fig. 1, E and F), which was in accordance with previous reports (48). The length of mitochondria was also significantly decreased in cells transfected with Parkin siRNAs (Fig. 1G). These results indicate that Parkin expression can significantly alter mitochondrial morphology in cells.

**Parkin Induces the Degradation of Drp1 through the Proteasome-dependent Pathway**—The remarkable effect of Parkin on mitochondrial morphology suggests that it may affect the level of either the pro-fission proteins or the pro-fusion protein, key regulators of mitochondrial dynamics. To test this possibility, we chose 293T cells which have high transfection efficiency for overexpression experiments. We examined the expression of Drp1, Mfn1/2 (mitofusion 1/2) and Fis1 (another pro-fission molecule) in cells transfected with GFP-Parkin by Western blotting. As shown in Fig. 2A, the level of Drp1 was significantly decreased by GFP-Parkin, whereas the levels of Mfn1/2 and Fis1 were not affected. siRNA-mediated knockdown of Parkin expression was performed in SH-SY5Y cells that have endogenous expression of Parkin. We found that Drp1 expression was significantly increased, but the expression of Mfn1/2 and Fis1 was not affected (Fig. 2B).

We further investigated whether the down-regulatory effect of Parkin on Drp1 was due to an increase in Drp1 degradation through the proteasome-dependent pathway, similar to the effect of Parkin toward other substrates. To test this possibility, cells were transfected with GFP-Parkin in the absence or presence of MG132, a specific proteasome inhibitor. We found that the reduction of Drp1 by GFP-Parkin was remarkably blocked by MG132 (Fig. 2C). A similar result was obtained by treatment of these cells with PS341, another proteasome inhibitor (Fig. 2D). In contrast, neither chloroquine, a lysosome inhibitor, nor PMSF and pepstatin, protease inhibitors, could inhibit the down-regulatory effect of Parkin on Drp1 expression (Fig. 2D).

We further examined the effect of Parkin on Drp1 stability by measuring the half-life of Drp1 via the cycloheximide (CHX)-
chase assay and [35S] methionine pulse-chase assay (supple-
mental Fig. S1). Our result revealed a striking decrease of Drp1
half-life in cells overexpressing GFP-Parkin (Fig. 2E and supple-
mental Fig. S1). Taken together, these data demonstrate that
Parkin induces Drp1 degradation through the proteasome-de-
pendent proteolytic machinery.

Parkin Interacts with Drp1 through Its Second Ring Finger Domain—Parkin, as an E3 ubiquitin ligase, can regulate the
ubiquitination and proteasome-dependent degradation of its
substrates. Our finding that Parkin is capable of regulating
Drp1 expression suggests that Drp1 might be a new substrate of
Parkin. To test this, we first examined whether these two pro-
teins interact. Cells were transfected with plasmids expressing
either Myc-Parkin or Myc-Vector, and cell lysates were then
analyzed by immunoprecipitation with anti-Myc antibody. As
shown in Fig. 3A, endogenous Drp1 was immunoprecipitated
by endogenous Parkin, and the immunoprecipitated Parkin protein decreased when endoge-
nous Drp1 protein level was down-regulated by Drp1 shRNA
(Fig. 3B). To study whether Drp1 and Parkin interact directly, in
vitro translated Drp1 was incubated with bacterially purified
MBP-Parkin or MBP immobilized on amylose beads, and MBP
pulldown assay was performed. Drp1 was detected in the pull-
down preparation of MBP-Parkin but not in that of MBP (Fig.
3C). Thus, our results demonstrate that Drp1 and Parkin can
interact both in cells and in vitro.

Parkin contains two ring finger domains separated by an in-
between ring finger domain important for its interaction with
most of the substrate proteins. We next determined the domain
mediating the interaction of Parkin with Drp1. A series of Par-
kin truncated constructs were constructed to determine the
domain of Parkin that interacted with Drp1 (Fig. 3D). Cells
were transfected with the plasmids that express various trunca-
tions of GFP-Parkin, together with Myc-Drp1, and immuno-

FIGURE 4. Parkin promotes the ubiquitination of Drp1 in cells. A, SH-SYSY cells were transfected with HA-UB together with control or Parkin siRNAs as
indicated. Cells were treated with MG132 before harvest. Cell lysates were then subjected to immunoprecipitation (IP) and Western blotting to examine the
ubiquitination of Drp1. Uniquitinated Drp1 protein level was quantified according to the results of three independent blots. B, 293T cells were transfected with
HA-UB together with GFP-Parkin or GFP vector. Cells were treated with MG132 for 8 h before harvest. Cell lysates were then subjected to immunoprecipitation
and Western blotting. Uniquitinated Drp1 protein level was quantified according to the results of three independent blots. C, 293T cells were transfected with
various HA-UB constructs together with GFP-Parkin or GFP vector. Cells were treated with MG132 for 8 h before harvest. Cell lysates were then subjected to
immunoprecipitation and Western blotting. All Western blot data shown in the bar graphs represent means ± S.E. (error bars) from three independent
experiments. *, p < 0.01 and **, p < 0.001.

Drp1 Is a Novel Substrate of Parkin

11654 JOURNAL OF BIOLOGICAL CHEMISTRY
VOLUME 286 • NUMBER 13 • APRIL 1, 2011
Drp1 Is a Novel Substrate of Parkin

precipitation assay was then performed. Such an experiment revealed that the second ring finger domain of Parkin mediated its interaction with Drp1 (Fig. 3E). CHX assay indicated that the decrease of Drp1 half-life by Parkin was inhibited by deletion of the second ring finger of Parkin (supplemental Fig. S1).

Parkin Promotes the Ubiquitination of Drp1 in Cells—We then examined whether Drp1 is ubiquitinated by Parkin. To test this possibility, SH-SY5Y cells were transfected with HA-UB and control or Parkin siRNAs and treated with MG132 before harvest. Immunoprecipitation analysis indicated that the down-regulation of Parkin expression significantly reduced the ubiquitination of Drp1 (Fig. 4A).

We then examined whether Drp1 ubiquitination was enhanced by Parkin overexpression. 293T cells were transfected with HA-UB together with Myc-Drp1 and GFP-Parkin and treated with MG132 before harvest. By immunoprecipitation and Western blotting, we found that Parkin significantly increased the ubiquitination of Drp1 (Fig. 4B). Lys48-linked polyubiquitination acts as the canonical signal for targeting the substrate to the proteasome for degradation (14). Based on our finding that Parkin could ubiquitinate Drp1 leading to its degradation by the proteasome, we hypothesized that Parkin may mediate the polyubiquitination of Drp1 via Lys48-linked ubiquitin chains. To test this, cells were transfected with Myc-Drp1, GFP-Parkin, and various HA-UB constructs that encode wild-type ubiquitin or ubiquitin mutants containing arginine substitutions of all the lysine residues except the one at position 29, 48, or 63, respectively. Immunoprecipitation analysis revealed that Parkin was able to induce the polyubiquitination of Drp1 in the presence of wild-type or Lys48 ubiquitin, but not Lys63 or Lys63 ubiquitin (Fig. 4C). Thus, our results suggest that Parkin mediates the polyubiquitination of Drp1 mainly via Lys48-linked ubiquitin chains.

Mutations in Parkin Affect Its Ability to Ubiquitinate Drp1 for Degradation—We then investigated whether familial PD-causing mutation (C431F) of Parkin, which is known to have attenuated E3 ligase activity (49), impaired its ability to ubiquitinate Drp1. 293T cells were transfected with HA-UB together with Myc-Drp1 and GFP-Parkin or GFP-Parkin (C431F), and treated with MG132 before harvest. By immunoprecipitation and followed by Western blotting, we found that Parkin mutation significantly decreased the ubiquitination of Drp1 (Fig. 5A). Similar results were achieved when the second ring finger domain of Parkin was deleted (Fig. 5A).

Next, we attempted to determine whether Parkin could directly ubiquitinate Drp1 and whether mutations in Parkin could result in the loss of its activity for Drp1 ubiquitination, by using an in vitro ubiquitination assay. To reconstitute the ubiquitin-conjugation-ligation reaction, in vitro translated Drp1 was incubated with purified ubiquitin, E1, E2 (UbcH7), and various Parkin proteins. MARCH5, an E3 ligase known to ubiquitinate Drp1, was used as a positive control for Parkin. The reaction products were analyzed by Western blotting with an anti-ubiquitin antibody. We found that wild-type Parkin, but not the C431F, T240R, or ΔR2 mutants, induced a significant increase in ubiquitinated protein bands (Fig. 5B). Western blotting with an anti-Drp1 antibody further confirmed that the high molecular weight ubiquitinated proteins represent the ubiquitinated Drp1 (Fig. 5B). We also found that the effect of Parkin on Drp1 ubiquitination was comparable with that of MARCH5 (supplemental Fig. S2). Taken together, these results demonstrate that Parkin is able to ubiquitinate Drp1 directly.

Mutations in Parkin account for 50% of all recessively transmitted early onset PD cases (50). We found that Parkin mutations R42P, K161N, T240R, R275W, and C431F could reduce its activity toward perinuclear mitochondrial clustering (Fig. 5, C and D) and Drp1 degradation (Fig. 5E). These data suggest that Parkin-induced ubiquitination and degradation of Drp1 might directly impact the regulation of mitochondrial morphology by Parkin and mutations of Parkin affecting its E3 ligase activity increase Drp1 expression, resulting in mitochondrial dysfunction.

DISCUSSION

In this study, we have found that Drp1 is a novel substrate of Parkin. Parkin effectively promotes the ubiquitination and proteasome-dependent degradation of Drp1. Our results implicate a potential role for Parkin dysregulation in mitochondrial dynamics and PD pathogenesis. Because the role of the Parkin mutation in the pathogenesis of PD has been well established, it has long been speculated that this event could result in the accumulation of its substrates affecting mitochondrial morphology and functions (18). A number of substrates of Parkin have been identified, and deregulation of these substrates are responsible for the symptoms of neurodegenerative disease such as PD (14, 18). However, the direct connection of these substrates with the mitochondrial dysfunction widely observed in PD is remains elusive. Our results thus suggest a straightforward mechanism for how mutations of Parkin impact mitochondrial integrity and functions, leading to PD.

Our results demonstrate that Parkin interacts with and subsequently ubiquitinates Drp1, a mitochondrial fission factor, resulting in the degradation of Drp1 by the proteasome-dependent pathway. The second ring finger domain of Parkin appears to be important for its interaction with Drp1. Mutations or deletion in this region may result in the loss of the E3 ligase...
activity of Parkin and/or affect its interaction with Drp1, thereby reducing the level of ubiquitination and leading to mitochondrial fragmentation. Strong evidence has shown that, in mammalian cells, increased level of Drp1 causes mitochondrial fragmentation (31–34). Accumulation of mitofusin leads to enhanced mitochondrial aggregation and is protective to the mitochondrial dysfunctions and apoptosis (51). These results are in contrast to the recent reports showing that, in Drosophila, Parkin promotes mitochondrial fission (39, 40), and knockdown of Parkin results in mitochondrial elongation and increase of Mfn levels for mitophagy in Drosophila cells (38). The reasons for this discrepancy may be because Parkin regulates mitochondrial fission and fusion differently in distinct systems, and this warrants further clarification. Nevertheless, our results are consistent with the recent report that knockdown of Parkin leads to mitochondrial fragmentation (48). Our data suggest that one of the important functions of Parkin is to keep the cellular Drp1 level under control for proper mitochondrial functions.

Drp1 is the major factor responsible for mitochondrial fission (29, 33), which is one of the early events for neuronal cell death and the development of PD and other types of neurodegenerative diseases (20). Our results uncover a novel regulatory pathway of Drp1 by ubiquitination and proteasome-dependent degradation involving a cytosolic E3 ligase. E3 ubiquitin ligases localized in mitochondria, such as MARCH5/MITOL and Mulan, have been shown to regulate mitochondrial fission by interaction with Drp1 and another mitochondrial fission factor Fis1 (52, 53). To our knowledge, Parkin is the first cytosolic E3 ligase identified to regulate Drp1 turnover. Parkin is known to interact with PINK1/DJ1 (54) to regulate protein ubiquitination and degradation. It would be interesting to examine whether this type of interaction is also important for monitoring the Drp1 level in mitochondria. Recently, strong evidence has shown that Parkin is recruited onto damaged mitochondria to mediate mitophagy, a process for eliminating damaged or unwanted mitochondria (55, 56). Loss of PINK1 function promotes mitophagy in a Parkin- and Drp1-dependent manner (46). It is possible that Parkin has dual roles for mitochondrial homeostasis and quality control. On one hand, it is able to keep cellular Drp1 levels in check to prevent mitochondrial fragmentation. Once it is recruited toward depolarized mitochondria, Parkin may help remove damaged mitochondria. It is of interest to note that ubiquitination of proteins promotes both protein degradation and selective removal of unwanted protein aggregates or damaged organelles. Dysregulation of these processes may cause mitochondrial fragmentation and accumulation of damaged mitochondria for subsequent cell death and neural diseases.

It is possible that dysregulation of the Parkin/Drp1 axis represents one of the early events predisposing susceptibility of neuronal cells to intracellular or environmental changes because mitochondrial fragmentation is tightly associated with the loss of mitochondrial functions and enhanced mitochondrial oxidative stress. Investigation of how the Parkin/Drp1 axis is regulated may help elucidate the molecular pathogenesis of PD and design new treatment strategies, in addition to a better understanding of mitochondrial homeostasis.

Acknowledgments—We are grateful to Drs. Ted Dawson and Jian Feng for generously providing the plasmids. We are also grateful to Dr. Aimin Zhou from Cleveland State University for a critical reading of the manuscript.

REFERENCES

1. Ishikawa, A., and Tsuji, S. (1996) Neurology 47, 160–166
2. Thomas, B., and Beal, M. F. (2007) Hum. Mol. Genet. 16, R183–194
3. Braak, H., Ghebremedhin, E., Rüb, U., Bratzke, H., and Del Tredici, K. (2004) Cell Tissue Res. 318, 121–134
4. Ferrer, I. (2009) Prog. Neurobiol. 88, 89–103
5. Ferrer, M. I. (2006) Nat. Rev. Genet. 7, 306–318
6. Dawson, T. M. (2007) Parkinsonism Relat. Disord. 13, S248–249
7. Dawson, T. M., and Dawson, V. L. (2003) Science 302, 819–822
8. Bossy-Wetzel, E., Schwarzbach, R., and Lipton, S. A. (2004) Nat. Med. 10, S2–9
9. Kitada, T., Asakawa, S., Hattori, N., Matsumine, H., Yamamura, Y., Minoshima, S., Yokochi, M., Mizuno, Y., and Shimizu, N. (1998) Nature 392, 605–608
10. Lesage, S., and Brice, A. (2009) Hum. Mol. Genet. 18, R48–59
11. Biskup, S., Gerlach, M., Kupsch, A., Reichmann, H., Riederer, P., Vieregge, P., Wüllner, U., and Gasser, T. (2008) J. Neurol. 255, 8–17
12. Shimura, H., Hattori, N., Kubo, S., Mizuno, Y., Asakawa, S., Minoshima, S., Shimizu, N., Iwai, K., Chiba, T., Tanaka, K., and Suzuki, T. (2000) Nat. Genet. 25, 302–305
13. Marín, I., Lucas, J. I., Gradilla, A. C., and Ferrús, A. (2004) Physiol. Genomics 17, 253–263
14. Tanaka, K., Suzuki, T., Hattori, N., and Mizuno, Y. (2004) Biochim. Biophys. Acta 1695, 235–247
15. Dev, K. K., van der Putten, H., Sommer, B., and Rovelli, G. (2003) Neuropharmacology 45, 1–13
16. Corti, O., Hampe, C., Koutnikova, H., Darios, F., Jacquier, S., Prigent, A., Robinson, J. C., Pradier, L., Ruberg, M., Mirande, M., Hirsch, E., Rooney, T., Fournier, A., and Brice, A. (2003) Hum. Mol. Genet. 12, 1427–1437
17. Ko, H. S., von Coelln, R., Sriram, S. R., Kim, S. W., Chung, K. K., Plentikova, O., Troncoso, J., Johnson, B., Saffary, R., Goh, E. L., Song, H., Park, B. I., Kim, M. J., Kim, S., Dawson, V. L., and Dawson, T. M. (2005) J. Neurosci. 25, 7968–7978
18. Kahle, P. I., and Haass, C. (2004) EMBO Rep. 5, 681–685
19. Ko, H. S., Kim, S. W., Srim, R. S., Dawson, V. L., and Dawson, T. M. (2006) J. Biol. Chem. 281, 16193–16196
20. Cho, D. H., Nakamura, T., Fang, J., Cieplak, P., Godzik, A., Gu, Z., and Lipton, S. A. (2009) Science 324, 102–105
21. Li, H., and Guo, M. (2009) J. Clin. Invest. 119, 442–445
22. Lin, M. T., and Beal, M. F. (2006) Nature 443, 787–795
23. Knott, A. B., Perkins, G., Schwarzbach, R., and Bossy-Wetzel, E. (2008) Nat. Rev. 9, 505–518
24. Orth, M., and Schapira, A. H. (2001) Annu. Med. Genet. 106, 27–36
25. Schon, E. A., and Manfredi, G. (2003) J. Clin. Invest. 111, 303–312
26. Schapira, A. H. (2008) Lancet Neurol. 7, 97–109
27. Yaffe, M. P. (1999) Nat. Cell Biol. 1, E149–150
28. Okamoto, K., and Shaw, J. M. (2005) Annu. Rev. Genet. 39, 503–536
29. Chen, H., and Chan, D. C. (2005) Hum. Mol. Genet. 14, R283–289
30. Detmer, S. A., and Chan, D. C. (2007) Nat. Rev. Mol. Cell Biol. 8, 870–879
31. Zhu, P. P., Patterson, A., Stadler, J., Seeburg, D. P., Sheng, M., and Blackstone, C. (2004) J. Biol. Chem. 279, 35967–35974
32. Wells, R. C., Picton, L. K., Williams, S. C., Tan, F. J., and Hill, R. B. (2007) J. Biol. Chem. 282, 33769–33775
33. Yoon, Y., Krueger, E. W., Oswald, B. J., and McNiven, M. A. (2003) Mol. Cell. Biol. 23, 5409–5420
34. Ishihara, N., Nomura, M., Jofuku, A., Kato, H., Suzuki, S. O., Masuda, K., Otera, H., Nakanishi, Y., Nonaka, I., Goto, Y., Taguchi, N., Morinaga, H., Maeda, M., Takayanagi, R., Yokota, S., and Mihara, K. (2009) Nat. Cell Biol. 11, 870–879
Drp1 Is a Novel Substrate of Parkin

Biol. 11, 958–966

35. Frank, S., Gaume, B., Bergmann-Leitner, E. S., Leitner, W. W., Robert, E. G., Catez, F., Smith, C. L., and Youle, R. J. (2001) Dev. Cell 1, 515–525

36. Van Laar, V. S., and Berman, S. B. (2009) Exp. Neurol. 218, 247–256

37. Whitworth, A. J., and Pallanck, L. J. (2009) J. Bioenerg. Biomembr. 41, 499–503

38. Ziviani, E., Tao, R. N., and Whitworth, A. J. (2010) Proc. Natl. Acad. Sci. U.S.A. 107, 5018–5023

39. Poole, A. C., Thomas, R. E., Andrews, L. A., McBride, H. M., Whitworth, A. J., and Pallanck, L. J. (2008) Proc. Natl. Acad. Sci. U.S.A. 105, 1638–1643

40. Deng, H., Dodson, M. W., Huang, H., and Guo, M. (2008) Proc. Natl. Acad. Sci. U.S.A. 105, 14503–14508

41. Yang, Y., Ouyang, Y., Yang, L., Beal, M. F., McQuibban, A., Vogel, H., and Lu, B. (2008) Proc. Natl. Acad. Sci. U.S.A. 105, 7070–7075

42. Park, J., Lee, G., and Chung, J. (2009) Biochem. Biophys. Res. Commun. 378, 518–523

43. Greene, J. C., Whitworth, A. J., Kuo, I., Andrews, L. A., Feany, M. B., and Pallanck, L. J. (2003) Proc. Natl. Acad. Sci. U.S.A. 100, 4078–4083

44. Clark, I. E., Dodson, M. W., Jiang, C., Cao, J. H., Huh, J. R., Seol, J. H., Yoo, S. J., Hay, B. A., and Guo, M. (2006) Nature 441, 1162–1166

45. Cui, M., Tang, X., Christian, W. V., Yoon, Y., and Tieu, K. (2010) J. Biol. Chem. 285, 11740–11752

46. Dagda, R. K., Cherra, S. J., 3rd, Kulich, S. M., Tandon, A., Park, D., and Chu, C. T. (2009) J. Biol. Chem. 284, 13843–13855

47. Kuroda, Y., Mitsui, T., Kunishige, M., Shono, M., Akaie, M., Azuma, H., and Matsumoto, T. (2006) Hum. Mol. Genet. 15, 883–895

48. Lutz, A. K., Exner, N., Fett, M. E., Schlehe, J. S., Kloo, K., Lämmermann, K., Brunner, B., Kurz-Drexler, A., Vogel, F., Reichert, A. S., Bouman, L., Vogt-Weisenhorn, D., Wurster, W., Tatzelt, J., Hass, C., and Winklhofer, K. F. (2009) J. Biol. Chem. 284, 22938–22951

49. Giasson, B. I., and Lee, V. M. (2001) Neuron 31, 885–888

50. Mata, I. F., Lockhart, P. J., and Farrer, M. J. (2004) Hum. Mol. Genet. 13, R127–133

51. Sugio, R., Shimizu, S., and Tsuchimoto, Y. (2004) J. Biol. Chem. 279, 52726–52734

52. Li, W., Bengston, M. H., Ulbrich, A., Matsuda, A., Reddy, V. A., Orth, A., Chanda, S. K., Batalov, S., and Jozeiro, C. A. (2008) PloS One 3, e1487

53. Nakamura, N., Kimura, Y., Tokuda, M., Honda, S., and Hirose, S. (2006) EMBO Rep. 7, 1019–1022

54. Xiong, H., Wang, D., Chen, L., Choo, Y. S., Ma, H., Tang, C., Xia, K., Jiang, W., Ronai, Z., Zhuang, X., and Zhang, Z. (2009) J. Clin. Invest. 119, 650–660

55. Narendra, D., Tanaka, A., Suen, D. F., and Youle, R. J. (2008) J. Cell Biol. 183, 795–803

56. Geisler, S., Holmström, K. M., Skujat, D., Fiesel, F. C., Rothfuss, O. C., Kahle, P. J., and Springer, W. (2010) Nat. Cell Biol. 12, 119–131