Wild-type PINK1 Prevents Basal and Induced Neuronal Apoptosis, a Protective Effect Abrogated by Parkinson Disease-related Mutations*

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Mutations in the PTEN-induced kinase 1 (PINK1) gene have recently been implicated in autosomal recessive early onset Parkinson Disease (1, 2). To investigate the role of PINK1 in neurodegeneration, we designed human and murine neuronal cell lines expressing either wild-type PINK1 or PINK1 bearing a mutation associated with Parkinson Disease. We show that under basal and staurosporine-induced conditions, the number of terminal deoxynucleotidyltransferase-mediated dUTP nick end labeling (TUNEL)-positive cells was lower in wild-type PINK1 expressing SH-SY5Y cells than in mock-transfected cells. This phenotype was due to a PINK1-mediated reduction in cytochrome c release from mitochondria, which prevents subsequent caspase-3 activation. We show that overexpression of wild-type PINK1 strongly reduced both basal and staurosporine-induced caspase 3 activity. Overexpression of wild-type PINK1 also reduced the levels of cleaved caspase-9, caspase-3, caspase-7, and activated poly(ADP-ribose) polymerase under both basal and staurosporine-induced conditions. In contrast, Parkinson disease-related mutations and a kinase-inactive mutation in PINK1 abrogated the protective effect of PINK1. Together, these results suggest that PINK1 reduces the basal neuronal pro-apoptotic activity and protects neurons from staurosporine-induced apoptosis. Loss of this protective function may therefore underlie the degeneration of nigral dopaminergic neurons in patients with PINK1 mutations.

Parkinson disease (PD) is the most common neurodegenerative movement disorder, affecting ~1% of the population by age 65 years (3, 4). It is characterized by the predominant degeneration of midbrain dopaminergic neurons. Although most patients with PD are sporadic, familial cases represent ~10% of all diagnoses. To date, six genes responsible for inherited forms of PD have been identified. Mutations in the α-synuclein (5), LRRK2 (leucine-rich repeat kinase 2) and UCH-L1 (ubiquitin C-terminal esterase L1) genes cause dominant forms of familial PD. In contrast, mutations in parkin (6), DJ-1 (7, 8), and the newly identified PTEN (phosphatase and tensin homologue on chromosome 10)-induced kinase 1 (PINK1) (1, 2) are responsible for recessive forms of familial PD.

PINK1 encodes a highly conserved, 581-amino acid, putative serine-threonine protein kinase and is a member of a small family of novel kinases including CLIK1 (CLP-36 interacting kinase)/PDLIM1 kinases. Bioinformatic analysis suggests that residues Gly-193 to Leu-507 comprise the catalytic domain, residues Gly-193 to Lys-219 form the ATP-binding cassette (with Tyr-166 as an autophosphorylated regulatory residue), and residues Asp-384 to Glu-417 form an activation loop (Fig. 1). PINK1 is transcriptionally transactivated by the PTEN gene (9) and is expressed at variable levels in different cancer cell types. Valente et al. showed that overexpressed, epitope-tagged PINK1 localized to mitochondria and may have a protective function against cell death (1).

To further investigate the role of PINK1 in neuronal apoptosis, we overexpressed this protein in SH-SY5Y human neuroblastoma cells and N2a murine neurons. Here we report that wild-type PINK1 prevents neuronal apoptosis under both basal and staurosporine-induced apoptotic conditions. We demonstrate that PINK1 reduces neuronal apoptosis by reducing the release of cytochrome c, thereby limiting the subsequent activation of caspases-9, -7, and -3 and PARP. We show that this protective effect is abrogated by PD-related mutations and by rendering the kinase inactive through removal of a key lysine residue in the catalytic domain (Lys-219).

EXPERIMENTAL PROCEDURES

Cloning and Reverse Transcription PCR—Human PINK1 cDNA was obtained from OriGene TrueClone and mutated by PCR-mediated mutagenesis using appropriate oligonucleotide primers to generate human PINK1 bearing E240K and/or L489P clinical mutants and the K219M mutant. After validation by DNA sequencing using an automated DNA sequencer (ABI PRISM 3100 genetic analyzer from Applied Biosystems), the cDNAs were subcloned into the pcDNA4 or 6 (Invitrogen).

Design of PINK1 Antibody—Polyclonal rabbit anti-PINK1 antisera was raised against the 24-residue sequence (AVRQALGRGQLGRAALLRTGFK) at the N terminus of PINK1. Following affinity purification, the antisera (LWP2) was diluted to 1:2500 for Western blots. The other antibodies are described in the following paragraphs.
Wild-type PINK1 Prevents Neuronal Apoptosis

Cell Culture Conditions and Transfections—SH-SY5Y human neuroblastoma cells and N2a murine neurons were grown in 5% CO₂ in Dulbecco’s modified Eagle’s medium (Invitrogen) supplemented with 10% fetal calf serum containing penicillin (100 units/ml) and streptomycin (50 μg/ml). Stable and transient transfectants expressing wild-type untagged PINK1 were obtained after transfection with 4 μg of PINK1 cDNA by means of Lipofectamine 2000 (Invitrogen) according to the manufacturer’s recommendations. Positive transfectants were screened for their PINK1 immunoreactivity as described below. Cells were used 48 h after transfection. For stable transfections, at least 10 clones were analyzed for each construct.

Human Skin Fibroblasts Primary Cultures—With informed consent, forearm skin biopsies were performed on both a 74-year-old male Parkinson disease patient with a pathogenic compound heterozygous E240K/L489P mutation in the PINK1 gene and a 76-year-old neurologically normal male (2). ~300 mg of tissue were immediately placed in 10 ml of transport media (α-minimal essential medium supplemented with 20% fetal calf serum (Wisent) and 1% penicillin and streptomycin). Under sterile conditions, the biopsies were each placed in a 60-mm tissue culture dish and cut with a scalpel into small pieces. 5 ml of serum-free medium (Wisent) containing 394 units of collagenase (C9263 from Sigma) were added to the tissue and incubated for 1 h at 37 °C. The tissue and medium were then transferred to a 15-ml centrifuge tube and centrifuged at 800 g for 10 min. The supernatant was then removed, and the pellet was resuspended in 5 ml of citrate saline solution (50 mM KCl, 1.5 mM MgCl₂, 1 mM EGTA, 1 mM dithiothreitol, and a 10 mM Tris-buffered saline. PINK1 immunoreactivity was detected using Alexa Fluor 488-conjugated goat anti-mouse antiserum (Molecular Probes), and serial z-stacked images were collected using the Radiance2000 (Bio-Rad) confocal microscope and compiled with Confocal Assistant 4.01.

Induction of Cell Death and Caspase-3 Activity Measurements—Stable or transient transfectants were preincubated with or without staurosporine (2 μM) for 3 h, and then caspase-3 activity was fluorometrically measured as detailed extensively (11). Caspase 3 activity is considered as the Ac-DEVD-al-sensitive Ac-DEVD-7AMC hydrolyzing activity (11).

Cell Fractionation and Cytochrome c Purification—Proteins were extracted 3 h after treatment with or without 2 μM staurosporine. Cells were lysed in buffer containing 250 mM sucrose, 20 mM Heps, pH 7.5, 10 mM KCl, 1.5 mM MgCl₂, 1 mM EGTA, 1 mM dithiothreitol, and a protease inhibitor set (Roche Applied Science). Homogenates were centrifuged at 750 × g for 10 min at 4 °C, and supernatants were recentlyfuged at 10,000 × g for 15 min at 4 °C. The pellet was resuspended and contained mitochondrial proteins. The resulting supernatants were further centrifuged at 100,000 × g for 1 h at 4 °C. The resulting pellet contained microsomal fractions. The remaining supernatants contained cytosolic proteins. The protein blots were incubated with a monoclonal anti-V5 antibody (Invitrogen) for PINK1-V5 detection, a monoclonal anti-OxPhos complex IV subunit II antibody (Molecular Probes) as a molecular marker for mitochondrial fraction purification, a polyclonal anti-GRP78 (glucose-regulated protein, 78 kDa) antibody (Sigma) for a microsomal marker, and a polyclonal anti-caspase-3 antibody as a cytosolic marker. Immunoreactivity was detected with the ECL chemiluminescence detection kit (Amersham Biosciences).

Western Blot Analyses—For the detection of wild-type untagged PINK1, 50 μg were separated by electrophoresis on a 10% SDS-polyacrylamide gel and Western blotted with the LW2 anti-rabbit polyclonal antibody. V5-tagged PINK1 and FLAG-tagged PINK1 were detected on Western blot with a monoclonal anti-V5 antibody (Invitrogen) and a monoclonal anti-FLAG antibody (Sigma), respectively. Anti-actin antibody was purchased from Sigma. Active caspase-3, -7, -9 and PARP were separated on 10–20% gradient Tricine gels (Invitrogen), and a Western blot was performed using anti-active caspase-3, anti-active caspase-7, anti-active caspase-9, and anti-cleaved PARP antibodies (R&D Systems). Immunological complexes were revealed as described previously (12). Protein concentrations were established by the Bradford method.

TUNEL Method—For DNA nick end labeling, SH-SY5Y cells were grown on slides. Slides were immersed in 4% methanol-free formaldehyde diluted in phosphate-buffered saline, and cells were permeabilized with Triton® X-100 and then processed for the dUTP nick end labeling TUNEL technique according to manufacturer’s protocol (DeadEnd™ Colorimetric TUNEL system from Promega). The DNA label corresponds to brown spots (Fig. 2B).

Bioinformatic Studies—PSORT and MitoProt web-based tools were used to assess the presence of protein sorting and localization signals in PINK1. PSORT predicts protein sorting signals and localization sites from input amino acid sequences. PSORT predicts that PINK1 has a 34.8% probability of being targeted to the mitochondria (psort.nibb.ac.jp/). MitoProt is designed to identify the N-terminal regions of input proteins that have the ability to support a mitochondrial targeting sequence and predict a cleavage site (ihg.gsf.de/ihg/mitoprot.html). The MitoProt tool suggests that there is a 0.9948 probability that the first 35 amino acid residues of the human PINK1 protein target it for mitochondrial localization.

Statistical Analysis—Statistical analysis was performed with PRISM software (GraphPad Software, San Diego, CA) by using the Newman Keuls multiple comparison test for one-way analysis of variance.

RESULTS

We have recently identified the first reported compound heterozygous mutation (E240K and L489P) in a family with early onset Parkinson disease (2). We therefore created PINK1 cDNA constructs containing the following: 1) a human wild-type PINK1; 2) the PD-related E240K PINK1 mutant; 3) the PD-related L489P PINK1 mutant; 4) a double mutation (E240K/L489P); and 5) an artificial kinase dead mutant (K219M) that eliminated the Lys-219 residue that is predicted to be critical for the putative kinase activity of PINK1 (Fig. 1).

Human SH-SY5Y neuroblastoma cells stably transfected with wild-type human PINK1 express a ~63-kDa protein recognized by our rabbit polyclonal antibody (named LWP2) that is directed against the first 24 amino acids at the N terminus of human PINK1 including the mitochondrial targeting motif (Fig. 2A). The observed molecular mass of ~63 kDa corresponds to the expected molecular mass for an unprocessed PINK1 holoprotein.
To investigate the role of PINK1 in cell death, we first used the TUNEL method to assess the percentage of apoptotic cells under basal and staurosporine-treated conditions in SH-SY5Y cells that were either stably expressing an empty vector (mock) or stably expressing wild-type PINK1. Under basal conditions, mock-transfected SH-SY5Y cells displayed only a small proportion of TUNEL-positive cells (7.8 ± 1.5%). However, as expected, the proportion of TUNEL-positive cells (26.3 ± 3.5%) was strongly increased after a 3-h treatment with 2 μM staurosporine, a widely used inductor of apoptosis (Fig. 2, B and C). In contrast, in SH-SY5Y cells stably expressing wild-type PINK1 the number of TUNEL-positive cells was significantly reduced under both basal (2.3 ± 0.6%) and staurosporine-stimulated conditions (8.3 ± 2.1%) (Fig. 2, B and C).

To examine the effect of PINK1 expression on caspase-3 activity, we first transiently transfected SH-SY5Y cells with the following: 1) wild-type untagged PINK1; 2) C-terminally V5-tagged wild-type PINK1; or 3) N-terminally FLAG-tagged wild-type PINK1 (Fig. 3A). On Western blots the N-terminally directed LWP2 antibody in lysates of mock-transfected and wild-type PINK1 stably transfected SH-SY5Y cells. B and C, mock-transfected and wild-type PINK1-transfected SH-SY5Y cells were treated for 3 h in the absence (basal) or presence of 2 μM staurosporine and then analyzed by TUNEL method. The histogram (panel C) represents the quantification analysis of three independent determinations expressed in percentage of TUNEL-positive cells (brown-labeled cells, panel B). White bars represent mock stably transfected cells, and black bars represent wild-type PINK1 stably transfected SH-SY5Y cells. *, p < 0.05; **, p < 0.005 (panel C).

Wild-type PINK1 Prevents Neuronal Apoptosis

FIGURE 1. Amino acid sequence of PINK1 protein. The serine/threonine protein kinase catalytic domain is boxed. The codons mutated in a PD family segregating two (compound heterozygous) mutations are highlighted in yellow. The putative critical Lys-219 residue is highlighted in green. The conserved Cys-166 (putative redox cysteine) and Tyr-171 (putative regulatory autophosphorylated tyrosine) residues are highlighted in aquamarine. The N-terminal 24-amino acid epitope for the LWP2 rabbit polyclonal anti-PINK1 antibody is highlighted in red.

FIGURE 2. Wild-type PINK1 stable expression decreases the number of basal and staurosporine-stimulated TUNEL-positive SH-SY5Y cells. A, PINK1 holoprotein levels detected by the N-terminal LWP2 antibody in lysates of mock-transfected and wild-type PINK1 stably transfected SH-SY5Y cells. B and C, mock-transfected and wild-type PINK1-transfected SH-SY5Y cells were treated for 3 h in the absence (basal) or presence of 2 μM staurosporine and then analyzed by TUNEL method. The histogram (panel C) represents the quantification analysis of three independent determinations expressed in percentage of TUNEL-positive cells (brown-labeled cells, panel B). White bars represent mock stably transfected cells, and black bars represent wild-type PINK1 stably transfected SH-SY5Y cells. *, p < 0.05; **, p < 0.005 (panel C).
overexpressing untagged wild-type PINK1. The stable overexpression of PINK1 also caused a 30% reduction in the caspase-3 activity following staurosporine stimulation of apoptotic pathways (Fig. 3G). Taken together, these results show that the effect of wild-type PINK1 in blocking the activation of apoptotic signaling pathways is highly reproducible and not cell type-specific.

Bioinformatic studies using PSORT and MitoProt web-based tools predict a mitochondrial localization of PINK1. In agreement with this, Valente et al. (1) have shown that C-terminally Myc-tagged wild-type PINK1 transfected in COS-7 mammalian cells localized to mitochondria. In SH-SY5Y cells, immunocytochemical studies showed that C-terminally V5-tagged PINK1 distributes to punctate cytoplasmic structures, some of which overlap with perinuclear MitoTracker staining (Fig. 4A). The pattern of immunofluorescence was not affected by either the artificial K219M kinase-inactivating mutation or the PD-associated mutations (Fig. 4A). In accord with this, subcellular fractionation also revealed that C-terminally V5-tagged PINK1 distributes to mitochondrial and microsomal fractions, but only minimally to the cytosolic fraction (Fig. 4B). This mitochondrial localization, together with the reduction of caspase-3 activity triggered by PINK1 overexpression, led us to investigate the effect of PINK1 on cytochrome c translocation from mitochondria into the cytosol. As expected, the induction of apoptosis by staurosporine leads to an increase in cytosolic cytochrome c in SH-SY5Y cells (Fig. 4C). There was a significant reduction in the staurosporine-induced translocation of cytochrome c into the cytosol of cells overexpressing either C-terminally V5-tagged (74%) or
untagged wild-type PINK1 (50%) (Fig. 4C). However, the same staurosporine treatment did not trigger a translocation of PINK1 between mitochondrial, microsomal, and cytosolic fractions. This latter result suggests that the effect of PINK1 is likely mediated directly through the release of cytochrome c from mitochondria rather than by altering the subcellular distribution of PINK1 itself (Fig. 4B).

The release of cytochrome c from mitochondria allows its binding to the apoptotic protease-activating factor 1 (APAF1)-caspase-9-dATP complex and triggers activation of caspase-9, which then triggers the apoptotic cascade by successively activating other caspases like the apoptotic protease-activating factor 1 (APAF1)-caspase-9-dATP complex and triggers activation of caspase-9, which then triggers the apoptotic cascade by successively activating other caspases like caspase-3 (Fig. 5A). Thus, we wondered whether the reductions in cytochrome c translocation, caspase-3 activity, and caspase-3 immunoreactivity were associated with changes in the activation of caspase-9 and caspase-7 and cleavage of PARP. Using specific antibodies, we analyzed by Western blotting the amounts of active, cleaved caspase-9, -7, and -3 and PARP in SH-SY5Y cells that were expressing either empty vector (mock) or stably transfected untagged PINK1 under basal and staurosporine-stimulated conditions (Fig. 5B). Under basal conditions, SH-SY5Y cells with wild-type untagged PINK1 (Fig. 5B) tended to display lower levels of cleaved caspase-9, -7, and -3 compared with mock-transfected cells. As expected, under staurosporine-induced apoptotic conditions we observed elevated immunoreactivity of cleaved caspase-9, -3, -7 and of the active form of PARP, but this increase was significantly attenuated in PINK1-expressing cells (Fig. 5B). Cumulatively, these results suggest that the reduction of cytochrome c translocation due to PINK1 overexpression limits the activation of the other downstream members of the apoptotic cascade (Fig. 5, A and B). The levels of actin (Fig. 5B, bottom section) and PINK1 were not affected by a 3-h treatment with staurosporine (Fig. 5C).

To investigate the effect of PD-related mutations in PINK1 and to determine whether PINK1 kinase activity was required for the anti-apoptotic effect of PINK1, we transiently transfected SH-SY5Y cells with the following: 1) wild-type PINK1; 2) PD-related mutant PINK1 cDNAs (E240K PINK1, L489P PINK1, or E240K/L489P PINK1); or 3) catalytically dead PINK1 (K219M). Mutant PINK1 proteins were detected by Western blotting with anti-FLAG or V5 antibodies, which again identified either the ~63-kDa unprocessed holoprotein (anti-FLAG; Fig. 6B, right) or the holoprotein and a series of shorter peptides, reflecting the normal processing of the putative mitochondrial targeting sequence (anti-V5; Fig. 6B, left). These experiments again replicated the inhibitory effect of wild-type PINK1 on basal caspase-3 activity (70.4% ± 7.3 in wild-type V5-PINK1-expressing cells and 62.3% ± 6.2 in

Wild-type PINK1 Prevents Neuronal Apoptosis

FIGURE 4. Wild-type PINK1 stable expression reduces cytochrome c translocation. A, confocal analyses of SH-SY5Y cells transiently transfected with V5-tagged PINK1 (wild-type, K219M, or E240K) show co-localization of PINK1 (green) with MitoTracker (red) mitochondrial stain. Nuclei were identified with ToPro3 (blue). B, transiently expressing V5-tagged PINK1 SH-SY5Y cells were treated in the absence (−) or presence (+) of 2 μM staurosporine for 3 h, and the abundance of V5-tagged PINK1 was then measured in cytosolic, mitochondrial, and microsomal fractions, which were confirmed by immunoreactivity to markers of cytochrome c oxidase, GRP78 (78-kDa glucose-regulated protein), and caspase-3, respectively. The subcellular distribution of PINK1, as determined by biochemical fractionation, was unchanged by staurosporine treatment. C, cells transiently expressing the indicated construct of PINK1 were treated in the absence (−) or presence (+) of staurosporine. Cytosolic fractions were purified, and equal amounts of protein were electrophoresed and Western blotted with cytochrome c antibody (upper section). The lower section shows the staurosporine-induced change in cytosolic cytochrome c in mock-transfected cells (n = 3) or cells transiently expressing PINK1-V5 (n = 3) or untagged PINK1 (n = 2). Bars represent the mean ± S.E. of the percent change from basal (staurosporine-un-treated) expression. Wt, wild-type.
Wild-type PINK1 Prevents Neuronal Apoptosis

FIGURE 5. Wild-type PINK1 expression reduces staurosporine-induced caspase-9, -3, and -7 and PARP activation. A, the mitochondrial stress pathway begins with the release of cytochrome c from mitochondria, which then interacts with Apaf-1, causing self-cleavage and activation of caspase-9. The effector caspases, caspase-3 and caspase-7, are downstream of the activator caspases and act to cleave various cellular targets, including PARP, which leads to DNA fragmentation and cell death by apoptosis. B, equal amounts of lysates from non-treated (−) or staurosporine-treated (+) mock or wild-type (wt) untagged PINK1 stably transfected SH-SY5Y cells were loaded on Tricine gels, and Western blotting was then performed with cleaved caspase-9, caspase-7, and caspase-3-specific antibodies as described. Protein loading was monitored by actin analysis. C, wild-type (Wt) PINK1 expression was analyzed by Western blotting with an anti-PINK1 LWP2 antibody in total lysates of non-treated (−) or staurosporine-treated (+) mock and PINK1 stably expressing SH-SY5Y cells. The upper band is a non-specific band identified by the LWP2 antibody.

PINK1-FLAG cells compared with mock-transfected cells (Fig. 6A). In contrast, the presence of PD-related mutations (single E240K, L489P, or double E240K/L489P) totally abolished the inhibitory effect of wild-type PINK1 on caspase-3 activation. Indeed, E240K V5-PINK1 mutant-expressing cells displayed 111.1% ± 8.1 caspase-3 activity, L489P V5-PINK1-expressing cells displayed 118.5% ± 13.2 caspase activity, and E240K/L489P V5-PINK1-expressing cells displayed 93.5% ± 6.9 caspase-3 activity values, which were not statistically different from those of mock-transfected control cells (p = not significant (ns); Fig. 6A). Similar results were observed in cells expressing FLAG-tagged PINK1, where double E240K/L489P PINK1-FLAG mutant-expressing cells displayed caspase-3 levels comparable with those of mock-transfected cells (p = not significant (ns); Fig. 6A). These results were not explained by the differing levels of expression of the mutant PINK1 proteins.

In the same experiment, we also investigated the effect of the C-terminally V5-tagged or N-terminally FLAG-tagged “kinase dead” K219M PINK1 mutant constructs. These kinase dead mutant constructs behaved in SH-SY5Y cells in exactly the same way as the PD-related PINK1 mutants. Indeed, they totally abolished the inhibitory effect of PINK1 on caspase-3 activation (98.4% ± 6.1 and 85.5 ± 4.5 for K219M V5-PINK1 and K219M PINK1-FLAG, respectively, p = not significant (ns); Fig. 6A).

Finally, we obtained primary cultures of human skin fibroblasts from a neurologically normal elderly male and from a 74-year-old male familial PD patient with the compound E204K/L489P mutation in PINK1 (2). Reverse transcription PCR studies confirmed that PINK1 mRNA is robustly expressed in the fibroblasts of both the normal control and the PINK1 mutant carrier. The analysis of basal caspase-3 activity in cell lysates of the fibroblasts from the PD patient showed much higher basal caspase-3 activity than the lysates of fibroblasts from the age-matched control (147.7% ± 12.9 in E204K/L489P mutant PINK1 fibroblasts compared with control; Fig. 6C). Although this result is limited to primary cultures of fibroblasts from a single PINK1 mutation carrier, it is nevertheless consistent with the results of the other studies using cell lines overexpressing exogenous PINK1.

DISCUSSION

The etiology of Parkinson disease is complex and poorly understood. Toxins such as rotenone and N-methyl-4-phenyl-1,2,5,6-tetrahydropyridine (MPTP), which inhibit complex I activity in the mitochondrial electron transport system, can induce parkinsonism. Reduced complex I activity has been reported in PD not only in the substantia nigra but also in the skeletal muscle and platelets. These and other observations have led to the hypothesis that mitochondrial dysfunction, which generates oxidative stress, contributes to the etiology of PD (reviewed in Ref. 13).

The mitochondrial localization of PINK1 and the putative role that mitochondria play in PD neurodegeneration suggest that PINK1 may have a modulating effect on mitochondrial dependent cell death pathways. In the present study we therefore investigated the role of PINK1 in basal and staurosporine-induced neuronal apoptosis. Our results establish that in neuronal cells both transient and stable expression of PINK1 did the following: 1) prevented basal and staurosporine-induced cytochrome c translocation from mitochondria to the cytosol; 2) reduced activation of caspase-9, -7, and -3 and PARP; 3) prevented TUNEL-positive DNA fragmentation; and 4) strongly reduced both basal apoptotic activity and staurosporine-induced cell death. These results are likely to be highly relevant to the role of PINK1 mutants in vivo, because primary cultured human skin fibroblasts expressing PD-related PINK1 also displayed a much higher basal apoptotic activity compared with fibroblasts from a normal individual or neuronal cells expressing wild-type PINK1. Importantly, PINK1 localization itself does not appear to be modified by apoptosis induction, suggesting that the anti-apoptotic
effect of PINK1 is likely to be mediated by direct or indirect effects on the mitochondrial apoptotic cascade.

We have previously predicted that both of two missense mutations (E240K and L489P) observed in a compound heterozygous patient with PD affect residues within the predicted catalytic domain of PINK1 and are likely to alter PINK1 activity (2). In the present study, we have shown that the presence of either one of these mutations totally abrogates the basal PINK1 protection against cell death, confirming that they do indeed affect PINK1 function, either through loss of the catalytic activity and/or through instability of the mutant protein. This conclusion is supported by the observation that the artificial K219M kinase dead PINK1 mutant is also totally inactive against basal and induced apoptosis. These results are strikingly similar to those recently demonstrated for another protein involved in PD, namely α-synuclein. Wild-type but not mutant α-synuclein also limited cell death triggered by serum withdrawal (14).

The anti-apoptotic function of wild-type PINK1 suggests that altered phosphorylation of one or more target proteins might be involved in the neuronal degeneration of PD. PINK1 was originally discovered by transcription profiling experiments and is one of several genes that are transcriptionally transactivated by the PTEN gene (9). PINK1 shows variable levels of expression in different cancer cell types. The PTEN protein acts as a tumor suppressor through direct antagonism of phosphatidylinositol 3-kinase signaling (15, 16). PTEN promotes apoptosis by inhibiting activation of the cell survival kinase Akt in neurons (15, 16), and PTEN overexpression causes increased sensitivity to excitotoxic cell death in hippocampal neurons (17). Thus, PINK1, which is up-regulated by PTEN, appears to have an opposite effect on apoptosis. We hypothesize that PINK1 may serve as a feedback modulator of the pro-apoptotic activity of PTEN, although PINK1 might also regulate other genes, proteins, and cell death pathways independently of PTEN. The recent observation of a functional link between PTEN and DJ-1 (18), another gene in which recessive mutations also cause PD (7, 8), raises the question of whether PINK1 and DJ-1 function in the same signaling pathway.
The identification of proteins phosphorylated by PINK1 will be an important step in the understanding of PD pathogenesis.

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