α-Synuclein Aggregates Interfere with Parkin Solubility and Distribution

ROLE IN THE PATHOGENESIS OF PARKINSON DISEASE

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Parkinson disease (PD) belongs to a heterogeneous group of neurodegenerative disorders with movement alterations, cognitive impairment, and α-synuclein accumulation in cortical and subcortical regions. Jointly, these disorders are denominated Lewy body disease. Mutations in the parkin gene are the most common cause of familial parkinsonism, and a growing number of studies have shown that stress factors associated with sporadic PD promote parkin accumulation in the insoluble fraction. α-Synuclein and parkin accumulation and mutations in these genes have been associated with familial PD. To investigate whether α-synuclein accumulation might be involved in the pathogenesis of these disorders by interfering with parkin solubility, synuclein-transfected neuronal cells were transduced with lentiviral vectors expressing parkin. Challenging neurons with proteasome inhibitors or amyloid-β resulted in accumulation of insoluble parkin and, to a lesser extent, α-tubulin. Similarly to neurons in the brains of patients with Lewy body disease, in co-transduced cells α-synuclein and parkin colocalized and co-immunoprecipitated. These effects resulted in decreased parkin and α-tubulin ubiquitination, accumulation of insoluble parkin, and cytoskeletal alterations with reduced neurite outgrowth. Taken together, accumulation of α-synuclein might contribute to the pathogenesis of PD and other Lewy body diseases by promoting alterations in parkin and tubulin solubility, which in turn might compromise neural function by damaging the neuronal cytoskeleton. These studies provide a new perspective on the potential nature of pathogenic α-synuclein and parkin interactions in Parkinson disease.

Parkinson Disease (PD) belongs to a group of heterogeneous movement disorders jointly named Lewy body disease (LBD) (1). These conditions are associated with progressive and selective loss of dopaminergic and non-dopaminergic cells (2) and the formation of Lewy bodies (LBs) and Lewy neurites, which contain fibrillar α-synuclein (α-syn) (3–6).

Although the identification and distribution of α-syn-immunoreactive LBs is a useful neuropathological marker for the diagnosis of PD and LBD (7–9), recent studies suggest that abnormal neuronal accumulation of α-syn oligomers and protofibrils (10–12) might be centrally involved in the pathogenesis of the neurodegenerative process in these disorders. α-Synuclein is an abundant synaptic protein (13) that interacts with a variety of proteins (14, 15), including those involved in regulating the vesicular release of dopamine (16, 17).

While the cause of sporadic PD is still unclear, familial forms of PD have been linked to mutations in various genes including α-syn, parkin, DJ1, PTEN-induced kinase 1 (PINK1), and leucine-rich repeat kinase-2 (LRRK2) (18–21). Missense mutations (A30P, A53T, and E46K) and multiplications in the α-syn gene (22, 23) that accelerate aggregation and toxic conversion of α-syn have been described in a few families with autosomal dominant PD (24). Mutations in parkin are the most common cause of familial parkinsonism (25–27). Several reports indicate that parkin functions as an E3 ubiquitin ligase and that familial-linked mutations in parkin disrupt its ligase activity (28, 29) and de-stabilize its ubiquitin-like domain (30). In sporadic forms of PD and LBD, parkin accumulates in the insoluble fraction (31). In addition to incorporating ubiquitin to a number of substrates (32) such as the aminoacyl tRNA synthetase cofactor p38/JTV-1 (p38), α-tubulin, cell division control-related protein-1 (CDCrel-1, also known as the septin, Sept5), glycosylated α-syn (33), Parkin-associated endothelin receptor-like receptor (Pael-R) (34), and synphilin-1, parkin ubiquitiates itself as an early step in its proteasome-mediated degradation process (29, 35, 36). Of these substrates, more recent studies in parkin knock-out mice have shown that p38 is the most important parkin substrate (37); however, p38 is unlikely to be a target of parkin-mediated degradation because a previous study showed that p38 is largely mono-ubiquitinated in the presence of parkin, and poly-ubiquitinated p38 is difficult to detect (38).

Based on the genetic evidence and the known role of parkin as a ubiquitin ligase, most studies have focused at investigating the role of parkin alterations and proteasomal dysfunction on α-syn accumulation in the pathogenesis of PD (28, 39). However, recent evidence suggests that mutations (40) and stress...
factors might lead to the accumulation of parkin, and that translocation of parkin into the insoluble fraction might impair neuronal cell function (41–43). Moreover, because parkin and α-syn associate (44) and co-localize in LBs (31), and posttranslational alterations are often found in patients with sporadic PD and LBD (45, 46), then it is also possible that α-syn might interfere with parkin by promoting aggregation and accumulation in the insoluble fraction.

In this context, we explored the pathological interactions between α-syn and parkin. We found that α-syn accumulation interfered with parkin and α-tubulin solubility and distribution, leading the cytoskeletal alterations and neuronal dysfunction. This study provides a new perspective about the nature of the interactions between α-syn and parkin.

**EXPERIMENTAL PROCEDURES**

**Tissue Culture**—Briefly, as previously described (47), B103 neuronal cell lines stably transfected with either human α-syn, β-syn, or empty vector (pCEP4; Invitrogen, Carlsbad, CA) were used. These cells were routinely maintained in Dulbecco’s modified Eagle’s medium containing 10% fetal bovine serum (Irvine Scientific, Irvine, CA) in the presence of 50 μg/ml hygromycine B (Calbiochem, San Diego, CA) in a 5% CO2 atmosphere. This neuronal cell line, derived from rat neuroblastoma, was selected because α-syn overexpression results in the formation of discrete insoluble aggregates in the cell body and processes accompanied by reduced neurite outgrowth (47), mimicking another important aspect of LBD, namely compromised axonal plasticity (48).

Primary rat cortex (P0) was dissected, digested by trypsin, mechanically dissociated, and plated at 8 × 10^4–1 × 10^6 cells per dish/well under serum-free conditions in neurobasal A media supplemented with B27. Primary cultured neurons were maintained for a minimum of 14 divisions prior to co-transfection with lentiviruses expressing parkin, α-syn, or GFP.

**Lentivirus-meditated Transfection and Treatment with MG132**—Lentiviral vectors encoding α-syn (lenti-α-syn), β-syn (lenti-β-syn), parkin (lenti-parkin), the pcDNA(+)Myc-parkin was a kind gift from Dr. N. Hattori, Department of Neurology, Juntendo University School of Medicine, Japan), APP(sw), or green fluorescent protein (lenti-GFP) were prepared as previously described (49). Briefly, 2.5 × 10^6 cells growing in 6-well plates were incubated with either lenti-α-syn, lenti-β-syn, lenti-parkin, lenti-APP(sw), lenti-GFP (1:1000 dilution of preparations of 1.5 × 10^7 transduction units), or vehicles alone in 10% fetal bovine serum at 37°C and 5% CO2 for 3 days. Cells were then washed with phosphate-buffered saline and incubated with Dulbecco’s modified Eagle’s medium containing 10% fetal bovine serum with 10 μM MG132 (Calbiochem) for 20 h. The efficiency of transduction of each lentivirus was confirmed to be more than 90% by confocal microscopy. The cells were either harvested in buffer containing 1% Triton X-100 and used for immunoblot analysis and immunoprecipitation experiments or fixed in 4% paraformaldehyde for 20 min for immunocytochemistry.

**Antibodies**—Mouse monoclonal anti-α-tubulin was obtained from Sigma. Rabbit polyclonal anti-ubiquitin, mouse monoclonal anti-actin, and rabbit polyclonal anti-β-syn were from Chemicon (Temecula, CA). Mouse monoclonal anti-α-syn was from BD Transduction Laboratories (Lexington, KY). Mouse monoclonal anti-c-Myc was purchased from Santa Cruz Biotechnology (Santa Cruz, CA). Rabbit polyclonal anti-parkin was from Cell Signaling Technology (Beverly, MA). Mouse monoclonal anti-Aβ was obtained from Signet Laboratories (Dedham, MA).

**Immunoblot and Immunoprecipitation Analyses**—Immunoblot analysis was performed as previously described (50). Briefly, cells were lysed in buffer A (20 mM Tris-HCl, pH 7.5, 150 mM NaCl, 1 mM EDTA, 1 mM NaVO4, 50 mM NaF, with protease inhibitors (Roche Applied Science)) containing 1% Triton X-100. After incubation at 4°C for 20 min, cell lysates were separated into detergent-soluble and -insoluble fractions by centrifugation at 15,000 × g at 4°C for 20 min. Frozen tissues from the temporal cortex of six LBD and five age-matched control cases (Table 1) were analyzed for parkin accumulation in the insoluble fraction. Cases were obtained from the UCSD Alzheimer Disease Research Center. For each case, 100 μg of frozen superior temporal neocortical sample were homogenized in 3× volume buffer A and centrifuged at 1,000 × g for 15 min. 1% Triton X-100 was added to supernatant, and samples were separated into soluble and insoluble fractions by centrifugation at 15,000 × g for 20 min. Fractions were then used for immunoblotting.

The detergent-soluble and -insoluble fractions were resolved with SDS-PAGE and transferred to a polyvinylidene difluoride membrane filter (Immobilon P; Millipore, Bedford, MA). The membranes were blocked with Tris-buffered saline containing 5% skim milk, flowed by incubation with primary antibody in Tris-buffered saline containing 5% skim milk. After washing with Tris-buffered saline, proteins were visualized by enhanced chemiluminescence and analyzed with a Versadoc XL imaging apparatus (Bio-Rad). Analysis of actin levels was used as a loading control.

**Immunoprecipitation assays** were carried out essentially as previously described (51). Cells were lysed in buffer A containing 1% Triton X-100. The lysates were then centrifuged for 20 min at 12,000 rpm, and the protein concentrations were determined with a protein assay kit (Bio-Rad). 300 μg of the supernatants were incubated with 1 μg of antibody for parkin, α-tubulin, or α-syn overnight at 4°C, and then immunocomplexes were adsorbed to protein A- or G-Sepharose beads (Amersham Biosciences). After washing extensively with buffer A contain-
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FIGURE 1. α-Syn promotes the accumulation of parkin and α-tubulin in the insoluble fraction in a neuronal cell line. For all panels, cells were lysed in buffer containing 1% Triton X-100 and fractionated into detergent-soluble and -insoluble fractions, followed by immunoblot analysis. A, immunoblot analysis of the soluble fraction of synuclein-transfected B103 cells that were infected with lentivirus (lenti) expressing parkin or GFP and treated with 10 μM MG132 for 18 h. B, immunoblot analysis with an antibody against parkin shows that in α-syn-transfected cells, parkin accumulated in the insoluble fraction in the presence of MG132. C, parkin accumulation in the insoluble fraction of α-syn-expressing neuronal cells infected with lenti-Parkin and amyloid precursor protein (APP)sw (high level of Aβ42 expression) but not in cells infected with APPsw(−) (a mutant that cannot be cleaved and therefore no Aβ is generated). D, di-ubiquitinated (di-Ub) form of parkin was also detected in blots developed with long exposures. E, blocking α-syn expression with a specific siRNA resulted in decreased parkin accumulation in the insoluble fraction. Synuclein-transfected B103 cells were infected with lenti-parkin or GFP and treated with siRNA to block α-syn expression. Cells were subsequently treated with MG132. Cell lysates were probed by Western blot for α-syn and parkin. E, immunoblot analysis with an antibody against α-tubulin showed that in cells expressing α-syn, α-tubulin accumulates in the insoluble fraction in the presence of MG132. For each blot, actin levels were used as a loading control.

In Vitro Ubiquitination Assay—For immunoprecipitation, parkin was bound to anti-Myc IgG-linked protein G beads from extracts of 293T cells transfected with pcDNA3.1 (+) Myc-parkin. To prepare aggregates of α-syn, recombinant α-syn was incubated in 20 μl of distilled water at 65 °C for 24 h essentially as previously described (53). Reactions were performed in a 50-μl mixture containing 50 mM Tris-HCl, pH 7.5, 2 mM MgCl₂, 2 mM ATP, 10 μg of ubiquitin (Boston Biochem, Boston, MA), 100 ng of E1 (Calbiochem, San Diego, CA), 200 ng of UbcH7 (Boston Biochem, Boston, MA), immunoprecipitated Myc-tagged parkin, and 5 μg of purified α-tubulin (Cytoskeleton Inc, Denver, CO) with 0.25 nmol of bovine serum albumin, 0.25 nmol of recombinant soluble or aggregated α-syn. The reactions were carried out for 2 h at 37 °C before terminating with an equal volume of 2× SDS sample buffer. The reaction mixtures were then analyzed by immunoblotting for ubiquitin antibody.

Small Interfering RNA (siRNA) Studies—Both control siRNA and siRNA for α-syn were purchased from Santa Cruz Biotechnology. Transfection of the siRNA (final concentration, 100 nm) was carried out using OligofectAMINE (Invitrogen) for 72 h with lentivirus transfection. Cells were then lysed and used for immunoblot analysis.

RESULTS

α-Synuclein Promotes the Abnormal Accumulation of Parkin and Tubulin into the Detergent-insoluble Fraction of Neurons—To investigate the effects of α-syn on parkin accumulation and cellular distribution, stably transfected B103 neuroblastoma cells expressing α-syn-, β-syn-, or vector control were infected with a lentiviral vector expressing parkin. For this, we first analyzed the levels of α- and β-syn in the presence or absence of MG132 (Fig. 1A) because previous studies have shown that proteasomal inhibition facilitates the pathological effects of α-syn.
that the amount of insoluble parkin was dramatically decreased in experimental conditions, immunoblotting analysis revealed that with a lentiviral vector expressing familial-linked A53T mutant effects were more pronounced in primary neurons infected with vector- or β-syn-transfected cells (Fig. 1B). Similarly, insoluble parkin accumulated in α-syn-transfected cells when cells were treated with lactacystin, another proteasome inhibitor (data not shown), or infected with a lentiviral vector expressing the AD-related mutant amyloid precursor protein (APPsw) (Fig. 1C). Actin levels were not affected under either condition (Fig. 1C). To confirm the effects of α-syn on parkin, siRNA experiments were performed. When cells were transfected with siRNA targeting α-syn mRNA, expression levels of α-syn significantly decreased in α-syn-transfected cells both in the presence and absence of MG132 (Fig. 1D). Under these experimental conditions, immunoblotting analysis revealed that the amount of insoluble parkin was dramatically decreased by α-syn siRNA compared with control siRNA in α-syn-transfected cells, whereas insoluble parkin was not changed by α-syn siRNA in vector-transfected cells (Fig. 1D).

Recent studies have shown that parkin-mediated ubiquitination promotes degradation of target proteins such as α-tubulin (55). Therefore, we reasoned that, similarly to parkin, α-syn might interfere with α-tubulin clearance. Immunoblot analysis showed that in the presence of MG132, the levels of insoluble α-tubulin were considerably increased in α-syn-transfected cells even when parkin was overexpressed (Fig. 1E).

We next examined whether α-syn accumulation may promote similar effects on parkin and α-tubulin in primary neurons. For this purpose, rat cortical primary neurons were co-infected with lentiviral vectors expressing parkin and α-syn. Immunoblot analysis showed that in the presence of MG132, α-syn expression promoted accumulation of insoluble parkin and, to a lesser extent, α-tubulin in the detergent-insoluble fractions compared with lenti-GFP control (Fig. 2). These effects were more pronounced in primary neurons infected with a lentiviral vector expressing familial-linked A53T mutant α-syn (Fig. 2). β-Synuclein had no effect on insoluble parkin or α-tubulin accumulation in primary neurons (not shown). Together, these results support the possibility that α-syn might promote abnormal accumulation of parkin and α-tubulin in the detergent-insoluble fraction that might affect the neuronal cytoskeleton.

Parkin and α-Tubulin Are Increased in the Insoluble Fraction in the Brains of LBD—To investigate whether α-syn accumulation in patients with LBD results in similar alterations in parkin as observed in neuronal cell lines, immunoblot analysis with detergent-insoluble fractions was performed. This analysis showed that in patients with LBD, levels of parkin and α-tubulin in the detergent-insoluble fraction were significantly increased compared with unimpaired controls (Fig. 3, A–C). In the detergent-soluble fraction, no differences were detected between the two groups (Fig. 3, A–C).

Double immunocytochemical analysis confirmed that compared with controls (Fig. 3, D–F), in LBD α-syn and parkin were co-localized in the neocortical LBs and neurites (but not in synapses) (Fig. 3, G–I). These results support the notion that aggregated α-syn might lead to an increase of insoluble parkin and α-tubulin in vivo.

Parkin Self-ubiquitination and Ubiquitination of α-Tubulin Are Altered by α-Syn Overexpression—It has been previously shown that parkin self-ubiquitinates and that ubiquitinated parkin is rapidly degraded by the proteasome (29, 35, 36, 56). Therefore we reasoned that α-syn might interfere with parkin ubiquitination, resulting in reduced solubility. To examine this possibility, parkin immunoprecipitates were analyzed with an antibody against ubiquitin. This study showed that levels of ubiquitinated parkin were decreased in α-syn-transfected cells treated with MG132 compared with controls (Fig. 4, A and B). Consistent with previous reports (57–59), high molecular weight ubiquitinated parkin was only detected in the presence of a proteasome inhibitor.

Because insoluble α-tubulin also accumulates in cells co-expressing α-syn and parkin, it is possible that ubiquitination of α-tubulin might also be altered. To test this possibility, cell extracts were immunoprecipitated with an α-tubulin monoclonal antibody, followed by analysis with an antibody against ubiquitin (Fig. 4C). Immunoblot analysis showed that in the presence of MG132, ubiquitinated α-tubulin is reduced in α-syn-transfected cells when compared with vector- and β-syn-transfected cells co-transfected with parkin (Fig. 4, C and D). Thus, α-syn overexpression reduces ubiquitinated α-tubulin and this might lead to accumulation of insoluble α-tubulin. To confirm that total cellular levels of ubiquitinated proteins were not affected by α-syn overexpression, total cell extracts were subjected to immunoblot analysis for ubiquitin. These results demonstrated that synuclein expression did not alter the total levels of ubiquitinated proteins (Fig. 4, E and F). Taken together, these results suggest that under stress conditions α-syn might reduce ubiquitinated parkin and α-tubulin by interfering with parkin ubiquitin ligase activity.
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α-Synuclein and Parkin Co-immunoprecipitate in Neuronal Cell Lines—The alterations in parkin and α-tubulin solubility in α-syn-overexpressing cells might be related to direct interactions between α-syn and parkin. Because α-syn and parkin colocalize in LBs, and we have shown that α-syn promotes parkin and α-tubulin accumulation in the detergent-insoluble fraction of brain homogenates, we hypothesized that α-syn might interact with parkin. First, to investigate the cellular localization of α-syn and parkin, double immunolabeling experiments were performed (Fig. 5, A–L). These studies showed that in the absence of MG132, α-syn and parkin were colocalized to the cytoplasm and neuritic processes with a diffuse pattern (Fig. 5, G–I). In contrast, in the presence of MG132, increased parkin immunoreactivity was detected in α-syn-transfected cells. Furthermore, both of these molecules were colocalized to inclusion-like structures (Fig. 5, J–L).

To confirm the association between α-syn and parkin in α-syn-transfected neuronal cells, co-immunoprecipitation experiments were performed. For this purpose, cell extracts were subjected to immunoprecipitation with an anti-α-syn antibody, followed by immunoblotting with an antibody against parkin. Compared with vector and β-syn-transfected cells, α-syn was efficiently immunoprecipitated in α-syn-transfected cells, and parkin was found to consistently co-immunoprecipitate with α-syn in the presence of MG132 (Fig. 5M). Because of the inherent challenge in demonstrating the specificity of immunoprecipitation of insoluble proteins, co-immunoprecipitation of parkin and α-syn was confirmed by immunoprecipitation with an anti-parkin antibody, followed by immunoblotting with an antibody against α-syn. This confirmed that parkin and α-syn co-immunoprecipitate in the presence of MG132 (Fig. 5M). Parkin and α-syn also co-immunoprecipitated in the absence of MG132, albeit to a much lesser extent (Fig. 5M). Taken together, these results support the possibility that interactions between α-syn and parkin lead to the formation of insoluble aggregates that might directly damage the neuronal cytoskeleton rather than impairing parkin function.

α-Synuclein inclusions might affect the neuronal microtubule structure. To examine the pattern of the cellular microtubule network in synuclein- and parkin-transfected cells, we performed immunohistochemical studies for α-tubulin. A well organized α-tubulin-immunoreactive microtubule network was detected under baseline conditions (Fig. 6, A–C and M); in contrast, treatment with MG132 resulted in neurite retraction in α-syn-transfected cells, but not in vector or β-syn-transfected cells (Fig. 6, D–F, and M). In combination with parkin and MG132, the cytoskeletal alterations were exacerbated in the α-syn-transfected cells compared with vector control or β-syn (Fig. 6, G–L, and M).

DISCUSSION

Although both α-syn and parkin are linked to the molecular pathogenesis of familial PD (39), whether α-syn aggregation may affect parkin in PD and LBD has not been previously investigated. For the present study, we found that in the presence of stress conditions (namely proteasomal inhibition with MG132
or lactacystin, or Aβ treatment), α-syn promoted parkin and, to a lesser extent, α-tubulin accumulation in the insoluble fraction of neurons and resulted in cytoskeletal alterations.

These findings are consistent with recent studies showing that pro-oxidants (nitric oxide, iron, hydrogen peroxide), neurotoxins (1-methyl-4-phenylpyridinium, rotenone, paraquat), and dopamine analogs promote similar alterations in parkin solubility (42). Moreover, alterations in parkin solubility were associated with an increased tendency of parkin to aggregate and precluded parkin from exercising its protective functions (42). Similarly, mutations associated with familial PD that will be focused on in the future. Alternatively, and in view of recent studies showing that PD-related stress factors promote parkin insolubility (41–43), the parkin aggregates might be toxic and disrupt the neuronal cytoskeleton by compromising α-tubulin solubility.

In the present study, the effects of α-syn on parkin and α-tubulin solubility were observed primarily in the presence of proteasomal inhibitors or Aβ. α-synuclein exerted more prominent effects on parkin than on α-tubulin solubility. Because inhibition of proteasomal activity by itself promotes accumulation of insoluble parkin and interferes with parkin activity (42),
this suggests that stress conditions may be necessary for the pathological interactions between α-syn and parkin to occur. This is consistent with recent studies showing that proteasomal inhibitors interfere with parkin solubility (42). Furthermore, during the aging process and in the presence of Aβ (66), proteasomal activity is reduced (67) and insoluble α-syn and parkin levels are increased (42, 68). Therefore, the combination of oxidative stress, neurotoxins, and mutations associated with familial parkinsonism might provide the conditions to facilitate the pathological interactions between α-syn and parkin.

There are several possible ways through which cellular stress and α-syn aggregates might promote parkin accumulation. For example, recent studies have shown that nitrosative stress leads to S-nitrosylation of parkin, which in the long term results in a dramatic decrease in E3 ligase ubiquitin proteasome degradative activity (69, 70). In agreement with this possibility, previous studies have shown that insults associated with a PD-like phenotype induced similar alterations in microtubules resulting in neurodegeneration (71, 72), and the neurons in patients with PD and LBD exhibit significant cytoskeletal alterations (61, 73, 74). Moreover, a recent study showed that α-syn aggregates impair microtubule-dependent trafficking (60).

It was previously shown that wild-type parkin reduces the toxicity induced by dopamine in SH-SY5Y cells (75), by hydrogen peroxide, hydroxynonenal, and 1-methyl-4-phenylpyridinium in NT-2 cells (76), and is capable of rescuing the phenotype associated with α-syn overexpression in the rat (77, 78).
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and in Drosophila (79). In contrast, in our model system, α-syn overexpression altered parkin solubility and resulted in cytoskeletal alterations. The differences in models used and the relative levels and efficiency in the expression of α-syn and parkin with the viral vectors might explain the apparent discrepancy between our results and those of others. In addition, the effects of α-syn aggregates on parkin were detected primarily in the presence of proteasomal inhibitors, and compared with wild-type α-syn, mutant A53T α-syn had a more prominent effect on parkin. Moreover, although previous studies have shown that parkin protects against a variety of neurotoxins (76, 80), it has also been shown that parkin has no effects in delaying cell death associated with proteasome inhibition (76), suggesting that increased levels of cellular stress are necessary for the pathological interactions between parkin and α-syn to take place.

These findings have important implications for understanding the pathogenesis of PD and LBD as they provide a potential mechanistic link through which two molecules associated with familial PD-α-syn and parkin might interact to promote neurodegeneration. However, it remains controversial as to what extent parkin alterations precede or follow α-syn pathology in sporadic PD and LBD, and whether in familial cases with mutations in the parkin gene, the PD phenotype might emerge independently of α-syn and vice versa.

In conclusion, our results indicate that α-syn aggregates induce abnormal accumulation of parkin and, to a lesser extent, α-tubulin, resulting in cytoskeletal pathology. Moreover, these studies provide a new model for the pathological interactions between α-syn and parkin that might help better understand the pathogenesis of PD and LBD.

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