Molecular Pathogenesis of Parkinson Disease

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Parkinson disease (PD), the most common neurodegenerative movement disorder, is characterized by an extensive and progressive loss of dopaminergic neurons in the substantia nigra pars compacta. One of the pathological hallmarks of PD is the presence of Lewy bodies, intracellular inclusions of aggregated α-synuclein. Although the cause and pathogenesis of selective loss of dopamine neurons and the accumulation of α-synuclein in PD remain elusive, growing lines of evidence from environmental risk factors and early-onset genetics point to a convergence between energy metabolism and the disposal of damaged proteins in the development of PD. These findings suggest that impairments in mitochondrial and ubiquitin-proteasome system function can significantly contribute to the pathogenesis of PD. This review will summarize recent insights gained from genetic and environmental studies of PD that underscore this association.

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Parkinson disease (PD) is clinically defined as a neurological disorder characterized by 4 cardinal signs (resting tremor, bradykinesia, rigidity, and postural instability). Parkinson disease affects approximately 1% of the population by the age of 65 years, increasing to 4% to 5% of the population by the age of 85 years.1 The disease is chronic and progressive. Patients experience increasing difficulty in daily living functions along the course of the disease. Although environmental risk factors for PD have received significant interest, the importance of genetic factors underlying the likelihood of developing PD is increasingly recognized. Despite the overall rarity of the familial forms, which compose less than 10% of all cases, the identification of several genes causing early-onset PD (such as α-synuclein, UCHL1 [a ubiquitin carboxy-terminal hydrolase L1], parkin, DJ1 [a parkin-associated protein involved with oxidative stress], and PINK1 [a putative serine threonine kinase]) has yielded crucial insights into the possible pathogenic mechanisms.1 Several other genes are associated with the parkinsonian phenotype, but usually additional clinical signs such as dementia, dystonia, or ataxia are evident on neurological examination.

Parkinson disease is initially recognized by its clinical symptoms and ultimately diagnosed in postmortem analysis by the presence of Lewy bodies and the loss of dopaminergic neurons in the substantia nigra pars compacta.1 These neurons give rise to the nigrostriatal pathway, and, as a consequence of the neuronal loss, there is a depletion in striatal dopamine resulting in the clinical phenotype. Lewy bodies, found in significant quantities in PD brain specimens, consist of eosinophilic inclusions containing a loosely compacted core of aggregated α-synuclein and other proteins. Therefore, it was not surprising that the first genetic evidence to early-onset familial PD was linked to the α-synuclein gene (SYNCA-PARK1 locus). Linkage studies and subsequent mutation analysis of the SYNCA gene in the Contursi kindred and several Greek kindreds with autosomal dominant early-onset PD identified an autosomal dominant missense mutation (A53T).1

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Subsequent to this initial finding, 2 additional missense mutations in SYNCA, A30P and E46K, were respectively identified in a German and a Spanish family with autosomal dominant early-onset PD. The role of α-synuclein as a key player in the pathogenesis of PD was further revealed when Singleton and colleagues discovered a genomic triplication of SYNCA in the original Spellman-Muenter-Iowa kindred, another autosomal dominant PD family; a concomitant increase in the soluble α-synuclein protein confirmed this finding. An important implication of this study was that the aberrant metabolism (protein mis-handling) and increase of wild-type α-synuclein is likely to be a precipitating cause of dopaminergic neuronal loss in PD. While missense mutations in α-synuclein are likely to be pathogenic, it is unclear whether these mutations result in an altered functional protein or enhance the rate of cytoplasmic synuclein aggregation leading to disease.

Parkin mutations (PARK2) cause a form of autosomal recessive juvenile PD (ARJP); however, patients with ARJP are atypical in clinical presentation (dystonia is common) and in most cases there is absence of Lewy bodies. Parkin is a ubiquitin E3 ligase that functions to prepare substrates for protein degradation mediated through the ubiquitin-proteasome system (UPS). Initially, much effort was focused on the relationship between parkin and the putative substrates that might occur in Lewy bodies (ie, α-synuclein) because the accumulation of these substrates could be potentially toxic to cells. Our insight into the role of parkin in PD was changed when it was shown that fruit fly models of the disease, made by inactivation of parkin, had little effect on dopaminergic neurons, whereas muscle mitochondrial seemed compromised. In support of this finding, knocking out the gene in mice caused several physiological changes but no neuronal loss in the substantia nigra, despite the severe loss of dopamine neurons observed in ARJP. Moreover, steady-state levels of the known parkin substrates were surprisingly unaltered in the parkin knockout mouse. Two-dimensional gel electrophoresis followed by mass spectrometry analysis revealed a decrease in the abundance of several proteins involved in mitochondrial function or in the protection from oxidative stress and age-dependent increase in oxidative damage in the substantia nigra of parkin knockout compared with wild-type mice. In another parkin knockout mouse model, the loss of locus coaeruleus neurons and a marked reduction of the norepinephrine-dependent startle response were observed. The physiological changes in this model have yet to be addressed. Last, parkin was shown to protect against ceramide, an agent that induces mitochondria-dependent apoptosis by reducing mitochondrial swelling and cytochrome c release in vitro. Protection was blocked in the presence of a proteasomal inhibitor, suggesting that the protective action of parkin requires its ubiquitin E3 ligase activity and is mediated through the UPS.

In addition to parkin, mutations in 2 other genes were recently identified that were associated with mitochondrial impairment. Bonifati and colleagues identified a rare form of ARJP caused by mutations in the DJ1 gene (PARK7). The investigators found a large deletion comprising exons 1 to 5 and a point mutation (L166P) in one Dutch and one Italian family, respectively. The L166P missense mutation has been shown to alter the stability and function of the DJ1 protein. Although the exact role of DJ1 remains unclear, wild-type and mutant DJ1 differed in the colocalization to the mitochondria in transfected cells. DJ1 overexpression protects against mitochondrial damage and oxidative stress whereas mutant DJ1 (and DJ1 knockdown experiments) sensitizes cells to these effects.

Valente and colleagues recently identified a familial, early-onset, autosomal recessive form of parkinsonism caused by mutations in a putative mitochondrial protein kinase named PINK1 (phosphatase and tensin homolog–induced kinase–PARK6). PINK1 localizes to the mitochondria, where the protein is believed to have a role in maintaining mitochondrial homeostasis. Mutant PINK1 increases cell susceptibility to stress conditions, inducing mitochondrial dysfunction and apoptosis. Whether PINK1 or DJ1 has potential substrates and/or interactors has not been addressed. Given the convergence of function between these early-onset autosomal recessive genes, it is tempting to speculate whether parkin, DJ1, and PINK1 interact at the functional level. To our knowledge, there is no pathological confirmation of PD for either DJ1 or PINK1 families (because of lack of postmortem tissue). It will be interesting to see whether patients with these mutations present with Lewy body inclusions or present like patients with ARJP. The early-onset genetics brings together a common and recurring theme—mitochondrial impairment, oxidative stress, and proteotoxic stress are critical factors in the pathogenesis of PD. Proteasomal activity is highly dependent on adenosine triphosphate production, and inhibition of either mitochondrial or proteasomal function can lead to PD-like pathological features in experimental models. For example, administration of environmental toxins that disrupt mitochondrial complex I activity, such as N-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (a derivative of a synthetic opiate) and rotenone (a pesticide), induce PD-like symptoms. Rats treated with rotenone show a selective loss of dopaminergic neurons and the formation of Lewy bodies. Similarly, administration of proteasomal inhibitors induces PD-like symptoms in rodents. McNaught and colleagues have examined the effects of inhibiting the UPS in rats by administering synthetic and naturally occurring proteasome inhibitors. Within 2 weeks after receiving the proteasome inhibitors, the rats began to show clinical signs that were remarkably similar to PD patients, including bradykinesia, rigidity, and tremor; brain specimens from these animals had selective neuronal loss and Lewy body–like inclusions in the substantia nigra, locus coaeruleus, and other areas typically affected in PD patients. These findings place mitochondrial impairment, oxidative stress, and proteasome dysfunction at center stage in the pathogenesis of genetic and environmental forms of PD. Environmental factors, such as exposure to toxins that have been
linked to PD, seem to be more important in late-onset forms of the disease whereas in early-onset PD genetic factors assume predominant importance. Nevertheless, similar elements seem to link these patho-
genic factors to a convergent mechanism of cell death. Do events that affect mitochondrial energy production and decrease resistance to oxidative stress result in UPS dysfunction?

METHODS

Considerable evidence suggests the neuronal damage that occurs in early-onset PD may be caused by impairment in the UPS. One influential hypothesis in PD is that subsets of neurons are susceptible to failure in proteasome-mediated protein turnover. It has been previously reported that overexpression of mutant α-synuclein increases sensitivity to proteasome inhibitors by decreasing proteasome function. In addition, mutant α-synuclein also causes selective toxic reaction to catecho-
aminergic neurons in primary mesencephalic cultures, an effect that can be mimicked by the application of proteasome inhibitors. As previously noted, McNaught and colleagues provided compelling in vivo data that inhibition of the UPS resulted in the clinical symptoms and pathological features of PD.

Despite this evidence and the existence of excellent mouse models for PD, in vivo assessment of the involvement of the UPS and analysis of its activity remain major challenges. Specifically, our understanding of the link between proteolysis and disease would benefit from the ability to follow the activity of the UPS in affected brain tissues before and after administration of disease modifiers (eg, N-methyl-4-phenyl-1,2,3,6-tetrahydropyridine and epoxomicin) or in the presence of specific genetic abnormalities (eg, α-synuclein mutations). We and other groups have started to address the question of proteasomal impairment in neurodegenerative disease by developing transgenic mouse lines that allow the UPS to be monitored in vivo. These mice use similar constructs; they express a green fluorescent protein back-bone fused with a proteasome substrate. Impairment of the UPS using pharmacological manipulation leads to an accumulation of the reporter that can be conveniently assessed by fluorescent intensity. These novel mouse models are powerful research tools that will eventually allow investigators to directly test the relationship between mitochondrial activity and proteasomal activity as it relates to the development of PD. The results from these studies will hopefully lead to a better understanding of the pathogenic cascade and provide a rationale for development of therapeutic agents.

RELEVANCE TO THE STUDY OF NEUROSCIENCE

The active turnover of damaged cellular proteins is of major importance in the intracellular environment. By using the methods herein and other cellular and biochemical techniques, questions regarding the involvement of genetic and environmental triggers can be identified. A specific facet of cell death of interest is to monitor the UPS through the progression of a disease. Abnormal and misfolded proteins are degraded primarily by the UPS in an adenosine triphosphate–dependent manner (Figure). Impaired UPS activity, caused by depressed mitochondrial metabolism or through direct inhibition of UPS components, may potentially result in the accumulation of toxic proteins that contribute to the pathogenesis of PD. It has long been suggested that exposure to environmental toxins plays a role in the development of PD; the finding that exposure to rotenone and epoxomicin results in Lewy body inclusions in rodents bolsters this observation. The UPS itself may be inhibited by the accumulation of aggregation-prone proteins, including α-synuclein. Lewy bodies could inhibit the UPS by saturating and/or sequestering one or more molecular chaperones and/or ubiquitin ligases required for UPS function or by interaction with the proteasome. There is scientific evidence to support either of these hypotheses. Indeed, parkin, the molecular chaperones Hsp70/Hsp90, and proteasome subunits colocalize in Lewy bodies and mitochondrial proteins (eg, cytochrome c). Whether protein aggregation is a cause or a consequence of neurotoxicity remains an unanswered question. Nevertheless, a decline in UPS activity due to environmental or genetic reasons may explain the irreversible and precipitous loss of dopamine neurons that characterizes PD. These results are provocative because they suggest that proteasome inhibition might be a common link between the different early-onset genetic and environmental triggers of PD.

RELEVANCE TO THE PRACTICE OF NEUROLOGY

In many diseases, genetic testing offers the physician the ability to verify a differential diagnosis and select an appropriate route of treatment based on an individual’s genetic profile. In PD, accurate diagnosis of the disease cannot be confirmed only by clinical examination because different mutations (ie, SYNCA and parkin) may confer similar behavioral phenotypes but have different underlying neuropathological features. However, the use of genetic screening in individuals with a family history of PD should be carefully evaluated because it is not understood how most of these mutations can cause PD. For instance, a growing body of evidence suggests that, for the autosomal recessive genes, the presence of a single mutation in 1 of the 2 alleles may confer increased susceptibility to PD. Many patients have been described as having single heterozygous mutations in the parkin, DJI, or PINK1 gene. In these cases, the failure to detect a second mutation may have been an artifact of the screening procedure or a result of a genomic rearrangement of the other copy of the gene. However, a more parsimonious hypothesis is that a loss of a single copy of one of these genes results in haploinsufficiency, acting as a susceptibility factor toward PD. Given the finding that these genes only confer susceptibility to PD, epi-
genetic causes are likely significant contributing factors. Factors such as smoking, geographic area, family history, exposure to exogenous toxins
and agents (N-methyl-4-phenyl-1,2,3,6-tetrahydropyridine, pesticides, and infection agents), and aging may greatly influence the propensity for an individual to develop PD, potentially because of effects on other underlying biochemical processes. While there seem to be multiple causes of PD, recent lines of evidence suggest that the mechanisms and potential treatments of this disease are converging on potential pathological processes found in the interplay between the UPS and mitochondria.

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