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What causes cell death in Parkinson's Disease?

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Abstract

There is no proven neuroprotective or neurorestorative therapy for Parkinson's disease (PD) to date. Several advances in the genetics of PD have created an opportunity to develop mechanistic based therapies that hold particular promise for identifying agents that slow and even halt the progression of PD as well as restore function. Here we review many of the advances in the last decade regarding the identification of new targets for the treatment of PD based on understanding the molecular mechanisms of how mutations in genes linked to PD cause neurodegeneration.

Introduction

Parkinson's disease (PD) is a chronic progressive neurodegenerative disorder with no proven neuroprotective or neurorestorative therapy. Identification or proof that a drug is neuroprotective or neurorestorative will herald a new era of PD treatment, much in the same way that Levodopa altered the course and management of PD. To develop neuroprotective and neurorestorative therapies, it is essential to understand the molecular and biochemical mechanisms of PD pathogenesis. It is with this understanding that a fundamental basis for a mechanism-based rationale will be established that determines drug targets for modifying disease progression and tissue regeneration.

Human postmortem material, animal models, and genetic analyses have provided important clues to the etiology of PD. In particular, the genetic approach has recently unraveled a series of proteins that when mutated can cause familial forms of PD. This is insofar important, as this approach provides an entry into identifying the signaling pathways that go errant during the development of PD. Understanding the contribution of these signaling pathways to neurodegeneration in PD will undoubtedly allow for important access points for

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drug targeting. Importantly, understanding the interaction of the signaling pathways that are affected in the different forms of familial PD should help to identify crucial nodal points that may be prudent primary drug targets. In the current manuscript, key proteins mutated in PD are discussed in the context of their potential for drug development. Specifically, we start with the main proteins that cause genetic forms of PD (summarized in table) and conclude with proteins that play a significant role in sporadic disease that have come to the forefront mostly from non-genetic research avenues.

Gain of function mutations in Parkinson's Disease

Autosomal dominant PD is caused by mutations in α -synuclein and LRRK2 (leucine-rich repeat kinase 2), indicating gain-of-function mutations for both proteins¹. Interestingly, α -synuclein is a major component of Lewy bodies and Lewy neurites, which constitute two of the main pathological hallmarks of PD². This observation demonstrates how familial and sporadic forms of PD are molecularly interrelated, emphasizing the notion that insight into the pathogenesis of familial PD should critically advance our knowledge of sporadic PD (Figure 1). This, in turn, implies that drug therapies for familial PD should be applicable to the PD population at large, rather than be confined to a few rare cases.

α -Synuclein is a 140 amino acid protein that shows diffuse cellular localization, as it is found in the cytoplasm, perhaps in the cell nucleus, as well as associated with membrane structures³. α -Synuclein is thought to promote cellular toxicity in aggregate form⁴, although it is not clear whether the oligomeric or the polymeric form produces that effect. However, analogous to findings in the field of Alzheimer's disease, recent evidence suggests that oligomeric α -synuclein can promote cell death, while polymeric α -synuclein can be protective (latter point is discussed separately in the sporadic disease section). This observation has drawn attention to the possibility that reducing α -synuclein levels to avoid oligomer formation may constitute a disease-modifying treatment option, while interfering with conversion from oligomeric to polymeric α -synuclein may be disease-promoting. Accordingly, RNAi and interference with α -synuclein expression through selective transcriptional inhibition are two focused approaches that aim at preventing aggregation at the early stage^{5, 6}. Moreover, strategies that decrease the level of α -synuclein are attractive from the viewpoint that simple duplication or triplication of the wild type α -synuclein causes autosomal dominant PD⁷ and polymorphisms in the α -synuclein promoter that alter the level of expression of α -synuclein determines one's relative risk of developing PD^{8, 9}. Consistent with this notion is also the observation that knockout of α -synuclein dramatically protects against the loss of dopamine neurons in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) model of PD¹⁰. Alternatively, inhibiting α -synuclein oligomers may be an important approach to preventing disease initiation or progression in PD^{11, 12}. In fact, immunoantibodies against oligomeric α -synuclein were developed by active and passive immunization protocols. In parallel to their ability to decrease aggregate formation, these antibodies decrease PD pathology in mice models¹³, implying that injections of antibodies in PD patients may constitute a viable neuroprotective treatment modality of the future. Presently, human trials are in preparation or in the beginning phases. Aside from immunoantibodies, the antibiotic rifampicin has also been shown to inhibit oligomerization of α -synuclein^{14–17}. In a mouse model of multiple system atrophy, a synucleinopathy of

glial cells that can present with parkinsonian features, rifampicin improved motor performance in association with decreased protein aggregation. These findings are promising, as they provide a treatment alternative to the immunoantibodies, thereby allowing a comparison of the side effect profile between the two approaches as well as assessment of synergistic or additive effects in the setting of a combination therapy. Finally, it is known that α -synuclein is cleaved¹⁸, albeit mostly by as yet unidentified α -synucleinases. Given that cleavage of α -synuclein enhances its propensity to aggregate into toxic complexes, identifying the α -synucleinase(s) and developing drugs that inhibit their activity may provide great benefit to PD patients.

As the production of aggregated α -synuclein appears to contribute to neurodegeneration, a separate and independently valuable neuroprotective treatment approach is to facilitate clearance of these protein complexes. Accordingly, much research has been conducted on understanding what protein degradation pathways are involved in degrading wild type and mutant α -synuclein³. α -Synuclein is ubiquitinated, but whether ubiquitination plays a role in the clearance of α -synuclein is not clear. The ubiquitin proteasome system does seem to play a role in PD¹⁹ since defects in the UPS are found in sporadic PD²⁰, and the ubiquitin E3 ligase parkin²¹ (see below) is mutated in some familial forms of PD. Hence, the notion is conveyed that UPS dysfunction results in abnormal protein accumulation and aggregation of proteins, which subsequently leads to cellular dysfunction and neurodegeneration in PD²². In this manner, α -synuclein may accumulate in dopaminergic cells and cause cell death. However, aside from the UPS, other protein degradation pathways may also play a role in PD. The autophagy pathway is one of those alternative routes, where proteins are processed within lysosomes. Such self-digestion can occur in a chaperone-dependent or independent manner, with the former variant requiring the lysosomal receptor, lamp2a. Interestingly, the ill-defined ATP13a2 protein, a ATPase that localizes to the lysosome, is mutated in parkinsonism syndromes, including in the Kufor-Rakeb syndrome and potentially in some juvenile and young-onset Parkinson's disease^{23–25}. Given that in the disease state ATP13a2 redistributes to the endoplasmic reticulum²³, this protein may turn out to provide a first genetic link between lysosomal pathways and PD. In this context, it is notable that wild type α -synuclein can be degraded by chaperone-mediated autophagy (CMA), whereas mutated α -synuclein binds to lamp2a, but is not taken up by the lysosome, thereby preventing its digestion²⁶. Thus, the gain-of-function mutation of α -synuclein may impede CMA-dependent self-degradation, suggesting the ensuing protein accumulation and toxicity to be a secondary and inevitable by product²⁷. Current research is focused on assessing why lamp2a binding of mutant α -synuclein is not sufficient for degradation and how mutant α -synuclein may be degraded in the setting of PD. Some evidence suggests that mutant α -synuclein may also be degraded by autophagy, but potentially by the chaperone-independent pathway. Accordingly, promoting lamp2a-mediated uptake of mutant α -synuclein and improving chaperone-independent autophagy in the dopaminergic cell may be two very promising research avenues that should yield drug targets in the future. Consistent with this notion, lysosomal inhibitors are able to negate the effect of the immunoantibodies that target oligomeric α -synuclein¹³. These observations underscore the importance of the autophagy system in PD, regardless of which subtype is implemented for degrading the aggregate form of α -synuclein²⁸.

Several cellular targets of α -synuclein have been found. The protein is thought to be an important component of the presynaptic protein scaffold that regulates dopamine release. In fact, inhibiting α -synuclein or proteins that interact with it at the synapse alters dopaminergic transmission and synaptic function^{29–31}. This provides a direct link between α -synuclein function and PD, given that dopamine depletion is a hallmark of the disease. Furthermore, aggregated α -synuclein impacts on various membrane structures of the cell. It can form pore-like complexes in the plasma membrane that alter membrane excitability and calcium permeability^{32, 33}, affect the integrity of lysosomal and endosomal compartments, and inhibit vesicular trafficking from the ER to the Golgi apparatus in a Rab-dependent manner³⁴. α -Synuclein also functions as a chaperone to maintain the integrity of the presynaptic terminal since α -synuclein overexpression rescues the neurodegenerative phenotype of cysteine-string protein (CSP) knockout mice³⁵. Despite the progress made in delineating the various cellular functions that mutant α -synuclein may affect, it is not yet understood how much each of these perturbances contributes to overall neurodegeneration. Similarly, it is unknown what precise molecular properties of α -synuclein mediate these toxic effects. An attractive hypothesis suggests that oligomeric mutant α -synuclein may integrate into cellular protein complexes, much like prion proteins, to disrupt physiological protein-protein interactions. It is speculated that the PD mutations trigger changes in secondary protein structures of α -synuclein that promote such toxic protein binding behavior. Questions such as these are under intensive investigation, considering that their resolution will greatly help to prioritize cellular targets for neuroprotective and neurorestorative treatments.

LRRK2 is the other protein mutated in an expected gain-of-function manner. Similarly to the case with α -synuclein, there is evidence that knowledge about pathogenesis of familial PD will translate effectively into understanding sporadic PD. As LRRK2 is very commonly mutated in sporadic cases, with estimates ranging up to 4%, there is great enthusiasm in the research community that understanding LRRK2 function and contribution to PD pathogenesis will benefit a wide spectrum of PD patients¹.

Possessing a molecular weight of 280 kDa, LRRK2 constitutes a very large protein that is localized to the cytoplasm, associated with cellular membranes that include the outer mitochondrial membrane^{36, 37}. LRRK2 is arranged in multiple protein modules, including GTPase Roc and kinase domains. Based on the module analysis, it is thought that LRRK2 belongs to the Roco protein family, a novel group of the Ras/GTPase superfamily. Additionally, the kinase domain appears to have homology to the MAP kinase kinase kinases of the mixed lineage kinase subclass³⁸.

Despite the structural evidence of defined domains with known functions, the overall function of LRRK2 is not well understood. Much data, however, points to the possibility that the gain-of-function is linked to increased kinase activity. Indeed, individual LRRK2 mutations found in PD families, including the G2019S and I2020T mutations, show increased intrinsic kinase activity, when assessed with myelin basic protein (MBP) as substrate^{37, 39}. Moreover, increased LRRK2 kinase activity seems to go hand-in-hand with disease segregating genetic mutations³⁸. Reduction in kinase activity of mutant LRRK2 reduces neurotoxicity^{38, 40, 41}, consistent with the notion that toxicity is linked to enhanced

kinase activity. The neurotoxicity is, at least in part, reflected in apoptosis that is associated with prominent phospho-tau-positive inclusions with lysosomal characteristics and a reduction in neurite length and branching⁴². Altogether then, it appears that increased or abnormal phosphorylation of critical LRRK2 substrates is involved in mutant LRRK2 neurodegeneration.

Despite the support for the model of increased kinase activity, some line of research urges caution with regard to such conclusion, as kinase activity depends on the substrates tested. No definitive LRRK2 phosphorylation substrates have been identified. However, when using the cytoskeletal proteins moesin, ezrin and radixin as potential substrates, instead of MBP, the picture of LRRK2 kinase activity becomes rather complex⁴³. While the G2019S mutation continued to demonstrate increased kinase activity, the I2020T mutation differed from previous data by showing a reduced substrate phosphorylation. Furthermore, seven other PD mutations of LRRK2 showed either reduced or unchanged kinase activity. Hence, as authentic and physiologically relevant LRRK2 substrates are discovered, the PD mutations of LRRK2 may differentially affect its kinase activity on selective subsets of substrates. Implying a more complex picture then, specific PD mutations may affect LRRK2-dependent phosphorylation pathways differently to give rise to disease pathogenesis⁴⁴.

The principal link between LRRK2 kinase activity and PD pathogenesis directs the research focus on the mechanisms that regulate LRRK2 kinase activity. In this light, the kinase activity of LRRK2 appears to be regulated by its GTPase domain. GTP binding is critical for the kinase activity of LRRK2⁴⁵, and enhanced GTP binding appears to promote neuronal toxicity, just like increased LRRK2 kinase activity does³⁸. Recent structural evidence suggests that the GTPase domain may function as a dimer, with the PD-associated pathogenic residues R1441 and I1371 being positioned at the interface of the two monomers to critically contribute to dimer stability⁴⁶. Moreover, the PD mutation R1441C/G disrupts GTP hydrolysis^{47, 48}. Hence, judging from the effect of the PD mutations on the GTPase domain of LRRK2, GTP binding may positively regulate LRRK2 kinase activity and neurotoxicity in PD, while GTP hydrolysis may counteract this effect, with residue 1441 contributing to dimer stability to promote GTPase activity. Additional studies are needed to better dissect the relationship between the GTPase domain of LRRK2 and PD pathogenesis, in particular studies that address how GTP hydrolysis regulates kinase activity

Irrespective of the exact mechanism of LRRK2 kinase regulation, such research poses a great opportunity for the development of new drugs for PD. One avenue involves the modulation of GTPase activity, thereby perhaps fine tuning the kinase activity back to the normal range rather than just eliminating it. In this way, key aspects of the physiological function of LRRK2 may be preserved, which would allow for a very effective treatment. Alternatively, even selective inhibition of aberrant LRRK2 kinase as a whole offers a tremendous potential, judging from successes with kinase inhibitors in other fields of medicine, such as the use of the c-Abl kinase inhibitor for the treatment of specific cancer types⁴⁹. Lastly, given that the chaperone activity of HSP90 can stabilize LRRK2⁵⁰, regulating HSP90 or other LRRK2 stabilizers may yet provide a separate level of control for the kinase activity.

Recently, evidence indicates that some PD families may have mutations in the LRRK2 protein that are not localized to the kinase or GTPase domains. In particular, two disease-causing mutations were found in the N-terminal domain⁵¹. Unless these mutations have long-ranging effects on kinase or GTPase activity, this observation suggests that mechanisms distinct from altered kinase activity may account for PD pathogenesis in some PD families. How mutations in the N-terminal region cause disease is presently unknown, owing in part to a limited functional understanding of that area. Nonetheless, the notion that PD mutations may be situated across the entire protein lends support to non-kinase mechanisms, likely implemented through interacting proteins of the various LRRK2 domains. It may even be possible to speculate that some of these LRRK2 mutations may not reflect a gain-of-function state of LRRK2, as phosphorylation-independent pathways maybe affected in a cell selective manner.

Given the key role of LRRK2 in PD, strong efforts to identify LRRK2 interaction partners have been made. These efforts are now beginning to yield promising protein candidates. The discovery that parkin can molecularly interact with LRRK2 is of particular interest⁵², as parkin causes autosomal recessive PD (see below). Given that parkin is part of the UPS and LRRK2 is associated with the outer mitochondrial membrane, it may be considered that the molecular connection between these two proteins involves mitochondrial LRRK2 substrates that require carefully regulated degradation by the UPS. In any event, discovering the way in which LRRK2 intersects with the cellular signaling network will provide an understanding of how LRRK2 kinase activity drives neurodegeneration and, in extension, which protein interactions may represent effective drug target points. Restoration of physiological LRRK2 interactions or inhibition of abnormal LRRK2 interactions by drugs that regulate LRRK2 conformation may yield promising therapeutic results.

Loss-of-function mutations in Parkinson's Disease

Autosomal recessive PD is caused by mutations in parkin, PINK1, and DJ-1, indicating that each of these proteins likely loses its physiological function in the disease state. Parkin is an E3 ubiquitin ligase, which catalyzes the final step of protein ubiquitination to allow for successful targeting to and degradation by the UPS^{53–55}. Loss of function of parkin may result in protein accumulation as a consequence of lack of ubiquitination. Subsequent neurodegeneration may be caused by toxic effects of aggregated protein⁵⁶. Interestingly, PD patients with parkin mutations, in general, do not have any large inclusions, such as Lewy bodies. This may suggest that large-scale protein aggregation does require ubiquitination and processing beyond the parkin step^{57, 58}. Furthermore, the toxicity may be mediated by small oligomeric proteins rather than by large polymeric protein complexes. The oligomeric structures may not include α -synuclein, however, as mice mutant for α -synuclein (overexpressed A53T mutation of alpha-synuclein) did not change their phenotype in the background of a loss-of-function parkin mutation⁵⁹. Hence, parkin and α -synuclein may mediate independent mechanisms of PD pathogenesis, which is consistent with the absence and presence of Lewy bodies in parkin and α -synuclein mice, respectively. To some extent then, these results point out pathogenetic and molecular differences between some of the PD families and argue for a therapeutic approach that may need tailoring to specific PD subtypes.

Recent studies have identified distinct parkin substrates. One of them, the adaptor protein Eps15, implicates parkin in proteasome-independent ubiquitination, as parkin was shown to promote epidermal growth factor (EGF) signaling by delaying EGF receptor (EGFR) endocytosis⁶⁰. This effect was mediated by parkin-dependent ubiquitination of Eps15, which resulted in decreased binding of Eps15 to EGFR, thereby delaying EGFR internalization and degradation. Prolonged EGF signaling, in turn, extended PI(3)K-Akt signaling. This result implies that parkin may be centrally involved in modulating ubiquitin-dependent degradation of proteins, but in many ways other than merely regulating proteasome activity. Furthermore, given the crucial role of Akt in neuronal survival, it is plausible that loss of parkin function in PD may correlate with reduced Akt activity to explain decreased cell survival. Two other substrates that have been identified are aminoacyl-tRNA synthetase (ARS) interacting multifunctional protein type 2 (AIMP2) (p38/JTV-1) and the Far Upstream Element-binding Protein-1 (FBP-1). They seem to be authentic parkin substrates, as they accumulate in parkin-deficient mice and in autosomal recessive PD due to parkin mutations^{61, 62}. Such confirmatory data is valuable and missing for other proposed parkin substrates. Interestingly, AIMP2 is selectively toxic to dopaminergic neurons⁶². Both proteins are currently being investigated for their baseline functions and their functional changes in the absence of parkin.

Other proteins that bind to parkin are not necessarily substrates, but in fact modulate parkin function. In that capacity, they are heavily studied, not lastly due to interest in developing novel therapeutics. One of those proteins is heat shock protein 70 (Hsp70). It is intriguing that parkin interacts – albeit indirectly – with Hsp70⁶³, given the significance of the heat shock response in PD pathogenesis. In animal models of PD, overexpression of Hsp70 ameliorates disease progression⁶⁴, and drugs that activate the heat shock response, such as geldanamycin prevent neurodegeneration⁶⁵. Now, the interaction between parkin and Hsp70 begins to unravel how the protective effect of the heat shock proteins may be exerted in PD. Understanding the functional consequences of the parkin-Hsp70 interaction should aid in designing more specific therapeutic strategies that compensate for the fallouts of a compromised parkin-Hsp70 interaction in the disease state. Recently, the protein BAG5 was found in a complex with parkin and Hsp70⁶⁶. By inhibiting Hsp70, BAG5 could promote sequestration and suppression of parkin. BAG5 was also shown to directly inhibit parkin, as reflected by decreased ubiquitination activity and decreased suppression of UPS dysfunction and cell death. While the mechanism by which BAG5 exerts its inhibitory effect is not understood, it has been speculated that the RING finger domain of parkin is blocked. Independently, the E4-like protein CHIP was shown to dissociate parkin from Hsp70. This promoted ubiquitination of parkin substrates, such as the Pael receptor, that otherwise would induce cell stress⁶³. Hence, targeting BAG5, CHIP, or other yet unknown modulators of the parkin-Hsp70 interaction should yield therapeutic benefit in PD. Alternatively, parkin is modulated by S-nitrosylation^{67, 68} and developing drugs that regulate such modification may prove valuable. It has been demonstrated that nitrosylation inhibits parkin E3 ligase activity and thereby causes UPS dysfunction, which is consistent with increased nitrosylation of parkin in PD^{67, 68}. Novel drugs that inhibit parkin nitrosylation may therefore be able to delay or even reverse neurodegeneration in PD.

In contrast to parkin data that supports the importance of UPS dysfunction in PD pathogenesis, the discovery of mutations in PINK1 and DJ-1 underscores a critical role for mitochondrial dysfunction in the disease state. PINK1, which stands for PTEN (phosphatase and tensin homologue)-induced putative kinase 1, encodes a putative serine/threonine kinase that localizes to the mitochondria⁶⁹. Several lines of evidence suggest that it is part of a signaling network that regulates the mitochondrial response to various cellular stressors. Consistent with that notion, animal models of mutant PINK1 and inhibition of PINK1 expression in cell systems both demonstrate mitochondria with altered structure and defective function^{70–72}. These experiments have established at the same time that the PINK1 mutation is a loss-of-function mutation. Interestingly, PINK1 dysfunction can be rescued by overexpressing parkin, but not vice versa. This indicates a genetic interaction between the two genes and places PINK1 upstream of parkin in the signaling hierarchy^{70, 71}. Current research is focusing on elucidating the molecular nature of this crosstalk, entertaining the possibility that PINK1 and parkin may be components of a linear signaling pathway⁷³. Similar to the molecular connection between LRRK2 and parkin, the signaling interaction between PINK1 and parkin may be mediated through mitochondrial PINK1 substrates that require degradation by the UPS. This notion is insofar attractive to drug development, as it implies that certain key nodal points in signaling exist that connect several of the familial PD genes. Hence, targeting these nodal points should simultaneously improve multiple cellular defects during PD pathogenesis and be of great value to PD treatment across a wide range of PD patients.

Given the loss-of-function nature of the PINK1 mutation, it is hypothesized that the loss of phosphorylation of PINK1 substrates leads to mitochondrial dysfunction and triggers or promotes neurodegeneration. Thus, much effort has been invested in defining PINK1 interaction partners. TRAP1 and HtrA2 represent the two main PINK1 interactors that have been identified so far^{74, 75}. TRAP1 is a mitochondrial chaperone that is activated under oxidative stress. Under such conditions, PINK1 phosphorylates TRAP1, which decreases cytochrome c release from the mitochondria into the cytoplasm and thereby dampens an important mitochondrial trigger for apoptotic signaling pathways. HtrA2, a mitochondrial protein with serine protease activity, is also involved in stress signaling pathways. Notably, upon activation of MEKK3-p38, PINK1 phosphorylates HtrA2 to increase its proteolytic activity and confer resistance to mitochondrial stress⁷⁶. The targets of proteolytic activity and the type of apoptotic pathway that these targets regulate are not known at this point in time. Nonetheless, the findings on TRAP1 and HtrA2 both elucidate how PINK1 is intricately placed in mitochondrial stress-sensing pathways that protect the cell from oxidative stress. Conversely, loss of PINK1 function in PD may promote neurodegeneration, because protective responses of the mitochondria to oxidative stress are impaired, which results in mitochondrial dysfunction and aberrant activation of cell death pathways.

Current research is trying to understand whether the signaling pathways that involve TRAP1 and HtrA2 intersect. Along the same line, it is being evaluated whether the cell death pathways that these proteins impact on are related or even the same. Such knowledge is required for developing treatment approaches that affect oxidative stress responses. In case of signaling intersection, a nodal point in signaling may be identified that allows for a drug target with a broad protective range. In this context, it is interesting to note that HtrA2 not

only molecularly interacts with PINK1, but like PINK1 also appears to be genetically identified as causing a familial form of PD. HtrA2 mutations were shown to be linked to the PD-13 locus, unlike PINK1 inherited in an autosomal dominant pattern, and suggested to affect the serine protease function⁷⁷. Consistent with the notion that the proteolytic function is reduced by these mutations, mice that harbor familial HtrA2 mutations have a PD phenotype that is similar to HtrA2 knock-out mice^{78, 79}. Curiously, the PINK1 phosphorylation site is adjacent to the familial mutations, indicating that perhaps distinct mutations in the protease domain cause different alterations in protease activity that affect separate groups of effector proteins. However, despite this promising evidence for a genetic role of HtrA2 in PD, caution has to be exercised in light of recent data that points to significant frequencies of the HtrA2 mutations in control populations⁶⁹. Hence, further research requires sorting out these contrasting results to better assess the role of HtrA2 in PD. Perhaps some mutations will turn out to reflect benign polymorphisms, while other mutations in HtrA2 may be disease-promoting. By extension, on a molecular level, some reductions in the proteolytic function of HtrA2 may be compensated for, while others may be pathological. Moreover, it seems that PINK1 is topologically organized in a way that it can only phosphorylate physiologic PINK1 substrates that are outside the mitochondria in the cytosol, thus HtrA2 and TRAP may not be regulated by PINK1 phosphorylation *in vivo*⁸⁰.

Aside from PINK1 and HtrA2, another mitochondrial protein of great interest is DJ-1. Some evidence suggests that DJ-1 functions as an atypical peroxiredoxin-like peroxidase that scavenges mitochondrial peroxide (H_2O_2) through oxidation of its Cysteine 106⁸¹. Moreover, and consistent with DJ-1 being involved in regulating oxidative stress, DJ-1 was found to stabilize the antioxidant master regulator Nrf2. DJ-1 prevents Nrf2 binding to Keap1, which would otherwise promote Nrf2 degradation by the UPS⁸². Hence, DJ-1 aids in maintaining Nrf2 protein levels, which secures the efficient transcription of antioxidative proteins to preserve an adequate stress response. The exact molecular mechanism by which DJ-1 exerts its effect on Nrf2 is not yet known, as physical interactions with the involved proteins was not observed. Nonetheless, just like with PINK1 and HtrA2, DJ-1 appears to demonstrate a property to mount a response to oxidative stress, which may also arise from the mitochondria⁸³. In contrast to these two proteins, however, the protective response of DJ-1 appears to be targeted to a separate component of the oxidative stress. It is possible that in this way all three proteins coordinate their functions to protect the cell from oxidative damage.

A separate line of investigation suggests that DJ-1 is involved in dopamine signaling. Based on knock-out studies in mice, it was proposed that DJ-1 may regulate dynamic dopaminergic transmission in the nigrostriatal neuronal network, with particular affinity to the D2 receptor system⁸⁴. In line with that function, DJ-1 has been found to promote the transcription of tyrosine hydroxylase (TH). Precisely, DJ-1 inhibits the sumoylation of the transcriptional co-repressor PSF, which prevents sumoylation-dependent recruitment of histone deacetylase 1 (HDAC-1) to the TH promoter complex⁸⁵. This mechanism implies that DJ-1 acts as a transcriptional co-activator by inhibiting PSF-mediated transcriptional repression by HDACs. Given that DJ-1 physically interacts with PSF, it further implies that DJ-1

localization extends to the cell nucleus. It is plausible that such action of DJ-1 may explain, at least in part, the phenotype observed in the knock-out mice⁸⁴.

Yet another avenue of research has placed DJ-1 directly into the cell death pathway. DJ-1 can interact with the protein Daxx in the nucleus to prevent Daxx from entering the cytoplasm, where it otherwise would activate ASK-dependent apoptotic cell signaling⁸⁶. In that respect, cell stressors that promote Daxx-mediated neurodegeneration are counteracted. Interestingly, given the multitude of functions that have been associated with DJ-1⁸⁷, drug development for promoting DJ-1 function in PD patients may appear a challenging undertaking, as the spectrum of unwanted side effects may be significant.

Sporadic Parkinson's Disease

A prominent type of defect seen in sporadic PD is mitochondrial dysfunction^{88, 89}. Important evidence supporting this dysfunction is derived from the observation that inhibitors of complex I of the mitochondrial electron chain transport system can cause sporadic PD⁹⁰. Moreover mutations in the mitochondrial DNA polymerase gamma (POLG) is associated with sporadic PD and certain forms of hereditary parkinsonism illustrating the central role of mitochondria in the pathogenesis of PD⁹¹⁻⁹⁴. Neuronal dysfunction caused by complex I inhibitors is very complex and involves activation of most, if not all, cell death pathways, as an inadequate electron chain transport system produces significant mitochondrial dysfunction. The cell death pathways include caspase-dependent and -independent signaling, necrotic mechanisms, and neuronal injury that results from glial cell-induced inflammation⁹⁵. Perhaps somewhat surprising, the neurodegeneration caused by some of these agents is reasonably selective to dopamine neurons, which explains the prominent PD symptoms in affected patients.

The pesticide rotenone and MPTP are two prominent examples of complex I inhibitors⁹⁶. The discovery of MPTP, in particular, provided a good model for PD in patients, animals, and cell culture systems alike⁹⁷. Additionally, MPTP models have been effectively used to develop and test novel drugs for PD. In this way, several crucial candidates for neuroprotection were identified, which are currently being assessed in drug trials with PD patients. To produce dopaminergic neurodegeneration, MPTP must be converted to 1-methyl-4-phenyl-pyridium (MPP⁺) by monoamine oxidase B (MAO-B)⁹⁸. Accordingly, inhibition of MAO-B has been a long-standing target point for drug development. Selegiline and rasagiline are such MAO-B inhibitors, which are being rigorously tested for their neuroprotective effects in PD patients. Some findings indicate that their potential for neuroprotection may relate to inhibition of GAPDH translocation⁹⁹. Preliminary data appears promising, although interpretation of the data is confounded by the observation that these drugs also provide symptomatic relief¹⁰⁰. As such, an isolated neuroprotective effect for the MAO inhibitors has not been demonstrated to date. Other compounds that have shown protection against MPTP neurotoxicity are co-enzyme Q10 and creatine^{101, 102}. Co-enzyme Q10 is proposed to maintain the integrity of the mitochondrial electron transport system as part of its mechanism of action, while the exact mechanism for creatine is not yet fully understood. These two agents are also thought to preserve mitochondrial function and are currently in various stages of investigation as potential neuroprotective agents in

Parkinson's disease. Initial reports have failed to demonstrate a clear neuroprotective or disease-slowng effect in PD patients.

Another contributor to sporadic PD is oxidative stress. Superoxide anion, nitric oxide, and hydroxyl radicals can inflict neuronal damage in multiple ways, which includes damaging mitochondria that subsequently activates cell death pathways. Notably, additional compromises of the cell defense systems against oxidative stress, such as mitochondrial complex I inhibition or even aging, may provide increased susceptibility to neurodegeneration. This may begin to explain the onset of sporadic PD in the middle age and the progressive nature of the disease. Inflammation also seems to play a role in PD¹⁰³, and α -synuclein that is oxidatively damaged through nitration of its tyrosine residues can bypass immunological tolerance, setting in motion adaptive immune responses that exacerbate PD¹⁰⁴. Prudently then, assessing medication that aims at curbing oxidative stress, inflammation and modulating immune function bears some prospect of neuroprotective potential. However, this theoretically promising approach has not yet yielded significant results in animal models or drug development.

Dopamine agonists are an important class of drugs that are being tested for their neuroprotective quality, as their hydroxylated benzyl ring structure confers antioxidative properties^{105, 106}. Moreover, they seem to reduce dopamine turnover and hence free radical generation through their action on presynaptic auto receptors. Consistent with their suggested anti-oxidant function, dopamine agonists protect against 6-hydroxydopamine, MPP+ or systemic administration of MPTP, although the exact mechanism of their action in these cellular or animal models is not yet understood¹⁰⁷. Nonetheless, two clinical trials with dopamine agonists have demonstrated a delay in the progression of imaging markers that are thought to reflect nigral striatal cell density. The trial findings are encouraging, as they indicate the possibility of neuroprotection, although a clear relationship between imaging markers and clinical outcome has not been established. Hence, a careful clinical assessment of the PD patients is needed to justifiably correlate delayed progression of imaging markers with delayed progression of PD. To date, such a correlation has not been shown convincingly in any trial.

What has always been poorly understood is why only certain neuronal cell populations are affected in PD. This seminal matter of selective neuronal vulnerability has recently received some unusual insight¹⁰⁸. Dopaminergic neurons in the substantia nigra (SN) of the mouse possess pacemaker activity, which is present since birth and thought to orchestrate voluntary motor control¹⁰⁹. A few weeks after birth, these neurons replace their sodium ion channels with 1.3 L-type calcium channels to maintain pacemaker activity. Interestingly, application of calcium antagonists to chemical PD models (MPTP, rotenone, 6-OH dopamine) demonstrated survival of dopaminergic SN neurons, which was associated with a reversal to sodium-channel regulated pacemaker activity¹¹⁰. This observation suggests that the selective vulnerability in PD may be related to unusual calcium ion channel activity in the affected neuronal population, with increased calcium influx into the cell triggering neuronal dysfunction and cell death. Spared neurons in PD, in turn, may not have similar plasma membrane permeability for calcium, which allows escape from neuronal dysfunction even in the presence of the same cell stressors that dopaminergic neurons face. Alternatively, as seen

in the ventral tegmental area (VTA) neurons that are not affected in PD but have abundant L-type calcium channels, effective calcium sequestration by calcium binding proteins may provide sufficient resistance to calcium-induced cell stress. Indeed, the calcium binding protein calbindin is expressed at much higher levels in the VTA neurons than in dopaminergic SN neurons.

Consistent with the notion that the mouse data may be transferable to PD patients, a retrospective epidemiological analysis revealed that patients taking the dihydropyridine class of calcium antagonists have a lowered incidence of PD¹¹¹. While prospective trials with PD patients are needed to confirm these results, calcium antagonists have been moved to the forefront of new neuroprotective PD drugs. The calcium antagonists used in the mouse models and epidemiological analysis were preferentially targeting L-type calcium ion channels, but interestingly enough had a greater affinity for the 1.1–1.2 than the 1.3 L-type channel. Hence, there seems to be still room for improved efficacy, which has sparked great interest to develop specific 1.3 L-type calcium channel inhibitors to maximize the benefit-to-side effect ratio. However, improving the drug selectivity for 1.3 L-type calcium channels may not be so straightforward as anticipated, given that knockout mice for the 1.3 L-type calcium channel present with deafness¹¹². It will be a challenge to design drugs where increased selectivity does not produce a serious side effect, while decreasing the side effect profile overall.

A perhaps less expected mechanism of how sporadic PD may come about involves histone deacetylation by the sirtuin protein family (SIRT2s). SIRT2 has received the most attention. Its activity appears to be neurotoxic in PD, as inhibition of SIRT2 by the compound AGK2 diminished α -synuclein mediated toxicity in primary midbrain cultures as well as in a *Drosophila* model of PD¹¹³. Curiously, SIRT2 inhibition also results in larger α -synuclein aggregate size in cell culture¹¹⁴, pointing to the possibility that polymeric α -synuclein may be neuroprotective and oligomeric α -synuclein the primary neurotoxic form. The mechanism by which inhibition of SIRT2 may confer neuroprotection is poorly understood, but promoting the formation of enlarged inclusion bodies may be one mechanism¹¹⁵. SIRT2 deacetylates α -tubulin and histones¹¹⁶. Since, histone deacetylation is required for autophagy¹¹⁷, SIRT2 may be neurotoxic by virtue of regulating protein clearance, including α -synuclein protein turnover and aggregate size. As autophagy involves lysosomal recruitment to aggregated cellular proteins that are associated with the microtubule organizing center, inhibition of SIRT2-mediated deacetylation of histones and α -tubulin may play a role in neuroprotection.

Aside from SIRT2, the sirtuin family member SIRT1 has also been investigated in PD. Interestingly, in contrast to SIRT2, increased activity of SIRT1 showed a delay in α -synuclein toxicity¹¹⁸, indicating that different sirtuin proteins may have complex and at times opposing effects on neurodegeneration in PD¹¹⁹. While SIRT2 may promote α -synuclein to exist in the oligomeric form, it is currently being investigated whether SIRT1 may disassemble oligomeric α -synuclein into monomers. These findings emphasize that while drug targeting of the sirtuins may warrant important therapeutic consideration in PD, much attention has to be paid to selective drug targeting and type of regulation, given the complex interactions of histone deacetylases during neurodegeneration.

Based on the research findings, considerable intersection between sporadic and familial PD can be appreciated. It may be argued that a commonality is particularly seen with respect to mitochondrial dysfunction. As outlined previously, several proteins mutated in familial PD, including PINK1, HtrA2 and DJ-1, play a role in dampening oxidative stress by upholding an appropriate mitochondrial stress response. Thus, what connects sporadic and familial PD is the observation that increased production of reactive oxygen species, decreased stress responses, and complex I inhibition all cause dysfunction of the mitochondrial electron chain transport system; when severe enough, such mitochondrial dysfunction reaches a threshold that activates the various cell death pathways. It is not surprising then that inhibition of excitotoxicity from oxygen radicals also protects against MPTP neurotoxicity. In fact, loss of parkin function has been implicated in neuronal death in the MPTP model⁶⁶. Furthermore, the detrimental *in vivo* role of BAG5 was tested in this mouse model, where the degeneration of the substantia nigra in response to MPTP was markedly increased when BAG5 was expressed selectively in that area. In support of the critical role of mitochondrial integrity in sporadic and familial PD is also the finding that calcium antagonists may be neuroprotective in PD patients. As increased intracellular calcium may trigger neurodegeneration, a link to mitochondrial function is not unlikely, given that mitochondria are very effective buffers of cellular calcium¹²⁰. Further, increased intracellular calcium can induce oxidative stress, which likely mounts mitochondrial stress responses that likely involves the proteins PINK1, HtrA2 and DJ-1. Altogether, these findings are yet another example where pathogenetic insight into sporadic and familial PD can be cross-fertilizing in many aspects. It may not come as a surprise when novel drugs, derived independently from research in sporadic and familial PD, will act synergistically or additive with respect to restoring mitochondrial function in either category of PD patients.

Conclusions

As we enter the 21st century, it is expected that we will identify and have substantial proof of neuroprotective therapies for PD. It is yet to be determined whether the MPTP or related toxin models will be predictive for neuroprotective therapies for PD, as no proven therapy for PD has yet arisen from these models. The most promising neuroprotective therapies will probably emerge from discoveries and testing of hypotheses that are based on insights from the genetic causes of PD. Indeed, since the dawn of the 21st century, enormous advances have been made in identifying how the proteins affected in familial PD are incorporated into signaling pathways to exert their cellular functions. However, it remains to be determined whether these pathways have common nodal points that are compromised in all forms of sporadic and genetic PD or whether these pathways represent distinct entities that are compromised individually in the various forms of idiopathic PD. Perhaps agents that are protective in both the genetic and MPTP models might yield the greatest therapeutic reward. Based on the extent of progress that has been made over the last few years alone, the continuous pursuit of mechanism-based studies will undoubtedly make the day come when we will be able to offer patients neuroprotective treatment options.

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Figure 1.

Molecular Mechanisms of Neurodegeneration in PD. There are multiple potential pathways of cell death in PD. It is unclear at the present time whether these pathways converge to one overarching central cell death mechanism in PD or whether they are independently and distinctly involved in different forms of genetic and sporadic PD. On the left are potential mechanisms and therapeutic targets for sporadic PD. Central to sporadic PD is mitochondrial complex I deficiency, which is modeled by MPTP and other complex I inhibitors. MPTP is converted by monoamine oxidase B (MAO_B) to MPP⁺ where it is concentrated in dopamine neurons due to its high affinity for the DA transporter (DAT) followed by transport and concentration into the mitochondria due to its positive charge. Once in the mitochondria, MPP⁺ poisons the mitochondria by inhibiting complex I. Selegiline and rasagiline were initially used as neuroprotective agents due to their inhibition of MAO_B, but it is now thought that they may actually inhibit GAPDH-dependent-cell death pathways downstream of complex I inhibition. Calcium channel antagonists of the dihydropyridine class may rejuvenate DA neurons to the point where they use sodium channels for their characteristic pacing, thus rendering them resistance to toxic effects of complex I inhibition. Co-enzyme Q10 and creatine have neuroprotective properties in animal models of PD through augmenting the function of mitochondria. Complex I

inhibition leads to cell death through classic apoptotic pathways, endoplasmic reticulum (ER) stress as well as neuronal nitric oxide synthase (nNOS) activation, DNA damage and poly (ADP-ribose) polymerase (PARP) activation. Accompanying derangements in mitochondrial complex I is the subsequent activation of microglia. Strategies aimed at reducing the inflammatory process in PD holds particular promise. On the right are potential therapeutic opportunities for familial PD. DJ-1 appears to function in controlling the level of reactive oxygen species (ROS). Reducing oxidative stress and/or restoring DJ-1 function may be protective. PINK1 is a mitochondrial kinase and disruption of PINK1 may lead to alterations in the function of TRAP1 and HtrA2 setting in motion cell death pathways. Thus, strategies aimed at inhibiting TRAP1 and/or HtrA2 as well as enhancing PINK1 may be beneficial. Parkin is an E3 ubiquitin (Ub) ligase and strategies aimed at maintaining its catalytic activity are particularly attractive, as well as, lowering the burden of toxic substrates such as aminoacyl-tRNA synthetase (ARS) interacting multifunctional protein type 2 (AIMP2) and the Far Upstream Element-binding Protein-1 (FBP-1), which are degraded by the ubiquitin proteasome system (UPS) in a parkin-dependent manner. S-nitrosylation (SNO) of parkin inhibits its activity and interfering with this process may also be protective. PINK1 and parkin appear to function in a common genetic pathway with PINK1 acting upstream of parkin, thus maintaining parkin function will in theory inhibit PINK1 dependent cell death pathways. LRRK2 kinase and/or GTPase inhibitors may be beneficial against cell death due to LRRK2 mutations. Reducing ROS and reactive nitrogen species (RNS), modulating α -synuclein (α -syn) phosphorylation (PO_4), reducing α -synuclein levels through RNAi or immunotherapy, reducing α -synuclein oligomerization, inhibiting α -synuclein cleavage or enhancing the degradation of α -synuclein through inhibition of SIRT2 are potential targets to reduce neurodegeneration by derangements in α -synuclein.

Table

Genetic forms of Parkinson's Disease (PD)

Form	Pattern of inheritance	Chromosome region	Name of gene	Gene identified	Name of protein	Function of protein
Familial PD	AD	4q21-q22	PARK1	Yes	alpha-synuclein	synaptic protein
Young onset PD	AR / AD	6q25.2-q27	PARK2	Yes	parkin	ubiquitin-protein ligase
Familial PD	AD	4q region	PARK4	Yes	multiplication of alpha-synuclein chromosomal region	excess alpha-synuclein protein
Young onset PD	AR	1p35-p36	PARK6	YES	PINK1	mitochondrial stress-induced degeneration
Young-onset PD	AR	1p36	PARK7	YES	DJ-1	sumoylation pathway, oxidative stress protection
Familial PD	AD	12p11.2-q13.1	PARK8	YES	LRRK2 Dardarin	protein phosphorylation
Familial PD	AD	1p36	PARK 9	YES	ATP13A2	lysosomal Protein
Familial PD	AD	2p12	PARK13	Controversial	serine protease HTRA2	mitochondrial stress response

Included in table are only those genetic forms of Parkinson's Disease that have identified genes and are discussed in text. AD = autosomal dominant; AR = autosomal recessive; N/I = not identified. Modified from: A comprehensive review of movement disorders for the clinical practitioner (Vol. 1); Fahn, Jankovic, Hallett, Jenner (2007).