Molecular pathogenesis of Parkinson’s disease

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Parkinson’s disease (PD) is a common and incurable neurodegenerative disease, affecting 1% of the population over the age of 65. Despite a well-described clinical and pathological phenotype, the molecular mechanisms which lead to neurodegeneration remain elusive. However, there is a wealth of evidence from both toxin based models and genetic based models, which suggest a major etiologic role for mitochondrial dysfunction, protein aggregation, the ubiquitin–proteasome system and kinase signalling pathways in the pathogenesis of PD. Ultimately, an understanding of the molecular events which precipitate neurodegeneration in idiopathic PD will enable the development of targeted and effective therapeutic strategies. We review the latest evidence for the proposed molecular processes and discuss their relevance to the pathogenesis of sporadic PD.

INTRODUCTION

Parkinson’s disease (PD) is a common neurodegenerative disease first described in 1817 (1). Subsequently, the clinical triad of bradykinesia, tremor and rigidity came to be recognized as core clinical features. One hundred years later, the neuropathological hallmarks underlying the clinical phenotypes were characterized as the loss of dopaminergic neurons in the substantia nigra, together with the presence of intraneuronal inclusions termed Lewy bodies (2). However, despite these early descriptions, the etiology of PD remains unclear. In the last decade, the identification of several genes that cause rare familial forms of PD has revealed novel proteins and pathways that may produce both dopaminergic neuronal degeneration and a clinical parkinsonian syndrome. The genetic burden of actual mutations in the idiopathic or sporadic form of PD is small, accounting for only 5–10% of the overall PD population. However, the strikingly consistent, specific phenotype of familial and sporadic PD has led researchers to believe that one common molecular mechanism may underlie PD. It is hoped that the same pathways underlying familial forms of the disease may have major relevance in the pathogenesis of the sporadic forms. Ultimately, understanding the pathogenesis of the sporadic form of PD will have the greatest impact on advancing novel therapies for this common incurable neurodegenerative disorder.

This review highlights the evidence for the major pathways that precipitate neurodegeneration in PD from genetic analyses, in vitro models of protein function, experimental animal models and postmortem brain studies. We explore the convergence of these pathways and their potential contribution to sporadic PD.

OXIDATIVE STRESS AND MITOCHONDRIAL DYSFUNCTION

The earliest hypothesis of PD pathogenesis was based on the finding that three mitochondrial complex 1 inhibitors, namely MPTP, rotenone or paraquat, were able to reproduce parkinsonism with selective dopaminergic neuronal loss in vitro, as well as in vivo mice (3,4) and primate (5) models. The initial models did not fully reproduce the features of PD, mainly because there was an absence of one of the major hallmarks of PD, the Lewy body. However, a chronic infusion of rotenone in rodents (6) and, more recently reported, a chronic infusion of MPTP in mice (7) have recapitulated the pathological features of PD with alpha-synuclein positive aggregates. This supports the initial theory that sporadic PD may be caused by a combination of environmental toxins acting via inhibition of the mitochondrial respiratory chain to produce selective dopaminergic cell loss and inclusion bodies.

Inhibition of complex 1 has two major consequences: the depletion of ATP, hence impairment of all ATP dependent cellular processes, and the generation of free radicals that cause oxidative stress. There is clear evidence of oxidative stress in postmortem PD brain: elevated levels of lipid peroxidation markers (4-hydroxynonenal and malondialdehyde) and

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protein nitration have been found in the substantia nigra and Lewy bodies (8). Reduced levels of glutathione and oxidized glutathione, which act as antioxidants, are the earliest marker of nigral cell loss in PD brain (9). Furthermore, a reduction of complex 1 activity by 30% has been described in brain, muscle and platelets of idiopathic PD patients (10,11).

Evidence for oxidative stress is indeed found in many neurodegenerative diseases and it is questionable whether it is truly causal or consequent upon diseased neurons. However, support for a primary role of oxidative stress has emerged from the study of rare familial forms of PD. A variety of missense, truncating, splice site and deletion mutations have been identified in the gene DJ-1 (12), which cause a form of autosomal recessive parkinsonism (Park 7). The exact function of DJ-1 is unclear, but overexpression of DJ-1 appears to protect cells against mitochondrial complex 1 inhibitors and oxidative stress induced by hydrogen peroxide. This effect is abrogated by DJ-1 mutations (13) or by DJ-1 knockdown using siRNA (14). DJ-1 may be able to act directly as an antioxidant because it can be oxidized at the cysteine residue C106. Moreover, it has been demonstrated that endogenous DJ-1 is localised to the mitochondrial matrix and the mitochondrial intermembrane space in addition to its cytoplasmic pool (15). Interestingly, a quantitative proteomic study of the substantia nigra of mice treated with MPTP revealed a significant increase in the protein DJ-1 in mitochondrial fraction of the substantia nigra (16). Together, this evidence suggests that DJ-1 may play an important role in neuroprotection against oxidative stress caused by mitochondrial toxins.

In 2004, missense and truncating mutations within the PINK1 gene were found to cause autosomal recessive PD (17). Bioinformatic analysis reveals that the PINK1 protein consists of a highly conserved kinase domain and a
mitochondrial targeting motif. The presence of this N-terminal mitochondrial targeting motif combined with the demonstration by two groups that PINK1 localizes to the mitochondria in transfected cells is noteworthy (18). As with the other genes that cause autosomal recessive parkinsonism, PINK1 has been suggested to have neuroprotective properties against a variety of cellular stresses, a function which is lost by the mutation G309D identified in certain families (17). If future experiments prove definitively that PINK1 has a role within the mitochondria that protects cells from degeneration, this would further strengthen the hypothesis that mitochondria are critically involved in the pathogenesis of PD.

PROTEOTOXIC STRESS: AGGREGATION AND MISHANDLING

Many of the neurodegenerative diseases share a common pathogenic process: the abnormal accumulation and processing of mutant or damaged proteins. There are two stages in this event: (1) the process of protein aggregation and (2) the cellular response to abnormal proteins. First, as is seen with several other neurodegenerative diseases, PD pathology is characterized by the tendency of a highly soluble native neuronal protein to progressively polymerize and develop an altered conformation, resulting in intracellular aggregation—a process associated with neuronal dysfunction and loss. In other inclusion body diseases, it remains unclear whether the presence of such inclusions is pathogenic, protective or incidental. Secondly, the abnormal or misfolded proteins are normally targeted via ubiquitination to the proteasome, where they are degraded in an ATP dependent manner. Thus, dysfunction of the ubiquitin–proteasome system (UPS) could lead to the accumulation of cytoxic damaged proteins, ultimately resulting in neuronal death.

Protein aggregation

The identification of the first familial PD gene as alpha-synuclein (19) led to the important discovery that the major constituent of Lewy bodies in sporadic PD is alpha-synuclein (20). Missense mutations of the alpha-synuclein gene, as well as the increased gene dosage effect of alpha-synuclein gene triplication (21), cause autosomal dominant familial PD. The human pathology associated with these mutations reflects a widespread and fulminant disease process with nigral cell loss, alpha-synuclein positive Lewy bodies in the brainstem, cortical Lewy bodies (22) and glial cell inclusions (23).

In its native state, alpha-synuclein is a soluble and unfolded protein. Owing to a central hydrophobic region in the protein, alpha-synuclein has a high propensity to aggregate and initially forms an intermediate annular structure called an oligomer or protofibril and ultimately forms insoluble polymers or fibrils (24). These insoluble fibrils are the major constituent of Lewy bodies. It is unclear whether the fibril, the protofibril or the soluble species is the most toxic species in neurons. A variety of factors promote the aggregation of alpha-synuclein; modifications of the protein such as phosphorylation, nitration and glycosylation may contribute to aggregation (26); and dopamine itself is able to stabilize the alpha-synuclein protofibril by forming a dopamine–alpha-synuclein adduct (27).

Reproducing the features of PD in experimental animal models has met with mixed success: there is no significant loss of neurons in the substantia nigra in transgenic mouse models (28). In contrast, viral mediated overexpression of alpha-synuclein induces nigral degeneration in rats (29). Similarly, Drosophila models based on the expression of normal and mutant forms of human alpha-synuclein show selective loss of dopaminergic neurons and the formation of alpha-synuclein inclusions (30). Moreover, this model has confirmed that phosphorylation at the Ser129 residue is crucial to the toxicity of alpha-synuclein and mutations of this serine residue, which prevent phosphorylation, also abolish the toxicity (31). Interestingly, the reduction of toxicity in this model is associated with increased inclusion body formation, suggesting that inclusion bodies may protect neurons by reducing the amount of diffuse toxic protein by sequestering it in inert bodies.

Ubiquitin–proteasome system

The first evidence of a direct role of the UPS in neurodegeneration emerged after the identification of the parkin gene. Mutations in the parkin gene are known to cause a large proportion of early onset autosomal recessive parkinsonism (32). There is a wide spectrum of parkin mutations ranging from large homozygous deletions to multiplications, small deletions/insertions and missense mutations. Pathologically, parkin mutations are associated with significant dopaminergic neuronal loss in the substantia nigra and the locus coeruleus. However, in the few parkin related PD cases that have come to autopsy, there is a notable absence of Lewy bodies in patients with the homozygous deletions of parkin, although Lewy bodies are present in patients with compound heterozygous parkin mutations (reviewed in 33). These findings suggest that parkin may play a significant role in Lewy body formation, but conversely, nigral cell loss and clinical parkinsonism can occur in the absence of inclusion body pathology.

Parkin encodes an E3 ubiquitin ligase with the characteristic two RING (really interesting new gene) finger domains separated by an IBR (in-between ring) domain that is common to other E3 ligases (33). These enzymes catalyze the addition of ubiquitin chains to target proteins before its destruction by the proteasome. Many putative parkin substrates have been identified including synphilin-1, O-glycosylated alpha-synuclein, Pael-R, CHIP, cdc-Rel1A, cyclin E, synaptotagmin X1 (reviewed in 35). Loss of parkin function may lead to accumulation of its substrates, which would ultimately lead to neuronal cell death. Indeed, overexpression of the parkin substrate Pael-R produces dopaminergic cell death in vitro, which can be rescued by parkin overexpression (36).

Despite these in vitro findings, modelling parkin associated PD in vivo has proved challenging: mice with targeted deletion of exon 3 of parkin do not show nigral neuronal loss (37). The parkin knockout model in Drosophila clinically shows locomotor dysfunction due to peripheral muscle degeneration rather than dopaminergic neuronal loss (38).
Further support for the role of the UPS was provided by the identification of UCHL-1, another gene implicated in causing dominant PD (39). UCHL1 is a ubiquitin C-terminal hydrolase L1, which aids the recycling of polyubiquitin chains back to monomeric ubiquitin. The genetic evidence for UCHL-1 is less strong than for genes such as parkin, because the initially identified mutation has only been demonstrated in two siblings with PD and has not been reported in any additional families. A mouse model with an infarmed deletion of exons 7 and 8 of UCHL-1 demonstrates gracile axonal dystrophy, sensory and motor ataxia with accumulation of beta amyloid and ubiquitin deposits, but without evidence of nigrostriatal neuronal loss (40).

There is growing evidence that the UPS may be important in the pathogenesis of sporadic PD. Postmortem brain tissue from patients with idiopathic PD show functional deficits in the 20S proteasome activity (41). Administration of synthetic and natural inhibitors of the UPS to rodents for 2 weeks produces selective nigral cell loss and Lewy body-like inclusions, together with clinical signs of bradykinesia, rigidity and tremor (42). Thus, a primary aberration in the UPS induced in vivo (as described earlier for complex 1 inhibitors) is able to reproduce many of the specific features of PD.

**EMERGING PATHWAYS: KINASES IN PD**

The discovery of the PINK1 gene focused on the potential role of kinases in the neurodegenerative process. Kinases are known to have major roles in cell cycle signalling and are the most common domain encoded by cancer genes. PINK1 encodes a serine/threonine kinase with significant homology to the calcium-calmodulin protein kinases. PINK1 was initially identified as a kinase that was upregulated on overexpression of PTEN, a tumour-suppressor gene, suggesting that PINK1 may play a role in cell cycle regulation (43). Moreover, transient knockdown of PINK1 renders cells susceptible to apoptosis on exposure to taxol (44). Thus, the neuroprotective function of PINK1 may actually lie in the direct regulation of a programmed cell death pathway, occurring as much later event than either oxidative stress or UPS dysfunction.

The subsequent identification of the LRRK2 gene as the Park 8 locus added further interest in the importance of kinases in mediating neurodegeneration (45,46). Missense mutations in this large gene were found to cause autosomal dominant PD in pedigrees from Basque and the UK. LRRK2 mutations cause a range of differing pathologies including neuronal loss in the substantia nigra either in the absence of Lewy bodies or in the presence of widespread Lewy body disease or in the presence of neurofibrillary tangles. The predicted product of the LRRK2 gene is a 286 kDa protein called dardarin: dardarin is a member of a novel family of protein kinases which have sequence similarity to both tyrosine and serine/threonine kinases. In addition to the kinase domain, there are several other conserved domains such as the leucine-rich repeats, the WD40 domain and a Ras/small GTPase superfamily domain (47). As yet, little is known about the function of LRRK2, although the presence of these novel domains suggests a unique function in dopaminergic survival and perhaps a hitherto unknown pathway that leads to nigral cell loss.

**MOLECULAR CONVERGENCE AND DIVERGENCE**

The inherited forms of parkinsonism (Table 1) demonstrate how a single molecular aberration is sufficient to independently reproduce the clinical and pathological features of PD. Moreover, the mutation is present in all cells of the body, and yet, the cell loss is restricted to the substantia nigra. Mitochondrial and associated structures, informing that these molecular events must be cell specific to precipitating PD-type neurodegeneration. However, with the growing wealth of genetic clues, comes increasingly complex puzzles: how can one reconcile the fact that the PD phenotype can occur in the absence of Lewy bodies (for example, in LRRK2 and parkin-associated parkinsonism) and yet a biochemical excess of normal alpha-synuclein as a result of alpha-synuclein gene triplication is also sufficient to cause PD? Is it possible to place the UPS, mitochondrial function, oxidative stress, protein aggregation and kinase signalling in one unified pathway that leads to nigral cell death? Furthermore, if there are instead several distinct pathways that are separately able to precipitate nigral cell death, which of these is the most significant in sporadic PD?

If all the described cellular processes (Figure 1) do have relevance in sporadic PD, then one would expect to find evidence of molecular convergence between them. Certainly, the mitochondrial system and the UPS do not exist separately: complex 1 inhibitors cause a reduction in proteasomal activity (47), and conversely, proteasomal inhibitors can cause mitochondrial damage (49). Inhibiting proteasome function renders cells more susceptible to oxidative stress through complex 1 inhibition (48). In addition to its defined role in the UPS, parkin plays a less well understood role in the mitochondria: for example, the most prominent feature of the parkin knockout in Drosophila is mitochondrial pathology in the flight muscles, resulting in apoptosis. Furthermore, a proteomics approach in the parkin knockout mouse revealed that the most notable changes were in mitochondrial proteins in the electron transport chain, an event associated with a reduction in mitochondrial respiratory capacity (50). There is also evidence of a relationship between mitochondrial dysfunction and protein aggregation: complex 1 inhibition and other forms of oxidative stress lead to alpha-synuclein aggregation (51). Moreover, mice lacking the alpha-synuclein gene are resistant to the toxic effects of MPTP, suggesting that dopaminergic neuronal degeneration requires both mitochondrial dysfunction and subsequent alpha-synuclein aggregation to occur (52). In turn, aggregated alpha-synuclein is able to inhibit the proteasome and thus interfere with the UPS (53,54). Thus, there are multiple levels of interaction between these pathways such that a relatively minor abnormality in one or more cellular processes can be amplified by its interaction with other cellular processes to potentially result in several forms of stress (proteasomal, oxidative and aggregation) that would ultimately force a cell into programmed cell death.

Sporadic PD is a late-onset neurodegenerative disease that has been traditionally thought to be due to exposure to an environmental toxin on the background of a genetically susceptible individual. In this model, it could be conceivable that genetic variation in genes encoding proteins along
several pathways, such as the UPS, the mitochondrial respiratory chain or alpha-synuclein handling, would predispose individuals to low levels of chronic, relative dysfunction of these molecular processes. There is growing evidence from genetic association studies that genetic variation in such genes may contribute as susceptibility factors in sporadic PD (55,56). Cumulative ‘normal’ stress over a long period of time, such as the ageing process (which is known to be associated with reduction in the UPS function and increased levels of oxidative stress), or exposure to toxins, could then tip the balance from a genetically determined stressed cellular state to programmed cell death in that particular individual. This explanation would unite all the molecular pathways implicated in Mendelian forms of PD into a complex multifactorial model for sporadic PD.

CONCLUSION

The themes of protein aggregation, mitochondrial dysfunction and proteasomal stress continue to recur in many of our models, and therefore, may play a central role at some stage of PD pathogenesis. However, there remains a host of questions in this field that need to be addressed. For example, primary events must be distinguished from secondary consequences of stressed diseased neurons. Defining prerequisite events from contributory events in the PD pathway may eventually yield potential therapeutic targets. A clearer understanding of exactly how and when these pathways overlap and converge to produce nigral neuronal degeneration will be vital to understanding the pathogenesis of sporadic PD. To date, neither genetic studies nor postmortem brain studies are able to inform on the temporal sequence and relationship of the various cellular processes. These questions may only be convincingly answered by in vivo models, although hitherto no single toxin or gene-based model has fully recapitulated the pathological and clinical features that define human PD so clearly. Perhaps, we will finally conclude that sporadic PD encompasses a heterogeneous spectrum of disease, whereby several distinct molecular mechanisms may converge to produce a final common pathological and clinical phenotype in different individuals, a model that may have implications for our understanding of the aetiology of all neurodegenerative diseases.

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