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Review

Mitochondrial Dysfunction in Parkinson's Disease

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Abstract. It is clear from a striking convergence of human tissue studies, neurotoxin models, and genetic models that mitochondrial dysregulation plays a central pathogenic role in Parkinson's disease (PD) and related neurodegenerative conditions. Impaired mitochondrial quality could result from both increased damage and decreased ability to repair or clear damaged mitochondria. In particular, common deficits in mitochondrial respiratory chain function, oxidative stress, morphology/dynamics, and calcium handling capacities have been described in multiple PD model systems employing complex I inhibitors, 6-hydroxydopamine and molecular manipulation of Parkinsonian genes including α-synuclein, PTEN-induced kinase 1, Parkin, DJ-1, and, to a lesser extent, leucine rich repeat kinase 2. The most recent and exciting work implicates alterations in the regulation of macroautophagy and likely of selective mitophagic clearance of damaged mitochondria, although additional studies are needed to resolve some issues in this area. Future studies emphasizing the normal mitoprotective function(s) of proteins associated with recessive loss-of-function causes of familial PD, as well as compensatory mechanisms operating in their absence, may offer particularly valuable insights into strategies to enhance mitochondrial health.

Keywords: Autophagy, calcium dysregulation, electron transport chain, 1-methyl-4-phenylpyridinium (MPP+), mitochondria, mitophagy, neurodegeneration, oxidative stress, Parkinson's disease, PTEN-induced kinase 1

INTRODUCTION

Increasing evidence points to a central role of mitochondria in the pathogenesis of Parkinson's disease (PD). The early hints resulted from the observation that human exposure to 1- methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine (MPTP), an inhibitor of complex I (NADH/ubiquinone oxidoreductase) of the mitochondrial electron transport chain, causes a PD-like syndrome in humans [1]. Subsequent studies reported a decrease of complex I activity in the substantia nigra [2, 3], platelets [4], and skeletal muscle [5] of PD patients. The pesticide rotenone, another mitochondrial complex I inhibitor, also elicits pathological, biochemical, and behavioral features of PD in rats [6,7]. Even the nonselective oxidative toxin 6-hydroxydopamine exerts much of its toxicity through a delayed phase of mitochondrial reactive oxygen species (ROS) generation [8,9]. In recent years, gene mutations associated with familial forms of PD have been shown to either directly or indirectly affect mitochondria, including the PTEN-induced kinase 1 (PINK1), parkin, DJ-1, α-synuclein, and leucine-rich-repeat kinase 2 (LRRK2) [10–15]. In subsequent sections, we will discuss the literature on mitochondrial respiratory complex deficits, mitochondrial calcium buffering, mitochondrial morphology, and mitophagy in human PD patient tissues and in PD models.

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MITOCHONDRIAL RESPIRATORY COMPLEX DEFICIENCY IN PD

Since the discovery that mitochondrial complex I inhibitors MPTP and rotenone could be used to model PD, the most consistent findings with relation to human PD-related alterations in mitochondrial respiratory complexes have been decreased complex I activity or protein level, although the degree of inhibition varied within a wide range. However, it is still controversial whether complex I defects are confined to the substantia nigra or reflect global complex I deficits in PD. Some studies reported that complex I deficiency is only observed in the substantia nigra [16–18] or is more severe in nigral neuronal mitochondria than in platelet mitochondria [19]. Other studies report complex I abnormalities in mitochondria isolated from muscle [5, 20,21], platelets [4,22–25], the striatum [3], lymphocytes [23,26], cortical brain tissues [27], and fibroblasts [28] of human PD patients. Changes in subunit proteins or function of complex II, III, IV, and V have also been reported in PD [3,5,25,27,29,30], but these changes are less consistently observed and could be secondary to other factors. Similar changes in oxidative phosphorylation proteins have been reported in cybrid cells containing PD patient mitochondria [31] and in neurotoxin models involving MPTP/MPP+ [32], rotenone [6,33], and 6-OHDA [34].

Primary fibroblasts from patients deficient in PINK1 exhibit decreased complex I activity [35]. Likewise, complex I deficits have been observed in a number of genetic models of familial PD. Accumulation of wild-type α-synuclein in the mitochondria of human dopaminergic neurons was shown to cause reduced mitochondrial complex I activity and increased production of ROS [14]. Likewise, shRNA knockdown of PINK1 causes mitochondrial depolarization and increased mitochondrial superoxide production [36]. In PINK1 deficient mice or fruit flies, isolated mitochondria exhibit age-related decreases in complex I activity, mitochondrial depolarization, and increased sensitivity to apoptotic stress [37,38]. Similarly, parkin knockdown in zebrafish embryos causes a specific reduction in the activity of mitochondrial complex I [39].

While respiratory dysfunction is widely observed in multiple PD related settings, it remains debatable whether or not this represents a primary mechanism in PD or is secondary to other underlying pathogenic mechanisms. A primary role for mitochondrial calcium dysregulation and secondary limitation of mitochondrial substrates has been proposed in the setting of PINK1 deficiency [40]. Interestingly, MPP+, rotenone, or paraquat are able to cause cell death in primary neuron cultures from mice lacking Ndufs4 [41], a non-catalytic subunit of complex I whose phosphorylation enhances complex stability and activity.

The cause(s) of electron transport chain deficiencies in PD are not well understood. There are 13 oxidative phosphorylation-related proteins that are encoded by mitochondrial DNA (mtDNA), with the remainder encoded by nuclear DNA. Several studies reported increases in the number of mtDNA deletions/rearrangements in isolates from the SNc of PD patients or reductions in mtDNA gene levels for complex I and IV proteins in sporadic PD and in PD cybrid cells [42,43]. However many other reports indicate that changes in mtDNA reflect age-dependent damage to the mitochondrial genome, with no significant differences noted between control and PD patients [20,44–47].

One study showed 47% more protein carbonyl modifications on catalytic subunits in cortical mitochondria isolated from PD brain tissues, indicating increased burdens of oxidative damage. This change can be reproduced by incubation of control brain mitochondria with NADH in the presence of rotenone, but not by administration of exogenous oxidants [48]. Decreases in antioxidant capacity may also contribute, as acute reduction of cellular and mitochondrial glutathione levels results in decreased mitochondrial complex I function through a nitric oxide-mediated mechanism [49,50]. S-nitrosation of mitochondrial proteins has also been observed in a chronic glutathione depletion model [51], and S-nitrosation of complex I is correlated with a significant loss of activity [52]. In MPTP treated mice, the inhibition of striatal and midbrain complex I activities can be reversed by thiol antioxidants [32]. Recent studies further indicate that oxidative modulation of mitochondrial proteases could contribute to dysfunction. The Lon protease plays an important role in localized degradation of oxidized matrix proteins [53], but it is vulnerable to inactivation under conditions of GSH depletion and elevated oxidative stress [54]. In this manner, mechanisms of mitochondrial quality control can be impaired, leading to misfolding and aggregation of electron transport chain components.

ALTERED MITOCHONDRIAL MORPHOLOGY IN PD

There are only a few papers describing mitochondrial morphology in various tissues from PD patients. In

PD patient muscle tissues, a reduction in mitochondrial number and disruptions of the mitochondrial membrane are noted [55]. Abnormal mitochondrial distribution involving formation of small clusters has been reported in the SNc of a juvenile PD case [56]. Intramitochondrial inclusions were observed in the stellate ganglion of patients with PD [57], and there is variation in mitochondrial sizes and mitochondrial swelling in caudate nucleus biopsies from PD patients [58–60].

In human PD cybrids, more dramatic alterations have been reported, including mitochondrial swelling, rarefaction of cristae, discontinuous outer membranes, and, occasionally, intramitochondrial inclusions [60]. Mitochondrial abnormalities and intramitochondrial inclusions have also been reported in several studies of dog, monkey, and mouse SNc after MPTP exposure [61–64]. Mitochondrial swelling and cristae disruption is usually an early change in acute animal or culture neurotoxin models [64–66]. However, in a chronic MPTP mouse model, some enlarged mitochondria with packed matrix and intramitochondrial inclusions were observed [67]. The functional significance of these changes is unknown, but there may be a greater potential for mitochondrial repair in the chronic setting.

In recent years, several PD related genes have been shown to play critical roles in maintaining normal mitochondrial morphology and function. In human cell lines, PINK1 deficiency results in mitochondrial swelling, reduced cristae, and fragmentation that is dependent upon the fission protein dynamin related protein 1 (Drp1) [36], with similar morphologies to those described in PD cybrids [60]. Calcineurin-mediated dephosphorylation of Drp1 activates fission in PINK1 deficient cells [68]. However, in Drosophila model systems, knockout of dPINK1 causes either fusion or aggregation of mitochondria that is alleviated by increasing the fission-fusion balance [69]. As fission promotes autophagic clearance of PINK1 deficient mitochondria [36], some of the effects on steady state mitochondrial morphology may be indirect, accounting for differences in different model systems. PINK1 knockdown elicits mitophagy [36], and double PINK1/A53T α-synuclein mutation or Parkin/α-synuclein knockdowns lead to more severe mitochondrial alterations [70,71].

MITOCHONDRIAL CALCIUM BUFFERING IN PD

SNc DA neurons are autonomously active, generating action potentials [72]. Most neurons rely on monovalent cation channels to drive pacemaking, while SNc DA neurons also engage ion channels that enable Ca^{2+} to enter the cytoplasm [73]. Ca^{2+} fluxes across the plasma membrane and between intracellular compartments play critical roles in fundamental functions of neurons, including the regulation of neurite outgrowth and synaptogenesis, synaptic transmission, plasticity and cell survival. Ca^{2+} is under very tight homeostatic control, predominantly through the buffering actions of mitochondria and the endoplasmic reticulum [74]. Impairment of calcium buffering in cells enhances the vulnerability of SNc DA neurons to genetic and environmental challenges [74].

Cybrid cells containing mtDNA from PD patients exhibit altered calcium homeostasis [75], with decreased mitochondrial calcium sequestration and slower calcium recovery following administration of carbachol, an inositol 1,4,5-trisphosphate (InsP3)-inducing agonist that causes a transient elevation in cytosolic calcium. MPP+ also causes slower calcium recovery in SH-SY5Y cells [75] and stimulates the release of Ca^{2+} from mitochondria in presence of dopamine or 6-OHDA, leading to cytosolic calcium overload [76]. Rotenone causes a rise in intracellular free Ca^{2+} due to diminished mitochondrial calcium uptake [33,77]. Intracellular Ca^{2+} overload seems to be related to ROS production [77,78].

Recent studies indicate that calcium dysregulation is also present in genetic PD models. PINK1 knockdown or PINK1 mutation can cause mitochondrial accumulation of calcium, resulting in mitochondrial calcium overload, increased ROS production [40,70], and enhanced mitochondrial fission [68]. α-Synuclein oligomerization further exacerbates Ca^{2+} dysregulation, increasing the likelihood of mitochondrial permeability transition and commitment to neuronal cell death [79]. It has been proposed that decreased mitochondrial function caused by complex I inhibitors could impair the regulation of calcium flux [33,80]. Conversely, dysregulation of mitochondrial calcium has been linked to altered glucose transporter activity and substrate limitation [40]. These mechanisms could play into a feed-forward loop and likely reflect an underlying failure of mitochondrial quality control. The final tier of mitochondrial quality control is mediated by mitophagy [81], defined as the selective autophagic targeting of mitochondria to lysosomes for degradation. Indeed, impaired lysosomal function in other neurodegenerative diseases results in accumulation of fragmented mitochondria with reduced calcium buffering capacity [82].

REGULATION OF AUTOPHAGY IN PD

Mitochondrial autophagy (mitophagy) is a major mechanism for turnover of entire segments of mitochondria, although localized protein degradation pathways also exist [83]. In human PD and PD with dementia tissues, both increased autophagy [59] and mitophagy [84] have been reported. Alterations in autophagy or mitophagy are observed in several toxin and genetic models of PD, including MPTP/MPP+ [66], 6-OHDA [10,85], rotenone [86], PINK1 [10,87–90], parkin [87,88,90,91], and dopamine toxicity [92].

Autophagy is a regulated process mediated by the products of a series of conserved autophagy-related genes (Atg) and a variety of nutrient sensing and stress response signaling pathways [93]. RNA interference designed to reduce expression of Atg5, Atg7, or Atg8 (MAP1-LC3) can effectively inhibit the MPP+ or rotenone induced autophagy and mitochondrial protein loss [66,86]. The extracellular signal-regulated kinases (ERK1/2) are conserved serine/threonine protein kinases that have emerged as important regulators of neuronal responses to both functional and pathologic stimuli [94]. In human PD/DLB and neurotoxin PD models, ERK2 was activated and located to mitochondria [9,84,85]. Inhibiting the activation of ERK1/2 using U0126, which inhibits its upstream kinase, significantly reduces autophagy, and inhibits mitochondrial protein degradation analyzed by both immunofluorescence and biochemical methods [66]. Increasing mitochondrial targeting of transfected ERK is correlated with induction of mitophagy in the absence of toxin injury [85]. In contrast to acute MPP+ injury, where mitochondrial morphology is not rescued by U0126, U0126 promotes both morphologic and functional recovery in a chronic two-week MPP+ culture model (J Zhu & CT Chu, unpublished data). Thus, alterations in mitochondrial activity of ERK1/2 modulate both mitochondrial function and turnover.

Rapamycin is an autophagy enhancer that acts by inhibiting the mammalian target of rapamycin (mTOR), which tonically inhibits autophagy under conditions of adequate nutrition. Rapamycin can stimulate autophagy induced by rotenone, enhancing the clearance of damaged mitochondria [86]. However, rapamycin also synergistically exacerbates the autophagic neurite retraction caused by expression of the dominant G2019S mutation in LRRK2 [95], which currently represents the most common genetic cause of PD [96]. Other autophagy-independent effects of rapamycin, including effects on Akt signaling or protein synthesis, may also contribute to its neuroprotective properties [97]. Interestingly, LRRK2 G2019S expressing flies exhibit increased sensitivity to rotenone, and co-expression of parkin confers protection [98]. As discussed below, parkin has recently been implicated in mitophagy of depolarized mitochondria [91,99]. As some pathobiologic effects of LRRK2 G2019S are mediated through the ERK1/2 signaling pathway [95], which also plays a role in regulating mitophagy [85], it is possible that LRRK2 mutations may also influence mitochondrial homeostasis.

REGULATION OF MITOPHAGY IN PD

Mitophagy is defined as the selective clearance of mitochondria through autophagic mechanisms. In most cases, the selectivity is inferred based on the nature of the injury stimulus. Ideally, a combination of morphologic and biochemical methods are used to not only show association with the macroautophagy machinery, but also demonstrate flux or turnover of mitochondria that can be specifically reversed by inhibiting autophagy. Criteria for defining mitophagy have varied between studies, and these differences merit some consideration.

PARK2/Parkin is an E3 ubiquitin ligase. Mutation of Parkin causes an autosomal recessive form of juvenile PD [100]. A recent landmark study indicates that Parkin can be recruited to depolarized mitochondria to promote mitophagy as evidenced by disappearance of mitochondrial signal from fluorescent images [91]. A requirement for Atg5 was demonstrated, but selectivity was inferred as the stimulus caused global depolarization of all mitochondria. A series of further studies indicate that co-overexpression of full length PINK1 and Parkin enhances Parkin recruitment to mitochondria and PINK1 knockout fibroblasts show impaired recruitment of tagged Parkin to carbonyl cyanide m-chlorophenylhydrazone (CCCP) chemically-uncoupled mitochondria [87,89]. When overexpressed together, full length PINK1 can interact directly with the autophagy protein beclin 1, with contributions of both C-terminal and N-terminal PINK1 sequences [101]. Reciprocal anti-tag immunoprecipitation of co-overexpressed PINK1 and LC3 has also been demonstrated [88]. Other studies suggest that the effects of PINK1-Parkin co-overexpression on mitophagy proceeds through modulation of mitochondrial trafficking to form mitochondrial perinuclear aggregates [88,90], although direct effects on

rates of microtubule-dependent mitochondrial trafficking remain to be studied. The mitochondrial clustering is correlated with formation of poly-ubiquitin chains and recruitment of the autophagic adaptor protein p62/SQSTM1/sequestosome-1 [87]. Altered trafficking that enriches potential autophagic cargo near microtubule-organizing centers can contribute to relative selectivity of autophagy mechanisms.

In these PINK1-parkin studies, a cell line that does not endogenously express parkin was used, but even in cell types that do express both PINK1 and Parkin, co-overexpression of both proteins was often needed to induce mitochondrial perinuclear aggregation and autophagy. While this system has been useful for showing that mutant forms of Parkin vary in their ability for mitochondrial recruitment and subsequent mitochondrial loss [87,89], these cells are also known for robust expression of exogenous proteins and the ability to subsist on glycolysis, limiting extrapolation to neurons. Additional studies to determine if these mechanisms operate with endogenous expression levels in neurons, and the influence of mitophagy on neuronal function and survival are critically needed to expand this fascinating area of study.

While co-overexpressed PINK1 and Parkin serve to promote perinuclear mitochondrial clustering, additional mechanisms of selective mitophagy can be triggered in the setting of chronic PINK1 deficiency. Mitophagy is a major neuroprotective mechanism in stable PINK1 shRNA cell lines [10], and overexpression of Parkin enhances this mitophagic response through a mechanism dependent upon key components of the autophagy machinery [81]. Interestingly, wild type PINK1 lacking the N-terminal mitochondrial targeting sequence is capable of reversing the effects of PINK1 shRNA on mitophagy [10]. In contrast, full length PINK1 is required for Parkin recruitment [88]. It is possible that different processed forms of PINK1 mediate different effects. It is also possible that while knockdown of PINK1 reduces stable Parkin association with clustered mitochondria, PINK1 itself is not needed for the ability of Parkin to promote mitophagy. As with other enzyme-substrate combinations, stable association of Parkin with its substrate(s) on the mitochondrial surface may not be required, particularly under physiological conditions when only a subset of mitochondria will undergo autophagy.

It is also possible that different experimental settings could initiate different responses, such as the use of transient or inducible cell lines [87,88,90,101] versus stable cell lines [68,81]. Chronic reduction of PINK1 expression could activate pathways, including upregulation of Parkin itself [81], that counterbalance the damaging effects of PINK1 deficiency.

In contrast to PINK1-Parkin co-expression studies, our stable knockdown studies of PINK1 in SH-SY5Y cells and unpublished studies in primary neurons implicate PINK1 in stabilizing mitochondrial networks, suppressing toxin-induced fragmentation and autophagy [81]. Knockdown studies of endogenous PINK1 produce a moderate mitochondrial deficit, while studies of mitochondrial clearance involve chemical depolarization of all the mitochondria. We hypothesize that PINK1 functions at multiple levels in mitochondrial quality control, maintaining extended functional networks of mitochondria under normal cellular conditions, but promoting mitophagy under conditions of widespread, acute mitochondrial injury (Fig. 1). Given that PINK1 is both a cytosolic and mitochondrial-localized serine/threonine kinase, and there are different processed forms of PINK1 present in human cell lines and human brain tissues, it is intriguing to hypothesize that the ratio of isoforms and/or altered subcellular distribution of PINK1 may determine its different functions.

Indeed, two recent studies indicate that depolarized mitochondria are deficient in importing and processing PINK1 [89,102], as membrane potential is important for these basic mitochondrial functions. While this suggests that stabilization of full length PINK1 at the surface of depolarized mitochondria could serve as the signal for mitochondrial damage, it also highlights additional caveats to be experimentally addressed in the future. Several studies use disappearance of signal related to the translocase of the outer membrane (TOM20), cytochrome c, HtrA2/Omi, or mitochondrially targeted fluorescent protein as the assay for mitophagy, without confirming involvement of the core autophagy machinery. Yet, intermembrane space proteins are lost with permeability transition in severely damaged mitochondria, and proteins such as TOM20 and Mito-GFP are targeted for proteasomal degradation under conditions that disrupt mitochondrial import [103]. Thus, alternative methods to visualize mitophagy, including ultrastructural confirmation, and analyzing the effects of inhibiting autophagy induction, lysosomal degradation, and the proteasome, would benefit this growing area.

CONVERGENCE ON MITOCHONDRIAL QUALITY CONTROL

It is clear that issues of mitochondrial quality control to include prevention of damage, local repair, and recy-

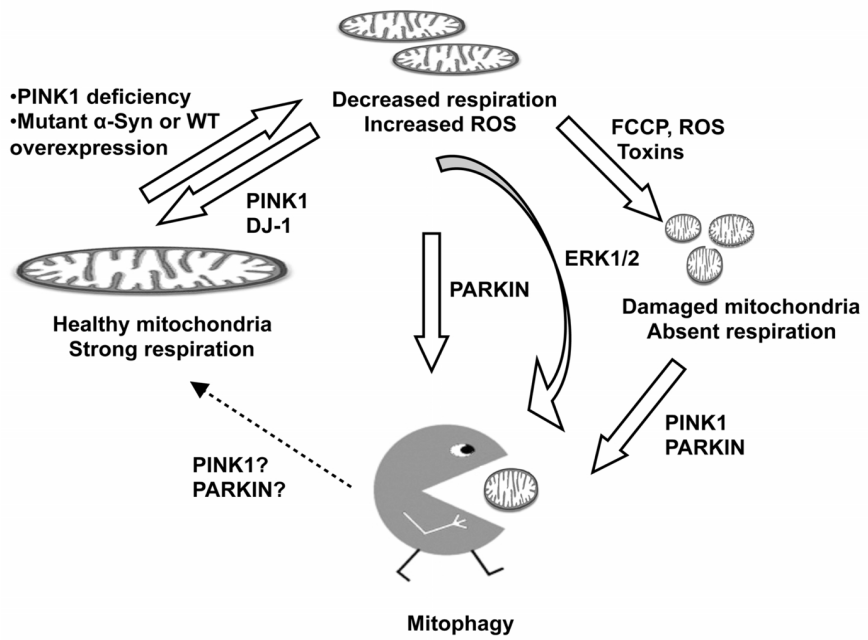

Fig. 1. Schematic illustrating potential crosstalk between toxin-activated pathways and PD-related genes in regulating mitochondrial quality. Under physiological conditions, mitochondria undergo fission-fusion transitions reflecting reversible alterations in mitochondrial respiratory function, with a small percentage of effete mitochondria cleared by autophagy. Knockdown of endogenous PINK1 or enhanced mitochondrial localization of mutant or overexpressed WT α-synuclein produce moderate mitochondrial deficits, which are opposed by WT PINK1 or DJ-1. Under these conditions, there is typically a mixture of damaged and functional mitochondria, and parkin-enhanced mitophagy confers neuroprotection in PINK1 deficient cells. Toxin-mediated activation of ERK1/2 also serves to promote mitochondrial dysfunction and mitophagy. Severe, widespread mitochondrial dysfunction, as induced by mitochondrial uncouplers and toxins, elicits complete fragmentation of the mitochondrial network and/or perinuclear mitochondrial aggregation. Under these conditions, full length PINK1 is stabilized by the inability of depolarized mitochondria to import and process proteins, and PINK1-Parkin interactions result in apparently complete mitochondrial clearance by autophagy. While it is unclear whether PINK1-Parkin are degraded due to irreversible association with mitochondrial aggregates, a few reports indirectly suggest a possible role in regulating mitochondrial biogenesis to complete the mitochondrial quality control cycle.

cling of damaged mitochondria may be central to understanding PD and potential future therapeutic avenues. To summarize: There is extensive evidence supporting the concept that mitochondrial dysregulation plays a central pathogenic role in PD. Data from human PD tissue studies, toxin models, and genetic models all indicate that understanding regulatory mechanisms governing mitochondrial quality control may be central to elucidating pathogenic mechanisms of chronic neurodegeneration (Fig. 1). In particular, overexpression or enhanced mitochondrial localization of α-synuclein, deficiency of PINK1, Parkin and DJ-1, and toxin-mediated activation of ERK1/2 serve to enhance mitochondrial dysfunction [12,13,66,85,104,105]. Parkin has also recently been implicated in the selective clearance of damaged mitochondria [91], and interactions with PINK1 and DJ-1 may regulate facets of Parkin function [88,89,106]. Future areas of research emphasis should include mechanisms of increased dysfunction with aging and disease, as well as age- or disease-related impairment of compensatory mechanisms that serve to repair or clear damaged mitochondria.

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