

Review Article

Mitochondrial Dysfunction: The Road to Alpha-Synuclein Oligomerization in PD

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While the etiology of Parkinson's disease remains largely elusive, there is accumulating evidence suggesting that mitochondrial dysfunction occurs prior to the onset of symptoms in Parkinson's disease. Mitochondria are remarkably primed to play a vital role in neuronal cell survival since they are key regulators of energy metabolism (as ATP producers), of intracellular calcium homeostasis, of NAD⁺/NADH ratio, and of endogenous reactive oxygen species production and programmed cell death. In this paper, we focus on mitochondrial dysfunction-mediated alpha-synuclein aggregation. We highlight some of the findings that provide proof of evidence for a mitochondrial metabolism control in Parkinson's disease, namely, mitochondrial regulation of microtubule-dependent cellular traffic and autophagic lysosomal pathway. The knowledge that microtubule alterations may lead to autophagic deficiency and may compromise the cellular degradation mechanisms that culminate in the progressive accumulation of aberrant protein aggregates shields new insights to the way we address Parkinson's disease. In line with this knowledge, an innovative window for new therapeutic strategies aimed to restore microtubule network may be unlocked.

1. Introduction

Parkinson's disease (PD) was first associated with the loss of the brown pigment neuromelanin from the substantia nigra. Later, it was postulated that the progressive loss of dopamine-producing cells in the substantia nigra pars compacta of the ventral midbrain caused PD symptomatology. In addition, PD is also associated with the presence of intracytoplasmic inclusions known as Lewy Bodies (LBs), which are composed largely of alpha-synuclein (alpha-syn). The function of alpha-syn is still unclear, but its involvement in PD pathogenesis is further indicated by a subset of familial cases of PD that carry either a missense mutation in *snca* (alpha-syn) gene or have a duplication or triplication of alpha-syn locus [1–4].

To date, mutations in at least 16 PD-genetic loci (PARK) have been linked to the pathogenesis of PD [5]. The discovery of these genes in which mutations cause early or late-onset forms of PD has greatly accelerated research

progress. These include *pink-1*, *dj-1*, *parkin*, *lrrk2*, *uchl-1*, *omi/htra2*, *atp13a2*, *pla2g6*, *fbx07*, and *snca*, as previously mentioned. OMI/HTRA2, PINK-1, DJ-1, LRRK2, and Parkin are either mitochondrial proteins or are associated with mitochondria and may be involved in pathways related to oxidative stress and free radical damage. Pink-1 encodes a putative serine/threonine kinase with a mitochondrial targeting sequence [6], and DJ-1 is a redox sensor which is involved in the response to oxidative stress [7]. Although Parkin is a putative E3 ligase in the ubiquitin proteasome system that localizes predominantly to the cytosol, it also associates with the mitochondrial outer membrane [8]. Most recently mutations affecting the mitochondrial serine protease OMI/HTRA2 have been linked to increased risk of PD [9]. Mice entirely lacking expression of *omi/htra2* due to targeted deletion of its gene, *Prss25*, show a decrease in a population of neurons in the striatum and have a parkinsonian phenotype that leads to death of the mice around 30 days after birth [10]. In addition, loss of Omi protease

activity increases the susceptibility of mitochondria to induce the permeability transition pore [11]. LRRK2 is a kinase that colocalizes with the mitochondrial outer membrane [12] and may regulate the response to mitochondrial inhibitors [13].

PD etiopathogenesis includes genetic, epigenetic, and environmental factors. However, in sporadic cases of PD, in which ageing is the major risk factor, the initial event that causes the pathology is not clear. Nevertheless, it is known that several mechanisms are involved in the development of PD pathogenesis such as mitochondrial dysfunction, oxidative damage, autophagic alterations, proteasome impairment, microtubule network disruption, and protein aggregation. Efforts have been made in order to discover what triggers the pathogenesis of PD that culminates in the death of a specific group of neurons, dopaminergic neurons. Nowadays several studies highlight mitochondrial dysfunction as the leading event. In this paper, we will underscore mitochondrial-mediated mechanisms and their involvement in PD pathogenesis.

2. Parkinson's Disease: A Mitochondrial Disorder?

There is mounting evidence for mitochondria involvement in neurodegenerative disorders, including PD. Mitochondria play a fundamental role in energy metabolism; their primary function is to provide energy (in the form of ATP) for intracellular metabolic pathways. Mitochondrial electron transport chain deficiencies are thought to underlie defects in energy metabolism and have been implicated in the neurodegenerative process. Moreover, brain cells are highly dependent on the oxidative phosphorylation (OXPHOS) machinery in mitochondria, which involves the respiratory complexes [14].

The first evidence of mitochondrial dysfunction involvement in the pathogenesis of PD emerged following the observation that accidental exposure of drug abusers to 1-methyl-4-phenyl-1,2,3,4-tetrahydropyridine (MPTP), an inhibitor of complex I (NADH/ubiquinone oxidoreductase) of the mitochondrial electron transport chain, induced parkinsonism [15]. Moreover, Parker and colleagues found a significant reduction of complex I activity in platelet mitochondria, purified from patients with idiopathic PD [16]. Evidence for dysfunctional mitochondrial metabolism in PD arises from studies performed in the substantia nigra of postmortem PD brain showing a deficient complex I activity [17, 18]. Additionally, a selective decrease in complex I subunits was demonstrated in PD brain [19]. This was corroborated by Keeney and coworkers that reported some abnormalities in the assembly and oxidation of complex I subunits in mitochondria from PD patients cortex [20]. Complex I deficiency has been also described in other PD tissues, namely, in peripheral cell models such as, platelets, lymphoblasts, muscle, and fibroblasts [21–26]. More recently, a specific complex I deficit was detected in highly purified mitochondria from the frontal cortex of PD patients [27]. To address mitochondrial involvement in the pathogenesis of PD, King and Attardi, in 1989, succeeded in

repopulating human cells depleted from mtDNA, a Rho0 cell line, with mitochondrial genes from donor cells [28]. Human cell lines (SH-SY5Y neuroblastoma or NT2 teratocarcinoma cells) depleted of mtDNA were fused with either control or PD platelets that contain mtDNA, but no nuclear DNA. Several authors showed that these cybrids harbour a complex I defect [29–32] and have a decrease in ATP levels and loss of mitochondrial membrane potential [29]. Further support for mitochondrial involvement in PD comes from studies using toxins [33–35] like rotenone, paraquat, and maneb that appear to reproduce some of the features of PD in animal models. Although several of these toxins are mitochondrial, they induce nigrostriatal lesions motor and postural deficits by different mechanisms of action [36–44].

High levels of mtDNA deletions have been observed in dopaminergic neurons from the substantia nigra of post-mortem human brains from aged individuals and idiopathic PD patients [45, 46]. mtDNA deletions in these neurons were correlated with a decrease in cytochrome c oxidase activity, one of the key enzymes of electron transport chain. This suggests that accumulation of mtDNA deletions in dopaminergic neurons from substantia nigra above a certain threshold can cause mitochondrial defects associated with PD. It is well established that mtDNA accumulates mutations during the ageing process which correlates with decline in mitochondrial function in late-onset PD. TFAM is a phylogenetically conserved protein and is imported to mitochondria which is essential for mtDNA maintenance and directly regulates mtDNA copy number and is absolutely required for transcription initiation at mtDNA promoters [47–49]. The most compelling evidence regarding TFAM involvement in PD was reported by Ekstrand and colleagues showing that the conditional and selective inactivation of both TFAM alleles in substantia nigra of transgenic mice induced respiratory chain deficiency in dopaminergic neurons leading to a Parkinsonism phenotype with adult onset of slowly progressive impairment of motor function accompanied by the formation of inclusions and neuronal death [50]. Taking into consideration the above findings, it was hypothesized that potentially functional polymorphic TFAM variants could influence PD risk depending on haplogroup background. However, Alvarez and coworkers study in 250 patients report no TFAM-mutations/polymorphisms that could contribute to the risk for PD [51, 52]. Other enzyme that regulates mtDNA replication and plays an important role in mtDNA maintenance is the mtDNA polymerase gamma 1 (POLG1). This enzyme is a nuclear-encoded protein involved in the synthesis, replication, and repair of mtDNA that is imported to mitochondria and localizes in the inner mitochondrial membrane. POLG1 mutations might impair mtDNA replication, which leads to gradual accumulation of multiple mtDNA deletions, resulting in respiratory chain dysfunction [53]. Luoma and colleagues have found that missense mutations of POLG1 cosegregate in patients with a phenotype that includes progressive external ophthalmoplegia and parkinsonism [54, 55]. Moreover, POLG1 mutations have also been described in case studies, in which parkinsonism was part of the clinical spectrum [56]. Deletion of functional *ndufs4*, a gene

encoding one of the subunits required for complete assembly and function of complex I, led to abolished complex I activity in midbrain mesencephalic cultures derived from *ndufs4* knockout mice [57]. However, this deletion did not affect the survival of dopaminergic neurons in culture. Nonetheless, studies performed in rats that expressed an enzyme called "alternative NADH dehydrogenase" (*Ndi1*) in the substantia nigra, showed a reduced toxic effect of MPTP and rotenone [58, 59].

2.1. Oxidative Stress: ROS-Related Consequences. Mitochondrial OXPHOS is known to be the major source of ROS. Cells in normal conditions are able to cope with excessive ROS because they are endowed with robust endogenous antioxidant systems. However, when ROS production overwhelms the endogenous antioxidant systems, due to mitochondrial dysfunction, this can potentially damage various types of biomolecules, including proteins, lipids, and nucleic acids. Markers of oxidative stress, such as products of lipid peroxidation, protein oxidation, and oxidation of mtDNA and cytoplasmic RNA, are increased in postmortem samples of substantia nigra in PD brains as compared to controls [60–64]. In PD patient's postmortem substantia nigra, there is evidence for decreased levels of reduced glutathione and altered iron metabolism [65]. Furthermore, proteomic studies revealed that several mitochondrial and ROS scavenging proteins expressed in the substantia nigra of PD patients had oxidative modifications supporting a role of oxidative stress in PD [66]. It is strongly believed that proximity of mtDNA to ROS generation site, the lack of histones, and diminished capacity for DNA repair increase mitochondrial vulnerability to mutations and oxidative stress [67, 68]. It appears that substantia nigra is more vulnerable to impairments of complex I activity than other brain regions and peripheral blood cells, possibly due to increased levels of iron and to dopamine metabolism [69]. The intracellular auto-oxidation of dopamine generates H_2O_2 and dopamine-quinone species which will exert cytotoxicity in dopaminergic neuronal cells [70, 71].

Several papers showed an involvement of oxidative stress in alpha-syn toxicity. Nitration and nitrosylation of proteins and especially of alpha-syn and parkin in PD have been documented [72–74]. Leong and coworkers demonstrated that methionine oxidation of dopamine generates soluble alpha-syn oligomers highlighting the potential role for oxidative stress in modulating alpha-syn aggregation [75]. Interestingly, studies performed in SH-SY5Y cells overexpressing alpha-syn A53T mutant or wild type and in isolated rat brain mitochondria showed that alpha-syn localizes at the mitochondrial membrane, and this interaction of alpha-syn with mitochondria causes oxidative modification of mitochondrial components [76].

The involvement of oxidative stress in PD is also corroborated by the fact that two of the PD-related genes, *dj-1* and *pink-1*, have important roles in maintaining the balance between oxidative stress and antioxidant defenses. DJ-1 functions as a redox-sensitive chaperone as well as a sensor for oxidative stress and apparently protects neurons

against oxidative stress and cell death. Mutations in this gene are the cause of autosomal recessive early-onset PD [77]. Hayashi and colleagues suggested that DJ-1 is an integral mitochondrial protein that directly plays a role in maintenance of mitochondrial complex I activity [78]. PINK-1 is a serine/threonine protein kinase that localizes to mitochondria. It is thought to protect cells from stress-induced mitochondrial dysfunction. Mutations in this gene cause one form of autosomal recessive early-onset Parkinson disease [6].

Taken together these findings suggest a pivotal role for mitochondrial-mediated oxidative stress in PD pathogenesis.

2.2. Mitochondrial Metabolism: NAD⁺/NADH Ratio. Several studies suggest that changes in energy metabolism in the brain are not merely a consequence of neuronal loss but rather a contributory factor to the progression and development of the disease [79–83]. The decrease in mitochondrial bioenergetics capacity and altered redox status is linked through various mechanisms including interconvertible reducing equivalent pool: NAD⁺ and reduced β -nicotinamide adenine dinucleotide (NADH) [84]. NAD⁺ and NADH are major factors for numerous energy metabolism-associated redox reactions and mitochondrial functions but also calcium homeostasis, ageing, and cell death [85]. Additionally, NAD⁺/NADH ratio is a powerful regulator of glycolysis, TCA cycle, and oxidative phosphorylation. Our group recently observed that NAD⁺/NADH ratio is significantly increased in PD cybrids when compared with CT cybrids (Esteves et al., submitted). By using an oxygen electrode, we observed that although whole cell basal oxygen consumption was comparable between the PD and CT cybrids, the proton leak was increased and maximum respiratory capacity was decreased in the PD cybrids [86].

Multiple families of enzymes catalyze various biochemical reactions by consuming NAD⁺. Among these are the recent identified sirtuins (SIRT) that have a catalytic domain characterized by its requirement for NAD⁺ as a cofactor. SIRTs are a family of deacetylase enzymes that are able to remove the acetyl group from ϵ -amine of lysine and have been found in a wide variety of subcellular locations. So far, in humans there are seven identified SIRTs (SIRT1 to SIRT7) that play a role in a wide variety of important biological processes, including transcriptional silencing, DNA recombination and repair, apoptosis, axonal protection, fat mobilization, and ageing [87]. The deacetylase and ADP ribosylase activity of SIRTs suggests that they could act as metabolic or oxidative sensors, regulating cellular machinery according to the information they receive. Resveratrol, an activator of SIRT1 [88], protected SH-SY5Y cells against dopamine-induced cytotoxicity by reducing intracellular oxidative stress [89]. Additionally, it was shown to exert a neuroprotective effect on 6-OHDA rat model of PD [90] and to significantly protect mice from MPTP-induced motor coordination impairment, hydroxyl radical overloading, and neuronal loss [91]. On the other hand, a work from 2009 showed that SIRT1 overexpression did not exert any neuroprotective effect against neuronal damage induced by MPTP

in mice [92]. A dramatic reduction in the expression of SIRT1 occurred when an acute stress was induced by addition of several neurotoxins (MPP⁺, rotenone, KA, or 3NPA) to the culture medium. However, in human samples of PD and dementia with LBs, there were no changes in SIRT1 expression [93]. So far, no work has been published regarding SIRT1 involvement in alpha-syn oligomers clearance in PD. In an MPTP-treated primate model of PD, caloric restriction, which upregulates SIRT1, prevented the function of locomotor function and preserved striatal dopamine [94]. SIRT1 is known to deacetylate approximately a dozen of known substrates, such as peroxisome proliferator-activated receptor gamma coactivator-1 alpha (PGC1 α). PGC1 α is of major importance for PD etiopathogenesis because it plays a central role in the regulation of cellular energy metabolism and stimulates mitochondrial biogenesis. Work from our group demonstrated that PD cybrids with inherent mitochondrial dysfunction have a decrease in SIRT1 activity that was correlated with a decrease in PGC1 α levels indicating that mitochondrial biogenesis is affected in our PD model [86]. However, more studies need to be performed in order to substantiate this data. The role of SIRT2 in neurodegenerative disorders is less established. Nevertheless, North and coworkers provided data revealing that SIRT2 is involved in cell cycle regulation via the deacetylation of α -tubulin [95]. More recently, Outeiro and coworkers reported an intriguing connection between SIRT2 and PD that suggested that inhibition of the human SIRT2 rescues cells from alpha-syn-mediated toxicity [96]. The exact mechanism whereby SIRT2 inhibition affects alpha-syn aggregation remains uncertain. One proposed mechanism is through alpha-syn interaction with alpha-tubulin [97–99]. Moreover, it was shown that PD cybrids have an increased free/polymerized tubulin ratio indicating that microtubule destabilization may mediate alpha-syn oligomers accumulation [98]. Alpha-tubulin acetylation is associated with microtubule stabilization, so one possibility is that an increase in SIRT2 activation due to increase in NAD⁺ levels increases alpha-tubulin deacetylation leading to microtubules network disruption which potentiates alpha-syn oligomerization (Esteves et al., submitted). So far, little is known about the remaining SIRTs involvement in PD. Overall, SIRTs targeting may be therapeutically beneficial in PD pathogenesis by manipulating the pathways responsible for PD neurodegeneration not only regarding alpha-syn oligomerization but also mitochondrial biogenesis. However, despite exciting data, the feasibility of developing SIRTs-based therapy for PD and other neurodegenerative diseases needs to be demonstrated in animal models, and finally in human trials.

2.3. Calcium Homeostasis Deregulation. Disturbances in calcium homeostasis can affect neuronal functionality since calcium is one of the most important elements in cellular signaling. An increase in cytosolic calcium concentration can arise from two sources: the major intracellular calcium store which is the endoplasmic reticulum and the extracellular space. Moreover, mitochondrial function is based on the

ability to uptake calcium and to accumulate it in the matrix, being this process dependent on the mitochondrial membrane potential. The primary role of mitochondrial calcium is the stimulation of OXPHOS [100]; thus, any perturbation in mitochondrial or cytosolic calcium results in profound alteration of cellular function. Rotenone was shown to increase the susceptibility of cells to calcium overload with subsequent cell death in SH-SY5Y neuroblastoma cells [101]. Sheehan and coworkers showed that PD cybrids sequestered less calcium in their mitochondria than CT cybrids, due to their decreased mitochondrial function [102]. Subsequently, our work showed that PD cybrids have a significantly higher concentration of cytosolic calcium as compared to CT cybrids and in addition show less capacity in mitochondrial calcium buffering, indicating that mitochondrial normal function is impaired [103].

In addition, an increased activity of calcium-stimulated phospholipase A₂ in the putamen is reported to be due to either decreased dopaminergic striatal input or degenerative processes in dopamine nerve terminals [104]. Dopaminergic neurons from substantia nigra express calcium buffering proteins which effectively sequester calcium without using ATP. Expression of the calcium buffering protein, calbindin, reduces vulnerability to mitochondrial toxins [105] and seems to confer resistance to MPTP [106]. Interestingly, dopaminergic neurons that do not express the Ca²⁺-buffering protein, calbindin-D28k, are selectively lost during PD progression [107]. In addition, dopaminergic neurons that express relatively high levels of another calcium-buffering protein, calretinin, appear to be resistant to degeneration in PD [108].

So far, it has been demonstrated that alpha-syn regulates calcium entry pathways and, consequently, that abnormal alpha-syn levels may promote neuronal damage through deregulation of calcium homeostasis [109].

3. Mitochondrial Metabolic Control of Cellular Traffic: Microtubules Involvement

Cytoskeletal injury is likely to be responsible for altered rearrangement and movement of organelles, being a common feature of several neurodegenerative diseases including PD. Microtubules are highly dynamic polar structures that play critical roles in diverse cellular functions. These include cell motility and division, organelle transport, cell morphology, and organization. Intracellular transport of protein and organelle cargo is one of microtubules' most important function, and is an essential requirement for all mammalian cells. In addition, microtubule-mediated transport is crucial to maintain the function and structure of neurons [110]. Microtubules dynamic instability requires an input of energy to undergo polymerization and depolymerization cycles. This dynamic process is extremely regulated and is influenced by many factors, where the concentration of free tubulin is the driving force [111].

Several lines of evidence suggested that microtubules disruption may be involved in PD. In fact, tubulin was shown to colocalize with alpha-syn in LBs. Also, tubulin folding is

dependent on ATP and GTP hydrolysis, and mitochondrial impairment with subsequent energy failure is one of PD hallmarks [112, 113]. Toxins that affect ATP production may affect the tubulin polymerization/depolymerization process leading to an increase in free tubulin. In fact, MPP⁺, a known complex I inhibitor that induces PD-like symptoms, depolymerizes microtubules in PC12 cells [114, 115]. Later, Cappelletti and coworkers provided the first evidence that microtubules instability is specifically affected by MPP⁺ [116]. Moreover, rotenone, another complex I inhibitor, was also shown to potentiate microtubules depolymerisation *in vivo* and *in vitro* [117] by binding to the colchicine site on tubulin heterodimers [118]. Ren and Feng found that microtubule depolymerization induced by rotenone caused vesicle accumulation in the soma and killed serotonergic neurons through a mechanism dependent on serotonin metabolism in the cytosol [119].

Microtubule depolymerization induces disruption in axonal transport, which leads to an accumulation of damaged organelles, aggregated/misfolded proteins, and vesicles. What happens in dopaminergic neurons is that dopamine leakage from the vesicles to the cytosol promotes an increase in oxidative stress induced by dopamine oxidation which culminates in cell death [120]. Treatment of cotransfected cells or primary mesencephalic neurons with colchicine, vinblastine, or nocodazole reversed alpha-syn-mediated inhibition of DAT activity providing insights for alpha-syn regulation of DAT activity, namely, by tethering the transporter to the microtubular network [121]. Furthermore, treating cells with nocodazole, a microtubule-disrupting agent, resulted in an increase in the number of small aggregate particles. Indeed, perturbation of microtubule system due to inhibition of microtubule assembly or due to deletion of genes involved in microtubule biogenesis stimulates alpha-syn aggregation and toxicity [122]. Also Webb and coworkers found that microtubule disruption with nocodazole inhibits autophagosome-lysosome fusion, what can decrease the degradation of alpha-syn oligomers [123]. Our group demonstrated that taxol, a microtubule stabilizer, was able to reduce alpha-syn oligomers accumulation in PD cybrids [98]. Intriguingly, the process of alpha-syn fibril formation was shown to be stimulated by tubulin and two other microtubule-associated proteins [124–126]. This means that when microtubule network is disrupted, the amount of free tubulin increases triggering alpha-syn fibrillization. Indeed alpha-tubulin directly interacts with alpha-syn [127]. On the other hand, Alim and collaborators found that alpha-syn is a functional microtubule-associated protein, and mutant forms of alpha-syn lose this potential [97]. Alpha-syn overexpression induces not only microtubule disruption but also impairs microtubule-dependent trafficking and induces neuritic degeneration in SH-SY5Y cells [128]. Moreover, Saha and colleagues found that the movement of alpha-syn mutant forms associated with PD through axons of cultured neurons has reduced transport rates. In addition, phosphorylation of alpha-syn in serine-129 also reduces its axonal transport [129].

Mitochondria use cytoskeletal proteins as tracks for their directional movement. Interaction of mitochondria with

microtubules and their movement along microtubule tracks depends on MAPs [130]. The cytoskeletal system regulates not only mitochondrial movement but also their morphology and function. Therefore, damage to microtubules interferes with mitochondria movement through axons. On the other hand, mitochondria damage perturbs transport of mitochondria through axons, increasing their retrograde movement. These changes in mitochondria dynamics lead to a decrease of mitochondria numbers in axons and mitochondria accumulation in cell bodies [131, 132]. Studies from 2002 revealed that depletion of mitochondria numbers and function in axons occurs in neurodegenerative disorders [133, 134]. Since mitochondria are ATP suppliers and microtubules need ATP to accomplish their function, damage to mitochondria will have a profound effect on axonal transport and as a consequence axonal maintenance and function [135]. Normal mitochondria are expected to generate sufficient amounts of ATP but also to divide [136]. Indeed, mitochondrial biogenesis occurs by fission of pre-existing mitochondria. Fission allows the dilution of damaged macromolecules and also prevents excessive mitochondria enlargement. In fact, enlarged mitochondria are less likely to be degraded by mitophagy, which potentiates their progressive failure [137]. Initial enlargement may occur because of oxidative damage to proteins responsible for mitochondrial fission or mutations in the corresponding nuclear genes. Moreover, mitochondrial turnover may also decrease due to decreased lysosomal capacity associated with lipofuscin overload. It is also called “ageing pigment” and has been considered a reliable biomarker for the age of neurons. Lipofuscin is an intralysosomal, polymeric substance, primarily composed of cross-linked protein residues and formed due to iron-catalyzed oxidative processes. Because it is undegradable and cannot be removed via exocytosis, lipofuscin accumulation is inevitable during ageing [138, 139]. The accumulation of lipofuscin within postmitotic cells is a recognized hallmark of ageing occurring with a rate related to longevity.

An increasing body of data emphasizes the crucial role of mitochondrial dynamics in mitochondria function. Mitochondria are not a static and autonomous organelle but, instead, a highly dynamic one. This organelle undergoes continual cycles of fusion (the combination of two mitochondria into a single organelle) and fission (the separation of long, tubular mitochondrion into two or more smaller parts) [140, 141]. These dynamic processes regulate mitochondrial function by enabling mitochondrial recruitment to critical subcellular compartments, content exchange between mitochondria, mitochondrial shape control, mitochondrial communication with the cytosol, and mitochondrial quality control. In fact, this highly dynamic balance between mitochondrial fission and fusion controls mitochondrial morphology, length, size, and number and also regulates mitochondrial function and distribution. Increasing evidence suggests that abnormal mitochondrial dynamics is involved in mitochondrial dysfunction which may mediate neuronal death in PD models. Interestingly, MPP⁺ and rotenone were shown to induce mitochondrial fragmentation dependent on the fission protein GTPase

Dynamin-related protein 1 (Drp1) [142–144]. Similarly, 6-OHDA-induced mitochondrial fragmentation in SH-SY5Y cells suggests that excessive mitochondrial fission might be mediating neurotoxicity induced by complex I inhibition [145]. In human fibroblasts, acute exposure to high-dose rotenone resulted in decreased mitochondrial membrane potential that caused mitochondrial fragmented morphology [146]. Also chronic exposure to rotenone was reported to reduce mitochondrial movement in differentiated dopaminergic SH-SY5Y cells [147]. These results suggest that PD toxins hamper fission/fusion machinery. Work obtained with two PD-related genes also highlighted microtubules involvement in PD as well as mitochondria dynamics impairment. For instance, parkin was shown to strongly bind to microtubules and to ubiquitinate tubulin [120]. In addition, PINK-1 was found to have a role in the trafficking of mitochondria along microtubules suggesting that loss of PINK-1 function besides altering mitochondrial morphology and dynamics can also alter microtubule function [148]. It was also suggested that the PINK-1/Parkin pathway promotes mitochondrial fission and/or inhibits fusion by negatively regulating fusion proteins function, such as Mitofusin-1 and -2 (Mfn1 and Mfn2) and Optic atrophy 1 (Opa1), and/or positively regulating Drp1 [149]. Moreover, the loss of mitochondrial integrity in PINK-1 and parkin mutants can be due to reduced mitochondrial fission [150]. In *Drosophila*, PINK-1 and parkin mutant phenotypes were markedly suppressed by overexpression of Drp1 or downregulation of Opa1 indicating that the PINK-1/Parkin pathway regulates mitochondrial remodeling process by promoting mitochondrial fission [151]. Additionally, PINK-1 deficiency is also linked to mitochondrial pathology in human cells and *Drosophila*, which can be rescued by Exner et al. [152]. Lutz and colleagues demonstrated that an acute downregulation of parkin in human SH-SY5Y cells severely affects mitochondrial morphology and function, a phenotype comparable with that induced by PINK-1 deficiency. Furthermore, they showed that loss of parkin or PINK-1 function increases Drp1-dependent mitochondrial fragmentation [153]. Narendra and colleagues demonstrated that dysfunctional mitochondria, with a low membrane potential, recruit parkin to the mitochondria and induce the mitochondria to undergo fusion and mitophagy [154]. Furthermore, the loss of PINK-1 function elicited oxidative stress and mitochondrial turnover that was coordinated by the autophagic and fission/fusion machineries [155, 156].

Overall, mitochondrial-mediated microtubule disruption leads to a rapid accumulation of protein aggregates and/or damaged organelles such as mitochondria contributing to neurodegeneration as seen in PD.

4. Protein Aggregation: Alpha-Synuclein Oligomerization

When misfolded proteins cannot be refolded or degraded (by the proteasome or by the lysosome), the cell activates an alternative way of defence, sequestering abnormal and/or toxic proteins into aggregates. There is convincing data

pointing to protein aggregation role in both familial and sporadic PD pathogenic process. Indeed, the presence of spherical protein inclusions is found in the cytoplasm of surviving nigral neurons, named LBs. It is thought that fibrillar alpha-syn is the building block of LBs. Indeed, alpha-syn is also the most sensitive marker for LBs, implying that it is necessary for LBs formation [157]. Nevertheless, LBs contain many proteins other than alpha-syn, including neurofilaments and other cytoskeletal proteins, suggesting that they might be important in aggresome formation. Alpha-syn is a small, highly charged 140-amino acid residue protein predominantly expressed in CNS, whose function is still poorly understood. However, several functions have been pointed out. It is thought that alpha-syn plays a role in the regulation of dopamine biosynthesis via activation of protein phosphatase 2A that reduces tyrosine hydroxylase phosphorylation, the rate-limiting enzyme in dopamine production [158, 159], and reducing the amount of available active mitogen-activated protein kinases [160] and phospholipase D [161]. Furthermore, several studies suggest that alpha-syn is involved in modulating synaptic transmission, the density of synaptic vesicles, and neuronal plasticity [162–165]. In addition, alpha-syn can act as molecular chaperone preventing aggregation of other proteins *in vitro*. In fact, alpha-syn can activate Hsp70, and more interestingly its structure is very similar to heat shock proteins [166, 167]. It also provides a supportive role in the folding/refolding of SNARE proteins critical for neurotransmitter release, vesicle recycling, and synaptic integrity [168]. Interestingly, alpha-syn knockout mouse showed no signs of neurodegeneration suggesting that alpha-syn is not required for neuronal development [165, 169]. SNCA gene mutations are very rare (three missense point mutations A30P, A53T, and E46K) however, they have helped to elucidate molecular mechanism of intracellular accumulation of alpha-syn. Transgenic mice expressing human wild-type alpha-syn gene develop several of clinical and pathological features of PD, such as accumulation of LBs, the loss of dopaminergic terminals in the basal ganglia, and associated motor impairments [170]. Overexpression of alpha-syn in substantia nigra results in loss of dopaminergic neurons, phosphorylation of alpha-syn at Ser129, and activation of caspase-9 [171]. Moreover, A53T and A30P alpha-syn transgenic mice develop neuronal mitochondrial degeneration and cell death [172]. Interestingly, A30P and A53T mutant alpha-syn are known to self-associate more rapidly than the wild-type forms facilitating aggregation into fibrils [173–176]. However, transgenic mice with A30P alpha-syn mutation do not exhibit alpha-syn inclusions [177]. In contrast, A53T alpha-syn transgenic mice develop neuronal synucleinopathy [178].

Alpha-syn can interact with many proteins, like calpain 1 that can cleave alpha-syn in the C-terminal region further enhancing its fibrillization process [179]. Likewise, alpha-tubulin induces alpha-syn fibrillization in yeast, rat brain, and human brain [122, 125]. Moreover, work from our lab demonstrated that in PD cybrids with a complex I defect and ATP depletion both calpain 1 over-activation and free alpha-tubulin increase trigger alpha-syn oligomerization [98, 103]. Numerous studies now support the hypothesis that

alpha-syn oligomerization is the key step driving neuronal damage in PD. Indeed, the idea that oligomers, rather than the fibrils, are the pathogenic aggregate species that are responsible for neuronal cell death in PD is emerging. In fact, based on several observations, it has been suggested that LB formation does not cause but, instead, protects against neurodegeneration [180, 181]. Interestingly, neuropathological analysis of PD patients' brains has shown that neurons containing LBs seem healthier than neighbouring neurons [182, 183]. Although the mechanism underlying the cytotoxicity of the oligomers is not clear [184, 185], earlier studies showed that alpha-syn protofibrils disrupt synthetic vesicles *in vitro* [186, 187] causing imbalance of cellular ions and, thus, cell death. Several alpha-syn posttranslational modifications lead to the formation of stable oligomers. These include nitration, oxidation, phosphorylation, and interaction with iron. Oxidative modification of alpha-syn via dopamine adducts may facilitate aggregation [175]. Similarly, recent data demonstrated that stable overexpression of alpha-syn in SH-SY5Y cells or exposure of cells to dopamine facilitated alpha-syn oligomerization [188]. Dopamine was shown to modulate differently the stability of protofibrils and fibrils composed of wild-type or mutant forms of alpha-syn [189]. Wakamatsu and coworkers showed that truncated human alpha-syn is deleterious to the development and survival of nigral dopaminergic neurons [190]. Likewise, a work from 2007 demonstrated that aggregated alpha-syn mediates dopaminergic neurons toxicity *in vivo* [191].

Other mechanisms showed that alpha-syn aggregation might be closely related to oxidative reactions which may play a critical role in PD neurodegeneration [192]. For instance, abnormalities in copper and H₂O₂ homeostasis were shown to play critical roles in the metal-catalyzed oxidative oligomerization of alpha-syn [193]. Additionally, by using antibodies to specific nitrated tyrosine residues in alpha-syn, extensive and widespread accumulation of nitrated alpha-syn protein inclusions was demonstrated in protein inclusions of PD brains [72]. Subsequently, it was found that in HEK 293 cells stably transfected with wild-type and mutant alpha-syn, nitrative and oxidative insults induced the formation of alpha-syn aggregates [194]. Our results in PD cybrids indicate that mitochondrial-driven ROS production induces alpha-syn oxidation and oligomerization. Alpha-syn aggregation was significantly decreased upon antioxidant treatment (CoenzymeQ or GSH) [30].

Other important covalent promoter of aggregation is phosphorylation. Alpha-syn carries a number of potential sites for phosphorylation. Indeed, Ser129 phosphorylation of alpha-syn strongly modulates interactions between alpha-syn and synphilin-1 and the formation of inclusions. The levels of soluble alpha-syn oligomeric species are increased by phosphorylation at Ser129 [195]. It was shown that alpha-syn is extensively phosphorylated in aggregates from synucleinopathies patients [196]. In addition, polyunsaturated fatty acids were reported to promote oligomerization, suggesting that alpha-syn may aggregate via interaction with cell membranes [197]. One proposed mechanism for oligomers toxicity, as previously mentioned, is the formation of pores by ring-like intermediates. Moreover, iron was shown to

specifically induce alpha-syn aggregation suggesting its role in aggregate formation [198].

Evidences suggest that mitochondrial dysfunction could induce alpha-syn misfolding. In fact, treatment with rotenone resulted in an increase of detergent-resistant alpha-syn aggregates in COS-7 cells expressing wild-type alpha-syn [199]. Moreover, mutant and wild-type alpha-syn interacts with mitochondrial cytochrome c oxidase, a key enzyme of the mitochondrial respiratory system [200]. Li and coworkers demonstrated that a portion of alpha-syn is present in the membrane of mitochondria in normal dopaminergic neurons [201]. Under overexpression conditions, alpha-syn may translocate to the mitochondria and cause enhanced toxicity in response to subtoxic concentrations of mitochondrial toxins [202]. Using SHSY cells overexpressing alpha-syn A53T mutant or wild-type, as well as isolated rat brain mitochondria, it was shown that alpha-syn localizes at the mitochondrial membrane causing oxidative stress [76]. More recently, Devi and colleagues showed that mitochondria of PD-vulnerable substantia nigra and striatum had significant accumulation of alpha-syn and decreased complex I activity [203]. Another interesting study demonstrated that during the ageing process in yeast, functional mitochondria are required for alpha-syn toxicity [204]. Furthermore, our work provided data showing that PD cybrids harbouring inherent mitochondrial impairment developed alpha-syn oligomers accumulation. Alpha-syn oligomerization in our studies was triggered by several different pathways all dependent on mitochondria malfunction (reviewed by [205]).

Although more studies need to be performed in order to elucidate alpha-syn function, it is quite undeniable that its oligomerization seems to be the key step that drives both pathology and cellular damage in PD.

5. Intracellular Mechanisms of Degradation

5.1. Calpain-Mediated Proteolysis. Sustained mitochondrial dysfunction in PD can release calcium, contributing to aberrant calcium homeostasis in the cytosol leading to activation of calpains. Indeed, mtDNA-depleted cells exhibit an increase in calpain activation [206]. Calpains are calcium-activated neuronal proteases that belong to a highly conserved family of cysteine proteases, thus, they are widely expressed in the CNS and are preferentially involved in the degradation of short-lived proteins [207]. Its structure consists of a cysteine-proteinase domain combined with a calmodulin-like Ca²⁺-binding domain. In fact, the activities of the representative classical, ubiquitous mammalian calpains, μ -calpain (calpain 1), and m-calpain (calpain 2) are regulated by Ca²⁺ concentration. They exist as a proenzyme heterodimer (80 kDa and 29 kDa) in resting cells but are activated by calcium through autolytic processing (to produce a heterodimer of 78 kDa and 18 kDa). Furthermore, *in vitro* studies showed that calpain 1 and 2 differ in the calcium concentration required to become activated; calpain 1 requires a calcium concentration in the μ molar range, and calpain 2 requires mmolar range [208]. It is widely known that calpastatin, a ubiquitously expressed 110 kDa protein,

is the sole endogenous inhibitor of calpains [209, 210]. Overall, calpains are intracellular nonlysosomal calcium-regulated cysteine proteases that mediate regulatory cleavages of specific substrates and are involved in a number of processes during differentiation, life, and death of the cell. These proteases cleave diverse proteins species, including receptors, ion channels, cytoskeletal components, proteases, oncogenic proteins, and cell signalling proteins. Although calpain activation was initially implicated in the necrotic process, several studies have indicated that these proteases play a prominent role in apoptotic processes. In fact, calpains are able to be activated via caspase-mediated cleavage of calpastatin during initiation of apoptotic execution [211]. Active caspase-3 can cleave calpastatin, thereby promoting uncontrolled calpain activation. Although calpains may enhance caspase activity, they can also function to block the activation of caspases. For example, calpains can cleave caspase-9 rendering it incapable of activating caspase-3 and preventing the subsequent apoptotic cascade [212]. Moreover, procaspase-3 is a calpain substrate. In PC12 cells, MPP⁺ induced calpain activation and AIF release which was prevented by calpain inhibitors [213].

Cdk5 belongs to a group of serine/threonine kinases best characterized for their role in cell cycle progression. This kinase was shown to have a role in the pathogenesis of PD. Cdk5 has been colocalized with LBs in PD patients' brains. Conversion of the cdk5 activator p35 to the more stable p25 form may lead to increased cdk5 activity. Consistent with this possibility, an increase in p25 levels was shown after MPTP treatment. Several reports suggest that the p35-to-p25 conversion is mediated through calpain activation [214–216]. Increased expression of calpain in the mesencephalon of PD patients and primate PD models has been demonstrated, suggesting a calpain involvement in PD pathogenesis [217]. Additionally, enhanced calpain expression and activity is observed in the substantia nigra and locus coeruleus of PD patients. Increased calpain expression and cell death in spinal cord in C57BL/6N mice were also reported following MPTP treatment [218]. The presence of calpains in morphologically abnormal dopaminergic fibers as well as in LBs further supports calpains involvement in PD. Most interestingly, inhibition of calpain blocks neuronal degeneration and restores behavioural function in experimental models of PD [219]. Likewise, the inhibition of mitochondrial complex I by MPP⁺ and rotenone activated calpain and seems to constitute an early event in the neurodegenerative process prior to caspase-3 activation [220, 221]. Moreover, rotenone-induced degeneration of spinal cord motor-neuron in male Lewis rats progressed with upregulation of calpain and caspase-3 [222].

It is now well accepted that alpha-syn oligomerization is pivotal in progression of PD pathology and cellular damage. Subsequently, it has been described in other diseases that involve protein aggregation, the protective effect of calpain inhibition. Haacke and coworkers showed that coexpression of the highly specific cellular calpain inhibitor calpastatin abrogated fragmentation and the formation of inclusions in cells expressing pathological ataxin-3 suggesting a critical role of calpain in the pathogenesis of Spinocerebellar ataxia

type 3 [223]. Moreover, Goñi-Oliver and colleagues provided data that established the first direct evidence that calpain promotes GSK-3 truncation in a way that has implications in signal transduction, and probably in pathological disorders such as AD [224]. In 2003, it was shown that parkin accelerates the degradation of alpha-syn via the activation of calpain leading to the prevention of alpha-syn-induced cell death [225]. Also in 2003, it was demonstrated that alpha-syn was a substrate of calpain 1 [179]. Latterly, the same group showed data revealing that calpain-mediated cleavage near and within the middle region of soluble alpha-syn with/without tyrosine nitration and oxidation generates fragments that are unable to self-fibrillized [226]. As opposed, Dufty and colleagues in 2007 showed that cleavage of alpha-syn by calpain occurs in PD brain and calpain-cleaved fragments of alpha-syn colocalized with activated calpain [227]. These results suggest that calpain may link alpha-syn to its disease-linked oligomerization, aggregation, and subsequent formation of LBs. Our group showed that PD cybrids have increased cytosolic calcium levels correlated with an increase in calpain activation and expression which subsequently potentiates the formation of toxic alpha-syn oligomers. In addition, our results showed that calpains inhibition decreased the toxic alpha-syn oligomers and increased the nontoxic insoluble alpha-syn aggregates, preventing caspase-3 activation [103].

Demarchi and collaborators showed that calpain modulates macroautophagy in mammalian cells [228]. Indeed, in calpain-deficient cells, autophagy is impaired, thus contributing to the apoptotic switch. Recent data showed that in AD the lysosomal autophagic system may not degrade tau in the normal adult rat brain. However, inhibition of autophagy may induce tau proteolysis through calpain activation [229]. So far, no data was shown confirming the correlation between autophagy and calpains in PD.

Overall, a role for calpains-mediated proteolysis is strongly postulated in the pathogenesis of PD. More studies need to be performed to address if calpains inhibition offer a potential therapeutic target in the neurodegenerative process of PD.

5.2. Autophagy: A Quality Control System. Age causes a progressive accumulation of wornout organelles and sub-cellular structures that reduces the cellular and molecular efficiency of various biological processes that are required for maintaining homeostasis and survival. This accumulation of extracellular/intracellular damaged proteins has been frequently used as a biomarker of ageing [230]. Moreover, it seems to reflect an unbalance between the capability/activity of the quality control systems and the amount of altered proteins in the cells. Autophagy is ubiquitous in eukaryotic cells and is the major mechanism involved in the clearance of oxidatively or otherwise damaged/worn-out macromolecules and organelles, controlling thus cell death. Autophagy is a process traditionally regarded as a cellular response to stress typically related to nutrient deprivation, toxin exposure, infection, or oxidative stress. In contrast to the ubiquitin-proteasome pathway which degrades mostly short-lived proteins, autophagy is primarily involved in breaking down proteins with long half-lives and damaged

organelles [231]. Moreover, is a highly organized and probably quite specific intralysosomal degradation pathway regulated by a large family of genes, the ATG family [232, 233]. Over 30 atg genes have been identified in yeast and at least 11 have orthologs in humans. For instance, atg6 is also known as beclin and atg8 is commonly called LC3 in mammals [234, 235]. There are three main types of autophagy in mammalian cells: macroautophagy, microautophagy, and chaperone-mediated autophagy (CMA) [236]. The differences among the various autophagic mechanisms depend of the type of cargo, route, and mechanism for its delivery to lysosomes. Macroautophagy involves any type of cellular material, including large organelles, such as mitochondria, being sequestered within a double-membrane-bounded vacuole called autophagosome. Autophagosomes receive acid hydrolases by fusion with late endosomes or lysosomes. Subsequently, the degradation of its content progresses and matures to a secondary lysosome or residual body depending on whether degradation is complete or partial, respectively [237–239]. Microautophagy involves macromolecules and small organelles that enter lysosomes through invagination of its membrane [240, 241]. CMA is a mechanism for selective digestion of a particular group of soluble cytosolic proteins with a KFERQ motif [233, 242]. Once this sequence is recognized, these proteins are directly translocated to the lysosomal membrane.

Alterations in the autophagic machinery have been implicated in the pathogenesis of PD. Indeed, an increased number of autophagic vacuoles and related structures of autophagy have been found in PD patients [243]. In addition, autophagic vacuoles are also described within mesencephalic dopaminergic neurons in several experimental models of PD [244, 245]. LC3-II and Beclin-1 expressions were significantly increased in brains from humans with Dementia with LBs and in transgenic mice overexpressing A53T alpha-syn, as compared with respective controls [246]. However, the lysosome-associated membrane protein 1 (LAMP1), cathepsin D (CatD), and Heat Shock Protein 73 (HSP73) immunoreactivity were significantly decreased within PD nigral neurons when compared to age-matched controls [247]. There is controversy over whether this increase in autophagic markers is protective or instead causes neuronal death. It has been suggested that this increase is responsible for neuronal death. However, enhanced degradation of deleterious aggregates or disrupted organelles can be protective [248].

Autophagy is indeed an important process in a variety of human diseases caused by toxic, aggregate-prone, intracytosolic proteins which became inaccessible to the proteasome when they form oligomers [249, 250]. Depending on its conformational state and cellular conditions, alpha-syn can be degraded by autophagy [251]. CMA and macroautophagy were shown to be important pathways for wild-type alpha-syn degradation in neurons [252]. Being a soluble protein, alpha-syn can be degraded by CMA because it contains the CMA recognition targeting motif [253]. None of the 3 mutations in familial forms of PD affect the CMA targeting motif. Nevertheless, the wild-type protein, contrary to the mutant forms, is readily translocated into the lysosomal lumen

whereas the mutant forms remain bound to the complex not only blocking its degradation but also the degradation of other substrates. Moreover, posttranslational modifications of alpha-syn that promote its oligomerization have a similar effect, suggesting that alpha-syn oligomers may block lysosomal translocation and CMA [248]. For example, in mouse ventral medial neuron cultures, SH-SY5Y cells, and isolated mouse lysosomes, dopamine-modified alpha-syn is not only poorly degraded by CMA but also blocks degradation of other substrates by this pathway [254]. While the narrow proteasome barrel excludes the entry of oligomers/aggregates of alpha-syn, such substrates can be degraded efficiently by macroautophagy. The ability of alpha-syn to inhibit proteasome activity is related to its propensity to assemble into filaments [255]. Indeed, fibrillar forms of the protein usually get jammed inside proteasome blocking its activity [256]. Interestingly, disruption of autophagy by RNA interference significantly increased alpha-syn oligomer accumulation *in vitro*, confirming the significance of macroautophagy in alpha-syn clearance. In addition, rotenone-induced alpha-syn aggregates were cleared following rapamycin stimulation of autophagy [246]. Rapamycin inhibits the mammalian target of rapamycin (mTOR), a negative regulator of autophagy [257]. Rapamycin has been shown to degrade all forms of alpha-syn in a stable inducible PC12 cell model [251]. These findings support the concept that neuronal autophagy is essential for protein homeostasis as well as for the reduction of the potentially pathogenic alpha-syn oligomers. Coexpression of Beclin-1, a regulator of the autophagic pathway, was found to activate autophagy, reduce accumulation of alpha-syn, and ameliorate associated neuritic alterations in a neuronal cell line that overexpressed alpha-syn. This neuronal cell line showed lysosomal accumulation and alterations in autophagy [258].

Ageing seems to affect mitochondria particularly. Mitochondria undergo the most dramatic age-related changes, such as structural deterioration like swelling and loss of cristae, sometimes complete destruction of the inner membrane, decreased respiration, and low ATP production [137]. Often they are extremely enlarged and are called “giant” mitochondria [259, 260]. Nonfunctional mitochondria are important sources of ROS which contribute to the accumulation of damaged and nonfunctional components in the cells which is characteristic of ageing. In addition, because of mitochondrial ROS generation, protein damage occurs and mtDNA mutations accumulate at an accelerating rate. Subsequently, this leads to the synthesis of abnormal mitochondrial proteins exacerbating mitochondrial dysfunction.

During recent years, accumulating evidence has been gathered showing that mitochondria also may be subject to selective degradation through mitophagy. Mitophagy is important to the elimination of dysfunctional mitochondria and mutated mtDNA since DNA repair in mitochondria is much less robust than in the nucleus [261]. There are many reports showing mitochondria within autophagosomes [262–264]. It was then proposed that damaged mitochondria are captured and digested by the lysosome.

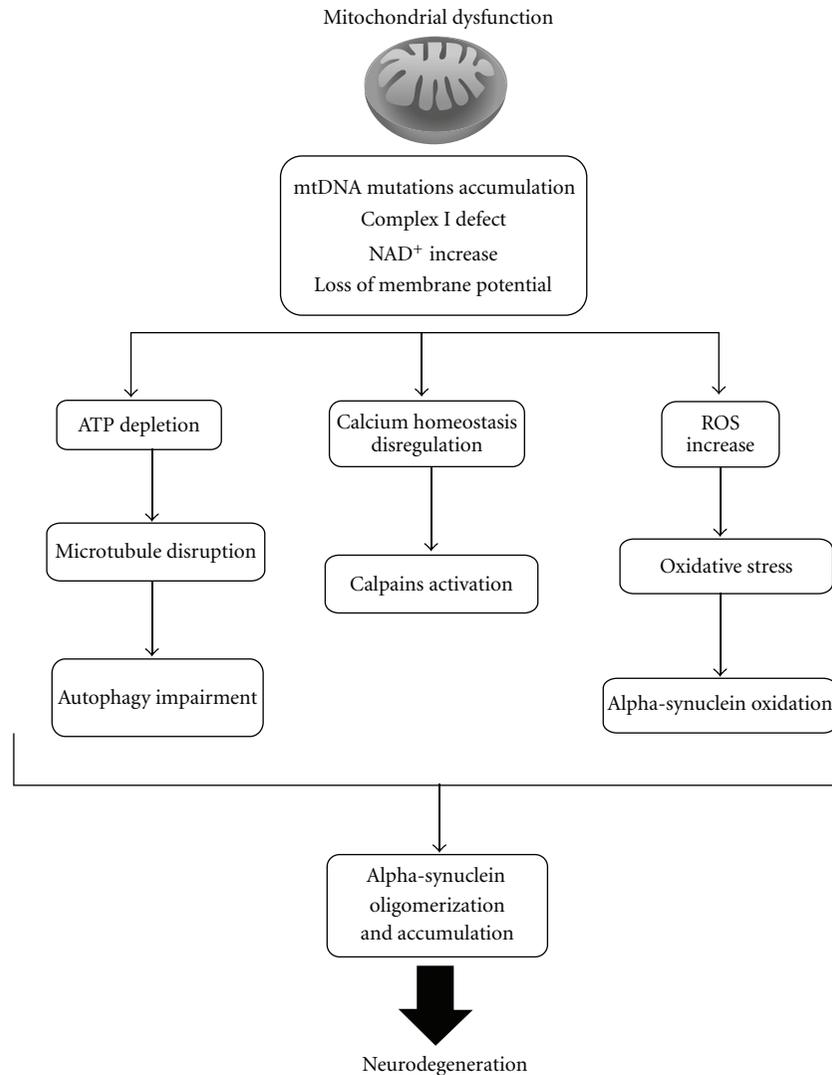


FIGURE 1: The mitochondrial cascade hypothesis for PD states that the inherited electron transport chain gene combinations determine basal ETC efficiency and ROS production. This defines the rate at which acquired mtDNA alterations occur determining when mitochondrial impairment reaches a threshold that activates the pathologic characteristics of PD. Mitochondrial impairment in PD is characterized by a complex I defect, which leads to ATP depletion and NAD^+/NADH ratio imbalance promoting microtubule disruption. Moreover, mitochondrial membrane potential is lost and calcium homeostasis is deregulated, which leads to calpains activation. In addition, the levels of mitochondrial ROS are significantly increased triggering oxidative stress. These all prompt alpha-syn oligomerization either by interaction with free tubulin, calpains or by oxidation. The end result is alpha-syn oligomerization and accumulation of alpha-syn oligomers as well as of disrupted organelles culminating in neurodegeneration.

The evidence for mitophagy comes from a work in yeast that identifies a specific protein in the mitochondrial outer membrane Uth1p, whose presence is necessary for mitochondrial macroautophagy. Degradation of mitochondrial proteins did not occur in mutant strains carrying null mutations of Uth1p [265]. Additionally, autophagic sequestration of mitochondria is further supported by the observation that protein markers of mtDNA are lost together with protein markers of mitochondrial inner membrane such as cytochrome oxidase subunit IV and subunit I. Parkin and PINK-1 PD-related proteins were recently associated to mitophagy. Chu and coworkers showed that MPP^+ induced

autophagy and mitochondrial degradation that was inhibited by siRNA knockdown of autophagy proteins Atg5, Atg7, and Atg8 [266]. Moreover, it was shown that Parkin promotes autophagy of damaged mitochondria and further supports that PD pathogenesis implicates a failure in the elimination of dysfunctional mitochondria [154]. Furthermore, stable knockdown of pink-1 induced oxidative stress and mitochondrial fragmentation in SH-SY5Y cells through autophagic and fission/fusion machineries [156]. A report from 2010 demonstrated a new interaction between PINK-1 and Beclin1, a key proautophagic protein. PINK-1 significantly enhanced basal and starvation-induced autophagy,

which was reduced by knocking down beclin1 expression [267]. Another report provided data linking PINK-1 and parkin to the selective autophagy of mitochondria [268].

In PD, autophagy is of extreme importance once it allows cells to rid themselves of damaged and superfluous mitochondria, as well as other organelles and most interestingly of protein aggregates accumulation.

6. Concluding Remarks

PD affects 1% of the population at age of 65 and up to 5% of the population by age 85 [269] and is the second most common neurodegenerative disorder after AD. Efforts have been made in order to elucidate PD trigger mechanisms within the cell and further consequences. Compelling data suggests that mtDNA alterations may lead to a massive mitochondrial impairment and thus triggering the death of dopaminergic neurons. When mitochondrial dysfunction reaches a critical threshold inside the cell it rouses ATP depletion, oxidative stress, and calcium homeostasis deregulation that in turn induces microtubule disruption, alpha-syn oxidation, and calpains activation. In addition, the fusion and fission machinery is impaired as well as autophagy leading to the accumulation of alpha-syn oligomers and disrupted organelles (Figure 1). This accumulation creates a positive feedback loop causing the pathology and ultimately the death of dopaminergic neurons. Further studies need to be performed to understand how and when mitochondrial dysfunction starts and why it affects a specific group of neurons. The therapeutic challenge might start acting at an mtDNA level or at a microtubule level improving the degradation of alpha-syn oligomers and disrupted organelles.

Abbreviations

PD:	Parkinson's disease
alpha-syn:	Alpha-synuclein
LBs:	Lewy Bodies
MPTP:	1-methyl-4-phenyl-1,2,3,4-tetrahydropyridine
mtDNA:	Mitochondrial DNA
OXPHOS:	Oxidative phosphorylation
ROS:	Reactive oxygen species
MPP ⁺ :	1-methyl-4-phenylpyridinium
AD:	Alzheimer's disease
6-OHDA:	6-hydroxydopamine
POLG1:	mtDNA polymerase gamma 1
NAD ⁺ :	β -Nicotinamide adenine dinucleotide
NADH:	Reduced β -NAD ⁺
SIRT1s:	Sirtuins
PGC1 α :	Peroxisome proliferators activated receptor gamma coactivator-1 alpha
CMA:	Chaperone-mediated autophagy
CNS:	Central Nervous System
Mfn1 and Mfn2:	Mitofusin-1 and -2
Opal:	Optic atrophy 1
Drp1:	GTPase Dynamin-related protein 1

LRRK2: Leucine-rich repeat kinase 2

NAM: Nicotinamide

CNS: Central Nervous System

BDNF: Brain-derived neurotrophic factor

GDNF: Glial cell line-derived neurotrophic factor.

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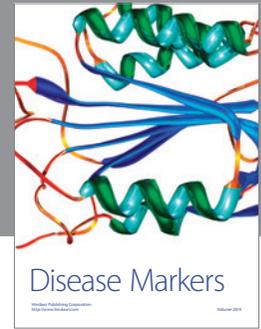
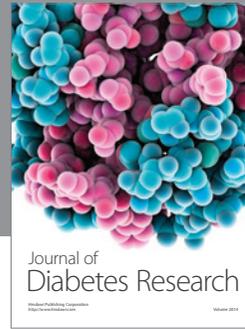
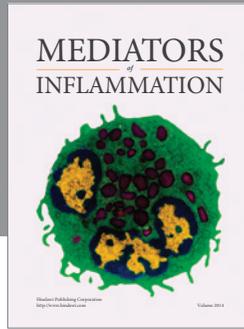
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