Parkinson’s Disease and Mitotherapy-Based Approaches towards α-Synucleinopathies

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Abstract

Parkinson’s disease (PD) is a neurodegenerative disorder characterized by the progressive loss of dopaminergic neurons in the substantia nigra pars compacta region of the midbrain and the formation of intracellular protein aggregates known as Lewy bodies, of which a major component is the protein α-synuclein. Several studies have suggested that mitochondria play a central role in the pathogenesis of PD, encompassing both familial and sporadic forms of the disease. Mitochondrial dysfunction is attributed to bioenergetic impairment, increased oxidative stress, damage to mitochondrial DNA, and alteration in mitochondrial morphology. These alterations may contribute to improper functioning of the central nervous system and ultimately lead to neurodegeneration. The perturbation of mitochondrial function makes it a potential target, worthy of exploration for neuroprotective therapies and to improve mitochondrial health in PD. Thus, in the current review, we provide an update on mitochondria-based therapeutic approaches toward α-synucleinopathies in PD.

Keywords: Parkinson’s disease; α-Synucleinopathies; Lewy bodies; mitochondria; mitochondrial dynamics; mitotherapy

1. Introduction

Parkinson’s Disease (PD) is the second most common progressive neurodegenerative disorder (NDD) after Alzheimer’s disease (AD). It was originally described by James Parkinson in his article “An essay on the shaking Palsy” in 1817, after which it was renamed PD [1–9]. PD is pathologically characterized by the loss of dopaminergic (dopamine-producing) neurons in the substantia nigra pars compacts (SNpc) of the midbrain and the formation of neuronal Lewy bodies (LBs) composed of α-synuclein protein (SNCA) [1,3,6,8–10]. Several studies have reported the clinical importance of SNCA in the pathogenesis of PD. LBs (intraneuronal proteinaceous cytoplasmic inclusions), the hallmark of PD, were first identified by Lewy in 1912 [11,12]. After many decades, in 1996, the first link between the PD phenotype and SNCA was made with the identification of an A53T point mutation on chromosome 4q21-23 [11,13]. LBs contain misfolded SNCA, a protein that also aggregates in related disorders such as Dementia with Lewy Bodies (DLB), Multiple System Atrophy with Lewy Bodies (MSA), Corticobasal Degeneration (CBD), and Progressive Supranuclear Palsy (PSP) [8,9,12,14]. Several studies have shown that aging and environmental stress (e.g., pesticides, water pollutants, etc.) may increase the neuropathology of PD [3,9,15]. This inflammatory process over time generates cellular senescence in neurons [9,16,17]. PD is an idiopathic disorder of the nervous system linked with both motor and non-motor symptoms [3,4]. The motor symptoms of PD include rigidity, resting tremor, balance impairment, postural instability, gait, and bradykinesia (slow movement) [1,3,4,8,9,11,18–22]. Non-motor symptoms of PD include cognitive decline (dementia), neurobehavioral disorders (anxiety, depression), sensory and sleep disturbances, and autonomic impairment (e.g., hyperhidrosis and orthostasis) [4,9,11,19,20,23]. PD is a chronic progressive NDD that occurs mostly in older persons but can also present in much younger patients [9].

1.1 Molecular Mechanism of PD

PD is a multifactorial disease, in which genetic and non-genetic environmental factors are involved [24–27]. Several biological processes are known to contribute to PD pathogenesis, such as accumulation of misfolded proteins [24,28–30], oxidative stress [24,31], mitochondrial dysfunction [24,32], and neuroinflammation [33,34]. However, the mechanistic details underlying these processes are not well understood. Eighteen genetic loci and twelve familial PD genes—ATP13A2, DNAJC6, DJ-1, EIF4G1, FBXO7, LRRK2, PARK2, PINK1, PLA2G6, SNCA, SYNJ1, and VPS35—have been identified to date [24,30,35].

1.2 Alteration of Cellular Processes and PD

It is widely reported that changes in several cellular processes play a pivotal role in the onset of PD. The details of some key processes are described below.

1.2.1 α-Synuclein Aggregation

The accumulation of misfolded SNCA and other proteins are established primary hallmarks of PD [36–38]. Several genetic, molecular, and biochemical studies conducted
on postmortem human brains from patients neuropathically diagnosed with PD with dementia and DBL suggest that the accumulation of misfolded proteins such as amyloid beta (Aβ), phosphorylated tau (p-tau), and SNCA [36,39] plays a role. Further, it has been demonstrated that fibrils of SNCA, proto-fibrils, oligomers, or other misfolded amyloid proteins can create pores in cellular membranes, leading to neuronal death through energy failure, inducing oxidative stress, neuroinflammation, and excitotoxicity [36]. Similarly, a mutation in the SNCA gene causes familial PD with early onset, a high correlation with dementia, and rapid progression [36,40]. In animal and cell culture models, overexpression of SNCA results in aggregation of SNCA in mitochondria, deficits in mitochondrial motility, and reduced mitochondrial membrane potential [41], while SNCA knockout mice show mitochondrial lipid and electron transport chain abnormalities [36]. Moreover, the A53T transgenic mouse model of PD develops degeneration of mitochondria in neurons, with SNCA aggregation in mitochondria and reduced activity of complex IV [36,42]. In addition, several studies have reported that in the human PD brain, dopamine (DA) neurons showed DNA damage in mitochondria, dysfunction of the respiratory chain complexes, and oxidative stress [36,43].

1.2.2 Tau

The accumulation of hyper-phosphorylated tau can lead to the formation of paired helical filaments known as “neurofibrillary tangles” (NFTs), which are a hallmark of several neurodegenerative diseases such as AD, PSP, and frontotemporal dementia with parkinsonism (FTDP) [36]. FTDP is associated with a locus at chromosome 17 (FTDP-17), where p-tau aggregation occurs in the SNpc and cortex of the brain [36,44]. P-tau can also be found in association with LBs and mutation of the LRRK2 gene, which are correlated with the development of sporadic PD [36,44,45]. Similarly, in FTDP cases, alteration in the gene coding for microtubule-associated protein (MTPP) leads to an increase in p-tau aggregation [36,45]. Moreover, while NFTs are associated with AD, they can co-localize with SNCA in LBs and play a significant role in destabilizing DA neurons, ultimately resulting in their degeneration and death [36,44,46].

1.2.3 Oxidative Stress

Oxidative stress (OS) plays a regulatory role in the aging process and directly affects the central nervous system (CNS) [47]. In addition, the oxidative stress theory is one of the most popular theories in PD and other neurodegenerative diseases such as AD, Huntington’s Disease (HD), and Amyloid Lateral Sclerosis (ALS) [36]. Under physiological conditions, reactive oxygen species (ROS) or free radicals are very important for gene transcription, regulation of synaptic plasticity, and apoptosis [47,48]. Moreover, oxidative stress occurs when ROS increases the activity of cellular antioxidant enzymes. Due to the continuous increase of ROS, an aggregation of cytotoxic compounds occurs that results in lipid toxicity, protein collapse, failure of key enzymes activities, and induction of cell death in several neurons including DA-neuronal tissue [49]. Recently, nicotineamide adenine dinucleotide phosphate (NADPH) oxidase (NOX) has been proposed as a key ROS generator [50] and plays a pivotal role in the triggering of oxidative stress and inducing neurotoxicity [51], including within mitochondria [52]. Complex I and II of the electron transport chain (ETC) are thought to house most of the ROS produced in mitochondria [47]. Moreover, increased oxidative stress can reduce lysosomes and affect the lysosomal autophagy system, which is connected with oxidative stress to the buildup of SNCA [47]. Another hypothesis proposes that extracytosolic DA can be oxidized to generate DA-quinones. Further, DA-quinine-modified SNCA may partially inhibit chaperone-mediated autophagy and promote SNCA to self-assemble [47,53,54], while the accumulation of intracellular SNCA increases mitochondrial oxidative stress [55].

1.2.4 Mitochondrial Dysfunction

Mitochondrial dysfunction is known to be important in PD pathogenesis [47]. Moreover, several studies have shown that mitochondrial dysfunction induces chronic ROS production and dopaminergic neurodegeneration. Further, overexpression of SNCA in mice results in increased susceptibility to toxins as compared with SNCA knockout mice, suggesting the toxicity of mitochondrial SNCA [56,57]. As mentioned above, PD affects mitochondrial complex I activity, which is directly related to adenosine triphosphate (ATP) production and, ultimately, leads to apoptosis [36,58]. Further, dysregulation of transcription factors causes changes in mitochondrial biogenesis, which leads to mitochondrial dysfunction [47]. Peroxisome-proliferator-activated receptor gamma coactivator-1α (PGC1-α) is a key player in mitochondrial biogenesis. In a knockout mouse model of PGC1-α, dopaminergic cells are the most sensitive to MPTP, while overexpression of PGC1-α protects against neurotoxicity [47,59]. Further, variation in various genes such as parkin, DJ-1, LRRK2, and PINK1 induces mitochondrial defects and altered mitochondrial function [47,56,60,61]. Moreover, a mutation in PINK1 induces the onset of an autosomal-recessive form of PD by reducing both mitochondrial respiration and ATP production, while increasing the accumulation of SNCA [47,62]. Dysfunction of Phosphatase and Tensin homolog (PTEN) induced kinase 1 (PINK1) also impairs mitophagy and mitochondria localization [47,63]. Several studies have demonstrated that in Drosophila, PINK1 and Parkin belong to the same signaling pathway alongside PINK1, upstream to Parkin [47]. In damaged and depolarized mitochondria, there is activation of kinases and aggregation of PINK1, and this induces cytosol to recruit Parkin to mediate autophagy [47,64,65].
1.2.5 Neuroinflammation

Molecular and cellular analysis of the human brain has revealed neuroinflammation-related damage in PD patients [47,66]. Both innate and adaptive immune responses are implicated in the progression of PD [47,67–69]. They regulate nuclear factor kappa-B (NF-κB), innate immune cells, and NOD-like Receptor (NLR) family pyrin domain-containing 3 (NLRP3), leading to an increase in cytokines, including interleukin (IL)-1β and tumor necrosis factor (TNF)-α [47,70,71]. In early PD patients, the putamen (associated with learning and memory) and midbrain exhibit a denser population of activated microglia, which is associated with reduced activity of DA transporter ligands [47,72,73]. SNCA and damage-associated molecular patterns (DAMPs) may induce a proinflammatory shift when entering cells via toll-like receptor 2 (TLR-2) [47,74–76]. When cells are damaged or dying, they release IL-1α, mitochondrial ROS, and DAMPs, triggering an innate immune response upon interaction with pattern recognition receptors (PRRs). Furthermore, in PD, the activation of microglial cells may result from PRR-mediated responses to DAMPs. It has been shown that DAMPs derived from mitochondria cause innate immunity and neuroinflammation in PD due to mitochondrial dysfunction [77]. This is evidenced by active microgliosis and NLRP3 inflammasome activation, which occurs postmortem in PD patients’ brains in the SNpc, confirming that PD is a state of neuroinflammation. In addition, blood and cerebrospinal fluid analyses have indicated increased levels of classical cytokines such as TNF-α, IL-6, and IL-1β through the classical innate immune pathway in PD patients, compared with age-matched controls [78]. Mitochondria have distinctive features such as different ribosomes and circular DNA, and are very important in maintaining innate immunity through their replication, leading to DAMP release that activates toll-like receptors and initiates sterile inflammation [79]. Importantly, the inflammatory milieu of PD is predominantly controlled by the T cells of the adaptive immune system. SNCA, a key protein of PD, can be produced by several immune cells including T cells [80]. Several studies have shown that T cell populations are disturbed in PD patients. Animal models expressing human wild-type SNCA have increased CD4 and CD8 T cell frequencies. Leucine-rich-repeat kinase 2 (LRRK2), associated with familial PD, modulates T cell activation through adaptive immune responses toward leading to PD pathogenesis [77,80]. These results underscore the complex relationship between mitochondrial dysfunction and adaptive immune responses, suggesting opportunities for the development of targeted immunomodulatory strategies within PD therapeutics.

In an animal model of 6-Hydroxydopamine (6-OHDA)-induced neuronal degeneration, microglial cells gradually shift from an anti-inflammatory M2 phenotype to a proinflammatory M1 phenotype [47,81]. Following this repolarization, NF-κB initiates the production of cytokines in M1 cells, resulting in the transcription of pro-caspase-1 and interleukin. These processes give rise to the NLRP3 inflammasome, which, in collaboration with caspase-1, activates proinflammatory IL-1β. In PD, other proinflammatory proteins released from M1 cells, such as TNF and inducible nitric oxide synthase (iNOS), also contribute to neurodegeneration [47] (Fig. 1).

1.2.6 Synaptic Dysfunction

Several studies have reported that SNCA binds with synaptic vesicle membranes and regulates neurotransmitter production [82]. In synucleinopathies, there is formation of SNCA aggregates in the brain that ultimately results in neuronal dysfunction [83,84]. Further, pathological SNCA species lead to presynaptic deficits through their interference with the soluble N-ethylmaleimide-sensitive factor attachment protein receptor (SNARE) complex required for synaptic vesicle fusion and neurotransmitter release. SNCA oligomers have been shown to disturb SNARE-mediated vesicular fusion by binding to vesicle-associated membrane protein 2 (VAMP2) and inhibit SNARE complex formation, affecting neurotransmitter release and ultimately resulting in synaptic failure [85,86]. Moreover, mitochondrial dysfunction is closely associated with presynaptic dysfunction caused by pathological SNCA. Importantly, SNCA can interact with mitochondria and induce mitochondrial fragmentation, impair mitochondrial fusion, and increase mitochondrial autophagy [39,86]. Such abnormalities can impair energy production and the calcium homeostasis vital for synaptic transmission and vesicle fusion. Furthermore, dysfunctional mitochondria due to SNCA are also involved in oxidative stress leading to neuronal damage, worsening pre-synaptic deficits, and increasing synaptic dysfunction [39].

Tau, a microtubule-binding protein, is commonly found in axons, but in neurodegenerative conditions such as AD it is mislocalized to dendritic spines. Consequently, synaptic function and plasticity is disrupted, leading to post-synaptic deficits. In addition, tau mislocalization to dendritic spines affects the structural and functional integrity of synapses [39,86,87]. It also disrupts post-synaptic density organization and affects synaptic protein trafficking, which eventually results in the impairment of synaptic transmission and plasticity [86,88].

2. Mitochondrial Dynamics and PD

Mitochondria are cytosolic free-floating organelles present in all eukaryotic cells and known as the “powerhouse” of the cell [6,89,90]. Mitochondria take care of cellular energy demands by generating cellular energy in the form of ATP by oxidative phosphorylation [6,91] and are involved in cell death regulation via apoptosis, the formation of iron-sulfur clusters, calcium homeostasis, and the metabolism of amino acids and lipids (Fig. 2) [6,92,93]. Mitochondria regulate calcium homeostasis and play a key
Fig. 1. Molecular mechanisms of PD. In PD, amyloid beta (Aβ) plaques and α-synuclein protein (SNCA) aggregation cause the formation of a pore in the cell membrane, leading to neuronal death (1). Overexpression of SNCA leads to SNCA accumulation in mitochondria, deficits in mitochondrial motility, and reduced mitochondrial membrane potential (2). Mutations in SNCA cause PD (3). Knockout of SNCA causes defects in the mitochondrial electron-transport chain (4). P-tau co-localizes with LBs leading to the development of PD (5). P-tau is also linked with mutations in LRRK2 and occurs in PD (6). ROS increases cellular antioxidant activity and generates oxidative stress, which leads to neuronal loss and neurotoxicity in PD (7). NOX is the most important ROS generator and plays a key role in the triggering of oxidative stress and neurotoxicity (8). Increased oxidative stress can affect the lysosomal autophagy system, which increases SNCA aggregation and results in PD (9). Abnormal mitochondrial complex I activity and reduced ATP production is seen in PD (10). Mutations in several genes such as LRRK2, DJ-1, Pink-1, and Parkin lead to mitochondrial dysfunction, oxidative stress, the generation of ROS, and neuroinflammation (11). In PD, Pink1 reduces ATP synthesis and mitochondrial respiration, and increases the accumulation of SNCA (12). Increased levels of NF-kB, innate immune cells, and NLRP3 generate cytokines such as IL1-β, TNF-α, and caspase1 that are associated with neuroinflammation in PD. NLRP3 induces 6-OHDA leading to neurodegeneration in PD (13). P-Tau, phosphorylated tau; PD, Parkinson’s Disease; ROS, reactive oxygen species; NF-κB, nuclear factor kappa-B; 6-OHDA, 6-Hydroxydopamine; NLRP3, NOD-like Receptor (NLR) family pyrin domain-containing 3; OM, outer membrane; IM, inner membrane; LB, lewy body; LRRK, leucine-rich repeat kinase; DJ-1, Parkinson disease protein 7; PTEN, phosphatase and Tensin homolog; Pink-1, PTEN-induced putative kinase 1; IL, interleukin; TNF-α, tumor Necrosis Factor-alpha; NADPH, nicotinamide adenine dinucleotide phosphate; NOX, NADPH oxidase.

Role in controlling programmed cell death and scavenging free radicals [6]. Structurally, mitochondria are double-membrane cell organelles composed of a lipid bilayer with a phospholipid, an inner membrane, and an outer membrane that surrounds the intracompartmental matrix [6]. The intermembrane space contains the major units of oxidative phosphorylation [6,91] and the mitochondrial matrix contains 10–100 copies of mitochondrial DNA. Mitochondria are dynamic organelles that continuously undergo fission and fusion processes and regulate cell survival, cell growth, cell division, and cell differentiation [90,94]. The balance between mitochondrial fusion and fission processes underpin the important roles of mitochondria, such as protecting mitochondrial DNA and controlling mitochondrial bioenergetics functioning [6,95].
Mitochondria are interconnected entities that fulfill cellular energy requirements by changing their morphology and mobilizing within the cell [96,97]. To ensure cellular energy demands, mitochondria pass through several processes such as the fission and fusion processes, axonal transport, and mitophagy [97–99]. There are several proteins available on the mitochondrial outer and inner membrane that regulate the mitochondrial fusion/fission process and mitochondrial transport. The fusion protein optic atrophy protein 1 (OPA1) is located on the inner mitochondrial membrane (IMM) while GTPase mitofusin1 and 2 (Mfn1/2) are located on the outer mitochondrial membrane (OMM) [97,100–104]. In addition to OPA1 and Mfn1/2, several other proteins such as dynamin-related protein 1 (Drp1), mitochondrial fission 1 protein (Fis1), mitochondrial dynamics protein of 49 kDa (MiD49), mitochondrial dynamics protein of 52 kDa (MiD52), and mitochondrial fission factor (MFF) participate in the fission process.

Intracellular mitochondrial dynamics involve mitochondrial movement from one cell compartment to another and are important in neuronal cells that are connected with long axons and dendrites [105]. The movement of mitochondria in axonal cells occurs by a process known as mitochondrial axonal transport, during which mitochondria travel through the axon to fulfill the neuronal energy requirements [106]. There are two types of mitochondrial axonal transport: anterograde and retrograde. During anterograde mitochondrial movement, mitochondria move from the cell body to the axon toward the microtubule (MT) plus end, mediated by the kinesin motor protein to provide energy at the synapses. In retrograde transport, mitochondria move from the axon to the cell body toward the MT minus end, mediated by the dynein motor protein to eliminate the damaged mitochondria [106–109]. The movement of mitochondria is mediated by a protein complex between the OMM protein mitochondrial rho GTPase (Miro), MT-bound motor proteins, and adaptor proteins such as trafficking kinesin protein 1 and 2 (TRAK1 and 2) [97,110]. Further, Miro1 also works with Drp1/Fis1-independent mitochondrial shape transition protein (Mist) for mitophagy [97,111]. MTs are polar α/β-tubulin polymers with the plus end (kinesin) to the cell dendrites and minus end (dynein).
within the cell body [97,112]. Under normal physiological conditions, mitochondria constantly undergo cycles of fission and fusion to maintain their quality control process (biogenesis and mitophagy) and balance between the morphology and energy needs of the cell [90,113–115]. From population and gene technology studies, more than 20 genes in monogenic forms of PD have been discovered, including SNCA, PINK1, Parkin, LRRK2, and DJ1. Some of these genes are correlated with mitochondrial dysfunction, for example PINK1 and Parkin play a key role in the regulation of the mitochondrial fission and fusion processes [1,116–118] (Fig. 3).

Further, triplication or mutation of SNCA; chemicals such as rotenone, maneb, and 1-methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine (MPTP); and mutations in PINK1, LRRK2, Parkinson disease protein 7 (DJ1), and vacuolar protein sorting-associated protein 35 (VPS35) can induce inhibition of complex 1 [6,119,120]. Inhibition of complex 1 induces apoptosis and produces ROS, resulting in the activation of intrinsic pro-apoptotic pathways, translocation of Bax into the mitochondria, and release of cytochrome c (cyt c) [6]. Phosphorylation of tumor necrosis factor receptor-associated protein 1 (TRAP1) or high-temperature requirement A2 (HTRA2), the substrate of PINK1, results in attenuation of cell death by mitochondrial PINK1 [6]. Mutations in PINK1 and Parkin are correlated with PD and can induce abnormal mitophagy resulting in the accumulation of damaged mitochondria, which eventually leads to cellular dysfunction and ultimately cell death [6]. SNCA has been shown to influence mitochondrial size, in both a dependent and independent manner in fission/fusion proteins, and transport and mitophagy processes [97,121–125].

Fig. 3. Mitochondrial dynamics. Schematic image showing mitochondrial dynamics including the mitochondrial axonal transport, and fusion and fission process. Mitochondrial axonal transport uses the mitochondrial outer membrane protein Miro, trafficking kinesin-binding protein (TRAK), an adaptor protein milton, and the motor proteins kinesin and dynein. Miro makes a protein complex with motor proteins with the help of milton (TRAK). Kinesin and dynein help in making the connection between microtubules and mitochondria and facilitates axonal transport. Anterograde transport occurs through kinesin from the cell body to the axon and retrograde transport is mediated by dynein from the axon to the cell body (A). The mitochondrial fusion process is induced by the mitochondrial outer membrane protein Mfn and the mitochondrial inner membrane protein optic atrophy 1 (OPA1), which help in the fusion of the outer and inner membrane, respectively. Mitochondrial fission occurs by the mitochondrial outer membrane protein mitochondrial fission 1 protein (Fis1) and the cytosolic protein dynamin-related protein 1 (Drp1). During fission, Drp1 forms high molecular weight oligomers with Fis1 on the mitochondrial membrane. Once Fis1 is detached from the mitochondria, the fission process is complete (B). Mfn, mitofusin1; MiD49, mitochondrial dynamics protein of 49 kDa; MiD52, mitochondrial dynamics protein of 52 kDa; MFF, mitochondrial fission factor.
Further, SNCA oligomers can bind to lipids in the OMM and distress the membrane bend which leads to reduced mitochondrial fusion [22,97]. Furthermore, upregulation of SNCA in a transgenic mouse model reduces the level of Mfn1/2 and is associated with a decrease in mitochondrial fusion rate [97,126], while down-regulation of SNCA was shown to be associated with mitochondrial elongation [97]. An increased concentration of SNCA was reported in a mitochondrial traffic jam well before the axonal degeneration that affects mitochondrial mobility (anterograde and retrograde) in the axon [22,123]. In anterograde transport, SNCA oligomers are disrupted by direct interaction between MTs and kinesin, which leads to an increased expression of tau and eventually affects MT structure [97,127,128]. SNCA induces fragmentation of MTs directly as well, hampering the movement of mitochondria from the distal cell area [129]. Furthermore, the PD-linked protein LRRK2 alters the MT polymerization cycles, affecting mitochondrial trafficking [130,131]. The parkinsonian toxin 1-methyl-4-phenylpyridinium (MPP+) induces impairment of mitochondrial transport and inhibits anterograde transport leading to an increase in dynemin-dependent retrograde transport [132]. The Complex I inhibitors of mitochondria, rotenone and MPP+, and parkinsonian phenotypes inducers were shown to increase the mitochondrial fission, and inactivation of Drp1 prevents the fission phenotype [97,133]. In the SNpc of PD patients, OPA1 is decreased in the absence of Mfn1 changes and mitochondrial fusion is deficient [97]. Thus, an increase in mitochondrial fission results in a fragmented mitochondrial network and alters the neuronal signaling in PD [97]. Mutations in PINK1 and LRRK2 in Drosophila alter mitochondrial calcium homeostasis involving the mitochondrial outer membrane protein Miro1 [97,134] (Fig. 4).

3. Mitotherapy: Future Medicine for α-Synucleinopathies/PD

As mentioned above, mitochondrial dysfunction results in decreased oxidative phosphorylation affecting cellular energy production and increased production of ROS, one of the key factors associated with aging and age-related diseases [135,136]. Thus, it is unequivocally accepted that mitochondrial quality control or mitochondrial health plays a vital role in the onset of several neurodegenerative disorders, and abnormal mitochondrial health/abnormal mitochondrial function has been documented in PD patients and various α-synucleinopathy models in animals such as rodents, nematodes, and Drosophila [1,137–140]. Further, it has been shown that aging and aging-related diseases are closely associated with abnormal functioning of mitochondria and available evidence suggests that alteration in mitochondrial function can be the first step in the onset of several neurological diseases [136,141–143]. Moreover, it has been reported that SNPce neurons are more susceptible to oxidative damage and thus, dependent on efficient mitochondrial function also, SNCA has been reported to play a regulatory role in altering ATP synthesis and complex I activity [144–146]. Further, several PD-associated genes (i.e., PINK1 and PARKIN) are linked with mitochondrial quality control/mitochondrial biogenesis and this strongly suggests a regulatory role of mitochondria in α-synucleinopathies/PD. Thus, identifying possible strategies targeting mitochondrial health is a promising approach for identifying therapeutic targets for α-synucleinopathies and neurodegenerative diseases.

In the past decade, mitochondria have been examined as a potential target for identifying possible therapeutic strategies for several conditions including neurological, metabolic, genetic, cancer, and viral diseases [140]. Mitochondria, the powerhouse of cells through generating ATP via the electron transfer from complex I to IV, become non-functional in several diseases which makes them a target for reviving cells affected by pathological conditions. Mitotherapy (mitochondrial therapy) utilizes approaches that can be used to improve mitochondrial health and subsequently helps to restore neuronal dysfunction and energy metabolism in neurons, promoting their capacity for energy regeneration [140,147].

Several strategies have been examined for their efficacy in improving mitochondrial health and disease conditions. These include the use of several molecules, antioxidants, nanoparticles, genetic therapy-based approaches, plant secondary metabolites, mitochondria-targeted drug molecules, efficient drug delivery methods, and mitochondrial transplantation. The details of some of these potential strategies are outlined in Fig. 5.

3.1 Use of Mitochondria Targeted Antioxidant Molecule

Coenzyme Q10 (CoQ10) is a potential target and has been used in several clinical trials for PD. CoQ10 acts as a lipid-soluble endogenous compound and serves as a cofactor for the ETC by accepting electrons from complexes I, II, and III [141,148]. In microsomal lipid membranes and inner mitochondrial membranes, CoQ10 acts as a free radical scavenger by regenerating α-tocopherol and reducing α-tocopheroyl radicals [141]. Furthermore, in PD patients, the level of CoQ10 in platelets is reduced and associated with altered mitochondrial complex I activity [142,143,149]. CoQ10 is also known to block cell death by blocking Bax in mitochondria [144,150], and oxidative stress [145,151] and mitochondrial permeability transition pore inhibition cause cell death by increasing mitochondrial calcium retention [146].

MitoQ (mitochondrial quinoline derivative), a mitochondria-targeted antioxidant, has shown potential for improving mitochondrial health in humans and animal models. MitoQ helps in the conversion of hydrogen peroxide (H$_2$O$_2$) to H$_2$O and O$_2$, reduces the toxicity induced due to free radicals, and acts against lipid peroxidation [152–156]. It also exerts neuroprotective effects against
MPTP-induced neurotoxicity in primary mesencephalic neuronal cells and cultured dopaminergic cells as well as in the MPTP mouse model of PD [157].

3.2 Use of Mitochondria-Targeted Neuroprotective Peptides

Mitochondria-targeted neuroprotective peptides are synthetic peptides of less than 10 amino acid residues in length. They are also known as Szeto-Schiller (SS) peptides as they were developed by Hazel H. Szeto and Peter W. Schiller at CRI, Quebec, Canada. SS peptides (SS02, SS31, SS20) are well studied and can easily transfer to mitochondria and scavenge the mitochondrial ROS, helping to improve mitochondrial health [154,158–160]. The use of SS peptides in different animal models of neurodegenerative diseases suggests that SS peptides (SS-02, SS-31) possess anti-apoptotic and necrosis effects [160]. Further, SS20 and SS30 have been reported to play a neuroprotective role by improving mitochondrial function in a PD model [160].

A study by Sekhar, 2022 [161] demonstrated that supplementation of Glycine and N-Acetylcysteine (GlyNAC) to mice and type 2 diabetic patients improves mitochondrial function by lowering oxidative stress [161].

3.3 Use of Lactoferrin-Functionalized Au-Bi2Se3 Nanodots

Lactoferrin-functionalized Au-Bi2Se3 nanodots (Lf-Au-Bi2Se3 NDs) is one of the promising molecules that have the potential for the treatment of PD by targeting the mitochondria and attenuating ROS [153,162,163]. Lf-Au-Bi2Se3 NDs exhibit strong blood-brain barrier (BBB) permeability and can efficiently cross BBB to reach the brain [154–156]. Within the brain, these nanodots (NDs)
Fig. 5. Mitotherapy-based strategies for α-synucleopathies. Lf-Au-Bi2Se3 NDs, Lactoferrin-functionalized Au-Bi2Se3 nanodots; PCG-1α, peroxisome proliferator-activated receptor-gamma coactivator (PGC)-1 alpha.

function as different enzymes, including superoxide dismutase, catalase, glutathione peroxidase, and reductase [164]. They help to regulate the level of cellular ROS and maintain mitochondrial membrane potential. In an in vivo study using a PD mouse model, it has been shown that use of Lactoferrin-functionalized Au-Bi2Se3 nanodots (Lf-Au-Bi2Se3 NDs) improves memory and motility, protected mitochondria, and reduced loss of dopaminergic neurons in the SNpc region of the brain [162, 165]. Further, it was demonstrated that Lf-Au-Bi2Se3 NDs mimic antioxidant enzymes to reduce ROS levels and protect cells from toxicity. Furthermore, Lf modification enhances receptor adsorption-mediated transcytosis in the BBB [156], thus increasing therapeutic specificity in PD. NDs were also found to be close to mitochondria, further supporting their mitochondria-protective effect [60, 166, 167]. Overall, Lf-Au-Bi2Se3 NDs show promise as a potential therapeutic target for PD [52, 162].

3.4 Use of Nanoparticles

Several studies have reported the utilization of gold nanoparticles (GNPs) conjugated with lipoic acid (LA) for the treatment of PD through the mitigation of oxidative stress [168–171]. Oxidative stress, caused by the excessive generation of ROS, is intricately linked to the gradual degeneration of neuronal cells in PD [171–173]. The study establishes the capacity of GNP-LA nanoconjugates to effectively shield SH-SY5Y cells (a cellular model for PD) from the detrimental effects induced by ROS [174–177]. In addition, it also emphasizes the biocompatibility of GNPs-LA nanoconjugates and their capacity to alleviate cellular damage caused by heightened levels of ROS [177, 178]. This promising treatment for PD takes advantage of the antioxidant attributes of LA, coupled with the drug delivery capabilities of GNPs. Furthermore, it highlights the use of atomic force microscopy to gain insights into the mechanical changes of living cells in response to physiological and pathological changes. This technique provides valuable insights into the effectiveness of GNP-LA nanoconjugates in protecting cells against oxidative stress [168, 177, 179, 180].

Schlichtmann et al. (2022) [181] and others discussed the use of nanoparticles as a targeted drug delivery system by using polyanhydride nanoparticles (NP) functionalized with a triphenylphosphonium derivative named (3-carboxypropyl) triphenylphosphonium (CPTP) [182–185] to improve the delivery of a neuroprotective drug called mito-metformin [186–191]. The objective of the study was to treat mitochondrial dysfunction, which is a significant factor in the progression of PD, by focusing on dopaminergic neuronal atrophy [192–195]. First, the researchers tested the internalization of nonfunctional and functionalized CPTP NPs by neurons [196–198]. They observed that functionalization of CPTP significantly enhanced the cellular internalization of NPs. After that, it was determined whether nanoformulas were effective in treating mitochondrial dysfunction caused by rotenone [189, 190, 192, 199, 200]. The results showed that mito-metformin-coated NPs, functionalized by CPTP, confer significant protection against rotenone-induced toxicity, while non-functional and soluble mito-metformin NPs did not have a similar pro-
ective effect [201–203]. The mechanism of action involves the activation of adenosine monophosphate-activated protein kinase (AMPK) by mito-metformin. AMPK plays an important role in maintaining cellular metabolism and is attenuated in PD [192,193,200,204,205]. By activating AMPK, mito-metformin helps restore cellular function and protect against dopaminergic-induced cell death [206]. Targeted delivery of mito-metformin using a CPTP-functionalized NP improves its efficacy and reduces the required therapeutic dose, thereby minimizing the risk of toxicity. Overall, this study demonstrates the potential of targeted nanocarriers, especially CPTP-functionalized polyanhydride NPs, in the treatment of PD. This approach could help slow the progression of the disease by enhancing drug delivery and mito-metformin’s neuroprotective effects and improving the quality of life for people with PD [57,182].

### 3.5 Use of Plant Secondary Metabolites/Plant Derived Components

**Plant secondary metabolites** modulate the oxidative stress and neuroinflammation in PD through numerous mechanisms [89,181,207–211]. They have antioxidant outcomes and modulate the complexes/enzymes of nerve cells, stimulating mitochondrial biosynthesis through pathways consisting of sirtuin 1 (SIRT1), peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1α), mitochondrial transcription factor A (TFAM), and nuclear factor erythroid 2-related factor 1 (Nrf1) [212–214]. Some unique plant secondary metabolites referred to in the article encompass pinocembrin, naringin, and naringenin [181]. Pinocembrin, a flavonoid, impacts mitochondrial features in PD by blocking off extracellular signal-regulated kinases 1 and 2 (ERK1/2) or silencing nuclear factor erythroid 2-related factor 2 (Nrf2) [215]. Naringin, another flavonoid, modulates mitochondrial features, stability, and bioenergetics within the SNpc through a Nrf2-mediated pathway [216]. Naringenin, a flavanone, has been shown to increase mitochondrial membrane ability and decrease ROS by affecting the Nrf2/antioxidant response element (ARE) pathway [217,218]. The demanding situations related to the healing properties of plant secondary metabolites in PD encompass negative bioavailability, instability/solubility/selectivity, speedy metabolism, and clearance [181]. These elements restrict their plasma concentration and efficacy. To triumph over those demanding situations, researchers endorse investigating suitable transport structures consisting of nanoparticles, micelles, liposomes, and solid-lipid nanoparticles [181]. These transport structures can increase mobile uptake, bioavailability, and specificity of neuroprotective plant-derived secondary metabolites, thereby enhancing their therapeutic ability in PD.

**Apocynin**, a non-poisonous plant molecule possesses neuroprotective effects and has the potential to treat PD [192]. Apocynin functions as an antioxidant, is centered on mitochondria, and allows less oxidative harm, which is an essential pathological mechanism that causes neurodegeneration in PD [177,179,192]. A study by Brenza et al. (2017) [176] in a preclinical animal model of PD demonstrated that the orally active apocynin spinoff decreased neuroinflammation and guarded toward dopaminergic neurodegeneration, thus playing a neuroprotective role [192,219,220]. Several other studies have also shown that the use of apocynin derivatives to target mitochondria plays a protective role in dopaminergic neurodegeneration in a preclinical animal model of PD [192,219–221]. Further, nano-formulated, mitochondrial-focused apocynin provides extraordinary safety in opposition to oxidative stress-caused mitochondrial disorder and neuronal harm in a variety of neurons, including the dopaminergic neuronal lineage [158–161]. Thus, available evidence suggests that apocynin can be a useful remedy for ameliorating PD with the aid of stopping neuronal damage by reducing oxidative stress [219], specifically when used in conjunction with nanoparticles. Detailed research is vital to evaluate the effectiveness of nanoparticle-mediated apocynin transport and its potential to cross the BBB [163,220].

### 3.6 Mitochondrial Transplantation/Mitochondrial Transfer Strategy

**Mitochondrial transplantation/mitochondrial transfer** a therapeutic modality that aims to enhance cellular function and mitigate damage by replacing damaged mitochondria with healthy ones [222–224]. This approach entails the isolation of functional mitochondria, which are then introduced into the damaged tissue. The transplanted mitochondria have the potential to restore bioenergetic function, augment ATP production [225,226], improve calcium regulation, and reduce oxidative stress, thereby promoting cellular survival and function [227–229]. However, mitochondrial transplantation as a therapeutic strategy poses potential risks and challenges. One of the challenges is the efficient delivery of mitochondria to the target tissue [230–233]. Various methods have been developed, including direct injection into the damaged organ or systemic administration of a bolus of mitochondria [137]. Direct injection allows for a focal concentration of mitochondria but is invasive and may require multiple injections. Systemic administration allows for global distribution but accessing certain organs such as the brain may be challenging [234]. Another challenge is understanding the incorporation and protective mechanisms of transplanted mitochondria. It is imperative to determine how the transplanted mitochondria integrate into the recipient cells’ mitochondrial network and whether they can maintain their functionality in the long term [227,235,236]. Additionally, there is a need to assess the potential risks associated with mitochondrial transplantation, such as immune responses, potential adverse effects, and the risk of introducing dysfunctional mitochondria [237–240]. In order to fully comprehend the efficacy,
safety, and long-term effects of mitochondrial transplantation as a therapeutic strategy, in-depth research is required to address these challenges and fully comprehend their effects.

3.7 Exercise/Physical Fitness and Mitochondrial Health

Several studies have reported that with aging and in neurodegenerative diseases there is a progressive decline in motor function due to reduced mitochondrial function and ATP biogenesis. Further, regular exercise and physical activity increase mitochondrial turnover and ATP biogenesis by reducing ROS [241–245]. It has been demonstrated that exercise restores aging-induced reduced PGC-1α transcription and expression of transcription factor EB (TFEB), whose activity regulates mitophagy-related processes [246,247].

3.8 Other Potential Molecules

SKQ1, consisting of plastoquinone in conjugation with tetraphenylphosphonium (TPP+), targets mitochondria and within mitochondria it helps to reduce reduced skulachev plastoquinone 1 (SkQ1H2) from the respiratory chain, preventing peroxidation of mitochondrial phospholipid and synthesis of superoxide [154,164–166].

Creatine is guanidine compound and acts as a crucial energy reservoir for ATP. Creatine is found in skeletal muscle and several organs, such as the brain, is transported by specific creatine transporters, and serves as a substrate for cytosolic and mitochondrial creatine kinase [152,167]. Creatine blocks apoptosis by inhibiting mitochondrial permeability transition pores [192]. Free radicals are induced by oxidative stress and promote the conversion of an octameric form of creatine kinase to a dimeric state, resulting in the opening of permeability transition pores to mediate cell death [152,219]. Moreover, creatine/phosphocreatine possess neuroprotective functions through the creatine kinase mitochondrial permeability transition pore system, suggesting that during oxidative stress induction, creatine and phosphocreatine enhances cytosolic, high energy phosphates, maintain the level of ATP and induce neurodegeneration [152,220,221].

In addition, other molecules for mitochondrial therapy such as exosomes, which are an emerging branch of drug delivery, and medicinal molecules such as curcumin have been shown to decrease neurodegeneration in traumatic brain injury and stroke, thereby improving mitochondrial function [140].

There are various genetic therapies for improving mitochondrial function, such as mitochondria-based transcription activator-like effector nucleases (TALENs), clustered regularly interspaced short palindromic repeat (CRISPR) approaches with mitochondria-specific mitochondria-targeted Cas9 (mitoCas9), mtTALENs, and gene therapy by targeting Cytochrome P450 Family 46 Subfamily A Member 1 (CYP46A1) in AD. Several drugs that facilitate the clearance of Aβ plaques and improve the function of mitochondria, including thiazolidinedione drugs (TZDs), peroxisome proliferator-activated receptor gamma (PPARγ) agonists, and hydralazine, have been used for the pathology of AD [140].

4. Conclusions

Neurodegenerative diseases are challenging issues worldwide, with no effective drugs available to treat them. Thus, researchers are trying hard to understand the molecular details of disease pathology, the cellular interactions of disease genes, and ways to positively alter the gene function to improve health. Mitochondria are vital cell organelles, responsible for fulfilling cellular energy demands and are affected in several neurological conditions including PD. Thus, mitochondrial therapy, aimed at improving mitochondrial health, has emerged as a potential therapeutic strategy. It is interesting to examine how mitochondrial dynamics and PGC-1α and Sirtuin-1 interact with each other and how they influence the pathogenesis of PD.

Author Contributions

AKT: conceptualized and designed the review, VB, HS & AKT: collected the information and drafted the manuscript, AKT: edited and approved the manuscript. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

Not applicable.

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Conflict of Interest

The authors declare no conflict of interest.

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