Review Article

Novel targets for mitochondria dysfunction and oxidative stress in Parkinson's disease

Karamjeet Kaur[1,2], Taranjit Singh[3], Dilpreet Kaur[1,2], Sania Grover[1,2], Shamsher Singh[1]*

[1]Department of Pharmacology, Neuroscience Division, ISF College of Pharmacy, Moga, Punjab, India, [2]Department of Pharmacy, IKG - Punjab Technical University, Jalandhar, Punjab, India, [3]Department of Pharmacy Practice, Indo Soviet Friendship College of Pharmacy, Moga, Punjab, India

Correspondence:
Dr. Shamsher Singh, Department of Pharmacology, Neuroscience Division, ISF College of Pharmacy, Moga - 142 001, Punjab, India.
Phone: +91-977998050.
E-mail: shamshersinghbajwa@gmail.com
How to cite this article: Kaur K, Singh T, Kaur D, Grover S, Singh S. Novel targets for mitochondria dysfunction and oxidative stress in Parkinson's disease. Pharmaspire 2019;11(4):97-106.

Source of Support: Nil,
Conflicts of Interest: None declared.

ABSTRACT

Parkinson is a progressive neurological disorder affecting neurons of basal ganglia. It generally consists of slowing down in initiation and execution of muscle movements generally characterized by tremors, muscle rigidity, bradykinesia, and gait abnormalities. Most of the cases of Parkinson's disease are idiopathic, but it is likely a result of interaction among aging, genetic factors, and environmental factors. The genes responsible for Parkinson can be alpha-synuclein (SNCA), PTEN-induced kinase 1, PARK8, etc. Autosomal dominant forms of Parkinson are associated with mutation of α-syn (PARK 1&4) and Leucine-rich repeat kinase 2. The most important factor in pathophysiology can be free radicals and mitochondrial dysfunction which is induced by mutation and deposition of SNCA protein. An increase in the levels of glutamate may also lead to the overproduction of free radicals and reactive oxygen species. Several antioxidants, such as glutathione (GSH), are present in substantia nigra pars compacta region of basal ganglia to limit the damage by free radicals. In the premotor stage of idiopathic Parkinson's disease, Lewy bodies are initially found in the medulla oblongata and some other regions. As the disease progress, Lewy bodies ascend to the midbrain, especially SNpc. The spread of Lewy body to cortex occurs in the advanced stage. The clinically detectable stage idiopathic Parkinson's disease is generally after 70–80% damage of the SNC neurons. The available therapies provide symptomatic benefits and choice of therapy is patient specific. The choice of therapy is much critical for optimizing short- and long-term outcomes.

Keywords: Parkinson's disease, mitochondrial dysfunction, oxidative stress, alpha-synuclein, Lewy bodies, substantia nigra pars compacta

INTRODUCTION

Parkinson's disease (PD) is the second-most common progressive neurodegenerative disease characterized by excessive degeneration of dopaminergic neurons in the substantia nigra which contains about 80% of the total brain dopamine (DA) that leads to non-motor and motor symptoms such as causing hypokinesia, tremor, rigidity, bradykinesia, and gait impairment (walking impairment). PD is allied with the occurrence of ubiquitin and α-synuclein (SNCA) -positive cytoplasmic inclusions in dopaminergic neurons (neuronal perikaryal) known as Lewy bodies. Epidemiological studies show that sporadic late-onset PD accounts for 90% of cases, whereas the remaining 10% are early-onset cases that mainly occur in familial clusters. By the time PD, motor symptoms are clinically diagnosed, 60% of dopaminergic neurons and 80% of putamen DA have been lost.[1]

A number of proposed mechanisms implicated in PD pathogenesis: SNCA proteostasis, mitochondrial function, oxidative stress, calcium homeostasis, axonal transport, and neuroinflammation, but mitochondrial dysfunction and oxidative stress have been constantly concerned as the cause of the death of DA neurons in PD.[2] Mitochondria are important for several cellular functions such as redox signaling, calcium homeostasis, cell proliferation,

Access this article online	
Website: www.isfcppharmaspire.com	**P-ISSN:** 2321-4732 **E-ISSN:** XXXX-XXXX

development, and cell death.[3] Mitochondrial dysfunction is mainly cause by the inhibition in the mitochondrial electron transport complex enzyme activities, generation of reactive oxygen species (ROS), adenosine 5′triphosphate (ATP) depletion, depletion of mitochondrial DNA (mtDNA), and caspase-3 released.[4] On the other hand, oxidative stress occurs because of the disproportion formed between generations of ROS and cellular antioxidant activity. Tyrosine hydroxylase and monoamine oxidase (MAO), ROS-generating enzymes are present in the DAergic neurons are predominantly prone to oxidative stress. Mutations in mtDNA as well as mitochondria are key regulators of programmed cell death by this apoptosis play an important role in mitochondrial dysfunction.[5] At present, there is no effective recuperative treatment available for PD, only symptomatic treatment is available. Thus, therapeutic approaches targeting mitochondrial dysfunction and related oxidative stress may grasp great assure of treatment for PD. The focus of this review is to discuss novel mitochondrial and oxidative stress targets that will promote the development of mitochondrial-directed therapeutics.

MITOCHONDRIAL DYSFUNCTION AND PARKINSON'S DISEASE

Anomalous mitochondrial function is one of the major causes in PD and has been broadly accepted as a central pathogenic mechanism for PD. Chronic systemic administration of rotenone and 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), a meperidine analog exerts its toxic effects through metabolism to 1-methyl-4-phenylpyridinium (MPP+) to specifically inhibit complex I of the electron transport chain (ETC), resulting in neuropathologic and behavioral changes in rats that are similar to human PD.[6] As a significance of impaired ETC through complex I inhibition, there is a decrease in mitochondrial ATP production with an increase in the generation of reactive oxygen and nitrogen species.[7] The downstream events appear to be multifaceted and involve activation of pro-apoptotic Bcl-2 family members, p53, c-Jun N-terminal kinases (JNK), and caspases as well as inflammation leading eventually to neuronal cell death.[8] Furthermore, mutations in genes encoding both mitochondrially targeted proteins and proteins involved in mitochondrial function and oxidative stress responses are allied with several familial forms of PD. These genes encoding proteins such as SNCA, PTEN-induced kinase 1 (PINK1) (PARK6), Parkin (PARK2), leucine-rich repeat kinase 2 (PARK8), and DJ-1(PARK7) have well distinct roles in mitochondrial homeostasis and mitophagy. Both PINK1 and Parkin are the main activators of mitophagy and DJ-1 being a redox sensor and chaperone.[9] Mutations in mtDNA play a role in the breakdown of dopaminergic neurons. Indeed, elevated levels of somatic mtDNA point mutations have also been reported in elderly PD patients.[10] The lower level of mtDNA expression reduced the expression of respiratory chain function in dopaminergic neurons in nigra and striatal DA depletion leads to behavioral impairments in PD patients.[11] Some proteins "apprehensively" associated with cell death pathways are believed to be important in PD. For example, a recently discovered component of complex I called gene associated with retinoid-IFN-induced mortality (GRIM-19) physically interacts with a serine protease high-temperature requirement protein A2 (HTRA2)/Omi and enhances its pro-apoptotic activity.[12] In humans, point mutations in HTRA2 are a vulnerability factor for PD (PARK13 locus). HTRA2 phosphorylation is decreased in the brains of PD patients carrying mutations in PINK1.[13] Several studies over many years indicate that mitochondria have an important role of in PD-associated pathology, the process by which the mitochondria become dysfunctional in PD and whether correction of mitochondrial defects could provide neuroprotection in PD remains to be determined.

Targets for mitochondrial dysfunction

Mitochondria are key organelles that perform essential cellular functions and play pivotal roles in cell death and survival signaling. Hence, they represent an attractive target for drugs to treat degenerative diseases. Targeting mitochondria with organelle-specific agents or prodrugs have proven to be an effective therapeutic strategy.

SNCA

SNCA is encoded by SNCA gene and its fibrillar form is the major component of Lewy bodies,[14] therefore providing the most obvious link between sporadic and familial PD.[15] Several PD-associated α-syn mutations occur at the amphipathic N terminus of the protein named as A30P, E46K, H50Q, and A53T. The A53T mutation is the most important α-syn mutation and has been reported to cause more fragmented mitochondria and increased ROS production.[16] A recent study demonstrated that cytosolic acidification enhanced the binding of SNCA to mitochondria and encouraged the translocation of SNCA from the cytosol to mitochondria. This translocation of SNCA was revealed to occur rapidly under pH changes during oxidative stress. Interestingly, the introduction of the mitochondria-specific lipid cardiolipin facilitated the SNCA binding at low pH. These consequences showed a direct role for SNCA in mitochondria, especially under pathological conditions.[17] Parihar *et al*. observed the association of SNCA with mitochondria-induced cytochrome C release and an increase in mitochondrial calcium and nitric oxide.[18] From a recent yeast study, it emerged that mitochondria play an important role in mediating the toxic effects of SNCA.[19]

Effect of SNCA aggregation

In vitro study evidence the dose-dependent loss of mitochondrial transmembrane potential without an effect on the function of the mitochondrial complex. When rat brain mitochondria were exposed with recombinant human SNCA, it showed that the N-terminal 32 amino acids of SNCA anchorage a mysterious mitochondrial targeting signal directing SNCA to the inner mitochondrial membrane where it links with complex I.[20] As the outcome, decreased complex I activity and increased ROS production due to the presence of SNCA in human fetal dopaminergic primary neuronal cultures overexpressing wild-type SNCA. In addition, pre-fibrillar forms of α-syn aggregates cause Ca2+ dependent mitochondrial dysfunction, including loss of mitochondrial membrane potential (MMP) and nicotinamide adenine dinucleotide (NADP) oxidation due to reducing mitochondrial Ca2+ retention in isolated mitochondria through complex I dysfunction.[9] Further evidence for increasing SNCA aggregation-induced mitochondrial alterations was found in overexpressing wild-type or mutant SNCA in cellular and mouse models, including ultrastructural abnormalities, complex IV activity, a decrease in the

MMP, oxidation of mitochondria-associated metabolic proteins, and increased sensitivity to mitochondrial toxins.[21]

Overexpression of SNCA with in cells has been shown to interfere with mitochondrial dynamics by disturbance of mitochondrial fusion, leading to mitochondrial fragmentation.[22]

Effect of SNCA on autophagy

SNCA is mainly degraded by Ubiquitin- Proteasome System (UPS),[23] but an increased level of wild type or A53T mutant SNCA can activate the macroautophagy pathway for degradation.[24] Abnormal SNCA levels can also cause mitophagy.[25] Overexpression of SNCA can occur in mitochondria and can activate mPTP through interacting with either adenylate translocator or voltage-dependent anion channel (VDAC) that interrupt MMP, initiating mitophagy, and cause PD symptom. Increasing evidence confirms SNCA as a mark of chaperone-mediated autophagy (CMA). One study showed that myocyte enhancer factor 2D (MEF2D), a transcription activator known as a neuronal survival factor, is the substrate of CMA. Wild type or mutant SNCA accumulation compromises the normal turnover of the MEF2D by CMA and leads to a decrease in the MEF2D DNA binding ability and neuronal stress, which underlies the neuronal loss of PD. Activating CMA activity through upregulating lysosome-associated membrane protein type 2A decreases SNCA turnover and protects against SNCA overexpression induced neurodegeneration in PD.[26]

Leucine rich repeat kinase 2 (LRRK2)

LRRK2 is a protein encoded by the PARK8 gene. It has a conserved serine-threonine kinase mitogen-activated protein kinase kinase kinase (MAPKKK) domain, a member of the Ras of complex GTPase family.[27] Thus far, well-known PD-associated mutations in LRRK2 are p.G2019S, p.R1441C/G/H, p.Y1699C, p.I2020T, and p.N1437H. Among them, G2019S is particularly common in PD patients.[28] There have been reports that mutation p.G2019S (Gly2019 to Ser), which is taking place in the MAPKKK domain and has been established to give ascend to a hyperactive kinase, increases autophosphorylation and phosphorylation of broad substrates.[29] A study suggests that the kinase activity of LRRK2 is increased due to the G2019S mutant, which is associated with augmented toxicity in dopaminergic neurons.[30] About 10% of overexpressed LRKK2 has been reported to associate with the outer mitochondrial membrane (OMM) in the mammalian brain.[31] It has also been demonstrated that reduced MMP and total intracellular ATP levels accompanied by increased mitochondrial elongation and interconnectivity may be exhibited with the p.G2019S mutation in PD patients. Increased LRRK2 level and p. G2019S mutation can enhance the intracellular ROS level, which was amplified by external oxidative stress. A combination of either LRRK2 WT or p.G2019S expression with oxidative stress synergistically increased cell death with the strongest effect being observed with overexpression of p.G2019S. Moreover, mutation p.G2019S has also been reported to cause loss of LRRK2-mediated defense against hydrogen peroxide, it could elevate oxidant level and potentially oxidative stress.[32]

DRP1

Drp1 is a large GTPase and a key mediator of mitochondrial fission. Drp1 is mainly found in the cytosol, but it translocates to the mitochondria in response to various cellular stimuli to initiate the mitochondrial membranes fission through GTP hydrolysis[33] and subsequently leads to mitochondrial depolarization. Specifically, phosphorylation of human dynamin-like protein 1 (DLP-1) at Ser637 (Ser656 in the conserved GTPase effector domain in rats) has been established to inhibit the function of DLP-1, thus reducing mitochondrial fission. This result is intervening by a cyclic AMP-dependent kinase (PKA) and the scaffolding protein AKAP1. Then, PKA moves to the mitochondrial outer membrane, where it phosphorylates and inhibits the activity of DLP-1.[34] A study demonstrated that toxin-induced dopaminergic neuronal death *in vitro* and *in vivo* supports a role for Drp1 hyperactivity and mitochondrial fission/fusion in the pathogenesis of dopaminergic neuronal death. The toxins such as 6-hydroxy DA (6-OHDA), rotenone, and MPP+ cause hyperactivity of Drp1 and mitochondrial fission, leading to dopaminergic cell death in neuronal cultures. Genetic inhibition of pro-fission Drp1 or overexpression of pro-fusion protein mitofusin-1 (Mfn1) prevents both neurotoxin-induced mitochondrial fission and neuronal cell death.[35] A study in which rat primary dopaminergic midbrain neurons show that MPP+-induced DLP-1-dependent mitochondrial fragmentation is an early and upstream event that makes worse the bioenergetic impairments and mediating other downstream adverse effects. These include increased ROS production, calcium disturbance, and increased mitophagy that leads to neuronal degeneration.[36] A traverse talk between ROS, calcium disturbance, and mitochondrial fragmentation forms a mitochondrial fission-initiated downward twirl that increases these adverse effects. Indeed, DLP-1 knockdown decreases mitochondrial fragmentation and prevents these downstream events induced by MPP+, suggesting that prevention of mitochondrial fragmentation is not only an attractive target to prevent MPP+-induced deficits but may also have significant relevance to the treatment of PD.

The data proved that LRRK2 may play an imperative part in regulating mitochondrial homeostasis.[37] Wild type LRRK2 interacts with several key regulators of mitochondrial fission/fusion representing it has several regulatory roles.[9] The endogenous LRRK2 was partly co-localized with mitochondrial fission DLP-1 either in the cytosol or on mitochondrial membranes in cortical neurons, which suggests that DLP-1 is very probable to be involved in LRRK2-induced mitochondrial fission in familial PD.[38] It has been established that murine primary neurons and human neuroblastoma increase the activity of Drp1 by phosphorylation of Drp1, when endogenous LRRK2 comes in contact with the fission regulator Drp1 at the mitochondrial membrane, causing mitochondrial fragmentation.[9] This LRRK2-Drp1-dependent mitochondrial fragmentation is improved by overexpressing wild type LRRK2 and by expressing the PD-associated G2019S mutant protein but may be restored by inhibiting Drp1 or increasing fusion.[39] Furthermore, dead kinase – domain or GTP-binding-deficient LRRK2 shows greatly decreased Drp1 interaction.[40] It has been observed that the phosphorylation of S616 of Drp1 is revealed to promote fission in sporadic PD patients.[9] LRRK2 also interacts with the mitochondrial fusion regulators Mfn1/2 and OPA1 and alters their activities and reduced levels of mature OPA1 exhibit in PD patients carrying the G2019S mutation.[41] Together, these data make it obvious that the

augmented kinase activity of LRRK2, consequences in decreased mitochondrial fusion as well as increased fission and recommends that regulation of LRRK2 kinase activity may be an important factor in maintaining mitochondrial dynamics in sporadic PD.

Parkin

Mutations in the Parkin (PARK2) gene are the most common cause of autosomal recessive PD.[42] Parkin functions as a ubiquitin E3 protein-ligase and contains a ubiquitin-like domain, two RING finger domains, and a conserved region between the RING domains. It has been reported that the neuroprotective activity of Parkin is allied with proteasome-independent ubiquitylation.[43] The gene codes for E3 ubiquitin ligase able of mediating polyubiquitination by means of different ubiquitin linkages through lysine 29, 48, and 63 of ubiquitin. Recent research demonstrated that more than 100 pathogenic Parkin mutations interrupt the protein's E3 ligase activity by altering the solubility or stability of the protein, leading to dopaminergic cell death.[44] Moreover, recent evidence from post-mortem PD brain samples and mouse models suggests that Parkin is inactivated by oxidation, nitrosylation, and phosphorylation by c-Abl, an important stress-activated non-receptor tyrosine kinase that is activated in sporadic PD brains and in animal models of PD.[45] Considerable reduction inactivity of mitochondrial complex I was found both in patients with Parkin mutations and sporadic PD patients, but complex IV activity was only decreased in sporadic PD patients.[46] LaVoie *et al*. could reveal that misfolded Parkin was present in the substantia nigra of sporadic PD patients.[47] Parkin can protect cells against degenerative mechanisms such as mitochondrial dysfunction, excitotoxicity, endoplasmic reticulum stress, proteasome inhibition, and overexpression of SNCA.[48] Recently, a study established that the ectopic appearance of Parkin is capable to increase replication of the mitochondrial genome and transcription of mitochondrial genes. In addition, Parkin was found to defend mtDNA from oxidative damage and to encourage mtDNA repair.[49] Activation of Parkin may play an important role for the treatment of PD by targeting mitochondria.

PINK1

PINK1 is a 581 amino acid protein encoded by a PARK6 gene. It has an N-terminal mitochondrial targeting sequence and a serine/threonine kinase domain.[50] Higher PINK1 expression gives protection from apoptotic cell death in an assortment of stress conditions, while the loss of PINK1 function increases the susceptibility of cells to stress-induced cell death.[51] PINK1 deficiency shows a damaging effect on mitochondrial functions such as a decrease in MMP, activities of the complexes I and IV, ATP production, mtDNA levels, and an increase in ROS production and abnormal ultrastructural mitochondrial morphology.[52] Alterations in calcium homeostasis were also observed in cultured cells co-expressing a pathogenic PINK1 mutant (W437X).[53]

Mitophagy and Parkin/PINK

Parkin and PINK mutants show a considerable slowing of mitochondrial protein turnover and mitophagy. Failure to remove the damaged mitochondrial proteins by mitophagy plays an important role in PD pathogenesis.[54] A study demonstrated that dimeric PINK1 on OMM can engage Parkin and in that way, it phosphorylates Parkin at Ser65.[55] Using mass spectrometry, VDACs were recognized as the docking site for Parkin recruitment to the OMM.[56] Behind Parkin translocation to mitochondria, Parkin ubiquitinates many OMM proteins and consecutively recruits other proteins to commence mitophagy. Subsequently, the Parkin-labeled mitochondria are transported to lysosomes for degradation.[26] Moreover, Parkin mutations lead to impaired mitophagy, but it can be rescued by upregulation of translocation of the OMM (TOMM), suggesting that TOMM acts as a key regulator in PINK1/Parkin-mediated mitophagy.[57]

DJ-1

DJ-1 gene, encoding a 189-amino acid protein, has been associated with rare cases of early-onset autosomal recessive PD.[58] Endogenous DJ-1 was found in the matrix and intermembrane space of mitochondria.[59] DJ-1 overexpression protects neurons from oxidative stress-induced damage and DJ-1 deficiency causes to be cells more vulnerable to oxidative stress.[60] Alanine substitutes at the site of cysteine 106 hampers with both mitochondrial localization and protection against mitochondrial toxins.[61] Isolated mitochondria from DJ-1 knockout mice showed a reduction in mitochondrial aconitase activity, leading to a two-fold increase in hydrogen peroxide escort, indicating insufficiency in scavenging mitochondrial ROS in PD.[62]

Endogenous DJ-1 is present in the mitochondrial inter-membranous space and binds to ETC complexes (NDUFA4 and ND1) for the ATP production.[63,64] Experimental studies have demonstrated that overexpressing wild-type DJ-1 protected against complex I inhibitor toxin like rotenone-induced mitochondrial dysfunction and that the knockdown of endogenous DJ-1 increased the susceptibility of neurons to rotenone and other complex I inhibitor.[65]

DJ-1 as a regulator of apoptosis and autophagy

Apoptosis signal-regulating kinase 1 (ASK1) is a potent apoptotic molecule. ASK1 plays a critical role in 6-hydroxydopamine (6-OHDA) and MPTP-induced apoptosis in cellular and animal models of PD.[66] One of the molecular events that activate ASK1 is its interaction with the death protein Daxx. Upon stress situations, Daxx, which is a nuclear protein under basal conditions, translocates to the cytoplasm, interacts, and activates ASK1 leading to cell death. DJ-1 potently blocks this death signaling pathway by interacting with Daxx in the nucleus and preventing it from gaining access to ASK1 in the cytoplasm.[67] DJ-1 may also interact with ASK1.[68] Under basal conditions, it is bound to thioredoxin 1 (Trx 1), which is a key redox modulator as well as a physiological inhibitor. Upon oxidative stress, however, Trx 1 dissociates from ASK1, thereby activating this kinase.[67] Considering the role of DJ-1 as a regulator of ASK1 activity, DJ-1 suppresses ASK1 activity by preventing the dissociation of Trx 1 from ASK1.

DJ-1 can serve as a regulator of autophagy. In dopaminergic neurons, DJ-1 overexpression induces ERK-dependent mitophagy and protects against neurotoxin-induced apoptosis.[69] Loss of DJ-1 leads to mitochondrial phenotypes, including reduced membrane potential, increased fragmentation, and accumulation of autophagic markers.

HTRA2

HTRA2 is a mitochondrial serine protease also known as OMI. Mutations in the PARK13 gene encoding HTRA2 protein are suggested to be a susceptibility factor for PD patients. Expression of a mutation that causes the Gly399Ser substitution and Ala141Ser substitution, both of which have been found in individuals with PD, leads to mitochondrial swelling, decreased MMP, and increased risk of toxin-induced cell death.[70] In the programmed cell death pathway, the release of HTRA2 might be due to the permeabilization of the mitochondrial membrane by pro-apoptotic molecules.[69] Moreover, HtrA2 interacts with an alleged mitochondrial protein kinase PINK1.[71] Upon activation of the p38 stress-sensing pathway, PINK1 phosphorylates HTRA2, conferring its protease activity, and HTRA2 phosphorylation is decreased in brain tissue from PINK1 mutations PD patients.[72] A recently revealed GRIM-19, a component of complex I physically interact with a serine protease HtrA2/Omi and augments its pro-apoptotic activity.[13]

mtDNA

mtDNA point mutations and mtDNA deletions both have separate mechanisms, even though the cause of both types of mutation are thought to be more frequently due to endogenous processes as opposed to exposure to exogenous agents.[73] mtDNA point mutations may take place mainly in two ways: First, base substitution mutations caused by mitochondrial polymerase γ replicating across damaged bases.[74] Second, the closeness of mtDNA to ROS, as well as the lack of protective histones,[75] may lead to oxidative damage to mtDNA, which can be highly mutagenic.[76] Indeed, levels of oxidative damage to mtDNA in the brain are especially pronounced in the SN, where DA metabolism, high levels of iron, and low levels of GSH (an important antioxidant) may produce an environment particularly high in oxidative stress.[77] Accordingly, it was hypothesized that oxidative damage to mtDNA and consequential mutations may play an important role for mitochondrial dysfunction in PD. The first published study to quantify the number of multiple mtDNA point mutations in the SN was to characterize the aggregate burden of mtDNA mutations in the SN of post-mortem tissue from PD patients as well as young and old control subjects.[78] In addition, the work from Bender *et al*. recognized that mtDNA deletions were higher still in individuals with PD compared to aged controls.[79]

The mitochondrial transcriptional factor A (TFAM) controls transcription of mtDNA and was allied to PD by the discovery that TFAM knockout mice (MitoPark mice) had reduced mtDNA expression and respiratory chain deficiency in SNpc DA neurons in Parkinson's disease.[80] Although some studies showed that TFAM mutations do not significantly increase the risk of PD,[81] an investigation into the influence of TFAM variants on PD depending on mtDNA haplogroup found certain variants increased the chances of developing PD,[82] suggesting a possible role for mtDNA, in some instances, and respiratory chain dysfunction in PD. mtDNA polymerase γ 1 (POLG1) is an enzyme involved in controlling the synthesis, replication, and repair of mtDNA and has shown to play a role in reduced activity of mitochondrial respiratory chain complexes in PD.[83] The mutations of this protein might lead to dysregulation of mtDNA and DNA deletions recommend to have a role in mitochondrial dysfunction in the disease. However, whether there is a common hereditary role for POLG1 in PD needs further study since a large-scale study does not support this hypothesis.[84]

OXIDATIVE STRESS AND PARKINSON'S DISEASE

Oxidative stress occurs when an imbalance is formed between the production of ROS and cellular antioxidant activity. Tyrosine hydroxylase and MAO are two main enzymes which are responsible for the more vulnerable DAergic neurons to oxidative stress. ROS can be generated through several pathways such as direct interactions between redox-active metals and oxygen species through reactions, including the Fenton and Haber-Weiss reactions, or by indirect pathways involving the activation of enzymes such as nitric oxide synthase or NADP-oxidase (NADPH) oxidases.[85] In PD, inhibition of mainly mitochondrial complexes I and III produces superoxide anion(O2−) which is highly reactive and can easily cross the inner mitochondrial membrane, where it is reduced to produce H_2O_2. Further, H_2O_2 can also be generated by peroxisomes.[86] As peroxisomes contain antioxidant enzyme like catalase, it helps to converted H_2O_2 to water and preventing its gathering. On the other hand, H_2O_2 is released to the cytosol, where it contributes to oxidative stress due to down-regulated peroxisomes enzymes. In addition, highly ROS is again generated by the conversion of H_2O_2 by the Fenton reaction into •OH in the presence of reduced metals like ferrous ion (Fe2+).[87] Indeed, free radical-mediated oxidative damage to lipids results in the generation of toxic products, including HNE and malondialdehyde, proteins oxidation, and DNA, RNA oxidation which has been detected in brain tissue of PD patients.[88]

Targets for oxidative stress

Oxidative DNA damage

At the level of DNA, the marker 8-hydroxy(deoxy) guanosine (8-OHdG/8-OHG) reveals the enhanced oxidative damage in the SN of PD brains.[89] The base modification 8-OHG is not a result of direct reactions of DNA with primary ROS such as superoxide (which are liberated as the species during electron transport) or hydrogen peroxide from superoxide dismutase (SOD_2) reaction.[90] Hydroxyl radicals, generated in the presence of Fe2+ by the Fenton reaction, can hydroxylate DNA bases, which generates oxidation products in PD. Since the specific mechanism of enhanced oxidative DNA damage remains vague, oxidative stress markers have been illustrated by various studies even in extracerebral tissues and bodily fluids of PD patients,[91] including an increase of 8-OHdG/8-OHG in the cerebrospinal fluid.[92] This points in the direction of a more generalized oxidative stress affecting and perhaps mutating nuclear and mtDNA. Since ROS generation in the ETC may induce mtDNA mutations, which in turn may inhibit ETC complexes and enhance mitochondrial ROS production, a long debate dealt with a possible "vicious cycle," which may lead to self-accelerating feedback between oxidative DNA damage and mutagenesis, leading to an accelerated (non-linear) accumulation of mutated mtDNA in aging dopaminergic neurons.

DA metabolism

DA itself may be a source of oxidative stress for selective degeneration of the DA neurons of the SNpc in PD.[93] However, after L-DOPA treatment, there is an excess amount of cytosolic DA outside of the synaptic vesicle in damaged neurons, DA is easily metabolized to cytotoxic ROS through MAO or by auto-oxidation.[94] In addition, reduced VMAT2 expression in DA exploitation in mice was enough to cause DA-mediated toxicity and progressive loss of DA neurons.[95] It has also shown to cause inhibition of the DA transporter and the TH enzyme[96] as well as the production of ROS and dysfunction in complex I activity causing mitochondrial dysfunction.[97] DA quinone covalently amends the SNCA monomer and encourages the SNCA to convert into cytotoxic protofibril form.[98] The DA quinine modified SNCA is not only poorly degraded but also reduces the CMA of other proteins.[99] Conversely, SNCA can bind to and permeabilize the vesicle membrane, causing the escape of DA into the cytosol[100] and this would sequentially induce the production of DA quinine. In addition, DA quinines can be oxidized to a monochrome, whose redox-cycling causes the production of the superoxide radical and the reduction of cellular NADPH. Again, a monochrome ultimately polymerizes to form the neuromelanin recognized to be accrued in the SNpc of the PD patients.[101]

GSH

A study shows a decline amount of GSH relative to GSH disulfide (GSSG), that is, GSH:GSSG ratio in the SN of post-mortem brain tissue from PD patients compared to controls.[102] GSH synthesis is downregulated in the rat brain that has revealed to result in progressive degeneration of nigral dopaminergic neurons. There are also reliable observations that inhibition of complex I activity causes a higher generation of ROS and following GSH levels is decreased. This decrease in GSH levels and increased levels of GSH disulfide can consequence from inhibition of GSH reductase.[103]

Grx1 is concerned for glutathionylation, a process described by the reversible formation of mixed disulfides between protein thiols and GSH.[104] Proteins can be glutathionylated under oxidative stress and protect the cell by inhibiting the irreversible oxidation of cysteine to cysteine sulfinic and sulfonic acid by this process.[105] In fact, knockdown of Grx in SH-SY5Y cells results in augmented apoptosis, supporting the concept that inhibiting protein glutathionylation may sensitize dopaminergic neurons to apoptosis.[106] In addition, decreased activity of isocitrate dehydrogenase (IDH) is exhibited with altered thiol-disulfide homeostasis in stressed dopaminergic neurons, mouse brains lesioned with MPTP.[67] IDH is an enzyme that converts isocitrate to alpha-ketoglutarate by oxidative decarboxylation, which requires either NAD+ or NADP+ with the subsequent generation of NADH and NADPH, respectively. NADPH is a vital reducing component in two systems which play important roles in protecting cells from oxidative damage.[97,98] Thus, IDH contributes to the supply of NADPH desired for GSH production against oxidative damage and is important for the regulation of cell survival.[107] Therefore, deactivation of IDH enzyme induced by oxidants can play a role in PD.

THERAPEUTIC IMPLICATION

LRRK2

Much concern has been produced by the modulation of the LRRK2 kinase domain as a potential therapy for LRRK2 mutation, causing dopaminergic degeneration in PD. Treatment of neuronal cultures with the LRRK2 inhibitors LRRK2-IN-1 and GW5074 directs to improved cell survival and reduced cell dysfunction in LRRK2 G2019S iPSC dopaminergic neurons.[108] In addition, PF-06447475 and GW5074 have been revealed to augment dopaminergic neuronal survival in primary neuronal cultures and neurons derived from patient iPS cells.[109] Nonselective LRRK2 inhibitors are tyrosine kinase inhibitor imatinib, mitogen-activated protein kinase kinase inhibitors PD98059 and U0126, Z8205, sunitinib, the Raf kinase inhibitors sorafenib and GW5074, and the anaplastic lymphoma kinase inhibitor, TAE684.45–49 selective Lrrk2 inhibitors are HG-10-102-1, GSK2578215A, JH-II-127, GNE-7915 9, GNE-0877 3, GNE-9605 19, PF-06447475, MLi-2.[110]

Importantly, increased mtDNA damage could be reversed with zinc-finger nuclease-mediated correction of the G2019S mutation back to WT. This result is suggestive of mitochondrial dysfunction, and indeed, another study using G2019S- and R1441C-induced pluripotent stem cells-derived neural stem cells further reporting reduced basal oxygen consumption, impaired mitochondrial dynamics.

PINK1 and Parkin

The autoinhibition exhibited by Parkin may be a target for small molecules that increase Parkin activation in a PINK1-independent manner.[9] Given that Parkin activation requires both mitochondrial translocation and ubiquitin phosphorylation, as evidenced by phosphomimetic Parkin,[111] it will be interesting to see whether merely decreasing autoinhibition restores regulation and impacts mitochondrial phenotypes in the absence of PINK1. The polyphenol resveratrol regulates mitochondrial energy homeostasis through augmentation of complex I activity and ATP production has been established in *in-vitro* primary fibroblasts cultures from patients with Parkin mutations (PARK2). These data show that a drug which causes activation of PINK1 and Parkin plays an important role for the treatment of PD.

DJ1

Using small molecules, the glyoxalase activity of DJ-1 can be increased to further increase catalytic rate/recycling of the protein. Furthermore, D-lactate and glycolate are the products of DJ-1 glyoxalase activity, can be a potential target to rescue the loss of MMP observed after PINK1 knockdown suggesting upregulation of DJ-1 glyoxalase which may be protective in PD.[9]

Im *et al*. proposed that the DJ-1 interacts with Daxx, a death-associated protein in the nucleus, by this means preventing its cytosolic translocation and activation of the ASK1, a pro-apoptotic protein which activates JNK1/2 and reduced cell death.[67] In addition, DJ-1 directly interacts with ASK1,[112,113] preventing Trx 1 dissociation from

ASK1, a factor which inhibits ASK1 activity and by reducing JNK activity in this way it may inhibit oxidative stress-induced apoptotic cell death.[67]

DJ-1 may act as an endogenous indicator of oxidative stress, signifying that it may play a role as an antioxidant. Many studies have established that oxidation occurs at the Cys-106 residue on the DJ-1 protein, consequential in the formation of cysteine-sulfinic acid, a process which emerges vital to its mitochondrial translocation and neuroprotective effect.[114] Supplementing DJ-1-deficit cells with GSH reverses both mitochondrial and autophagic changes suggesting that DJ-1 may act to maintain mitochondrial function during oxidative stress.[115]

Genetic supplement and neuropeptide

Genetic supplementation of deoxyribonucleoside kinase in Drosophila, or supplementation with deoxyribonucleosides or folic acid in human neuroblastoma cells with PINK1 knockdown, resulted in improvement in a number of markers of mitochondrial dysfunction.[9] The neuropeptide cocaine and amphetamine-regulated transcript protected mtDNA and cellular proteins and lipids of human neuroblastoma SH-SY5Y cells, HEK293 cells, and cultures of cortical and hippocampal neurons exposed to hydrogen peroxide.[116]

Drp1

Mdivi-1 treated cells are resistant to apoptosis induced by toxin, apparently due to an inhibition of mitochondrial membrane permeabilization.[117] This benefit necessitates further investigations into the therapeutic benefits of mdivi-1 in PD. PKCδ interacts and phosphorylates DLP-1 which encourages DLP-1 translocation to mitochondria to cause mitochondrial fragmentation. Importantly, using a selective PKCδ peptide inhibitor (δV1-1) which inhibits the action of PKCδ leads to reduced mitochondrial fission and fragmentation and presents neuronal protection, suggesting upstream signaling molecules may also be a good candidate.[118] Mitochondrial distribution of PGC-1α has lately been shown to maintain enough in neuritis.[119] PGC-1α is a transcriptional co-activator and plays an important role for the regulation of mitochondrial biogenesis and respiration.[120] PGC-1α represents a tremendous therapeutic target for mitochondrial dysfunction in PD. Indeed, overexpression of PGC-1α protects against oxidative stress-induced degeneration in neurons.[121] A study recently developed a peptide inhibitor P110, which is rationally considered to selectively inhibit Mitochondrial fission 1 protein/Drp1 interaction under oxidative stressed and other stressed conditions. Some *in vitro* and *in vivo* studies have shown that the treatment with inhibiting Drp1 translocation to mitochondria and Drp1 polymerization is inhibited by P110, without affecting Drp1 level.[118] These data reveal that inhibition of Drp1 hyperactivation by a Drp1 peptide inhibitor P110 alleviates the loss of dopaminergic neurons and attenuates the behavioral deficits induced by MPTP.

GSH and its precursor

As discussed earlier, enhanced oxidative stress and decreased levels of GSH have been illustrated in a number of PD models,[122] as well as in the SN of PD patients.[123] Based on these findings, it has been suggested that restoring the level of GSH in the brains of PD patients may be a capable therapy to protect the affected DA neurons from further injury.[124] A number of therapeutic compounds have been studied, including GSH alone (through delivery in liposomes and nanoparticles) and co-drugs such as GSH:L-Dopa or GSH:DA conjugates, as well as GSH analogs, and other hybrid compounds. Optimally, successful candidates should be stable during gastrointestinal digestion, undergo bioconversion to constituent compounds that are transported into the brain, navigate to the desired site of action, and protect against the oxidative damage. In addition, for effective treatment with these GSH analogs, they should be characterized by limited γ-GT metabolism, while also maintaining their reducing ability.[125]

Co-drug compounds, including GSH association that has similar or different modes of action of two different compounds in regulation to synergize their actions in the brain.[126] A number of these co-drugs have been developed as a potential treatment for PD. The most common cofactor for these co-drugs is L-dopa. Co-drugs have been made that directly link GSH and L-dopa.[127] L-dopa has been conjugated to a number of other agents, including entacapone (a COMT inhibitor marketed under the trade name Stalevo®),[128] cysteine,[129] N-acetyl cysteine,[130] l-Methionine,[131] lipoic acid,[132] caffeic acid, and carnosine.[133] Functionally, it is theorized that by joining L-dopa to GSH, the exogenous GSH can be aimed at the specific neurons within the SNpc that are affected in PD. Other recently generated co-drugs include flavonoid compounds that enhance the uptake of cystine/cysteine by uncoupling their uptake from the cystine/glutamate antiporter, xc−. Flavonoids are plant polyphenols and have free radical scavenging capacity. Amino acid moieties were added to flavanol compounds to test their effectiveness as neuroprotectants in conditions of glutamate toxicity. Conjugation of the flavonoid epicatechin (EC) with cysteine and cysteamine-EC increases cell survival and GSH level in a dose-dependent manner.[134]

CONCLUSION

The major goal of the therapy in Parkinson's disease is to improve the motor and non-motor symptoms of the patient so that patient's quality of life is maintained at the best possible state. Coenzyme Q10 (CoQ10) which is also known as Ubiquinone possesses potential usefulness in the treatment as it acts as an antioxidant in mitochondria and lipid membranes as well as it is a cofactor of ETC. Thus, the oxidation of lipids and proteins can be reduced. ROS induces the apoptosis by caspase-9 pathway. Thus, inhibition of the ROS by the scavengers such as CoQ10 or by overexpressing the antioxidants such as GSH can lower the neurodegeneration. CoQ10 also acts as an obligatory cofactor for mitochondrial uncoupling proteins; thus, by activation of these proteins, the generation of the mitochondrial free radicals can be reduced. SNCA which is the first causal for Parkinson's disease is found to have mutations, duplication, or triplication which progresses and extends the neurodegeneration. Mutations in the Parkin (PRKN) gene are common in autosomal recessive juvenile Parkinson's disease which is pathologically accompanied by no formation of the Lewy Bodies in most cases. PINK1 is also responsible for autosomal recessive and L-dopa responsive Parkinsonism. Gene PARK7 shows

three missense mutations (L166P, M26I, E64D) of exons 1-5 in some cases. LRRK2 showing G2019S mutation is the most significant cause of Parkinsonism, especially accounting for autosomal dominant cases.

REFERENCES

1. Bezard E, Gross CE, Brotchie JM. Presymptomatic compensation in Parkinson's disease is not dopamine-mediated. Trends Neurosci 2003;26:215-21.
2. Poewe W, Seppi K, Tanner CM, Halliday GM, Brundin P, Volkmann J, *et al*. Parkinson disease. Nat Rev Dis Primers 2017;3:1-21.
3. Marchi S, Patergnani S, Missiroli S, Morciano G, Rimessi A, Wieckowski MR, *et al*. Mitochondrial and endoplasmic reticulum calcium homeostasis and cell death. Cell Calcium 2018;69:62-72.
4. Roy A, Ganguly A, Dasgupta SB, Das BB, Pal C, Jaisankar P, *et al*. Mitochondria-dependent reactive oxygen species-mediated programmed cell death induced by 3, 3'-diindolylmethane through inhibition of F0F1-ATP synthase in unicellular protozoan parasite *Leishmania donovani*. Mol Pharmacol 2008;74:1292-307.
5. Lu J, Sharma LK, Bai Y. Implications of mitochondrial DNA mutations and mitochondrial dysfunction in tumorigenesis. Cell Res 2009;19:802-15.
6. Gerlach M, Riederer P, Przuntek H, Youdim MB. MPTP mechanisms of neurotoxicity and their implications for Parkinson's disease. Eur J Pharmacol 1991;208:273-86.
7. Liu Y, Fiskum G, Schubert D. Generation of reactive oxygen species by the mitochondrial electron transport chain. J Neurochem 2002;80:780-7.
8. Nakka VP, Gusain A, Mehta SL, Raghubir R. Molecular mechanisms of apoptosis in cerebral ischemia: Multiple neuroprotective opportunities. Mol Neurobiol 2008;37:7-38.
9. Ryan BJ, Hoek S, Fon EA, Wade-Martins R. Mitochondrial dysfunction and mitophagy in Parkinson's: From familial to sporadic disease. Trends Biochem Sci 2015;40:200-10.
10. Lin MT, Simon DK, Ahn CH, Kim LM, Beal MF. High aggregate burden of somatic mtDNA point mutations in aging and Alzheimer's disease brain. Hum Mol Genet 2002;11:133-45.
11. Ekstrand MI, Terzioglu M, Galter D, Zhu S, Hofstetter C, Lindqvist E, *et al*. Progressive parkinsonism in mice with respiratory-chain-deficient dopamine neurons. Proc Natl Acad Sci 2007;104:1325-30.
12. Banerjee R, Starkov AA, Beal MF, Thomas B. Mitochondrial dysfunction in the limelight of Parkinson's disease pathogenesis. Biochim Biophys Acta Mol Basis Dis 2009;1792:651-63.
13. Plun-Favreau H, Klupsch K, Moisoi N, Gandhi S, Kjaer S, Frith D, *et al*. The mitochondrial protease HtrA2 is regulated by Parkinson's disease-associated kinase PINK1. Nat Cell Biol 2007;9:1243-52.
14. Emamzadeh FN. Alpha-synuclein structure, functions, and interactions. J Res Med Sci 2016;21:29.
15. Coulom H, Birman S. Chronic exposure to rotenone models sporadic Parkinson's disease in *Drosophila* melanogaster. J Neurosci 2004;24:10993-8.
16. Choubey V, Safiulina D, Vaarmann A, Cagalinec M, Wareski P, Kuum M, *et al*. Mutant A53T α-synuclein induces neuronal death by increasing mitochondrial autophagy. J Biol Chem 2011;286:10814-24.
17. Cole NB, DiEuliis D, Leo P, Mitchell DC, Nussbaum RL. Mitochondrial translocation of α-synuclein is promoted by intracellular acidification. Exp Cell Res 2008;314:2076-89.
18. Parihar MS, Parihar A, Fujita M, Hashimoto M, Ghafourifar P. Mitochondrial association of alpha-synuclein causes oxidative stress. Cell Mol Life Sci 2008;65:1272-84.
19. Sampaio-Marques B, Felgueiras C, Silva A, Rodrigues M, Tenreiro S, Franssens V, *et al*. SNCA (α-synuclein)-induced toxicity in yeast cells is dependent on Sir2-mediated mitophagy. Autophagy 2012;8:1494-509.
20. Kaul SC, Deocaris CC, Wadhwa R. Three faces of mortalin: A housekeeper, guardian and killer. Exp Gerontol 2007;42:263-74.
21. Bosch M, Marí M, Herms A, Fernández A, Fajardo A, Kassan A, *et al*. Caveolin-1 deficiency causes cholesterol-dependent mitochondrial dysfunction and apoptotic susceptibility. Curr Biol 2011;21:681-6.
22. Nakamura K, Nemani VM, Azarbal F, Skibinski G, Levy JM, Egami K, *et al*. Direct membrane association drives mitochondrial fission by the Parkinson disease-associated protein α-synuclein. J Biol Chem 2011;286:20710-26.
23. Ebrahimi-Fakhari D, Cantuti-Castelvetri I, Fan Z, Rockenstein E, Masliah E, Hyman BT, *et al*. Distinct roles *in vivo* for the ubiquitin-proteasome system and the autophagy-lysosomal pathway in the degradation of α-synuclein. J Neurosci 2011;31:14508-20.
24. Cuervo AM, Stefanis L, Fredenburg R, Lansbury PT, Sulzer D. Impaired degradation of mutant α-synuclein by chaperone-mediated autophagy. Science 2004;305:1292-5.
25. Zhang J. Autophagy and mitophagy in cellular damage control. Redox Biol 2013;1:19-23.
26. Wang B, Abraham N, Gao G, Yang Q. Dysregulation of autophagy and mitochondrial function in Parkinson's disease. Transl Neurodegener 2016;5:19.
27. Guo L, Wang W, Chen SG. Leucine-rich repeat kinase 2: Relevance to Parkinson's disease. Int J Biochem Cell Biol 2006;38:1469-75.
28. Paisan-Ruiz C, Lewis PA, Singleton AB. LRRK2: Cause, risk, and mechanism. J Parkinson's Dis 2013;3:85-103.
29. Dev KK. PDZ domain protein-protein interactions: A case study with PICK1. Curr Top Med Chem 2007;7:3-20.
30. Ramonet D, Daher JP, Lin BM, Stafa K, Kim J, Banerjee R, *et al*. Dopaminergic neuronal loss, reduced neurite complexity and autophagic abnormalities in transgenic mice expressing G2019S mutant LRRK2. PLoS One 2011;6:e18568.
31. Winklhofer KF, Haass C. Mitochondrial dysfunction in Parkinson's disease. Biochim Biophys Acta Mol Basis Dis 2010;1802:29-44.
32. Li JQ, Tan L, Yu JT. The role of the LRRK2 gene in parkinsonism. Mol Neurodegener 2014;9:47.
33. Bossy-Wetzel E, Barsoum MJ, Godzik A, Schwarzenbacher R, Lipton SA. Mitochondrial fission in apoptosis, neurodegeneration and aging. Curr Opin Cell Biol 2003;15:706-16.
34. Bonda DV, Smith MV, Perry G, Lee HG, Wang X, Zhu X. The mitochondrial dynamics of Alzheimer's disease and Parkinson's disease offer important opportunities for therapeutic intervention. Curr Pharm Design 2011;17:3374-80.
35. Filichia E, Hoffer B, Qi X, Luo Y. Inhibition of Drp1 mitochondrial translocation provides neural protection in dopaminergic system in a Parkinson's disease model induced by MPTP. Sci Rep 2016;6:1-3.
36. Dias V, Junn E, Mouradian MM. The role of oxidative stress in Parkinson's disease. J Parkinson's Dis 2013;3:461-91.
37. Gaki GS, Papavassiliou AG. Oxidative stress-induced signaling pathways implicated in the pathogenesis of Parkinson's disease. Neuromol Med 2014;16:217-30.
38. Niu J, Yu M, Wang C, Xu Z. Leucine-rich repeat kinase 2 disturbs mitochondrial dynamics via dynamin-like protein. J Neurochem 2012;122:650-8.
39. Kaidery NA, Thomas B. Current perspective of mitochondrial biology in Parkinson's disease. Neurochem Int 2018;117:91-113.
40. Wang X, Yan MH, Fujioka H, Liu J, Wilson-Delfosse A, Chen SG, *et al*. LRRK2 regulates mitochondrial dynamics and function through direct interaction with DLP1. Hum Mol Genet 2012;21:1931-44.
41. Kang UB, Marto JA. Leucine-rich repeat kinase 2 and Parkinson's disease. Proteomics 2017;17:1600092.
42. Von Coelln R, Dawson VL, Dawson TM. Parkin-associated Parkinson's disease. Cell Tissue Res 2004;318:175-84.
43. Joch M, Ase AR, Chen CX, MacDonald PA, Kontogiannea M, Corera AT, *et al*. Parkin-mediated monoubiquitination of the PDZ protein PICK1 regulates the activity of acid-sensing ion channels. Mol Biol Cell 2007;18:3105-18.
44. Zhang Y, Gao J, Chung KK, Huang H, Dawson VL, Dawson TM. Parkin functions as an E2-dependent ubiquitin-protein ligase and promotes the

degradation of the synaptic vesicle-associated protein, CDCrel-1. Proc Natl Acad Sci 2000;97:13354-9.

45. Zhang D. Protein Kinase C-delta in Dopaminergic System and Experimental Models of Parkinson's Disease. Iowa: Iowa State University; 2008.
46. Büeler H. Impaired mitochondrial dynamics and function in the pathogenesis of Parkinson's disease. Exp Neurol 2009;218:235-46.
47. LaVoie MJ, Ostaszewski BL, Weihofen A, Schlossmacher MG, Selkoe DJ. Dopamine covalently modifies and functionally inactivates parkin. Nat Med 2005;11:1214-21.
48. Abou-Sleiman PM, Muqit MM, Wood NW. Expanding insights of mitochondrial dysfunction in Parkinson's disease. Nat Rev Neurosci 2006;7:207-19.
49. Exner N, Lutz AK, Haass C, Winklhofer KF. Mitochondrial dysfunction in Parkinson's disease: Molecular mechanisms and pathophysiological consequences. EMBO J 2012;31:3038-62.
50. Takatori S, Ito G, Iwatsubo T. Cytoplasmic localization and proteasomal degradation of N-terminally cleaved form of PINK1. Neurosci Lett 2008;430:13-7.
51. Wirawan E, Berghe TV, Lippens S, Agostinis P, Vandenabeele P. Autophagy: For better or for worse. Cell Res 2012;22:43-61.
52. Gautier CA, Kitada T, Shen J. Loss of PINK1 causes mitochondrial functional defects and increased sensitivity to oxidative stress. Proc Natl Acad Sci 2008;105:11364-9.
53. Marongiu R, Spencer B, Crews L, Adame A, Patrick C, Trejo M, *et al*. Mutant pink1 induces mitochondrial dysfunction in a neuronal cell model of Parkinson's disease by disturbing calcium flux. J Neurochem 2009;108:1561-74.
54. Deas E, Wood NW, Plun-Favreau H. Mitophagy and Parkinson's disease: The PINK1-parkin link. Biochim Biophys Acta Mol Cell Res 2011;1813:623-33.
55. Matsuda N. Phospho-ubiquitin: Upending the PINK-parkin-ubiquitin cascade. J Biochem 2016;159:379-85.
56. Narendra DP. Parkin/pink1 pathway for the selective isolation and degradation of impaired mitochondria. In: Mitochondrial Mechanisms of Degeneration and Repair in Parkinson's Disease. Cham: Springer; 2016. p. 159-82.
57. Martinat C, Shendelman S, Jonason A, Leete T, Beal MF, Yang L, *et al*. Sensitivity to oxidative stress in DJ-1-deficient dopamine neurons: An ES-derived cell model of primary parkinsonism. PLoS Biol 2004;2:e327.
58. Chan JY, Chan SH. Activation of endogenous antioxidants as a common therapeutic strategy against cancer, neurodegeneration and cardiovascular diseases: A lesson learnt from DJ-1. Pharmacol Ther 2015;156:69-74.
59. Kim RH, Smith PD, Aleyasin H, Hayley S, Mount MP, Pownall S, *et al*. Hypersensitivity of DJ-1-deficient mice to 1-methyl-4-phenyl-1, 2, 3, 6-tetrahydropyrindine (MPTP) and oxidative stress. Proc Natl Acad Sci 2005;102:5215-20.
60. Labbe G, Pessayre D, Fromenty B. Drug-induced liver injury through mitochondrial dysfunction: Mechanisms and detection during preclinical safety studies. Fundam Clin Pharmacol 2008;22:335-53.
61. Morissette M, Litim N, Di Paolo T. Natural phytoestrogens: A class of promising neuroprotective agents for Parkinson disease. In: Discovery and Development of Neuroprotective Agents from Natural Products. Netherlands: Elsevier; 2018. p. 9-61.
62. Mukherjee UA, Ong SB, Ong SG, Hausenloy DJ. Parkinson's disease proteins: Novel mitochondrial targets for cardio protection. Pharmacol Ther 2015;156:34-43.
63. Letts JA, Fiedorczuk K, Sazanov LA. The architecture of respiratory super complexes. Nature 2016;537:644-8.
64. Xiong N, Long X, Xiong J, Jia M, Chen C, Huang J, *et al*. Mitochondrial complex I inhibitor rotenone-induced toxicity and its potential mechanisms in Parkinson's disease models. Crit Rev Toxicol 2012;42:613-32.
65. Bové J, Perier C. Neurotoxin-based models of Parkinson's disease. Neuroscience 2012;211:51-76.
66. Karunakaran S, Diwakar L, Saeed U, Agarwal V, Ramakrishnan S, Iyengar S, *et al*. Activation of apoptosis signal regulating kinase 1 (ASK1) and translocation of death-associated protein, Daxx, in substantia nigra pars compacta in a mouse model of Parkinson's disease: Protection by α-lipoic acid. FASEB J 2007;21:2226-36.
67. Im JY, Lee KW, Junn E, Mouradian MM. DJ-1 protects against oxidative damage by regulating the thioredoxin/ASK1 complex. Neurosci Res 2010;67:203-8.
68. Waak J, Weber SS, Görner K, Schall C, Ichijo H, Stehle T, *et al*. Oxidizable residues mediating protein stability and cytoprotective interaction of DJ-1 with apoptosis signal-regulating kinase 1. J Biol Chem 2009;284:14245-57.
69. Gao H, Yang W, Qi Z, Lu L, Duan C, Zhao C, *et al*. DJ-1 protects dopaminergic neurons against rotenone-induced apoptosis by enhancing ERK-dependent mitophagy. J Mol Biol 2012;423:232-48.
70. Henchcliffe C, Beal MF. Mitochondrial biology and oxidative stress in Parkinson disease pathogenesis. Nat Clin Pract Neurol 2008;4:600-9.
71. Kuwana T, Newmeyer DD. Bcl-2-family proteins and the role of mitochondria in apoptosis. Curr Opin Cell Biol 2003;15:691-9.
72. Koh H, Kim H, Kim MJ, Park J, Lee HJ, Chung J. Silent information regulator 2 (Sir2) and forkhead box O (FOXO) complement mitochondrial dysfunction and dopaminergic neuron loss in *Drosophila* PTEN-induced kinase 1 (PINK1) null mutant. J Biol Chem 2012;287:12750-8.
73. Ma X, Kalakonda S, Srinivasula SM, Reddy SP, Platanias LC, Kalvakolanu DV. GRIM-19 associates with the serine protease HtrA2 for promoting cell death. Oncogene 2007;26:4842-9.
74. Taylor RW, Turnbull DM. Mitochondrial DNA mutations in human disease. Nat Rev Genet 2005;6:389-402.
75. Tuppen HA, Blakely EL, Turnbull DM, Taylor RW. Mitochondrial DNA mutations and human disease. Biochim Biophys Acta Bioenerget 2010;1797:113-28.
76. Bahr T, Welburn K, Donnelly J, Bai Y. Emerging model systems and treatment approaches for Leber's hereditary optic neuropathy: Challenges and opportunities. Biochim Biophys Acta Mol Basis Dis 2020;24:165743.
77. Bohr VA, Stevnsner T, de Souza-Pinto NC. Mitochondrial DNA repair of oxidative damage in mammalian cells. Gene 2002;286:127-34.
78. Fariss MW, Zhang JG. Vitamin E therapy in Parkinson's disease. Toxicology 2003;189:129-46.
79. Fujita KA, Ostaszewski M, Matsuoka Y, Ghosh S, Glaab E, Trefois C, *et al*. Integrating pathways of Parkinson's disease in a molecular interaction map. Mol Neurobiol 2014;49:88-102.
80. Chu Y, Kordower JH. Age-associated increases of α-synuclein in monkeys and humans are associated with nigrostriatal dopamine depletion: Is this the target for Parkinson's disease? Neurobiol Dis 2007;25:134-49.
81. Lim GG, Zhang C, Lim KL. Role of autophagy in Parkinson's disease. In: Autophagy-a double-edged sword: Cell Survival or Death? Vol. 17. London: IntechOpen; 2013. p. 353.
82. Gatt AP, Jones EL, Francis PT, Ballard C, Bateman JM. Association of a polymorphism in mitochondrial transcription factor A (TFAM) with Parkinson's disease dementia but not dementia with Lewy bodies. Neurosci Lett 2013;557:177-80.
83. Gaweda-Walerych K, Safranow K, Maruszak A, Bialecka M, Klodowska-Duda G, Czyzewski K, *et al*. Mitochondrial transcription factor A variants and the risk of Parkinson's disease. Neurosci Lett 2010;469:24-9.
84. Federico A, Cardaioli E, Da Pozzo P, Formichi P, Gallus GN, Radi E. Mitochondria, oxidative stress and neurodegeneration. J Neurol Sci 2012;322:254-62.
85. Tzoulis C, Bindoff LA. Acute mitochondrial encephalopathy reflects neuronal energy failure irrespective of which genome the genetic defect affects. Brain 2012;135:3627-34.
86. Valko M, Rhodes C, Moncol J, Izakovic MM, Mazur M. Free radicals, metals and antioxidants in oxidative stress-induced cancer. Chem Biol Interact 2006;160:1-40.
87. Lambert AJ, Brand MD. Reactive oxygen species production by mitochondria. In: Mitochondrial DNA. United States: Humana Press; 2009. p. 165-81.

88. Bergamini CM, Gambetti S, Dondi A, Cervellati C. Oxygen, reactive oxygen species and tissue damage. Curr Pharm Design 2004;10:1611-26.
89. Radak Z, Zhao Z, Goto S, Koltai E. Age-associated neurodegeneration and oxidative damage to lipids, proteins and DNA. Mol Aspects Med 2011;32:305-15.
90. Hayashi M. Oxidative stress in developmental brain disorders. Neuropathology 2009;29:1-8.
91. Boukhenouna S, Wilson MA, Bahmed K, Kosmider B. Reactive oxygen species in chronic obstructive pulmonary disease. Oxid Med Cell Longev 2018;2018:5730395.
92. Ferroni P, Barbanti P, Della-Morte D, Palmirotta R, Jirillo E, Guadagni F. Redox mechanisms in migraine: Novel therapeutics and dietary interventions. Antioxid Redox Signal 2018;28:1144-83.
93. Gmitterova K, Heinemann U, Gawinecka J, Varges D, Ciesielczyk B, Valkovic P, *et al*. 8-OHdG in cerebrospinal fluid as a marker of oxidative stress in various neurodegenerative diseases. Neurodegener Dis 2009;6:263-9.
94. Blesa J, Trigo-Damas I, Quiroga-Varela A, Jackson-Lewis VR. Oxidative stress and Parkinson's disease. Front Neuroanat 2015;9:91.
95. Miyazaki I, Asanuma M. Dopaminergic neuron-specific oxidative stress caused by dopamine itself. Acta Med Okayama 2008;62:141-50.
96. Choi SJ, Panhelainen A, Schmitz Y, Larsen KE, Kanter E, Wu M, *et al*. Changes in neuronal dopamine homeostasis following 1-methyl-4-phenylpyridinium (MPP+) exposure. J Biol Chem 2015;290:6799-809.
97. Arshad AR, Sulaiman SA, Saperi AA, Jamal R, Ibrahim NM, Murad NA. MicroRNAs and target genes as biomarkers for the diagnosis of early onset of Parkinson disease. Front Mol Neurosci 2017;10:352.
98. Vallone D, Picetti R, Borrelli E. Structure and function of dopamine receptors. Neurosci Biobehav Rev 2000;24:125-32.
99. Maruszak A, Żekanowski C. Mitochondrial dysfunction and Alzheimer's disease. Prog Neuropsychopharmacol Biol Psychiatry 2011;35:320-30.
100. Dev KK, Hofele K, Barbieri S, Buchman VL, van der Putten H. Part II: α-synuclein and its molecular pathophysiological role in neurodegenerative disease. Neuropharmacology 2003;45:14-44.
101. Hwang O. Role of oxidative stress in Parkinson's disease. Exp Neurobiol 2013;22:11-7.
102. Freeman D, Cedillos R, Choyke S, Lukic Z, McGuire K, Marvin S, *et al*. Alpha-synuclein induces lysosomal rupture and cathepsin dependent reactive oxygen species following endocytosis. PLoS One 2013;8:e62143.
103. Dickson DW, Braak H, Duda JE, Duyckaerts C, Gasser T, Halliday GM, *et al*. Neuropathological assessment of Parkinson's disease: Refining the diagnostic criteria. Lancet Neurol 2009;8:1150-7.
104. Tohgi H, Abe T, Saheki M, Hamato F, Sasaki K, Takahashi S. Reduced and oxidized forms of glutathione and α-tocopherol in the cerebrospinal fluid of parkinsonian patients: Comparison between before and after L-dopa treatment. Neurosci Lett 1995;184:21-4.
105. Smith AC, Boyd MR. Preferential effects of 1, 3-bis (2-chloroethyl)-1-nitrosourea (BCNU) on pulmonary glutathione reductase and glutathione/glutathione disulfide ratios: Possible implications for lung toxicity. J Pharmacol Exp Ther 1984;229:658-63.
106. Dalle-Donne I, Rossi R, Colombo G, Giustarini D, Milzani A. Protein S-glutathionylation: A regulatory device from *Bacteria* to humans. Trends Biochem Sci 2009;34:85-96.
107. Gallogly MM, Mieyal JJ. Mechanisms of reversible protein glutathionylation in redox signaling and oxidative stress. Curr Opin Pharmacol 2007;7:381-91.
108. Reitman ZJ, Yan H. Isocitrate dehydrogenase 1 and 2 mutations in cancer: Alterations at a crossroads of cellular metabolism. J Natl Cancer Inst 2010;102:932-41.
109. Yin F, Sancheti H, Cadenas E. Mitochondrial thiols in the regulation of cell death pathways. Antioxid Redox Signal 2012;17:1714-27.
110. Kang JF, Tang BS, Guo JF. The progress of induced pluripotent stem cells as models of Parkinson's disease. Stem Cells Int 2016;2016:4126214.
111. Luo Y, Hoffer A, Hoffer B, Qi X. Mitochondria: A therapeutic target for Parkinson's disease? Int J Mol Sci 2015;16:20704-30.
112. Atashrazm F, Dzamko N. LRRK2 inhibitors and their potential in the treatment of Parkinson's disease: Current perspectives. Clin Pharmacol 2016;8:177.
113. Shiba-Fukushima K, Imai Y, Yoshida S, Ishihama Y, Kanao T, Sato S, *et al*. PINK1-mediated phosphorylation of the Parkin ubiquitin-like domain primes mitochondrial translocation of Parkin and regulates mitophagy. Sci Rep 2012;2:1002.
114. Rosales-Corral S, Reiter RJ, Tan DX, Ortiz GG, Lopez-Armas G. Functional aspects of redox control during neuroinflammation. Antiox Redox Signal 2010;13:193-247.
115. McCoy MK, Cookson MR. DJ-1 regulation of mitochondrial function and autophagy through oxidative stress. Autophagy 2011;7:531-2.
116. Fernandez-Moriano C, González-Burgos E, Gómez-Serranillos MP. Mitochondria-targeted protective compounds in Parkinson's and Alzheimer's diseases. Oxid Med Cell Longev 2015;2015:30.
117. Gillies LA, Kuwana T. Apoptosis regulation at the mitochondrial outer membrane. J Cell Biochem 2014;115:632-40.
118. Qi X, Qvit N, Su YC, Mochly-Rosen D. A novel Drp1 inhibitor diminishes aberrant mitochondrial fission and neurotoxicity. J Cell Sci 2013;126:789-802.
119. Cheng A, Wan R, Yang JL, Kamimura N, Son TG, Ouyang X, *et al*. Involvement of PGC-1α in the formation and maintenance of neuronal dendritic spines. Nat Commun 2012;3:1-2.
120. Brown GC, Murphy MP, Jornayvaz FR, Shulman GI. Regulation of mitochondrial biogenesis. Essays Biochem 2010;47:69-84.
121. Johri A, Chandra A, Beal MF. PGC-1α, mitochondrial dysfunction, and Huntington's disease. Free Radic Biol Med 2013;62:37-46.
122. Townsend DM, Tew KD, Tapiero H. The importance of glutathione in human disease. Biomed Pharmacother 2003;57:145-55.
123. Alimonti A, Bocca B, Pino A, Ruggieri F, Forte G, Sancesario G. Elemental profile of cerebrospinal fluid in patients with Parkinson's disease. J Trace Elem Med Biol 2007;21:234-41.
124. Jenner P, Olanow CW. Understanding cell death in Parkinson's disease. Ann Neurol 1998;44:S72-84.
125. Cacciatore I, Cornacchia C, Pinnen F, Mollica A, Di Stefano A. Prodrug approach for increasing cellular glutathione levels. Molecules 2010;15:1242-64.
126. Marchese A, Arciola CR, Coppo E, Barbieri R, Barreca D, Chebaibi S, *et al*. The natural plant compound carvacrol as an antimicrobial and anti-biofilm agent: Mechanisms, synergies and bio-inspired anti-infective materials. Biofouling 2018;34:630-56.
127. Chao J, Leung Y, Wang M, Chang RC. Nutraceuticals and their preventive or potential therapeutic value in Parkinson's disease. Nutr Rev 2012;70:373-86.
128. Solla P, Cannas A, Marrosu F, Marrosu MG. Therapeutic interventions and adjustments in the management of Parkinson disease: Role of combined carbidopa/levodopa/entacapone (Stalevo®). Neuropsychiatr Dis Treat 2010;6:483.
129. Kerksick C, Willoughby D. The antioxidant role of glutathione and N-acetyl-cysteine supplements and exercise-induced oxidative stress. J Int Soc Sports Nutr 2005;2:38.
130. Friedel HA, Goa KL, Benfield P. S-adenosyl-L-methionine. Drugs 1989;38:389-416.
131. Packer L, Witt EH, Tritschler HJ. Alpha-lipoic acid as a biological antioxidant. Free Radic Biol Med 1995;19:227-50.
132. Decker EA. The role of phenolics, conjugated linoleic acid, carnosine, and pyrroloquinoline quinone as nonessential dietary antioxidants. Nutr Rev 1995;53:49-58.
133. Smeyne M, Smeyne RJ. Glutathione metabolism and Parkinson's disease. Free Radic Biol Med 2013;62:13-25.
134. Maher P, Lewerenz J, Lozano C, Torres JL. A novel approach to enhancing cellular glutathione levels. J Neurochem 2008;107:690-700.