Review

Significance of Mitochondrial Dysfunction in the Pathogenesis of Parkinson's Disease

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Abstract

Parkinson's disease (PD) is characterized by the degeneration of the dopaminergic neurons of the corpus striatum, which can be caused by the disruption of processes of mitochondrial homeostasis, including mitophagy, mitochondrial fusion and division, mitochondrial transport, accumulation of reactive oxygen species (ROS), and calcium signaling. Dopaminergic neurons are particularly vulnerable to mitochondrial dysfunction due to their polarized and expanded structure and high bioenergy needs. The molecular basis of these disorders is manifested in mutations of mitochondrial homeostasis proteins. Understanding the functions of these proteins and the disorders caused by these mutations can be used to create therapeutics for the treatment of PD and diagnostic biomarkers of PD. A comprehensive analysis of research papers to identify promising therapeutic targets and drug compounds that target them, as well as biomarkers of mitochondrial dysfunction that can be used in clinical practice for the treatment of PD has been conducted in the current review. This practical approach advantageously emphasizes the difference between this work and other reviews on similar topics. The selection of articles in this review was carried out using the following keyword searches in scientific databases: PubMed, Google Scholar, NSBI, and Cochrane. Next, the most relevant and promising studies were re-selected.

Keywords: Parkinson's disease; neuron; mitochondria; mitochondrial dysfunction

1. Introduction

Parkinson's disease (PD) ranks second in the world after Alzheimer's disease among neurodegenerative diseases [1]. PD is most characteristic for older people, its prevalence is 0.5–1% for persons aged 65–69 years, and 1–3% for persons aged 80 years [2]. In PD, dopaminergic neurons of the substantia nigra are affected, although it is also possible a degeneration of the other types of neurons, which leads to the disruption of motor reactions and causes the emergence of bradykinesia, muscle rigidity, and postural instability [2]. One of the key markers of PD is the accumulation in neurons of the so-called Lewy bodies, which are aggregated molecules of the protein α-synuclein [2].

The progression of PD is influenced by a number of both non-hereditary and hereditary factors, among which, mutations of the genes whose expression products are responsible for maintaining mitochondrial homeostasis play an important role [2]. These mutations lead to the emergence of mitochondrial dysfunction, which is expressed in bioenergy defects and disruption of the following mitochondrial dynamics processes: fusion, division, transportation, and disposal of mitochondria (mitophagy) [3]. Mitochondrial dysfunction is a critical condition for dopaminergic neurons that have significant bioenergy and metabolic needs [4]. Inflammation may play a role in the progression of PD [2]. In a study [5], it has been shown that inhibition of the glial cells action, which were resident macrophages of the central nervous system, led to a decrease in the destruction of dopaminergic neurons in PD. Initiation of inflammation can be associated with the disruption of the mitophagy process, which leads to an increase of the reactive oxygen species (ROS) concentration, which has been also shown in the studies of the pathogenesis for other chronic diseases [6]. Thus, the occurrence of mitochondrial dysfunction causes a whole complex of reactions leading to the destruction of dopaminergic neurons and the progression of PD. The mechanisms of PD pathogenesis caused by mitochondrial dysfunction will be described further in this article.

Currently, symptomatic therapy is mainly used to treat PD and does not lead to a complete cure of the disease. The used drugs are aimed at increasing the level of dopamine, whose concentration drops when dopaminergic neurons are destroyed. The main used preparations include dopamine precursor levodopa, dopamine receptor agonists, and type B monoamine oxidase inhibitors, which prevent dopamine

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consumption [7]. However, with the identification of new molecular targets for this disease, new drugs for the treatment of PD are tested. The roles of the candidate drugs that target mutant proteins responsible for the progression of mitochondrial dysfunction in dopaminergic neurons will be considered in this review.

2. Overview of Parkinson's Disease Pathogenesis

Conventionally, PD can be divided into familial and sporadic. In familial PD, the etiology of the disease is determined by a mutation in a specific gene, while in sporadic PD, the cause of the disease may be associated with mutations in several different genes or may not have a genetic component [8]. Despite the low frequency of occurrence of most mutations associated with the risk of developing PD, the study and identification of new mutations are necessary for a better understanding of the pathogenesis of this complex disease. More than 70 genetic loci are known to be associated with the risk of developing PD according to a genome-wide association studies (GWAS) study [9]. Proteins such as alpha-synuclein (multiple functions), Parkin (mitophagy), PTEN-induced kinase 1 (PINK1) (mitophagy), leucine-rich repeat kinase 2 (LRRK2) (multiple functions), DJ-1 (antioxidant function), and glucosylceramidase beta 1 (GBA-1) (lysosomal function) have the most characterized mutated forms involved in PD pathogenesis [10]. Notably, these and other proteins with pathological roles in PD are directly or indirectly related to mitochondrial function or homeostasis.

Due to the fact that in most cases, the development of PD is associated with mutations in several genes, which are further complicated by the influence of non-genetic factors, and the development of pathology in PD is associated with the disruption of various cellular and extracellular processes. Late detection of PD symptoms makes it difficult to accurately determine the dynamics of these impaired processes; however, the disorders underlying the pathogenesis of PD are quite well characterized. Thus, the development of PD is most often noted by the presence of α-synuclein protein aggregation in dopaminergic neurons. Mutations in α-synuclein lead to increased expression and accumulation of this protein, which has a toxic effect on neurons and interferes with various cellular processes, including protein transport and mitophagy [11]. Impaired mitophagy, directly associated with mutations in the *PINK1* and *Parkin* genes, leads to an increase in the number of dysfunctional mitochondria. Mutations in some genes, including α-*synuclein* and *DJ-1*, increase oxidative stress, which is one of the causes of mitochondrial dysfunction as a result of ROS damage to mitochondrial DNA (mtDNA) and proteins [10,11]. Mitochondrial dysfunction leads to energetic dysfunction of neurons and their subsequent exhaustion [12]. The result of these disorders is the initiation of apoptosis and neuroinflammation, which contributes to a more rapid spread of pathology in the substance nigra [13]. The purpose of this manuscript is to determine the importance of mitochondrial dysfunction in the pathogenesis of PD by assessing interactions with other pathogenic mechanisms and triggers.

3. Why are Dopaminergic Neurons Vulnerable to Mitochondrial Dysfunction?

Mitochondria are essential organelles that perform a large number of important functions in the cell. The most important function of mitochondria is the synthesis of adenosine triphosphate (ATP) through the process of oxidative phosphorylation. Mitochondria also take part in processes such as the formation of lipids and heme, which is the compound included in hemoglobin, Ca^{2+} signaling, and apoptosis [4]. Mitochondria are highly dynamic organelles, and their homeostasis involves the processes of fusion, division, intracellular transport, and mitophagy. A large number of proteins take part in these processes, and their mutations lead to the mitochondrial homeostasis disruption, and as a result, to the cellular homeostasis disruption and the development of some disease types, including PD [4].

Mitochondria play an important role in the proper functioning of neurons. Due to the heterogeneous polarized structure of neurons, mitochondria are unevenly distributed in them. A lot of mitochondria are contained in the cell areas with the greatest energy needs, including nodes of Ranvier, presynaptic, and postsynaptic terminals [8]. The synthesis of ATP is necessary for performing essential processes occurring in neurons, such as transportation of synaptic vesicles, creation of the actin cytoskeleton for the presynaptic development, generation of an electrochemical potential for the synaptic transmission of a nerve impulse, axonal transport, catching and recycling of neurotransmitters, as well as the regulation of Ca^{2+} dynamics [14,15].

The highly polarized and expanded structure of dopaminergic neurons requires accurate and correct functioning of mitochondrial dynamic processes to provide the energy and metabolic needs of different sections of neurons (Fig. 1). Transport is necessary for the delivery of mitochondria to the required areas of the neuron: retrograde transport delivers old or non-functional mitochondria from a synapse to the soma of a neuron, where they undergo mitophagy; and anterograde transport delivers functional mitochondria in the opposite direction, where energy is needed to transmit the nerve impulse [15]. Mitochondrial fusion occurring mainly in the neuron soma is necessary for the synthesis of new healthy mitochondria since new elongated healthy mitochondrions are formed as a result of the fusion of a defective and healthy mitochondrion [16]. Mitochondrial division usually precedes mitophagy, in case the damages are distributed asymmetrically; and then as a result of division, in addition to a defective mitochondrion, a healthy mitochondrion is formed [16].
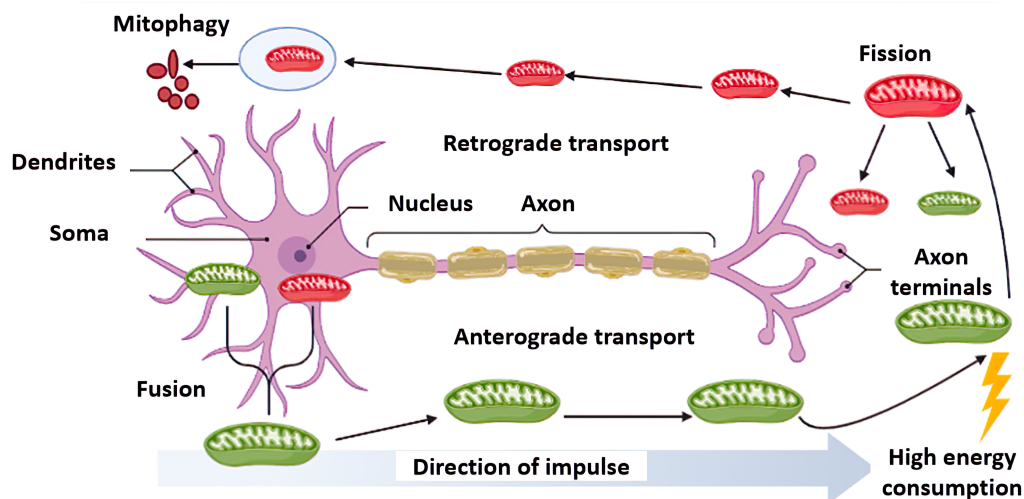
Fig. 1. Scheme of mitochondrial dynamics in neurons. Created with biorender.com.

Mitochondria are depicted by two colors: red—dysfunctional and green—healthy. The dynamics of mitochondria are strictly subordinated to the energy needs of neurons. With the fusion of dysfunctional and healthy mitochondria that occurs in the soma, healthy mitochondria are formed, most of which, with the help of mitochondrial transport proteins, migrate to the axonal endings, where an increased amount of ATP is required. The dysfunctional mitochondria formed here undergo division, which results in smaller mitochondria, most of which are dysfunctional. "Green" mitochondria remain in axon endings, while "red" ones migrate back to the neuron's soma, where they undergo final degradation called mitophagy.

The need for the proper functioning of mitochondrial dynamics processes in conditions of complex neuron structure and high bioenergy needs creates a high vulnerability of dopaminergic neurons to mitochondrial dysfunction. Additional threat factors are the accumulation of oxidized dopamine as a result of mitochondrial oxidative stress [17] and the low expression of electron transport chain (ETC) complex I, which is an indispensable component of the initial stage of oxidative phosphorylation [18]. An increased influx into dopaminergic neurons of Ca^{2+} through L-calcium channels also leads to mitochondrial oxidative stress and subsequent mitochondrial dysfunction [19].

4. Mechanisms of Neuronal Death in Parkinson's Disease

One of the mechanisms of dopaminergic neuron death is connected with mitochondrial dysfunction and, over time, it leads to the manifestation of PD clinical signs. The basic consequence of mitochondrial dysfunction is a decrease in ATP production; however, the main factor initiating neuronal apoptosis is not bioenergetic disorders in the cell caused by a decrease in ATP, but an increase in ROS level [14].

Normally, ROS are also formed during oxidative phosphorylation, but in a much smaller amount, in particular, due to the action of antioxidant enzymes [20]. The growth of ROS is associated with a decrease in the activity of complex I of the ETC. So it has been shown that the activity of complex I decreased by 25–30% in patients with PD [21], and, as noted above, the expression of the complex I was reduced in dopaminergic neurons. The growth of ROS increases due to the growing reduction of molecular oxygen to superoxide radical because of the disruption of electron transmission in ETC from complex I to ubiquinone [22]. High concentrations of ROS have disruptive effects on most types of biological macromolecules, including DNA, lipids, and proteins. Additional damage to complex I causes negative feedback with even greater ROS emissions. In addition, disruption of the ETC is associated with modulation of the function of other protein complexes of cellular respiration. Several studies have shown a decrease in the expression of subunits of complexes II–IV and ATP synthase [23]. Thus, the disruption of oxidative phosphorylation occurs at all stages of electron transfer in the ETC, which aggravates the development of mitochondrial dysfunction.

ROS have a direct effect on the initiation of apoptosis through the destruction of the membrane mitochondrial proteins, such as autosomal-dominant optic atrophy (OPA1) [24] and cardiolipin [25]; this interferes with the release of cytochrome C in cytosol, and as a result, cytochrome C interacts with the protein apoptotic protease activating factor 1 (APAF1), which transforms to the heptameric structure called the apoptosome that activates the caspase cycle already directly leading to cell death [26].

In addition to mitochondria-mediated apoptosis, neuronal death in PD can occur through other pathways, such as autophagy, necroptosis, ferroptosis, parthanatos, and pyroptosis. Autophagy is a pathway for the destruction of damaged organelles and is primarily aimed at cell survival

under stress conditions. However, sometimes autophagy can redirect its action towards cell death [27]. It is noted that autophagy is involved in the neurodegeneration in PD [28].

Another noted form of neuronal death in PD is parthanatos, which is associated with hyperactivation of poly(ADP-ribose) polymerase (PARP-1), which results from DNA damage by ROS and reactive nitrogen species (RNS) [27]. A marker for the development of parthanatos can be the translocation of the mitochondrial protein apoptosis inducing factor (AIF) from mitochondria to the nucleus [27]. Translocation of AIF into the nucleus has been observed in neurons of patients with PD [29]. In addition, PARP-1 inhibition prevents neuronal death and cytotoxicity caused by α-synuclein in *in vitro* and *in vivo* models of PD [30,31]. Another mechanism of the neuronal death noted in PD is ferroptosis, which is based on increased lipid peroxidation caused by iron accumulation [32].

It has been shown in a study [33] that different neuronal toxins could induce different pathways of neuronal death in PD models. It has been demonstrated that 48-hour exposure to 6OHDA and MPP + and 24-hour exposure to rotenone led to the activation of necroptosis in neurons. Necroptosis is associated with the formation of the necrosome, a structure consisting of Receptor-interacting protein kinase 1 (RIPK1), RIPK3, FAS-associated death domain protein (FADD), and caspase 8, which is in an inactive state. The necrosome activates the phosphorylation of Mixed lineage kinase domain like pseudokinase (MLKL), which leads to its transportation to the cell membrane and increases its permeability [33].

Pyroptosis in PD is associated with the development of neuroinflammation [34]. Moreover, its initiation begins with the activation of the NLR Family Pyrin Domain Containing 3 (NLRP3) inflammasome through interaction with damage-associated molecular patterns (DAMPs), including mitochondrial ones: mtDNA, cardiolipin, and cytochrome C, formed as a result of oxidative stress. This event causes the recruitment of the adapter protein ASC speck and the zymogen procaspase 1, which leads to autoproteolysis of procaspase 1 with the formation of the active form of caspase 1, which promotes the maturation of proinflammatory cytokines IL-1β, IL-18, and gasdermin-D as a result of cleavage of their precursors [34]. Gasdermin causes the formation of pores in the membrane, allowing the secretion of proinflammatory cytokines, which results in pyroptosis [34].

5. Mutant Proteins Responsible for the Development of Mitochondrial Dysfunction in Parkinson's Disease

Currently, several genes have been identified with mutations that lead to mitochondrial dysfunction and are unequivocally responsible for the progression of hereditary PD [35]. These mutations can be both autosomal dominant and autosomal recessive [36]. Each of the proteins coded by these genes takes part in mitochondrial homeostasis and can perform different functions affecting mitochondria.

5.1 Mitophagy Proteins

The key proteins involved in the mitophagy process are PTEN-induced kinase 1 (PINK1) and Parkin. Mutations in the genes of these proteins are the most common cause of PD development in individuals under 45 years old, accounting for 13% of cases among other etiological factors [35]. PINK1 is a serine/threonine kinase and is encoded by the *PARK6* gene, this protein serves as a detector molecule signaling mitochondrial dysfunction [37]. Parkin represents ubiquitin ligase E3 encoded by the gene *PARK2* and is responsible for mitophagy signal amplification [37]. Under normal conditions, PINK1 is transported to the inner membrane of a mitochondrion, where its cleavage occurs [38]. However, membrane depolarization occurs in a defective mitochondrion, and PINK1 is unable to transport to the inner membrane and accumulates on the outer mitochondrial membrane [39]. PINK1 phosphorylates Parkin, thus, activating it [39]. Activated Parkin performs ubiquitination of outer mitochondrial membrane proteins and attracts the autophagy receptors optineurin (OPTN) and nuclear dot protein 52 (NDP52) to a mitochondrion. These events lead to the formation of microtubule-associated protein 1A/1B-light chain 3 (LC3)-phagophores, which are involved in mitochondrial cleavage by lysosomes [37]. This process is shown schematically in Fig. 2.

Progression of PD is associated with emergent mutations of PINK1 and Parkin proteins. Thus, it has been shown that the PINK1-I368N mutant could not attach to the outer mitochondrial membrane due to conformational changes in its active site, which led to the arrest of mitophagy at the initial stage [40]. PINK1 function is associated with Parkin phosphorylation in the ubiquitin-like (UBL) domain. Phosphorylation leads to a change in the UBL conformation to open and become active [41]. It has been also shown that the mutations, G12R, R33Q, and R42P, in the UBL domain, caused reduced phosphorylation of Parkin, which led to impaired activation of this enzyme [42]. Two amino acid substitutions, G12R and T55I, in the UBL domain, activated Parkin autoubiquitination, which resulted in its degradation [42].

PINK1 and Parkin malfunctions are not only associated with mutations. Mitochondrial dysfunction can occur because of the appearance of S-nitrosylated PINK1 (SNO-PINK1), which is a post-translational modification that results in the arrest of the kinase activity of this enzyme. Increased SNO-PINK1 formation has been shown to lead to neuronal death [43]. Ubiquitin Specific Peptidase 30 (USP30), USP35, and USP15 are the inhibitors of Parkin, performing the role of deubiquitin ligases; they destroy the chains of ubiquitins created by Parkin and, in case of imbalance, can cause inhibition of mitophagy [44]. Phosphatase
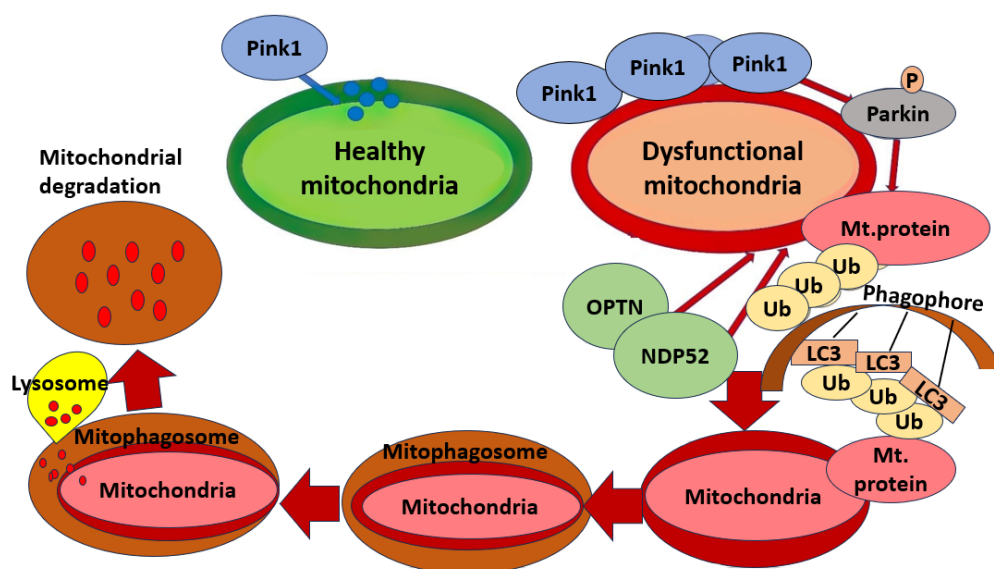
Fig. 2. Mitophagy initiated by PTEN-induced kinase 1 (Pink1) and Parkin participation. P, phosphorylated; Ub, Ubiquitin; LC3, Microtubule-associated protein 1A/1B-light chain 3; Mt. protein, mitochondrial protein; OPTN, Optineurin; NDP52, 52-kDa nuclear dot protein; Pink1, PTEN-induced kinase 1.

and tensin homolog (PTEN-L) also negatively regulates mitophagy by dephosphorylating Parkin and thus, inhibiting the action of PINK1 [45].

5.2 The Role of α-Synuclein in the Progression of Parkinson's Disease

α-synuclein is the protein that is highly expressed in the nerve tissue, comprising 1% of neuronal proteins [15]. α-synuclein performs many functions including the following: is responsible for the formation of vesicles, and the release of neurotransmitters into a synaptic cleft; takes part in intracellular transport of synthesized proteins from the endoplasmic reticulum to the Golgi apparatus; has an antiapoptotic effect; regulates the lysosomal degradation of proteins; and affects the processes of mitochondrial dynamics in neurons [15].

It is logical to assume that changing the expression of this protein and the appearance of mutant forms entails large-scale changes in the functioning of neurons. Indeed, mutations in the *SNCA* gene encoding α-synuclein lead to autosomal dominant PD [37]. Duplications and triplications of the *SNCA* gene are noteworthy; they lead to an increase in the expression of α-synuclein without changing its amino acid sequence. Increasing the concentration of α-synuclein in the cytoplasm of neurons leads to the formation of Lewy bodies, which are insoluble agglomerates of various proteins, among which α-synuclein occupies the prevailing position [15]. The presence of Lewy bodies is one of the main histological criteria for the diagnosis of PD, while their role in the pathogenesis of PD remains unclear; it is considered that Lewy body is a concomitant sign that does not directly affect the progression of the disease [15].

The accumulation of α-synuclein leads to the death of dopaminergic neurons through a large number of molecular pathways affecting the processes of mitochondrial dynamics. In *in vitro* and *in vivo* experiments, it has been found that wild-type α-synuclein—at an increased concentration—and mutant α-synuclein (to a greater extent) —with the mutations A53T, E46K, and H50Q—were able to cause fragmentation of mitochondria and increase the release of ROS [46]. In addition, the increased expression of wild-type α-synuclein and its mutant protein leads to the dissociation of mitochondria and endoplasmic reticulum, as a result of which Ca^{2+} metabolism is disrupted and the ATP production is reduced [47]. In addition, it has been shown that α-synuclein could cause mitochondrial dysfunction by directly penetrating a mitochondrion through the interaction with the proteins of the external mitochondrial membrane—translocase of the outer mitochondrial membrane (TOM40), TOM20, and voltage-dependent anion-selective channel 1 (VDAC1) [15]. Thus, α-synuclein is able to bind to the mitochondrial membrane protein VDAC1, modulating its function. Under pathological conditions, this leads to increased mitochondrial permeability, mitochondrial swelling, and initiation of apoptosis [48]. In elevated concentrations, α-synuclein is able

to inhibit the activity of proteins carrying out anterograde mitochondrial transport and physically block mitochondrial movement, forming conglomerates [15]. Furthermore, mutant α-synuclein is able to inhibit the mitophagy process, both directly affecting Parkin and indirectly through other proteins [37]. In addition, dysfunction of Parkin caused by other factors may lead to the pathological process caused by α-synuclein. In a study [49] on neuroblastoma cell culture, it has been shown that iron could have a toxic effect on mitochondria by causing α-synuclein aggregation. This mechanism was associated with iron inactivation of the Parkin protein, which led to the accumulation of α-synuclein, depolarization of the mitochondrial membrane, decreased ATP synthesis, and mitochondrial dysfunction with subsequent cell death. It is also notable that α-synuclein can reduce the levels of the "anti-aging" protein sirtuin 3 (SIRT3) in mitochondria. SIRT3 plays an important role in the regulation of oxidative stress and energy metabolism, and also has a neuroprotective effect. A study in a rodent model of PD revealed that a decrease in SIRT3 concentration caused by the accumulation of α-synuclein agglomerates led to disruptions of mitochondrial function and mitochondrial dynamics [50].

5.3 Mitochondrial Fusion/Division Proteins

Mitochondrial fusion is necessary to restore mitochondrial proteins and DNA in damaged mitochondria; as a result of fusion, the probability of mitophagy decreases [51]. The mitochondrial fusion process occurs with the joint participation of the proteins mitofusin 1 (Mfn1) and Mfn2 located on the outer mitochondrial membrane (OMM) and the protein OPA1, which is located on the internal mitochondrial membrane (IMM) [52]. The division of mitochondria, which leads to a decrease in their size and initiation of the mitophagy process, balances the fusion process [51]. The main protein performing mitochondrial division is dynamin-related protein 1 (Drp1), which is recruited on an outer membrane of mitochondria by the mitochondrial fission 1 protein (Fis1) and mitochondrial fission factor (Mff) proteins [15].

Although the direct role of the gene mutations of these proteins in the progression of PD has not been proven at the moment, a negative effect has been revealed in disorders of mitochondrial fusion and division processes caused by deletions in the genes *OPA1*, *Mfn1*, *Mfn2*, and *Drp1*, on the nigrostriatal pathway included in the dopaminergic system of the brain [15]. It has been shown that deletion in the *Mfn1* gene caused the disruption of the ETC functioning, leading to aberrant morphology of mitochondria and destruction of nerve terminals in the striatum [53]. Deletion in the *Drp1* gene in mice led to a decrease in the number of healthy mitochondria in the axons and nerve terminals of the striatum, which provoked the disruption of axon functioning and the death of dopaminergic neurons [54].

Various factors may act to modulate the balance of mitochondrial dynamics in PD. For example, microRNAs such as miR-34b/c activate Mfn1, which enhances mitochondrial fusion, and miR-205, through LRRK2, activates DRP1, which enhances mitochondrial fission [55]. It is known that some mutant proteins in PD—α-synuclein, VPS35, and LRRK2—as well as the S-nitrosylated form of Parkin are capable of indirectly enhancing mitochondrial fission [56]. At the same time, the balance of mitochondrial fission and fusion in PD requires further study.

5.4 Mitochondrial Transport Proteins

Anterograde transport in neurons from the soma to the synapse is carried out due to the activity of kinesins, and retrograde transport in the reverse direction is carried out due to the work of dyneins [57]. In the anterograde direction, new healthy mitochondria move to the area of nerve impulse transmission, which requires energy stored in ATP, and old, mainly dysfunctional mitochondria move in the retrograde direction for subsequent mitophagy. Kinesins and dyneins are motor proteins associated with microtubules. When carrying out mitochondrial transport with the reverse end, motor proteins interact with the Trafficking Kinesin (TRAK) protein, which in turn binds to the Mitochondrial Rho GTPase 1 (MIRO1) protein located on the outer membrane of mitochondria [9]. The protein MIRO1 is responsible for the binding of the motor proteins to a mitochondrion and choosing the direction of movement of a mitochondrion [58].

MIRO1 directly interacts with PINK1 and Parkin and is one of the first proteins to undergo ubiquitination and degradation in mitophagy [59]. In some mutations of PINK1 and Parkin proteins, MIRO1 does not degrade and its concentration increases on the outer mitochondrial membrane, as a result of which retrograde transport increases and defective mitochondria are not utilized, but accumulate in the cytoplasm, which over time leads to neuronal death and PD progression [60]. Mutations in the gene encoding the protein MIRO1—RHOT1—can also lead to neuronal death, which has been shown in a mouse model when the deletions were introduced into *RHOT1* [61].

A direct role in mitochondrial, as well as in general intraneuronal transport, is played by tubulin proteins, which form microtubules—strands along which intracellular cargo moves. It is noted that in PD, tubulin acetylation is impaired, which leads to the destabilization of microtubules and the stopping of anterograde and retrograde transport in the neuron [62].

5.5 Mitochondrial Biogenesis Proteins

The synthesis of new mitochondria is especially important when the cell needs energy. Mitochondrial biogenesis involves the replication of mitochondrial DNA and the synthesis of mitochondrial proteins, including ETC components. The key regulators of this process are the proteins

peroxisome proliferator-activated receptor γ coactivator-1α (PGC-1α) and peroxisome proliferator-activated receptor γ coactivator-1β (PGC-1β), which, through nuclear factor erythroid 2–related factor 2 (NRF-2), activate the mitochondrial transcription factor A (tfam), which is directly responsible for the transcription of mitochondrial proteins [63].

In PD, mitochondrial biogenesis is impaired, which significantly contributes to a decrease in energy metabolism and the subsequent death of dopaminergic neurons. In a study [64] it has been demonstrated that in brain samples from patients with PD, there was a reduced level of markers of mitochondrial biogenesis—PGC-1α and PGC-1β—as well as a decrease in the amount of mtDNA both in the blood and in the tissues of the prefrontal cortex of the patients with PD. According to the results of the study [65], two PGC-1α variants were identified in patients with PD, which were associated with the risk of PD (rs6821591 CC and rs2970848 GG). In addition, in brain samples from patients with PD, a truncated form of PGC-1α weighing 17 kDa has been found, which lacked the LXXLL site, responsible for interaction with several transcription factors, which also blocked mitochondrial biogenesis [66]. Decreased PGC-1α expression may be associated with increased gene promoter methylation. Thus, in a study on a mouse model of PD, it has been shown that hypermethylation of the PGC-1α promoter caused by palmitate led to a decrease in the levels of PGC-1α and other mitochondrial proteins [23].

5.6 ROS Reduction Proteins

ROS are a by-product of oxidative phosphorylation reactions; however, as a result of mitochondrial dysfunction, ROS accumulate and begin to destroy biological macromolecules, which leads to damage to cellular structures, initiation of inflammatory reactions, and apoptosis. Proteins responsible for protecting cells from oxidative stress act as the second block of defense, they have the ability to save neurons from death in case of disruption of mitochondrial dynamics. It was shown that the mutations in genes encoding Coiled-Coil-Helix-Coiled-Coil-Helix Domain Containing 2 (CHCHD2) and DJ-1 proteins led to an accumulation of ROS in the cytoplasm [4]. It is proved that these mutations are the cause of PD development—the mutant protein CHCHD2 causes autosomal dominant PD [67], and the mutant protein DJ-1 causes autosomal recessive PD [37]. CHCHD2 is located in an intermembrane space of a mitochondria, its main functions are maintaining the integrity of mitochondrial cristae and regulating the operation of the ETC [4]. In the case of the mutation in the *CHCHD2* gene, there is a disruption of electron transfer in the ETC, which leads to an increase in the rate of ROS formation [68]. The protein DJ-1 localizes in a matrix and intermembrane space of mitochondria, and in case of oxidative stress, it acts as a reductase and promotes ROS clearance [69].

5.7 Ca^{2+} Homeostasis Proteins

Ca^{2+} plays an important role in starting oxidative phosphorylation, acting as an enhancer of mitochondrial respiration [70]. However, as a result of the disruption of calcium metabolism, there is an unevenly increasing amount of Ca^{2+} in mitochondria, which turns to an acceleration of the cellular respiration process, depolarization of the mitochondrial membrane, and production of a high amount of ROS [71]. There are several reasons that link an increase in the concentration of mitochondrial Ca^{2+} and an increase in ROS output: firstly, when metabolism accelerates, there is a large leak of electrons with ETCs that are transferred to the ROS formation chain; second, Ca^{2+} activates nitric oxide synthase, which leads to the formation of nitric oxide, that inhibits the work efficiency of complex I and complex IV of ETC, which also contributes to faster ROS generation; and thirdly, the increased concentration of Ca^{2+} promotes the release of cytochrome c into the cytoplasm, which increases oxidative stress and initiates apoptosis [71]. Impaired calcium homeostasis is associated with mutations in the leucine-rich repeat kinase 2 (*LRRK2*) gene [70]. The protein LRRK2 has kinase and GTPase activity; it plays an important role in the functioning of neurons. Its functions are various, with some associated with the regulation of mitochondrial dynamics [70]. Thus, LRRK2 is the enzyme that phosphorylates DRP1, which modulates the process of mitochondrial division [72]. Mutations in the gene *LRRK2* are observed in 5% of familial PD cases and 1% of sporadic PD cases [73,74]. The most frequent mutation of LRRK2 is G2019S which leads to increased activation of calcium channels [75]. An additional protein affecting calcium homeostasis is the DJ-1 described above. Thus, it was shown that with a decrease in expression, there was an increase in the level of Ca^{2+} inside the mitochondria [76]. The roles of mutant proteins in the development of mitochondrial dysfunction in PD are shown in Table 1.

6. Biomarkers of Mitochondrial Dysfunction in Parkinson's Disease

Applying highly sensitive biomarkers is essential for effective therapy of PD besides the development of drugs. At present, very few biomarkers of any type related to PD are known [4]. The discovery of mitochondrial dysfunction biomarkers in PD can be helpful for patients in whom mitochondrial-targeted therapy is effective. Biomarkers of mitochondrial dysfunction can be used in conjunction with others for diagnosis, determination of disease severity, and prediction of its duration. Additionally, these biomarkers can be helpful for measuring drug efficacy and safety during the carrying out of clinical trials for drugs that are used for PD therapy.

Two of the most perspective biomarkers are the main mitophagy proteins PINK1 and Parkin, which undergo phosphorylation upon activation. The level of current mitophagy can be detected by the concentrations of these

Table 1. Mutant proteins responsible for mitochondrial dysfunction and mitochondrial dynamic disruption in PD.

Protein	Mutation/modification	Type of protein dysfunction	Pathological consequence
PINK1	I368N	PINK1 cannot attach to the OMM	Inhibition of mitophagy initiation; Increased dysfunctional mitochondria accumulation
	SNO-PINK1	Decreased kinase activity	Mitophagy inhibition
Parkin	G12R, R22Q, R42P	Decreased Parkin phosphorylation	Mitophagy inhibition;
	G12R, T55I	Parkin degradation	Increased dysfunctional mitochondria accumulation
α-synuclein	Duplication (triplication)	Increased expression and accumulation	Lewy bodies formation; Increased mitochondrial permeability and apoptosis; Decreased anterograde mitochondrial transport
	A53T, E46K, H50Q	Unknown	Ca^{2+} metabolism disruption; Decreased ATP production; Mitophagy disruption
Mfn1/2	Some deletions	Impaired activity	Disruption of the ETC functioning; Aberrant mitochondrial morphology; Destruction of nerve terminals in the striatum
Drp1	Some deletions	Impaired activity	Increased dysfunctional mitochondria accumulation; Disruption of axon functioning
MIRO1	Influence of PINK1 and Parkin mutations	MIRO1 accumulation	Increased retrograde mitochondrial transport; Increased dysfunctional mitochondria accumulation
PGC-1α	SNPs: rs6821591 CC and rs2970848 GG	Unknown	Unknown precise mecanism (Increased PD risk)
	Truncated form of PGC-1α without LXXLL site (alternative splicing)	Inability to bind with some transcriptional sites	Decreased mitochondrial biogenesis
	Increased PGC-1α promoter methylation	Decreased expression	
CHCHD2	A71P	Impaired activity	Disruption of the ETC functioning
DJ-1	Different mutations	Impaired activity	Increased ROS production
LRRK2	G2019S	Impaired activity	Increased Ca^{2+} accumulation in mitochondria; Increased ROS production

PINK1, PTEN-induced kinase 1; Mfn1/2, mitofusin 1/2; Drp1, dynamin-related protein 1; MIRO1, mitochondrial Rho GTPase 1; PGC-1α, peroxisome proliferator-activated receptor γ coactivator-1α; CHCHD2, Coiled-Coil-Helix-Coiled-Coil-Helix Domain Containing 2; LRRK2, leucine-rich repeat kinase 2; SNO-PINK1, S-nitrosylated PINK1; SNPs, single nucleotide polymorphisms; LXXLL, L is leucine and X is any amino acid; OMM, outer mitochondrial membrane; ATP, adenosine triphosphate; ETC, electron transport chain; PD, Parkinson's disease; ROS, reactive oxygen species.

phosphorylated proteins determined using the enzyme-linked immunosorbent assay (ELISA) in blood serum [4]. These biomarkers were already used in the diagnosis of other neurodegenerative diseases related to mitochondrial dysfunction such as Alzheimer's disease [77]. The other marker of the mitophagy process may be the protein phospho-Ser65-ubiquitin, whose concentration decreases in the serum of PD patients with mutations in the *PARK2* and *PARK6* genes, which encode the PINK1 and Parkin proteins, respectively [78]. The other potential biomarker protein of PD is Miro1, which participates in mitochondrial transport; it was shown that fibroblasts of patients with PD had impairment of clearance of this protein [79].

There are several biomarkers, related to the progression of oxidative stress in PD. It was found that coenzyme Q10 level (the component of ETC) was increased in blood plasm and thrombocytes of patients with PD while it was in deficit in lymphocytes [80]. Using ELISA based on monoclonal antibodies to the oxidized DJ-1 protein, it was found that the concentration of the oxidized protein was increased in patients with PD, who did not take medicine; while the control group and patients, who took dopamine replacement therapy, had no significant difference [81]. In another research, it was shown that the level of 8-hydroxydeoxyguanosine (molecule, which is produced under oxidative destruction of both nucleus and mitochondrial DNA) was increased in cerebrospinal fluid for patients with PD in comparison with the control group, and also correlated with the severity of the disease [82]. In the study [83], it was found that the level of extracellular mi-

tochondrial DNA, released by cells under oxidative stress, was increased in the blood plasma of the PD patients who showed mutations in the *PARK2* and *PARK6* genes. In a study [84], fibroblasts from PD patients with Parkin mutations revealed reduced levels of mitochondrial biogenesis regulators, namely the proteins Nrf1β, Nrf2, and Tfam. The same study showed that there was a significant decrease in the levels of the following proteins: Beclin-1 (autophagy), Parkin (mitophagy), and OPA-1 (mitochondrial fusion).

Since the onset of mitochondrial dysfunction may lead to changes in energy metabolism, some metabolites can be proposed as biomarkers in PD. Thus, in studies [85,86], decreased levels of apolyproteins A1, A4, B, C3, C4, and M were detected in plasma samples from patients with PD.

Although the role of inflammation as one of the causes of PD progression is not fully proven, one consequence of mitochondrial dysfunction is the initiation of proinflammatory factors, which allows them to be used as biomarkers. Thus, mutations in the gene *PARK2* in patients with PD led to the activation of the inflammasome NLRP3 in microglia and macrophages, as well as increased expression of the proinflammatory cytokines IL-1β, IL-6, and IL-18 [87]. The level of IL-6 was also correlated with the severity of PD [88].

Some cell types besides neurons may serve as model objects for studying PD progression. Thus, it was found that fibroblasts of patients with PD were characterized by extensive mitochondrial dysfunction and mitophagy disorder [89]. In addition, it was observed that peripheral blood mononuclear cells taken from patients with sporadic PD had an increased level of ROS production and mitochondrial dysfunction [90]. The presented biomarkers are listed in Table 2 (Ref. [4,78–86]).

7. Parkinson's Disease Therapy Targeting Proteins Involved in Mitochondrial Homeostasis

Since the treatment of PD is predominantly symptomatic, the development of new groups of drugs affecting the mechanism of the disease is a promising area of modern medicine. The creation of such drugs can slow down or even stop the progression of PD; however, it will not be possible to completely eliminate the negative impact caused by pathological factors, since at the time of the appearance of the first clinical symptoms of PD, about 60% of dopaminergic neurons are affected [3]. As promising targets for these drugs, proteins taking part in mitochondrial homeostasis—which, as described above, play an important role in the normal functioning of neurons—can be considered. At the moment, there are several types of drug developments whose mechanism of action is based on modulating disrupted mitochondrial homeostasis processes leading to mitochondrial dysfunction.

The most promising targets in PD therapy are the PINK1 and Parkin proteins, mutations in the genes that are the main causes of the progression of hereditary PD. Since the mutant proteins PINK1 and Parkin have reduced enzymatic activity, one strategy is to develop preparations based on the activators of these enzymes. Thus, in the study [91], it was shown that kinetin triphosphate (KTP), an analog of ATP, enhanced the activity of both mutant PINK1 (G309D) and wild-type PINK1, which led to a decrease in apoptosis caused by oxidative stress in a model of human neurons. Invention [92] represents the aromatic compound aimed at activation of Parkin protein by binding to the zinc finger domain and is intended for therapy of various groups of diseases, including PD.

Another strategy is to deactivate Parkin inhibitors. Since usp30 is a natural inhibitor of mitophagy that blocks it at early stages, the use of usp30 inhibitors has the potential to restore impaired mitophagy and help to improve clinical symptoms in PD. Thus, in a study [93], it has been shown that the introduction of usp30 inhibitors led to an increase in the level of p-Ser65-ubiquitin and enhanced mitophagy in *in vitro* neuronal models. In a study [94], the compound benzosulfonamide was proposed as a usp30 inhibitor and demonstrated increased selectivity for its target compared to other inhibitors. In the study [95], N-cyanopyrrolidine showed itself as a highly selective covalent inhibitor of deubiquitin ligase USP30, which prevents the ubiquitination of mitochondrial membrane proteins. Nilotinib used in the treatment of myeloid leukemia showed inhibitory activity against the other Parkin inhibitor, tyrosine kinase c-Abl [96].

The Miro1 protein, whose increased aggregation on the surface of mitochondria leads to the accumulation of dysfunctional mitochondria, also acts as a promising target in PD therapy. The study [79] identified the small molecule that caused Miro1 degradation and contributed to the inhibition of dopaminergic neurodegeneration.

Mutations in the genes of the LRRK2 and α-synuclein proteins are the reasons for an autosomal dominant variant of PD, the phenotypic manifestation of the mutations in this case appears in increased expression or increased enzymatic activity of these proteins. Accordingly, the use of inhibitory compounds regarding these proteins is an appropriate approach. Several different inhibitors have been found for α-synuclein, which showed efficacy in neuronal cell culture and transgenic mouse models [97–99]. In the study [100], the compound that reduced the GTP activity of LRRK2, which led to a decrease in neuronal degeneration, was described. An interesting work based on genome editing, in which the G2019S and R1441C mutations of the *LRRK2* gene were repaired using the zinc finger nuclease, led to a sharp decrease in damaged mitochondrial DNA in cells [101].

Table 2. Mitochondrial biomarkers in PD.

Biomarker	Sample type	Level changing	Reference
PINK1	Blood serum	↓	[4]
PARKIN	Blood serum, fibroblasts	↓	[4,84]
Phospho-Ser65-ubiquitin	Blood serum	↓	[78]
Miro1	Fibroblasts	↑	[79]
Q10	Blood serum, thrombocytes	↑	[80]
	Lymphocytes	↓	
Oxidized DJ-1	Blood serum	↑	[81]
8-hydroxydeoxyguanosine	Cerebrospinal fluid	↑	[82]
Extracellular mitochondrial DNA	Blood plasma	↑	[83]
Nrf1β	Fibroblasts	↓	[84]
Nrf2	Fibroblasts	↓	[84]
Tfam	Fibroblasts	↓	[84]
Beclin-1	Fibroblasts	↓	[84]
OPA-1	Fibroblasts	↓	[84]
Apoliprotein A1	Blood plasma	↓	[85]
Apoliprotein A4	Blood plasma	↓	[85]
Apoliprotein B	Blood plasma	↓	[86]
Apoliprotein C1	Blood plasma	↓	[86]
Apoliprotein C3	Blood plasma	↓	[86]
Apoliprotein M	Blood plasma	↓	[86]

One potentially promising strategy is to search for mPTP-targeted anti-PD drugs. Thus, in a study [102], it has been demonstrated that cyclosporine A, which is a mitochondrial permeability transition pore blocker, inhibited the loss of nigral dopaminergic neurons and improved motor activity in Wistar rats in a model of PD induced by rotenone. Other antibiotics whose mechanism of action involves inhibition of mPTP are also potential therapeutic agents for the treatment of PD, as shown for minocycline [103] and cyclosporine [104].

One mechanism of pathogenesis in PD is mitochondrial dysfunction. Evidence of this is the high significance of mitochondrial homeostasis for the proper vitality of dopaminergic neurons. The occurrence of mitochondrial dysfunction leads to the generation of ROS that take part in the initiation of apoptosis, which results in neuronal death. Gene mutations of mitochondrial homeostasis proteins are considered one of the main causes of mitochondrial dysfunction. These proteins can be used as targets for drug development and act as biomarkers of PD. The use of biomimetic nanoparticles that stimulate mitochondrial biogenesis, which, as noted above, is reduced in PD, has great potential for the treatment of PD [105]. The search for new mitochondrial proteins that have not previously been studied as neuroprotectors is a potentially promising direction for creating drugs with improved properties against PD. Thus, intranasal administration of the mitochondrial peptide humanin led to the restoration of mitochondrial biogenesis, neuroprotection, and restoration of behavior in an animal model of PD [106]. One of the promising ways to restore mitochondrial function in PD is the use of healthy mitochondria transplantation methods [107]. However, it is too early to talk about the success of this strategy since there is data only from studies on cell cultures and mouse models.

8. Conclusions and Future Perspectives

Mitochondrial dysfunction is the multi-directional pathological process involving a large number of molecular "participants". On the one hand, this creates many prospects for the development of new drugs, and on the other, it can lead to confusion in understanding the main pathways leading to clinical manifestations of PD through the occurrence of mitochondrial dysfunction. Therefore, we focused on key mitochondrial homeostasis proteins, in which mutation variants lead to the loss or enhancement of their basic functions, which is one of the reasons for the progression of PD. Drugs targeting mutant proteins can prevent neuronal death, and accordingly, further progression of PD, which is not preventable using the symptomatic drugs that are taken currently. However, on the other hand, these therapeutic agents cannot guarantee a complete cure for PD. Firstly, even in the case of the monogenic nature of PD, different mutations of the same gene can differently affect the properties of the protein and the degree of affinity of the effector molecule to the ligand. Secondly, and more importantly, clinical manifestations of PD occur only after damage to 60% of dopaminergic neurons [3], which makes it impossible to restore motor functions fully. In this situation, predictive medicine is no less important in the fight against PD than traditional therapy—early diagnosis allows starting advanced treatment, which can slow down or even

prevent PD. In addition, identifying new genes responsible for the progression of PD can help for a better understanding of this disease pathogenesis and find new protein targets for drugs. The additional use of mitochondrial homeostasis proteins as PD biomarkers allows them to be considered as useful tools not only in therapy, but also in the diagnosis of PD. An extremely important step in the fight against PD is to study the impact of mitochondrial dysfunction at various stages of PD, which will allow scientists to more accurately determine how exactly mitochondrial dysfunction is associated with the clinical manifestations of PD in addition to neurodegeneration, as well as how mitochondrial dysfunction manifests itself at different stages of the disease. Thus, in the study [108], it was demonstrated that mitochondrial dysfunction was one of the drivers of the disease, occurring at an early stage of PD even before the deposition of alpha-synuclein. In addition, it was shown that mitochondrial dysfunction also progresses as the disease intensifies, as a result of which, in the late stages of PD, new molecular pathways and targets that were not disturbed earlier were affected—loss of activity of all ETC complexes, impairment of the activity of the pyruvate dehydrogenase complex and the tricarboxylic acid cycle, increased catabolism of fatty acids, and impaired mitochondrial division.

Author Contributions

AO, JU and VS designed the review plan. AB, AP and MP aquired and analysed data and wrote the manuscript. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript.

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