Recovery of Impaired Mitochondrial Dynamics and Quality Control Mechanisms in Parkinson’s Disease

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Parkinson’s Disease (PD) is a progressive neurological disorder characterized by tremors, rigidity, and slowness of movements. Most often observed in those aged 60 and above, the perniciousness of this disease lies not just in its global reach, affecting many people worldwide, but also in how no current effective disease-modifying therapy has yet to been established. While the pathophysiology of PD is multifactorial, mitochondrial dysfunction plays an established central role. Through efforts directed towards understanding mitochondrial dysfunction as a precipitating factor of parkinsonism, it has yielded valued insights into the mechanisms governing this disease. Nevertheless, the established importance of maintaining mitochondrial health through mitochondrial dynamics and quality control mechanisms in neuronal survival, and its pervasive dysregulation in PD, represents a key therapeutic target for future therapies. This review will serve to elucidate the pertinence of mitochondrial dysfunction as a therapeutic target in PD and discuss evolving approaches taken to address the recovery of such impairments.

Abbreviations: PD - Parkinson’s Disease; Mito-QC - Mitochondrial Quality Control; ROS - Reactive Oxygen Species; Drp1 - Dynamin-related protein 1; OMM - Outer Mitochondrial Membrane; ALP – Autophagy Lysosomal Pathway; iPSCs - Induced Pluripotent Stem Cells; PMD - Peptide Mediated Allogeneic Delivery; bNIRS - Broadband Near-Infrared Spectroscopy

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Introduction

While the precise mechanism of neuronal death in PD is still not understood, mitochondrial dysfunction has been proposed to be crucial in the progression of neurodegenerative diseases (Grimm & Eckert, 2017). Dysregulation in dynamic changes in the mitochondrial network and effective Mitochondrial Quality Control (Mito-QC) mechanisms therefore show a prominent causative link with the progression of such diseases, especially PD (Gao & Zhang, 2018; Guedes-Dias et al., 2016; McCoy & Cookson, 2012; Wang et al., 2019). This is further reinforced by the multifarious roles of the mitochondrion in cell homeostasis and health, not only because the ATP they synthesize powers cellular metabolic reactions but also in how they are crucial regulators of phospholipid transfer, calmodulin-mediated cell signalling, and apoptosis (Duchen, 2000; Jeong & Seol, 2008; Kojima et al., 2016). Consequently, the impairment of pathways which preserve mitochondrial integrity is severely deleterious, resulting in a series of toxic events that impact cell survival such as impaired ATP production and accumulation of reactive oxygen species (ROS). Coupled with the extreme metabolic burden experienced due to the high density of synapses and dopamine metabolism, dopaminergic neurons are critically vulnerable to oxidative stress arising from mitochondrial dysfunction in PD. Moreover, considering the pertinence of mitochondria to cellular homeostasis and the ubiquity of mitochondrial dysfunction in PD pathogenesis, as compared to a series of isolated pathogenic pathways, the pathophysiology of PD is likened more to a nexus of interconnected pathways and events.
This in turn highlights the potential for future emphasis on synergy between targeted therapeutic agents during clinical trials rather than measuring their performance in isolation.

Additionally, curated selection of early-onset participants, alongside stratification of the study cohort based on genetic and neuroimaging results are further examples of the evolution of PD clinical trials. Compounded with more accurate biomarkers for PD progression, the remedying effects of potential therapeutic agents can be more precisely observed. This review will elaborate ongoing therapeutic strategies targeting the impaired mechanisms governing mitochondrial dynamics and quality control, as well as how the conceptual hurdles surrounding PD can be overcome in the near future.

**Mitochondrial Dynamics**

The mitochondrial network in neuronal cells is one that exists in perpetual change, constantly breaking apart, translocating, and reforming the network (Roy et al., 2015). While intricate, the dynamism displayed in the mitochondrial network is stringent mediated by an increasing collection of established and novel molecular mediators to achieve balance. Mitochondrial fusion and fission occur simultaneously to remodel mitochondrial membranes and sequester damaged mitochondrial cargo, enabling clearance through processes like mitophagy, that are critical to preserving healthy mitochondrial function.

Mitochondrial fission involves the splitting of a single mitochondrion into daughter organelles, a process mediated by the action of dynamin-related protein 1 (Drp1). Drp1 initiates the formation of a constrictive ring around the organelle to sever the outer mitochondrial membrane (OMM). This serves to isolate regions of mitochondrial damage, by allowing one of the daughter organelles to remain functional, while the other is degraded through Mito-QC mechanisms (Youle & van der Bliek, 2012).

Conversely, mitochondrial fusion allows for the merger of two mitochondria into a single organelle such that dysfunctional mitochondria can complement one another and achieve enhanced oxidative capacity. The dysregulation of mitochondrial dynamics therefore poses a pertinent problem in preserving mitochondrial integrity and operations, with fragmentation of such networks being a crucial observation in PD, cementing mitochondrial dynamics as a key therapeutic target (Youle & van der Bliek, 2012).

Going beyond, mitochondrial motility is also subject to dysregulation in PD. This subsequently impairs the trafficking of mitochondria between cells, a mechanism that alleviates cellular stress through the clearance of such defective organelles in cells with dysregulated Mito-QC pathways or by supplementing cells with functional mitochondria (Shanmugapriya et al., 2020). Targeting impaired mitochondrial trafficking may be a worthwhile approach to achieve recovery of mitochondrial dynamics.

**Impaired Mitochondrial Dynamics**

Chief among the precipitating factors leading to impaired mitochondrial dynamics is the association with excess alpha-synuclein. As one of the most well-characterized aspects of PD pathology, alpha-synuclein, an aggregation-prone protein that forms intracellular Lewy-body inclusions (Lee et al., 2014), has been shown to play a crucial role in propagating mitochondrial dysfunction. This is facilitated by the ability of alpha-synuclein to interact with the OMM, which then subsequently leads to mitochondrial fragmentation. Mitochondrial fragmentation is mediated through upregulation of the mitochondrial fission machinery as a result of alterations in the biophysical properties of the OMM when associated with alpha-synuclein (Krzystek et al., 2021). The association of alpha-synuclein is presumed to upregulate the recruitment and translocation of Drp1 to the OMM (Youle & van der Bliek, 2012).
Mitochondrial Quality Control

Mito-QC mechanisms naturally worsen with age, as both oxidative capacity and ATP production are reduced in mitochondria over the years. (Capel et al., 2005; Conley et al., 2000; Santanasto et al., 2015). In order to maintain mitochondrial integrity, mechanisms such as the formation of mitochondrial-derived vesicles (MDVs) take place. MDVs bud off from the organelle, sequestering locally damaged mitochondrial components and trafficking them away for degradation in lysosomes, facilitating the clearance of damaged proteins and lipids, while ensuring the remainder of the organelle is functional (McLelland et al., 2014). The process is facilitated through the action of microtubules associated with Miro1/2 and the membrane cleavage via Drp1-dependent scission to produce an independent vesicle (König et al., 2021); Miro proteins serve as integral regulator proteins involved in mitochondrial homeostasis and mitophagy.

Furthermore, in response to significant oxidative stress, the mitochondrial autophagy pathway, termed as mitophagy, will take place (Yang & Klionsky, 2010). This occurs following mitochondrial fission, whereby the more defective of the two daughter organelles is degraded for the selective clearance of damaged components (Twig et al., 2008). Among the currently established stress-induced mitophagy pathways, PINK1 and Miro1/2 are known to play a prominent role in regulation; the former being a mitochondrial kinase involved in signalling pathways that control mitochondrial biogenesis and mitophagy. The localization of PINK1 on the OMM upon oxidative stress, initiates the recruitment of the E3 ubiquitin ligase Parkin (Narendra et al., 2010; Rüb et al., 2017). The ubiquitylation of OMM proteins then facilitates recognition by autophagy receptors on the autophagosomal membrane, mediating engulfment of the damaged mitochondrion by the autophagosome and its degradation via the autophagy lysosomal pathway (ALP). The purpose behind such pathways is to curb the expansion of mitochondrial damage to the rest of the cell and is commonly utilized only under extensive levels of cellular stress given the exorbitant energy consumed to conduct mitophagy and replace degraded mitochondria (Cadete et al., 2016).

Impaired Mitochondrial Quality Controls

In a similar situation as mitochondrial dynamics, the deleterious and cascading nature of alpha-synuclein is evident in quality control mechanisms as well. When exposed to exogenous alpha-synuclein, neuronal cell models displayed reduced Parkin expression levels alongside with sustained mitochondrial dysfunction (Wilkaniec et al., 2021). The impairment of mitophagy in response to excess alpha-synuclein is further established given the elimination of ubiquitylation of OMM protein and the decrease in mitochondria present in autophagosomes as a result of alpha-synuclein associations (Wilkaniec et al., 2021). The downregulation of Parkin is potentially mediated through the post-translational modification of Parkin which facilitates its autoubiquitylation and proteosome-mediated degradation (Wilkaniec et al., 2019).

Alternatively and independent of PINK1/Parkin activity, alpha-synuclein may affect mitophagy through direct interaction with Miro proteins which are critical components of the mitochondrial motility machinery (Shaltouki et al., 2018). Alpha-synuclein association with the OMM has a stabilising effect on the Miro protein or preventing its removal from the OMM (Shaltouki et al., 2018). This in turn impairs the initiation of mitophagy and dysregulates Mito-QC mechanisms in neuronal cells as Miro degradation is prevented (Hsieh et al., 2016).
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Figure 2. Dysregulation of Mito-QC mechanisms in response to excess alpha-synuclein, impeding clearance of unhealthy mitochondria and facilitating expansion of mitochondrial dysfunction.

In addition to excess alpha-synuclein, genetic causes such as mutations in the PINK1 and Parkin genes are known causative factors associated with mitochondrial dysfunction in early-onset PD (Ham et al., 2020). The PINK1/Parkin pathway governs the ubiquitylation of OMM proteins which in turn triggers mitophagy of unhealthy mitochondria (Ivankovic et al., 2016). Dysregulation in key regulators of mitophagy in turn leads to impaired Mito-QC mechanisms which impede removal of unhealthy mitochondria and propagates mitochondrial dysfunction.

Therapeutic Approaches

Inhibition of Drp1 Recruitment and Translocation

Given the established role of alpha-synuclein in facilitating Drp1 mitochondrial translocation, and the downstream dysregulation of mitochondrial dynamics, Drp1 recruitment represents a potential target for candidate drug therapies. Inhibition of Drp1 through the selective peptide inhibitor P110 was shown to eliminate mitochondrial translocation of Drp1 and attenuated dopaminergic neuronal loss in classical MPTP animal PD models (Filichia et al., 2016). With attenuated recruitment and translocation of Drp1 to the OMM upon alpha-synuclein associations, the selective peptide inhibitor reduces the extent of dysregulation in the mitochondrial network and reduces mitochondrial fragmentation.

Enhancement of Mitochondrial Biogenesis

A comprehensive process incorporating the synthesis of mitochondrial proteins and translocation of nuclear-encoded components, mitochondrial biogenesis represents a key precipitating factor of mitochondrial dysfunction, more so than impaired mitophagy (Chandra et al., 2019; Kumar et al., 2020). Following the upregulation of PARIS, PGCl-1α expression is attenuated (Ge et al., 2020; Kumar et al., 2020); the former a zinc finger protein involved in the neurodegeneration of PD and the latter a regulator protein which stimulates mitochondrial biogenesis. This subsequent downregulation is the driving force behind the impairment of mitochondrial biogenesis and therefore represents the most nascent therapeutic target in eliminating mitochondrial dysfunction (Ge et al., 2020; Kumar et al., 2020). Drugs that activate PGCl-1α such as polyphenolic phytochemical ferulic acid displayed beneficial effects on the recovery of mitochondrial dynamics in 6-OHDA lesioned rat PD models where dopaminergic and non-dopaminergic neurons were selectively destroyed to form the negative control;
reinforcing the importance of the mode of action of PGC-1α targeting drugs as a critical therapeutic strategy for research (Anis et al., 2020; Bennett and Keeney, 2020). Moreover, upregulation of PGC-1α through novel approaches such as action of phosphatidylinositol 3-kinase or exenatide have exhibited enhanced mitochondrial biogenesis and preserved mitochondrial health (Chandra et al., 2018, 2019; Fan et al., 2010; Athauda et al., 2017). Furthermore, the induction of mitochondrial biogenesis through D1 receptor agonists also exhibits beneficial effects on enhancing mitochondrial biogenesis on top of dopaminergic neurogenesis in animal PD models (Mishra et al., 2020). The diversity of approaches available in targeting mitochondrial biogenesis buttresses the necessity for future research into this domain to elucidate the mechanistic principles and subsequently translate them into clinical application.

**Upregulation of Mito-QC Mechanisms**

Mounting evidence points towards the cruciality of mitophagy and other Mito-QC mechanisms in preserving mitochondrial integrity, as well as their prevalent dysregulation in the pathophysiology of PD, pointing to the enhancement of such mechanisms as a key therapeutic strategy for PD (Aman et al., 2020). Agents such as celastrol display neuroprotective effects through the activation of mitophagy accompanied by the attenuation of dopaminergic neuronal cell loss in cellular models of PD (Lin et al., 2019). Dopaminergic drug screening platforms have also been derived through the use of Induced Pluripotent Stem Cells (iPSCs) from patients with PD arising from Parkin or PINK1 mutations. These neuronal cells harvested from PD patients served to cultivate cells lines against which novel therapeutic agents could then be trialled against, allowing for the identification of 4 candidate drugs efficacious in alleviating dysregulated Mito-QC mechanisms (Yamaguchi et al., 2020). Results from candidate drug screening using such platforms demonstrated the utility of this method as a future identifier of candidate PD drugs and as a barometer for their success (Yamaguchi et al., 2020).

Moreover, UDCA is particularly promising in regulating mitochondrial dysfunction through the upregulation of PINK1/Parkin and AMPK/mTOR pathways. Such medications facilitate downstream cascading effects in attenuating selective loss of dopaminergic neurons and dysregulation of mito-QC mechanisms (Qi et al., 2020).

**Protein-Based Therapies**

In line with the depletion of Parkin associated with early onset PD arising from the accumulation of alpha-synuclein or loss-of-function mutation in the PARK2 gene, protein-based therapies such as the cell-permeable Parkin protein iCP-Parkin have been interrogated as a potential mode of therapy in PD cells and animal PD models (Chung et al., 2020). iCP-Parkin in turn compensates for the downregulated parkin response in PD models, acting as a strategy to supplement parkin activity *in vivo*. A potential disease-modifying therapy, iCP-Parkin is shown to initiate the recovery of mitochondrial health through the upregulation of Mito-QC mechanisms and mitochondrial biogenesis. This in turn attenuated mitochondrial dysfunction and the subsequent accumulation of alpha-synuclein in cellular and animal-based models (Chung et al., 2020).

**Micro RNAs**

Micro RNAs (miRNAs) are non-coding, short RNA molecules synthesized for the targeted silencing of genes and regulation of gene expression through Dicer/RNA-Induced Silencing Complex mechanisms. With copious amounts of literature delineating the association of dysregulation in miRNAs in the pathophysiology of PD (Martinez and Peplow, 2017), a pattern emerges, with the majority of miRNAs associated with PD linked closely to mitochondrial dysfunction (Sun et al., 2019; John et al., 2020). MiRNA 181a/b displays the capacity to influence Mito-QC mechanisms, antioxidant production and mitochondrial biogenesis (Indrieri et al., 2019). The selective targeting miRNA therefore represents a key therapeutic strategy for global treatment of PD in neuronal cells, independent of gene involvement.

With greater research into the intricate mechanistic principles of miRNA and its genetic association with the pathophysiology of PD, there is nascent therapeutic potential in governing miRNA expression to correct the array of dysregulated mitochondrial pathways which culminate in mitochondrial dysfunction.
The recovery of mitochondrial health through miRNA regulation is proposed to influence not only the attenuation of neuronal cell loss but also recovery of mitochondrial functions in bioenergetics and in prevention of the accumulation of ROS (Indieri et al., 2020). miRNA may also be used in identifying and diagnosing PD as specific miRNA in the brains of PD patients such as miR34b/c are mutated even in prodromal stages (Minones-Moyano et al., 2011; Ravanidis et al., 2020; Cressatti et al., 2020).

Supplementing Unhealthy Mitochondrial Networks

Cellular mitochondrial networks may also be recovered through the provision of healthy mitochondria directly into the brain or through intravenous injections. The complementation of dysregulated mitochondrial networks in neuronal cells is a promising therapeutic strategy for PD (Shanmughapriya et al., 2020). Peptide-Mediated Allogeneic Mitochondrial Delivery (PMD) when trialled against PC12 and PD rat models, the positive and negative controls respectively, displayed recovery of mitochondrial function and, in animals, enhanced locomotive capacity. Simultaneously, further studies have evinced the capacity for intravenous mitochondrial injections to enhance the activity of oxidative phosphorylation in mitochondrial networks, thereby decreasing ROS levels and restraining apoptosis of dopaminergic neurons (Shi et al., 2017). Furthermore, CNM-Au8, a suspension of gold-based nanocrystals, shows promise in supplementing the energy needs in neuronal cells and attenuating oxidative stress (CNM-Au8, n.d.). This in turn enhances nerve cell survival and subdues the progression of PD in cells plagued with mitochondrial dysfunction.

Therapeutic Usage of Induced Pluripotent Stem Cells

As a means of avoiding immune rejection, astrocytes differentiated from human iPSCs may facilitate the donation of healthy mitochondria in a sustained fashion (Cheng et al., 2020). A form of intercellular mitochondrial translocation, such therapeutic strategies allowed for the recovery of dopaminergic neurons and elimination of targeted cell death in in vitro PD models (Cheng et al., 2020).

Prodromal Biomarkers of Mitochondrial Dysfunction

Prodromal mitochondrial dysfunction can be identified through in-vivo assessment such as neuroimaging methods and blood biomarkers. Magnetic Resonance Spectroscopy Imaging (MRSI), a specific neuroimaging platform, provides a non-invasive approach in assessing mitochondrial bioenergetics and aspects of mitochondrial functions. Mediators such as phosphorous-MRSI (P-MRSI) can be used in conjunction to determine ATP levels among metabolites (Forestor et al., 2010). Furthermore, sustained improvements in the neuroimaging approach through optimization of MRI sequence or advancements in hardware will expand the potential of MRSI as a tool for research (Henchcliffe et al., 2008). Alternatives to MRI-based methods include broadband near-infrared spectroscopy (bNIRS), which is utilized to assess the oxidative status of cytochrome c through multiwavelength analysis beyond the standard range of optical microscopes. The usefulness of such approaches remains limited however, due to their commercial unavailability and specific hardware requirements (Dossi et al., 2019).

Blood-based analysis also shows promise in the detection of mitochondrial dysfunction, given the established position of mtDNA, glutathione-coupled mediators, malondialdehyde and lactate as biomarkers of mitochondrial dysfunction (McClintock et al., 2022). However, this would likely fail to indicate the selective death of dopaminergic neurons, given the relatively petite size of the substantia nigra to the human blood volume, unless significant problems in mitochondrial function are observed (Davis et al., 2020). Prodromal analysis of study cohorts will allow for the determination of participants expressing specific mitochondrial impairments in the mechanism targeted by therapies. Given the multi-faceted nature of the aspects governing mitochondrial dysfunction, selective stratification of participants for clinical trials will be necessary to address therapeutic targets and yield laudable results.
Discussion

The centralized role of mitochondria in the pathogenesis of PD cannot be understated given their far-reaching influences on the homeostatic control of neuronal cells. The dysregulation of mitochondrial dynamics and quality control pathways therefore represent a critical target for physical and virtual screenings as their recovery will unequivocally have an impact on disease progression. This paper delves into both the salience of uncovering the mechanisms which govern the progression of PD and how such mechanistic principles are transformed into practical therapeutic agents. Perhaps as a testament to their entrenched role, the interconnected and highly delicate homeostatic pathways driving mitochondrial dysfunction continue to impede the efforts of therapeutic strategies which are unable to address the multi-faceted nature of the problem, as highlighted by this review. With dysregulations in mitochondrial dynamics and QC being only a fraction of the biological mechanisms underpinning mitochondrial dysfunction, much less the pathophysiology of PD as a whole, a one-size-fits-all panacea to this malignant disease is unlikely to be a practical solution. In this vein, the future of clinical trials against PD will likely depend on a combinatorial cocktail of drugs catered to the varied dysregulated pathways of the individual patient (Titova and Chaudhuri, 2017). As such, rather than separately discussed and trialled, the focus of PD studies should be oriented towards synergism between existing and novel therapeutic agents to tackle the wide scope of causative factors. Moreover, ensuring that results of such therapeutic approaches are observable will require enrichment and stratification of study cohorts by incorporating prodromal biomarkers as a pre-requisite for participants in clinical trials. Participants who showcase significant dysfunction in these respective mechanisms must confirm the mechanistic principles undergirding targeted treatment approaches (Redensek et al., 2017).

Conclusion

This review paper evinces key therapeutic targets in ameliorating the progression Parkinson’s disease in patients, specifically regarding the precipitating factors of mitochondrial dysfunction which propagates the pathophysiology of the disease. Moreover, through stratifying study cohorts and adjusting the manner in which drug trials are conducted in future studies, the multi-faceted nature of Parkinson’s disease may be better addressed; yielding more promising disease-modifying treatments in the process.

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