Precision Neurology for Parkinson's Disease: Coupling Miro1-Based Diagnosis with Drug Discovery

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ABSTRACT: Parkinson's disease (PD) is a debilitating movement disorder, significantly afflicting the aging population. Efforts to develop an effective treatment have been challenged by the lack of understanding of the pathological mechanisms underlying neurodegeneration. We have shown that Miro1, an outer mitochondrial membrane protein, situates at the intersection of the complex genetic and functional network of PD. Removing Miro1 from the surface of damaged mitochondria is a prerequisite for mitochondrial clearance via mitophagy. Parkinson's proteins PINK1, Parkin, and LRRK2 are the molecular helpers to remove Miro1 from dysfunctional mitochondria destined for mitophagy. We have found a delay in clearing Miro1 and initiating mitophagy in postmortem brains and induced pluripotent stem cell–derived neurons from PD patients harboring mutations in *LRRK2*, *PINK1*, or *Parkin*, or from sporadic PD patients with no known mutations. In addition, we have shown that reducing Miro1 by both genetic and pharmacological approaches can correct this Miro1 phenotype and rescue Parkinson's-relevant phenotypes in human neurons and fly PD models. These results suggest that the Miro1 defect may be a common denominator for PD, and compounds that reduce Miro1 promise a new class of drugs to battle PD. We propose to couple this Miro1 phenotype with Miro1-based drug discovery in future therapeutic studies, which could significantly improve the success of clinical trials. © 2020 International Parkinson and Movement Disorder Society

Key Words: clinical trials; Miro1; mitochondria; mitophagy; Parkinson's disease; precision

Our world is experiencing an imbalanced demographic surge. With an aging population comes an increasing danger of late-onset neurodegenerative diseases to public health nationwide. Parkinson's disease (PD) is one of these disorders and a leading cause of disability that afflicts the elderly. PD is characterized by a selective loss of dopaminergic neurons in the substantia nigra. Developing an effective treatment for PD is impeded by the lack of understanding of core cellular mechanisms underlying neurodegeneration. Furthermore, the presence of sporadic cases makes it difficult to pinpoint a common target. As a result, there is no reliable biomarker or pharmacodynamic marker to aid in diagnosis and to monitor the response of possible disease-modifying medication.[1,2] These challenges stagnate the bench-to-bedside transition of experimental therapies.

Mitochondria have become a central theme for PD. They are highly dynamic organelles and frequently change shape and location in most cell types. The polarized structure and energetic need of a postmitotic neuron demand the proper transport of mitochondria within the extensions. When mitochondria are damaged, it is crucial for neurons to rapidly repair or clear them to prevent the devastating conseuquence from oxidative stress. The outer mitochondrial membrane (OMM) protein Miro1 is required both for maintaining mitochondrial motility and initiating mitophagy, a mitochondria-specific autophagy process that degrades the entire damaged mitochondria in lysosomes. Our recent work has shed light on Miro1's potential to act

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both as a molecular signature and as a drug target for PD. In this article, we will summarize our work on Miro1 and present our perspective on redefining the disease and reinnovating personalized treatment strategies for patients.

Miro1 Protein: A Linchpin for Mitochondrial Motility and Quality

Neurons demand mitochondria for a large amount of energy supply, a need that is constantly changing among each microdomain of a neuron in response to the fast fluctuation in neuronal activity. The unique architecture of neurons, which often contain extensive dendritic arborization and long axons, requires precise control of mitochondrial transport within this complex system. When mitochondria left at the terminals are damaged, they must either fuse with healthy mitochondria to fix the damage,[3] return to the soma via retrograde transport for disposal,[4,5] or be removed at the terminals.[4,6,7] Inefficient clearance of damaged mitochondria can cause oxidative stress to the neuron. However, the geometrical and energetical features of a neuron make it challenging for mitochondria to navigate and undergo self-destruction.

Miro plays a crucial role for maintaining both mitochondrial motility and quality. Miro is an atypical mitochondrial Rho GTPase (RhoT1/2). Mammals have 2 Miro paralogues (Miro1 and Miro2), whereas *Drosophila* has 1 orthologue (DMiro), which is highly conserved to human Miro1.[8] Miro has a carboxy-terminus transmembrane domain that is anchored to the OMM. Miro binds to milton (TRAKs), which in turn binds to kinesin heavy chain (KHC), and this Miro/milton/KHC complex recruits mitochondria to microtubules for anterograde transport[9,10] (Fig. 1A). When either Miro or milton is ablated in animal models, mitochondria are trapped in the soma and fail to enter the axons.[4,9-12] Miro has also been reported to directly bind to the retrograde microtubule motor dynein and the actin motor myosin XIX.[13-15] In contrast to the long-range mitochondrial movement in axons enabled by microtubules, actin-mediated mitochondrial movement allows instantaneous short-range redistribution of mitochondria, frequently observed in actin-enriched growth cones, dendritic spines, and synaptic boutons. Miro also contains a pair of EF-hands that bind to Ca^{2+} (Fig. 1B) and 2 GTPase domains. These functional activities of Miro allow upstream signaling pathways and messengers to influence mitochondrial motility via Miro.[9,16-18]

The Miro-dependent mechanisms underlying mitochondrial movement are applied to only healthy, functional mitochondria. Miro is also essential for clearing unhealthy, severely damaged mitochondria, but in an entirely different manner. Mitochondrial damages caused by various factors during disease and aging depolarize the proton potential across the mitochondrial inner membranes. Mitochondrial depolarization triggers the accumulation of the kinase PINK1 on the OMM. There, PINK1 phosphorylates multiple OMM proteins including Miro and Mitofusin,[4,19-21] and ubiquitin molecules that are on or adjacent to the OMM.[22-24] Phosphorylation of ubiquitin and OMM proteins[20] continues to activate and recruit the cytosolic E3 ligase Parkin to the surface of mitochondria,[25,26] which ubiquitinates Miro along with additional OMM proteins. Miro is subsequently removed from the OMM and sent to proteasomes for degradation (Fig. 2). The removal of Miro from the surface of damaged mitochondria results in the halting of their movement.[4,6] In addition to PINK1 and Parkin, we have shown that LRRK2, an enzyme with both GTPase and kinase activities, plays a role in removing Miro from damaged mitochondria.[7] Mitochondrial depolarization recruits cytosolic LRRK2 to mitochondria and initiates an interaction between Miro and LRRK2. This interaction is essential for the following Miro removal (Fig. 2).

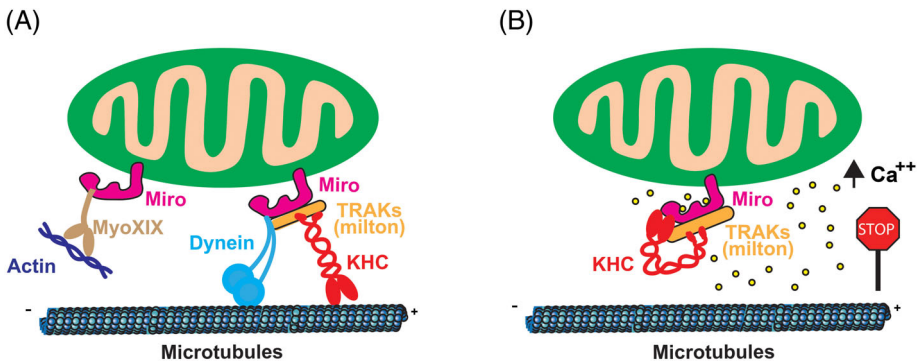

FIG. 1. Schematic representation of Miro-dependent mitochondrial transport. (A) Mitochondria move along microtubules either anterograde by means of KHC (red) or retrograde by dynein (blue). These motors recognize mitochondria by attaching to Miro on the OMM. Movement of mitochondria along actin filaments is mediated by Myosin XIX and Miro. (B) Ca^{2+}-dependent regulation of mitochondrial motility via Miro. The two pockets in Miro represent 2 EF-hands. The yellow dots represent Ca^{2+}. [Color figure can be viewed at wileyonlinelibrary.com]

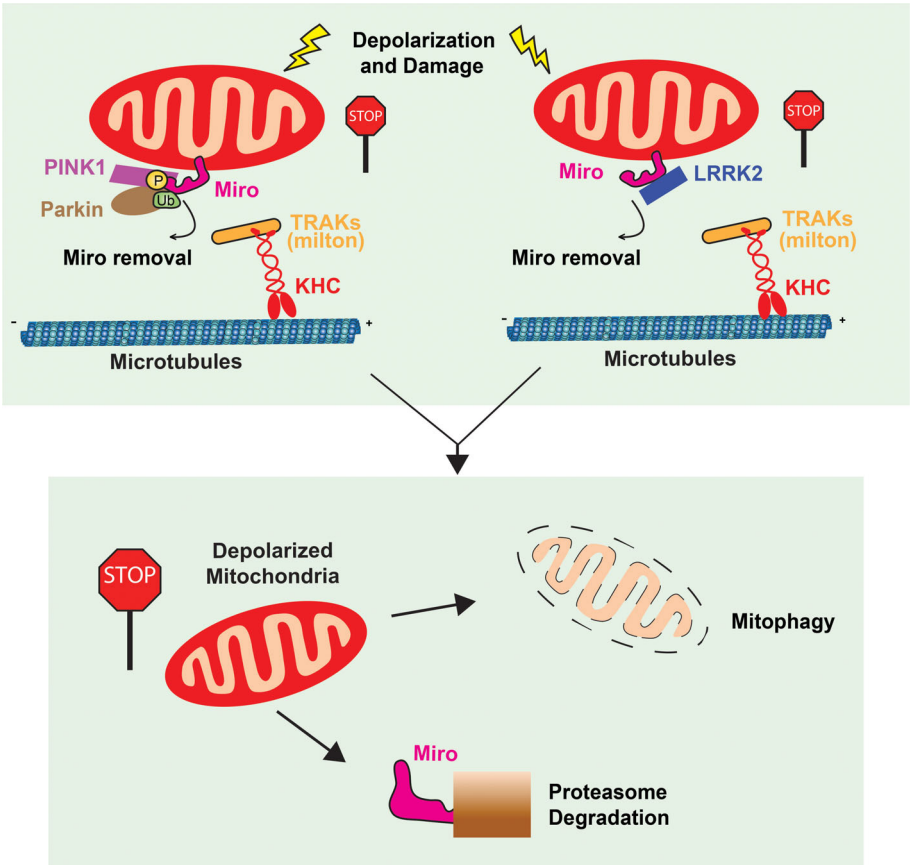

FIG. 2. Schematic representation of Miro-dependent mitochondrial quality control. Mitochondrial depolarization triggers 2 parallel pathways — PINK1/Parkin and LRRK2. Both pathways are required to remove Miro from damaged mitochondria to arrest mitochondrial motility and to permit mitophagy that follows. [Color figure can be viewed at wileyonlinelibrary.com]

Our data suggests that LRRK2 functions in parallel with the PINK1/Parkin pathway, and the kinase activity of LRRK2 is dispensable for this particular function. Collectively, arresting dysfunctional mitochondria by removing Miro is an essential first step in segregating mitochondrial damages from the rest of the healthy mitochondrial network and in quarantining these mitochondria for the following mitophagy.

The Miro1 Defect: A Surprising Molecular Denominator for PD

Mutations in *LRRK2*, *PINK1*, or *Parkin* cause familial PD.[27-30] An intriguing hypothesis is that in individuals harboring mutations in these genes, a failure to isolate and remove the damaged mitochondria likely contributes to neuronal cell death in PD. Notably, the majority of PD cases are sporadic without an inheritance pattern. An overarching question we have asked is whether a delay in removing Miro and clearing damaged mitochondria could be a common theme in both familial and sporadic PD. With this question, we have conducted extensive research in multiple PD models including postmortem brains, skin fibroblasts, induced pluripotent stemcell-derived neurons from PD patients, and fly PD models. We included PD patients harboring mutations not only in *LRRK2*, *PINK1*, or *Parkin*, but also in *GBA* or *SNCA*, and sporadic PD patients with no known mutations. By combining live-imaging methods, biochemistry, and genetic approaches, we have shown a unifying cellular defect in removing Miro1 from damaged mitochondria in neurons and skin cells derived from PD patients. Miro1 remains on damaged mitochondria for longer than normal, prolonging active transport and delaying the onset of mitophagy. This defect renders neurons vulnerable to mitochondrial stress, leading to neurodegeneration (Fig. 3).[7,31,32] Importantly, this molecular defect of Miro1 occurs not only in *PINK1/Parkin/LRRK2*-related PD cases, but also in familial PD patients harboring mutations in other genes including *GBA* and *SNCA* and even in idiopathic cases in which no known mutations are found (Fig. 3).

Our discovery is surprising in 2 ways. First, the failure to remove Miro1 from damaged mitochondria only exists in nonneuronal cells. We have established sensitive assays to measure Miro1 response to mitochondrial depolarization in skin fibroblasts from a total

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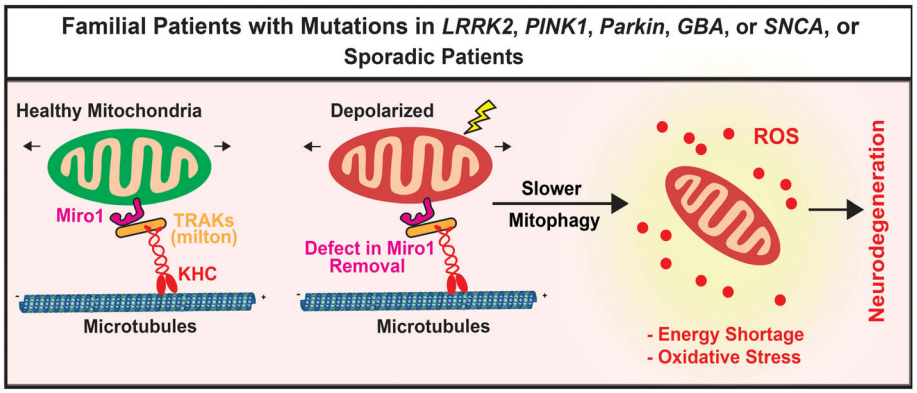

FIG. 3. A converging defect in removing Miro1 from damaged mitochondria in genetically distinct patients. The impairment in removing Miro1 from damaged mitochondria prolongs mitochondrial movement and delays mitophagy that follows, causing an energy shortage and oxidative stress. [Color figure can be viewed at wileyonlinelibrary.com]

of 83 PD patients and discovered that 94% of them show impairment in removing Miro1 from damaged mitochondria.[31] The convenience of acquiring skin cells from PD patients by a simple biopsy opens the door to novel biomarker research on Miro1. Our current detection methods only yield a "yes" or "no" answer to the Miro1 defect at a single time following mitochondrial depolarization. An exciting future direction is to establish a range of severity of this Miro1 phenotype and to correlate it with the symptomatic severity of patients.

Our result also raises questions about whether PD is a systemic disease. In fact, mitochondrial molecular defects have been widely reported in fibroblasts from PD patients,[33-37] but we have not observed skin cell death in PD patients. Why are dopaminergic neurons in the substantia nigra most vulnerable to the impairment of mitochondria? The answers lie in many layers, but all surround the unique features of these midbrain neurons.[38] They synthesize dopamine, which can be toxic to neurons under certain conditions. The axonal arborization is extensive and intertwined,[38,39] which poses a tough task for mitochondria and other organelles to travel. In addition, dopaminergic neurons in the substantia nigra are pacemaking, fast-firing neurons with high Ca^{2+} influx. This peculiar environment demands mitochondria to perform high respiratory activities and meanwhile exposes them to numerous toxins and stressors. It has been shown that mitochondria in dopaminergic neurons of the substantia nigra compacta are more energetic and under more oxidative stress than those of the ventral tegmental area cultured from mice.[39] It is possible that mitochondria in substantia nigra dopaminergic neurons are more prone to damage compared with those in neurons and non-neuronal cells elsewhere. Although healthy dopaminergic neurons have an effective quality control system to eliminate any dysfunctional mitochondrion quickly to prevent oxidative stress, neurons with molecular defects in mitophagy, such as those with mutations in *PINK1*, *Parkin*, or *LRRK2*, may be slow in clearing those damaged mitochondria over a long period of a human's life, which eventually contributes to the late-onset neurodegeneration.

The second surprise from our work is the mysterious Miro1 phenotype in idiopathic PD cells. Although we have provided molecular mechanisms linking pathogenic PINK1, Parkin, LRRK2, and α-synuclein to the removal of Miro1,[4,7,31,32,40] we cannot explain the same Miro1 phenotype in PD patients without any known mutations. Because we have observed the Miro1 defect in cultured fibroblasts, there could be genetic or molecular signatures preserved in those cells that affect Miro1. If these signatures are imprinted in the genome, it suggests the prevalence of PD cases caused by genetic mutations is much higher than expected. Furthermore, human genetic approaches have failed to detect a prominent genetic risk in sporadic patients, indicating even if those mutations exist, they must be extremely rare and variable among individuals.

The convergence on Miro1 in genetically and clinically distinct PD patients makes us rethink the name "Parkinson's disease." Since the 19th century, this name has been used to describe a class of patients solely based on their symptoms. However, patients with many other diseases such as progressive supranuclear palsy, multiple system atrophy, and corticobasal degeneration share similar clinical symptoms, especially in the earlier stages, which possibly contributes to the high rate of misdiagnosis of PD.[2] In our cohort, we have discovered that 6% of the PD patients are free of the Miro1 phenotype. It is unclear whether those patients are misdiagnosed as PD or if they just do not have the Miro1 defect. Redefining the disease based on the molecular underpinnings in individual patients would be timely and beneficial for designing future disease-modifying therapies.

Precision PD Neurology: Coupling the Miro1 Defect With the Miro1-Targeted Treatment

A common molecular theme in different types of PD suggests that there might be a mutual method to fight the disease. We have shown that reducing Miro1 by both genetic and pharmacological approaches can correct this Miro1 phenotype and rescue PD-relevant phenotypes in human neuron and fly PD models.[7,31,32] Combining the artificial intelligence technology in silico and functional screening in fruit flies in vivo, we have selected potential Miro1-binders that reduce Miro protein in flies. Treating fibroblasts from PD patients with the top compound eliminates the Miro1 molecular pathology, and treating PD flies and patient-derived neurons with the compound rescues their locomotor deficits and dopaminergic neurodegeneration.[31] These results demonstrate the clinical utility of the Miro1 phenotype in skin cells as a pharmacodynamic maker and the therapeutic promise of Miro1 reducers. We propose a strategy to couple the Miro1-based therapy with a Miro1-dependent companion diagnostic tool (Fig. 4). In future clinical trials, we could first determine whether a potential PD patient has the Miro1 phenotype using his or her cultured fibroblasts in a dish — if "yes," then use those fibroblasts to determine whether a particular drug can ameliorate the Miro1 defect, and if "yes," finally administer this compound to the patient. The strategy could significantly improve the success of future clinical trials because without a marker to stratify the right patients and monitor the drug effects, the studies would be essentially "blind."

It is important to note that both our genetic and pharmacological approaches (therapeutic dose) in human cell and fly PD models only cause a nominal reduction in basal Miro levels without significantly affecting the motility of healthy, polarized mitochondria.[7,31,32] This small reduction of basal Miro1 levels can assist Miro1 removal from unhealthy, depolarized mitochondria.[7,31,32] Because the depolarization-initiated mitophagy machinery that removes Miro1, including PINK1, Parkin, and LRRK2, is dormant in the basal condition and is only activated on depolarized mitochondria, our compounds at the therapeutic dose may also work in concert with the mitophagy molecular players upon depolarization to facilitate Miro1 degradation in proteasomes. Notably, we have shown that the Miro1-targeted approaches do not cause cell death of neurons from healthy subjects, but mitigate the sensitivity to stress of patient-derived neurons.[7,31,32] This information is useful to guide future drug discovery studies for how to minimize cytotoxicity. An ideal therapeutic dose for a lead compound should significantly reduce Miro1 following mitochondrial depolarization, but only subtly influence Miro1 protein at baseline.

PD is a multifactorial disease, and the Miro1 defect is unlikely to be the only causal factor. It is known that the penetrance of several PD-causing genes is incomplete, suggesting that there is more than one cause for each individual patient. The additional contributing components may include an environmental element, a second mutation, an immune response, or another disease. Therefore, the complex nature of PD welcomes a precision neurology treatment (Fig. 5). Each individual patient may need more than 1 therapy. The overall treatment strategy should be different among different patients because their clinical symptoms and genetic

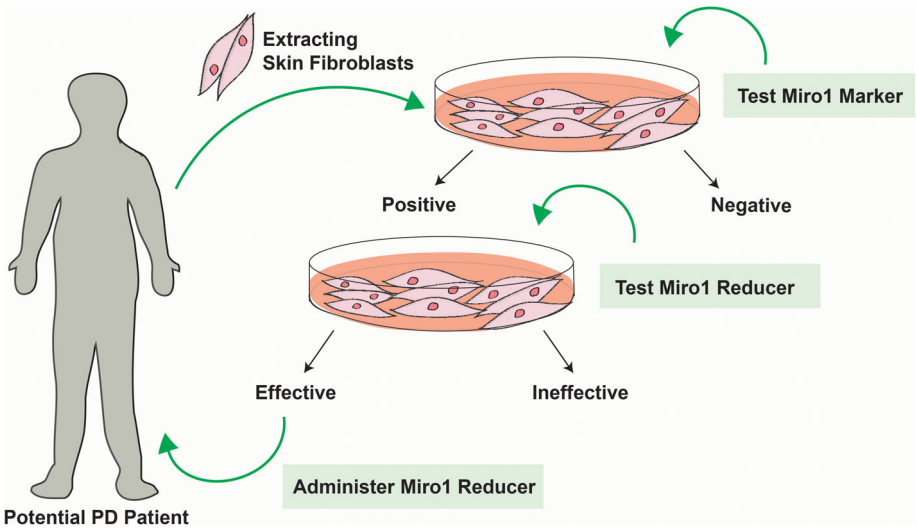

FIG. 4. Coupling Miro1-based therapy with Miro1-dependent companion diagnostic tool. In future clinical trials, we could first determine whether a potential PD patient has the Miro1 phenotype using his or her cultured fibroblasts in a dish — if "yes," then use those fibroblasts to determine whether a compound can ameliorate the Miro1 defect, and if "yes," finally administer this compound to the patient. [Color figure can be viewed at wileyonlinelibrary.com]

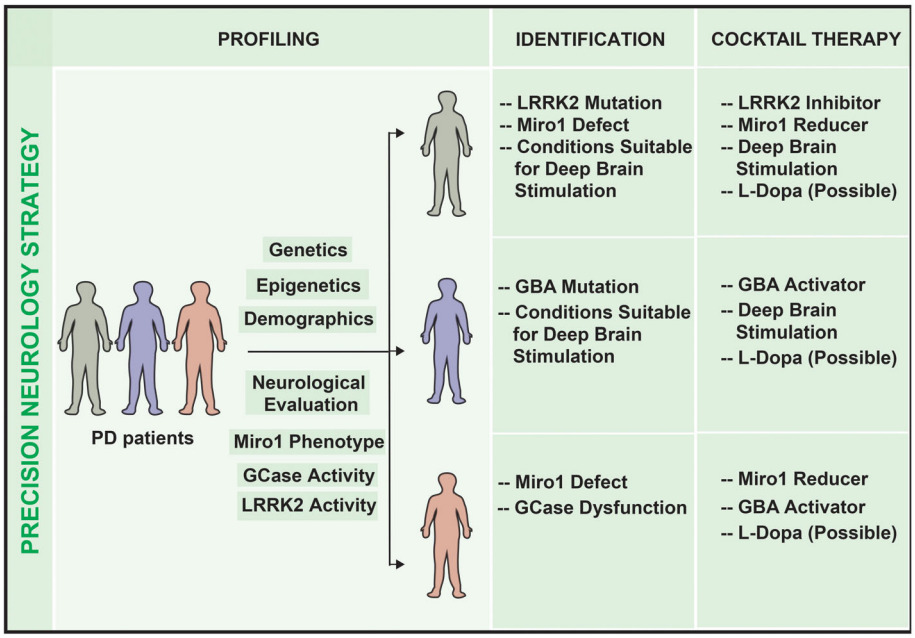

FIG. 5. Examples of future precision neurology for PD. Multiple aspects of a patient will be evaluated, and based on the profiling information, an individualized treatment plan will be generated for each patient. [Color figure can be viewed at wileyonlinelibrary.com]

compositions are not the same. For example, if a patient with genetic mutations in *LRRK2* or *GBA* also shows a Miro1 protein phenotype, a cocktail medication containing Miro1 reducers and compounds that target LRRK2 or GBA may work best. Additional management, such as physical therapies and deep brain stimulation, and traditional symptom-relieving therapies such as L-dopa should be considered part of the personalized plan as well.

We have tested the Miro1 marker in fibroblasts from 5 asymptomatic genetic carriers, and they all show the failure to degrade Miro1 following mitochondrial depolarization.[31] This observation suggests that we could use our methods to detect the presymptomatic population that will benefit most from early therapeutic intervention. We could administer Miro1-targeted compounds to individuals at risk for PD who test positive for the Miro1 marker, even before the symptoms occur, and this treatment may postpone or even prevent the onset of the disease. For late-stage patients, Miro1 reducers still have a good chance of stopping or slowing disease progression by rescuing the remaining neurons from dying.

Closing Remarks

Miro is a key molecular player to guide mitochondrial movement and degradation and to synergize cellular signaling pathways. The importance of Miro is magnified in neurons, as the need for mitochondria is regulated on a spatial and temporal scale to meet the constantly changing demands for energy and Ca^{2+} buffering in each part of a neuron. It is perhaps not surprising that Miro protein has been found to be misregulated in multiple types of PD, a late-onset neurodegenerative disease closely linked to mitochondrial dysfunction. Despite how much we have learned about Miro in health and disease, many questions remain to be answered, and a lot more work is warranted to be done. The urgency of finding a cure for PD prioritizes fast-tracking the translational research on Miro1 from laboratories to patients in hospitals, an effort that requires academic, industrial, and clinical collaboration. However, understanding the basic regulations of Miro in a normal cell and how the mechanisms are impaired in disease is equally paramount, which lays the foundation for any translational research. The increasing appreciation of Miro's importance in neuronal physiology and pathophysiology opens new frontiers for us to battle PD, the agonizing movement disorder that deeply burdens our society and afflicts our everyday life.

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Author Contributions

V.B. and X.W. wrote the article. V.B. made the figures.

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