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Parkinson's Disease: From Molecular Pathways in Disease to Therapeutic Approaches

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Abstract

Parkinson's disease (PD) is a complex multifactor disease marked by extensive neuropathology in the brain with selective yet prominent and progressive loss of midbrain dopamine neurons. Clinically PD is characterized by motor abnormalities including resting tremor, bradykinesia, altered gait, muscular rigidity, postural instability, together with autonomic dysfunctions. The etiological factors involved in the development of PD are still elusive, but there is considerable evidence that a combination of genetic susceptibilities and environmental factors plays a critical role in disease pathogenesis. The identification of single genes in the past decade linked to heritable forms of PD has challenged the previously held view of a nongenetic etiology for this progressive movement disorder. These genetic breakthroughs have revolutionized the research by providing unique opportunities to pursue novel mechanisms and identified new clues to disease pathogenesis in PD. This forum review provides an update on current hypotheses and recent findings in mitochondrial dysfunction, oxidative damage, innate and adaptive immune systems, protein misfolding and aggregation, and advances in translational approaches to PD. These aspects are reviewed with an aim to promote better understanding of molecular pathways prevalent in parkinsonian brain that will eventually aide in development of promising therapeutic strategies. *Antioxid. Redox Signal.* 11, 2077–2082.

Introduction

EVEN AFTER FOR ALMOST TWO CENTURIES since its first description by James Parkinson, Parkinson's disease (PD) remains an idiopathic disorder without cure and with limited symptomatic treatment. PD is the second most common neurodegenerative disorder that affects millions of elderly population worldwide. Clinically, most patients present with a motor dysfunction and suffer from slowness of movement, rest tremor, rigidity, disturbances in balance, in addition to autonomic dysfunctions and psychiatric problems. The pathological hallmark of PD which gives rise to constellation of clinical syndromes is the loss of neuromelanin-containing dopamine producing neurons within the substantia nigra pars compacta (SNpc). Loss of SNpc dopamine neurons results in a concomitant depletion of dopamine (DA) in the striatum, impairing the nigrostriatal system to prevent the execution of coordinated movements. Neuronal degeneration is widespread in the brain with SNpc getting involved only towards the middle stages of the disease and is also accompanied by the presence of fibrillary cytoplasmic inclusions, known as Lewy bodies, which contain ubiquitin and α-synuclein (17). Despite intensive research, the etiology of PD remains poorly understood, leaving with no effective neuroprotective and neurorestorative therapies. Current therapies are palliative at best by providing effective control of symptoms, particularly in the early stages of the disease. Clearly, the current symptomatic therapies cannot completely ameliorate later-stage symptoms, nor can they address the ongoing degeneration in the dopaminergic and nondopaminergic systems. This indeed emphasizes the urgency of developing more effective treatment modalities for PD patients. For this reason, a great deal of current research has focused on finding the cause of dopaminergic cell loss and on exploring protective, restorative, and replacement therapies. Although the etiology of PD remains incompletely understood, a broad range of studies conducted over the past several decades, including genetic analyses, epidemiologic studies, neuropathologic investigations in postmortem human samples, and experimental models of PD have provided us with several new clues to disease pathogenesis (1). This present forum discusses some popular theories that have emerged from these studies in an effort to better understand molecular pathways to disease and utilize this knowledge to develop more efficient interventional strategies.

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Pathways to Disease and Therapeutic Approaches

Epidemiological studies have identified age as the greatest risk factor for PD, and mitochondria through accumulation of mitochondrial DNA (mtDNA) mutations and production of reactive oxygen species is thought to contribute towards this process. Direct evidence for defects in mitochondrial function comes from studies of autopsy tissues from PD patients. A significant reduction in the activity of mitochondrial complex I, a defective assembly process of its protein subunits, and increased oxidative damage to these protein subunits are reported in substantia nigra and other brain regions (1, 20). There is also evidence from multiple independent studies of a systemic reduction in complex I activity in blood platelets and muscle biopsies. Mitochondrial DNA encoded defects have been demonstrated where complex I defects from PD platelets are transferable into mitochondrial deficient cell lines known as "cybrids" (1, 17, 20). A major question that arises is whether impaired complex I activity represents a primary defect contributing to PD pathogenesis or whether it is secondary to disease, or due to related issues, such as medication. The former seems to be true since complex I activity does not correlate with levodopa dosage, and is normal in other neurodegenerative diseases, such as multiple system atrophy, suggesting that it is not a nonspecific consequence of neurodegeneration (2, 18). The complex I deficiency in multiple regions suggests a systemic defect, at least in some cases, but it does not help differentiate acquired and genetic causes. There is also genetic evidence that abnormalities in mtDNA may contribute to PD pathogenesis. These involve the point mutation in mitochondrial 12SrRNA found in a pedigree with Parkinsonism, deafness and neuropathy, a marked age-dependent increase in mtDNA deletions in laser-captured dopaminergic neurons that are clonally expanded and associated with respiratory chain deficiencies (1). Additionally, many of the familial PD-linked genes also implicate mitochondria in disease pathogenesis. So far, mutations or polymorphisms in mtDNA and at least nine named nuclear genes have been identified in causing PD or affecting the risk of PD. These include *α-synuclein*, *parkin*, ubiquitin carboxy-terminal hydrolase L1, *DJ-1*, phosphatase and tensin homologue (PTEN)-induced kinase 1 (*PINK1*), leucine-rich-repeat kinase 2 (*LRRK2*), the nuclear receptor *NURR1*, *HTRA2,* and tau. Of the nuclear genes, *α-synuclein*, *parkin*, *DJ-1*, *PINK1*, *LRRK2,* and *HtrA2* either directly or indirectly link their pathogenic roles with mitochondrial dysfunction. Gene duplications and pathogenic mutations in *α-synuclein* are associated with rare forms of autosomal dominant PD. Biochemical and electron microscopic studies from human postmortem sporadic PD and mice overexpressing human A53T *α-synuclein* show increased mitochondrial accumulation of human *α-synuclein* (1). In sporadic PD there is evidence of association of *α-synuclein* with mitochondrial complex I, leading to its impaired activity. Transgenic mice overexpressing mutant *α-synuclein* develop significant mtDNA damage, impaired cytochrome oxidase (complex IV) activity, and show increased susceptibility to neurodegeneration induced by mitochondrial toxins (17). Both gene knockouts of *parkin* mouse and flies and overexpression of mutant *parkin* in flies and cells show mitochondrial abnormalities. Multiple independent studies have shown a direct link between *parkin* and its role in mitochondrial function, especially its ability to interact with mitochondrial transcription factor A (Tfam) to enhance mitochondrial biogenesis. This is significant since a conditional knockout of Tfam cause progressive loss of nigrostriatal dopaminergic neurons in mice (2, 17). *Parkin* also plays a role in maintaining mitochondrial homeostasis through targeting damaged mitochondria for mitophagy (13). Mitochondrial localization of PD associated gene *DJ-1* and its ability to impact mitochondrial functions by modulating oxidative damage induced by mitochondrial toxins further enforces the significance of mitochondria in PD (1, 20). Furthermore, *PINK1*, a mitochondrial kinase either due to loss of function or pathogenic mutations results in mitochondrial dysfunction and neurodegeneration due to abnormalities in mitochondrial morphology, bioenergetics, and by modulating cytochrome c release to impact apoptosis in numerous *in vivo* and cell culture models including patient cell lines (1, 20). Recent identification of PD cases due to loss of function mutations observed in the mitochondrial serine protease *HtrA2* and its functional role in the regulation of apoptotic pathways by interacting with inhibitory apoptotic proteins and *PINK1* further strengthens the importance of mitochondria in disease development. *Omi/HtrA2* knockout mice have a strong parkinsonian phenotype including rigidity, tremor, and striatal damage. Although a direct role of *LRRK2* mutations associated with PD and its role in mitochondrial dysfunction are yet to be established, association of a small fraction of *LRRK2* with mitochondria is suggestive of its role in mediating mitochondrial functions that may be key to disease development (1, 20).

Thus, multiple lines of studies suggest a pathogenic role of familial PD linked mutations in compromising normal mitochondrial functions in PD pathogenesis. Evidence for an abnormality in mitochondrial dynamics has also been strongly associated and is continuously accumulating in PD. Mitochondria are extremely dynamic organelles and are undergoing continual fusion and fission. A coordinated balance between fusion and fission not only determines the morphology of mitochondria in cells but also has a significant effect on mitochondrial functions. The unique characteristics of neurons that degenerate in PD may predispose midbrain dopamine neurons to susceptibility to alterations in mitochondrial dynamics. In addition, evidence from PD-related toxins supports that mitochondrial fission, fusion, and transport may be involved in pathogenesis (1, 20). Accumulating evidence suggests that two proteins linked to familial forms of PD, namely *parkin* and *PINK1*, interact in a common pathway to regulate mitochondrial fission/fusion. Taken together, the current data clearly indicate that mitochondrial dynamics may play a crucial role in PD pathogenesis, and a better understanding of mitochondrial dynamics within dopaminergic neurons probably will identify impaired dynamics as an early pathogenic event leading to PD. Additionally this line of research may help identify novel interventional targets for future therapeutic strategies.

Several epidemiologic studies suggest that pesticide and other environmental toxins that inhibit complex I are known to cause PD. The accidental discovery of the meperidine analogue MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) once used as a designer drug, has provided several insights on the potential role of mitochondrial complex I dysfunction in PD pathogenesis (1, 17). MPTP which is a protoxin is converted to the active neurotoxic metabolite 1-methyl-4-

phenylpyridinium ion (MPP^+) by monoamine oxidase B in glial cells which serves as a substrate for dopamine transporter. MPP^+ is selectively taken up by dopaminergic neurons and known to cause cell death via inhibition of complex I. In aged monkey and baboons, MPTP administration result in *α-synuclein* and ubiquitin-positive Lewy body like intracellular inclusions reminiscent of neuropathology seen in human PD. This toxin has since been extensively used in laboratory animals and further been refined, in which other mitochondria targeted pesticides such as rotenone, and paraquat result in pathology clinically similar to PD (1, 20). It has been suggested that the mechanism of toxicity in complex-I inhibition models involves oxidative stress which is a common underlying feature in PD pathogenesis. Mitochondria are known to consume a significant amount of oxygen via the electron transport chain and during oxidative phosphorylation some of the oxygen is reduced to ROS. Additionally DA-ergic neurons are also vulnerable to ROS induced toxicity since dopamine is known to generate ROS via monoamine oxidase B during its normal metabolism to generate oxidative stress. A great deal of interest has focused on the possibility that oxidative damage may play a role in the pathogenesis of PD. Several studies demonstrate increased levels of malondialdehyde, cholesterol lipid hydroperoxides, markers for lipid peroxidation, and increases in 8-hydroxy-2-deoxyguanosine, a marker of oxidative damage to DNA, are significantly increased in PD substantia nigra (10). Especially decrements in the levels of reduced glutathione in nigral dopaminergic neurons in incidental Lewy body disease (also known as pre-symptomatic PD) indicates that oxidative damage occurs much earlier than actual neuronal loss and, most importantly, that it is the triggering event in development of PD (5). There is evidence that depletion of glutathione may trigger inhibition of mitochondrial complex I which in turn lead to complex I-mediated neurodgeneration (9). In addition to its several known functions, reduced glutathione may function as a substrate for enzymes known to modulate glutathionylation and deglutathionylation of cysteine sulfahydryl groups of proteins to maintain thiol homeostasis. Since mitochondria are at the heart of both oxidative damage and redox signaling, the glutathionylation of mitochondrial proteins is of particular importance (7). This is consistent with the reports suggesting that loss of reduced glutathione with a concomitant inhibition of mitochondrial thiol homeostasis lead to mitochondrial dysfunction and increased iron accumulation by modulating iron regulating proteins to cause oxidative stress-induced neurodegeneration (9, 12). NADPH oxidase 1, a specialized superoxide generating system and Rac1, one of its active component found in midbrain dopaminergic neurons, has emerged as a new player in mediating neurodegeneration via oxidative damage (4). Accordingly, the discovery of novel strategies to mitigate oxidative stress has been a principal focus of current therapeutic initiatives. In the past, antioxidants such as vitamin E, vitamin C, N-acetylcysteine, and glutathione have been tested in numerous animal models and clinical trials for PD. Unfortunately most of these trials have shown little or no benefit to PD patients. Recently, strategies have been designed to target on novel ways to target antioxidants to specific organelles such as mitochondria, known to generate high levels of ROS. For instance, mitochondria targeted antioxidant peptides (19) and catalytic antioxidants are being actively studied for neuroprotective properties (11). Co-enzyme Q10 is a component of the respiratory chain that shuttles electrons between complexes I/II and III and it also functions as an antioxidant. A randomized, double blind, futility clinical trial conducted by the NINDS NET-PD investigators found that this compound is worth future therapeutic studies. In addition, modulation of endogenous antioxidants by transcriptional regulation has been identified as a promising target for neurotherapeutics (3, 8, 17). One of the principal transcription factors that modulate endogenous antioxidant expression is nuclear factor E2-related factor 2 (Nrf2). Activation of Nrf2 results in a diverse array of phase II antioxidants, detoxifying, and cytoprotective genes. Due to its pleiotropic nature, strategies to stimulate Nrf2 transcriptional activity could be beneficial. Several chemical modifiers of Nrf2 transcription are under development and their promising effects in animal models suggest that they could produce tangible benefits to PD patients (3). Yet another transcriptional regulator of endogenous antioxidant defense is the peroxisome proliferator-activated receptor-γ coactivator-1α (PGC-1α). Similar to Nrf2, PGC-1α is also a pleiotropic transcriptional coactivator known to modulate expression of antioxidant enzymes within mitochondria and genes that are involved in mitochondrial biogenesis (17). Agents known to stimulate PGC-1α activity similar to those outlined for Nrf2, may be also beneficial as novel interventional strategies for PD patients.

Besides oxidative stress and mitochondrial dysfunction there is now greater evidence that innate and adaptive immune systems play crucial roles in PD pathogenesis. Components of the innate immune system within the brain such as microglia and regulatory humoral elements, including the complement system and cytokines, are known to participate in the neuroinflammatory processes (15). Postmortem analyses of brains from human PD patients and animals models have revealed the presence of reactive microglia. Activated microglia are known to produce a variety of toxic substances that, in addition to killing infectious agents, can accelerate neuronal injury and death. These toxic substances include reactive oxygen species (ROS), reactive nitrogen species (RNS), proinflammatory cytokines, and prostaglandins. The substantia nigra contains the highest concentration of microglia in the brain, making this region especially susceptible to altered microglial activation responses. There is evidence for increased levels of pro-inflammatory cytokines such as TNF-α and IL-1β in postmortem PD tissues. Furthermore, increased levels of IL-6, a pro-inflammatory cytokine appears to increase the risk for developing PD. Given this possible causal link between neuroinflammation and PD, one would suspect that administration of anti-inflammatory drugs may render beneficial effects. Some epidemiological studies have revealed that individuals on a chronic regimen of nonsteriodal anti-inflammatory drug (NSAID) have significantly reduced risk for PD, while others failed to establish such a link. Recent studies have explored adaptive immune systems in pathogenesis of PD. They consist of highly specialized cells with specific immunologic effectors, the ones with regulatory, and memory capabilities (T and B lymphocytes) that specifically eliminate or prevent pathogenic insults; but is activated by the nonspecific innate immune system. Traditionally brain has been considered "immune-privileged" and protected through the blood-brain barrier, which prevents invasion of toxins and infectious agents. Naïve T cells and B cells are normally

precluded from entry into the brain, however, during neuroinflammatory processes, activated innate immune system secrete factors which facilitate entry of adaptive immune cells. Infiltration of T cells has been found in brains of PD patients. Adoptively transferred immune splenocytes in MPTP-treated mice results in significant infiltration of T-cells into brain and localize within the inflamed SNpc. There is also evidence of T-cell-induced exacerbation in MPTP-induced neurotoxicity following induced adaptive immune response towards nitrated but not native *α-synuclein*. This suggests a causal relationship between T cell infiltration, sustained microglial activation and oxidative stress in PD. Not all T cells are believed to be detrimental to brain neurons; there exists a naturally occurring T cell subtype that functions to prevent immune responses towards self-peptides known as the regulatory T cell (Treg). Tregs are generated in the thymus and constitutively express CD4 and CD25 in addition to the transcription factor FOXP3 (forkhead box p3) and are widely recognized to be capable of controlling innate immune reactivity and suppressing both CD4+ and CD8+ effector T cell responses. The Tregs are known to exhibit regulatory activities by suppression of immune responses by secretion of anti-inflammatory cytokines (15). A greater understanding of T cells and regulatory T cell function in the brain both in normal and disease conditions and identification of methods generating Tregs for therapeutic modalities will be of great interest in PD pathogenesis.

Yet another important aspect that has emerged in PD pathogenesis is protein misfolding and aggregation (16). Maintenance of cellular homeostasis is dependent in part on the stability of its protein components. There are three major cellular systems identified to cope with protein stability which includes the chaperone system, ubiquitin proteasome system (UPS), and autophagy. While the chaperone system is responsible for protein folding and refolding, both autophagy and UPS is part of the protein degradation machinery. The initial evidence of protein misfolding and aggregation in PD comes from the observation of Lewy bodies (LB) enriched with *α-synuclein* and ubiquitin in sporadic PD brains. *α-Synuclein* which is a natively unfolded protein is aggregation prone due to pathogenic familial mutations, exposure to exogenous neurotoxicants, and dysfunctional UPS. An area of intense investigation in the last decade has been to understand how aggregated *α-synuclein* evades all the three pathways of toxic protein clearance including chaperone system, autophagy, and UPS. There is evidence for alterations in the induction/and or functions of the chaperone system in PD. Overexpression of Hsp70 in *α-synuclein* cell and fly models reduces aggregation of *α-synuclein* and associated neurodegeneration, suggesting that chaperones might promote refolding and/or degradation of *α-synuclein*. This is consistent with blockade of *α-synuclein* toxicity through induction of Hsp70 by administration of geldanamycin in flies. Taken together, these studies suggest impaired chaperone function in PD pathogenesis. The UPS which normally identifies and degrades intracellular proteins when become dysfunctional is known to promote the toxic accumulation of proteins which significantly compromises normal functioning and survival of dopaminergic neurons. There is evidence of structural and functional impairment of the UPS in substantia nigra of sporadic PD patients. Furthermore, increase in age and exposure to environmental toxins especially the one's compromising mitochondrial functions are known to result in age related accumulation of toxic proteins via the disruption of UPS. Although controversy remains on modeling PD in rodents when administered with proteasome inhibitors, recent study where a genetic approach to conditionally deplete 26S proteasome in substantia nigra in mice resulted in loss of nigrostriatal dopaminergic neurons and *α-synuclein* positive inclusions (16). However, a more direct genetic evidence for the involvement of UPS disruption with that of PD comes from the discovery of genetic mutations of *parkin* resulting in autosomal recessive PD. *Parkin* has been identified as an ubiquitin E3 ligase involved in proteasomal degradation of misfolded proteins and disease-causing mutations result in accumulation of one or more of its substrates by impairing the ligase activity. In recent years there have been numerous studies demonstrating the ability of *parkin* mediating both degradation associated and nonproteolytic ubiquitin modifications. This suggests that both proteasome-dependent and proteasome-independent events could account for susceptibility of dopaminergic neurons in *parkin* related PD. Recent studies have also identified that polyubiquitinated proteins are removed via the mechanism known as autophagy that involves the lysosomes. There is evidence of decline in chaperone-mediated autophagy and macroautophagy with increase in age, impairing protein turnover and stability during stress response. This together with increased oxidative stress mediated by cytosolic dopamine and postsynthetic modifications of proteins such as *α-synuclein*, *parkin*, and *DJ-1* may lead to blockade of autophagy to result in accumulation of aggregated proteins. Taken together, maintenance of protein homeostasis within dopaminergic neurons via regulation of protein misfolding and aggregation is crucial since it shares close relationship with other intracellular pathways including oxidative stress, mitochondrial dysfunction to impact cell survival and death pathways (16, 17).

Identification of two autosomal dominant forms of PD due to gain of function mutations in *α-synuclein* and *LRRK2* gene and the current understanding of the functions of these two molecules suggests they might serve as possible avenue for target validation for PD therapeutics (14). The presence of *α-synuclein* as a major component of LB, familial *α-synuclein* mutations, gene multiplication, and protein misfolding suggests that toxic accumulation of *α-synuclein* plays a pathogenic role in PD. This suggests that approaches known to suppress *α-synuclein* accumulation may be a novel therapeutic strategy. Consistent with these studies *α-synuclein*-null mice showed heightened resistance to MPTP and other mitochondrial toxins (17). As indicated above, use of drugs that upregulate chaperones such as Hsp70 to block *α-synuclein* aggregation has shown efficacy in both *in vitro* and *in vivo* models of disease (14, 16). Other means to block *α-synuclein* aggregation includes use of synthetic peptides derived from the N-terminal amino acid sequence of α-synuclein, and human single-chain antibody fragments (scFv) that binds *α-synuclein* to inhibit formation of toxic *α-synuclein* fibrils. Another useful option that has emerged is to target *α-synuclein* expression itself as a neuroprotective therapy using RNA interference mediated knockdown of *α-synuclein* mRNA and by inhibiting transcriptional activation of GATA-2 known to induce *α-synuclein* expression. Taken together, these studies indicate that the idea of *α-synuclein* as a potential therapeutic target for PD is very promising. However, it will require more robust proof

from multiple target validation studies in several *in vivo* models of disease.

The notion of *LRRK2* as a potential drugable target in PD has originated from its resemblance to a "kinase" bearing structural similarity to mixed lineage kinases (14, 17). There are several missense mutations in the *LRRK2* gene identified to be associated with PD, of which the G2019S mutation is the most prevalent. The G2019S mutation has got a significant amount of attention since overexpression of *LRRK2* protein with the G2019S mutation results in significant increase in cell death markers, neurite shortening, and changes in membrane permeability. These detrimental phenotypic changes seem to correlate with increase in the *LRRK2* kinase activity, and mutations that ablate the kinase activities are known to the block the development of such phenotypes. This suggests that blocking the kinase function may serve as a desirable therapeutic approach to block *LRRK2*-mediated toxicity in PD. Hence, being a very provocative target, multiple laboratories and pharmaceutical companies are interested and working towards developing effective kinase inhibitors to block *LRRK2* kinase activity. Despite such an enthusiastic outlook towards this target, there needs to be a more comprehensive understanding of the known *LRRK2* kinase substrates and its downstream signaling cascades. In this regard studies targeted towards developing transgenic *LRRK2* models of PD will help decipher its precise function *in vivo*. Initial studies in flies suggest that overexpression of both *dLRRK* containing missense mutations in areas of the protein homologous to PD-linked mutations in human *LRRK2* gene results in dopaminergic toxicity. Interestingly flies expressing wild-type *dLRRK* or *dLRRK2* with mutations predicted to inactivate kinase activity failed to develop dopaminergic toxicity. This *in vivo* study suggests that the *LRRK2* kinase activity could serve as a valid target for neuroprotection for PD, although additional studies will be required from multiple *in vivo* models to definitively prove that *LRRK2* kinase activity is indeed an ideal target for drug development (14).

Translational approaches using cell based therapies have been long thought as an intuitive therapeutic approach for PD considering that the neuropathology involves selective loss of midbrain dopamine neurons (6). Numerous *in vivo* studies have shown that, unlike pharmacological and surgical approaches, neurorestorative therapies provide hope for the cure of PD. Studies in rodents and primate models of PD demonstrated that transplantation of dopaminergic neurons innervate the lesioned striatum to improve motor functions. This led to initial studies where patients received striatal infusion of autologous transplants of adrenal medullary cells with the rationale that self-transplants would be immunologically compatible and devoid of the risk of disease transfer between donor and host. This approach had some initial success but later multiple lines of studies found it to be futile due to poor survival of medullary grafts. This led to the transplantation using fetal midbrain tissues obtained from aborted fetuses, highly enriched in dopaminergic neurons. Several animal studies and small clinical trials using the fetal midbrain tissues showed variable degrees of success. Differences in surgical techniques, age of fetuses, and methods of cell preparation made it difficult to attempt comparisons across multiple studies and draw any viable conclusions. There have been several attempts to obtain alternative sources for dopaminergic neurons which included use of porcine xenografts, human retinal pigmented epithelial cells, autologous sympathetic ganglion and carotid cell bodies, in addition to alternative cells from neural lineage. Despite success in animal models, these approaches could not deliver significant neurorestorative effects in clinical trials. The most attractive option identified was the use of embryonic stem cells obtained from the blastocysts and have the potential to become any kind of cell. The use of human embryonic stem cells and their ability to differentiate into dopaminergic neurons using appropriate protocols for transplantation has multiple benefits, including decreased risk of tumor formation and the possibility that grafts containing large portions of DA neurons promote greater attenuation of PD symptoms. Despite the beneficial effects of embryonic stem cells, there are several controversies including affecting nonspecific behavior in hosts due to oversized transplants, retention of mitogenic progenitors in transplanted grafts that may be tumorogenic. A very promising discovery for cell-based therapy has been the creation of induced pluripotent stem cells (iPS) derived from skin fibroblasts that combines a specific set of genes capable of dedifferentiating cells committed to an adult phenotype. This technology was recently used where motor neurons and astroglia derived from iPS cells obtained from an 82-year-old female with familial SOD1 linked amyotrophic lateral sclerosis and demonstrated that it is feasible to obtain iPS cells from the fibroblasts of aged patients with progressive neurodegenerative disease. This technology resolved critical ethical questions associated with the use of embryos, cloning, and the availability of oocytes. Researchers have recently been able to obtain iPS cells from PD patients using viral reprogramming factor-free methods and differentiate them into dopaminergic neurons. Despite their immense potential, iPS cell transplants will still have to overcome all the major hurdles presented in other grafts survival, staying differentiated, and remaining genetically stable (6). Overall, there is enormous interest in the potential of cell-based therapies to provide an unlimited source of dopaminergic neurons for restorative therapies in PD, several basic issues would need to be resolved first before we could see the prospective use of cell-based therapies in the clinic.

Future Perspective

As discussed in this forum, one should appreciate that PD is a complex disease in terms of its etiopathogenic mechanisms. Given the multiplicity of genetic and environmental factors, together with inherent properties of the aging brain, it has become increasingly challenging to dissect the molecular pathways in PD pathogenesis. Substantial amount of evidence from genetic, animal models, human postmortem analysis, and epidemiology suggests a causative role of oxidative stress, mitochondrial dysfunction, neuroinflammation, and pathways that regulate protein misfolding and aggregation in disease pathogenesis. Therefore neuroprotective strategies targeting oxidative stress, mitochondrial dysfunction, neuroimmune pathways, inhibiting aggregation of *α-synuclein* and *LRRK2* kinase activities hold tremendous potential for PD therapeutics. Additionally, there is a high level of enthusiasm on the potential of cell-based therapies to provide an unlimited source of dopaminergic neurons to restore a functional nigrostriatal pathway.

However, many issues regarding such approaches remain to be addressed before it can become an ultimate choice of therapy. There is also an immediate need to develop tools that will identify valid biomarkers that will provide early identification of PD and allow implementation of intervention, as well as an unbiased assessment of disease progression. Future research needs to provide a greater understanding of the interplay and temporal relationship amongst various pathologic processes leading to the development of PD that will be most amenable to therapeutic intervention.

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