

Mitochondrial Deficiencies and Oxidative Stress in Parkinson's Disease: A Slippery Slope to Cell Death

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Summary

Parkinson's disease (PD) affects over 500,000 Americans. Most cases of PD are idiopathic, or occurring without a known cause. Two pathological features of PD, α -synuclein-rich Lewy bodies (LB) and oxidative damage, hint at the cause of the disease. Yet, disparities in recessive forms of PD increase the complexity of the disease mechanism. These recessive forms occur earlier in life and are devoid of LB. One common feature among these forms is the extensive presence of reactive oxygen species (ROS). Studies with the toxin MPTP produced similar pathologies to recessive PD but intriguingly showed inhibition of complex I in the mitochondria. These and other studies chased the mitochondria as the progenitor of oxidative stress. These investigations also uncovered several disparate mitochondrial proteins, one of which is a Krebs' cycle enzyme, α -ketoglutarate dehydrogenase (α -KGDH). Interestingly, α -KGDH activity is reduced in both Alzheimer's disease (AD) and PD. Links to both diseases may be due to its role in the inactivation of complex I. This review will focus on how mitochondrial impairments enhance neuronal toxicity in PD.

Introduction

Neurodegenerative diseases (NDD) are incurable, progressive, and fatal disorders of the central nervous system (CNS; Muchowski, 2002). Alongside this commonality, a culprit protein is frequently found tangled in symptomatic patients. Protein accumulation and subsequent aggregation is correlated with cell death in brains of the afflicted. Alzheimer's disease (AD) and Parkinson's disease (PD) are both NDD that have key similarities and differences. Investigations in both diseases have elucidated complementary mechanisms involving different genes.

In both AD and PD brains, insoluble protein deposits of tau and α -synuclein are, respectively, found (Caughey et al., 2003; Dauer and Przedborski, 2003). In conjunction with protein aggregation, the accumulation of toxic oxidants, like superoxide and hydrogen peroxide, is another hallmark of both diseases (Mizuno et al., 1995). Yet, the specificity of degeneration is unique to each disorder; AD has global neuronal death in the CNS, whereas PD has localized death (Caughey et al., 2003). These similarities and differences have led scientists on a search to find the genes implicated in both diseases. This review will span the discoveries made in PD that point to dysfunctions in the mitochondria and its respiratory chain, some of which are also found in AD.

Parkinson's Disease Etiology

PD is a movement disorder characterized by a triad of symptoms: bradykinesia, postural rigidity, and resting tremors (Dauer and Przedborski, 2003). Voluntary movement is disrupted by the loss of dopamine in the intact striatum. Specific death of dopamine-producing neurons in the substantia nigra *pars compacta* (SNpc) leads to this loss-of-function phenotype (Dauer and Przedborski, 2003). α -Synuclein-dominant protein inclusions, or Lewy bodies (LB), are suspect in causing neuronal death in PD (Spillantini et al., 1997). Additionally, post-mortem PD reveals oxidative damage partially due to redox-sensitive dopaminergic neurons (Beal, 2003). Yet, an exact mechanism that engages α -synuclein-toxicity with oxidative stress has not been determined.

Sporadic mutations in α -synuclein constitute 95% of PD cases. The other 5% of occurrences are attributed to a mixture of dominant and recessive genes. Dominant forms of PD have familial mutations in α -synuclein which increase its tendency to aggregate and form LB. Yet, recessive forms of PD are LB negative where the onset of symptoms occurs much earlier than in familial dominant forms. Two of these recessive genes, DJ-1 (Bonifati et al., 2003) and PINK1 (Valente et al., 2004), are involved in mitochondrial protection. Perturbations in these genes can impair mitochondrial function and lead to apoptosis and rapid generation of reactive oxygen species (ROS; Dauer and Przedborski, 2003). Whether or not α -synuclein is involved in the mitochondrial disease pathway is unresolved. Dominant and recessive forms of PD may involve two different mechanisms that achieve the same symptoms. Both mechanisms, however, will have oxidative stress as a key player in PD pathogenesis.

Oxidative Stress: The Main Cause of PD?

The question remains: does the accumulation of ROS initially cause specific nigrostriatal death in PD? Studies performed with the toxin MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) have yielded support for this hypothesis (Langston et al., 2003). The elucidation of PD pathogenesis has been largely dependent on the introduction of MPTP.

MPTP gains toxicity when converted to MPP⁺ (1-methyl-4-phenylpyridinium ion) by monoamine oxidase B (Chiba et al., 1984). MPP⁺ is transported in neurons through a dopamine transporter (DAT; Chiba et al., 1985). This is the only way MPP⁺ can enter a cell; thus, specific dopaminergic cell death can be ascribed by this quality. Complex I of the electron transport chain is inhibited by MPP⁺ (Nicklas et al., 1985). The inhibition of oxidative phosphorylation has many harmful effects including: decreased ATP production and increased oxidant production. This toxic state is further insulted by increased intracellular calcium, which enhances the release of dopamine to further promote oxidative damage (Fiskum et al., 2003). It is uncertain whether idiopathic PD is causally related to dopaminergic loss by complex I inhibition (Abou-Sleiman et al., 2006). Regardless of the

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pathway, oxidative modification plays an intimate role in the PD pathogenesis.

Oxidative stress may play a role in SNpc specific atrophy. Neuromelanin, and its high iron content which pigments the SNpc, may provide a necessary oxidative stress mechanism to specifically destroy the SNpc (Zecca et al., 2006). Iron may aggressively catalyze ROS generation from oxidized substrates by the Fenton reaction (Mizuno et al., 1995). The increase of reactive oxidants can be measured by cellular responses. One such response is superoxide dismutase activity (SOD). This enzyme's activity is elevated in the SNpc in PD (Saggu et al., 1989). Saggu and colleagues (1989) reported that Mn SOD activity was elevated over Cu-Zn SOD levels. This finding is significant because Mn SOD is localized in the mitochondria. Another indication of increased oxidant presence is the reduction of antioxidants. Glutathione is found reduced in PD (Perry and Yong, 1986). These data suggest that increased ROS are present in the mitochondria in PD. MPP⁺, SOD, and glutathione all point to the mitochondria as a putative producer of ROS.

Respiratory failure and increased oxidative stress both characterize PD. Yet, which event comes first? Jenner and colleagues (1992) studied an analogous system, Lewy body disease, to answer this question. They found loss of both glutathione and complex I substrates, yet glutathione was slightly less than complex I. They concluded that oxidative stress precedes respiratory failure in a PD. Other groups, however, disagree with Jenner and colleagues' conclusions.

PD has a twofold loss of respiratory activity, which may exceed glutathione loss. The Krebs' cycle enzyme, α -ketoglutarate dehydrogenase (α -KGDH), is also found deficient along with inhibition of complex I (Mizuno et al., 1994). In fact, reduction of respiratory activity may be even more deleterious than oxidative stress alone. Chance et al., (1979) suggest that α -KGDH and complex I inactivity triggers enhanced generation of oxidative stress. When the ETC slows down due to inadequate activities of complex I and α -KGDH, the leaky mitochondrial membrane generates increased free radicals. Thus, oxidative stress alone is not enough to explain PD pathogenesis. A pathway including mitochondrial respiratory failures must be engendered to understand how radicals are produced and sustained. In the next section, radical production from dopamine metabolism further explains SNpc specific degeneration

The SNpc is Sensitive to ROS: Dopamine Auto-Oxidation

Dopamine auto-oxidation has long been an attractive hypothesis for SNpc selective death due to its endogenous metabolism in the SNpc (Graham, 1978). Normal metabolism of dopamine produces hydrogen peroxide and superoxide radicals, which oxidize dopamine to form dopamine-quinone (Dauer et al., 2003). Dopamine-quinone then proceeds to disrupt all proteins with disulfide linkages (Dauer et al., 2003). Auto-oxidation has thus had a three-fold effect on the cell. One, the disassembly of proteins with disulfide linkages places an unnecessary stress and load on the ubiquitin-proteasome protein degradation system (UPS; Dauer et al., 2003). Two, ROS generated by dopamine metabolism increases general protein misfolding in the cell, thus further increasing the load onto the UPS. And

three, dopamine-quinone, like MPP⁺, may inhibit mitochondrial complex I activity. Cumulatively, dopamine metabolism primes SNpc neurons for self-destruction. Thus, programmed cell-death, induced by ROS and mitochondrial dysfunction, may lead to specific death of SNpc neurons.

In mitochondrial respiratory dysfunction, ATP production is decreased. The lack of energy supports the necrotic cell death hypothesis of PD neurons (Mizuno et al., 2005). However, since PD is a progressive disease, occurring over a long period of time, cellular necrosis cannot be the only theory for SNpc atrophy in PD. The decrease of ATP levels perturbs calcium homeostasis, which activates apoptotic pathways. Low levels of ATP decrease sodium ion transport to the outside of the cell. Thus, sodium must be expelled by the exchange of extracellular calcium (Reeves et al., 1992). As stated above, high levels of intracellular calcium create an excitotoxic environment. Calcium is a second messenger that activates many cellular signaling pathways. Most notably, degenerate proteases are activated which induce apoptosis (Mizuno et al., 2005). Furthermore, disruption in calcium homeostasis increases ROS production by the mitochondria. Imbalances in calcium homeostasis from mitochondrial respiratory failure feedback into the mitochondria and further degrade its normal activity (Mizuno et al., 2005).

To further support the apoptosis based cell-death scheme in PD, Hartmann et al. (2001), describe the proapoptotic mitochondrial mediator, Bax, a member of the Bcl-2 family of proteins. Its primary role in cell death is to release cytochrome c from the inner mitochondrial membrane, in effect, purging its potentiation. This facilitates the activation of caspases, a group of cysteine proteases, which cleave numerous cellular proteins. They found that Bax levels were significantly higher in dopaminergic neurons containing LB than in overall melanized areas. These and other studies have strongly suggested dopamine's role in SNpc specific cell death. These data support that dopamine metabolism enhances toxic ROS levels.

Collectively, mitochondrial deficiencies and dopamine metabolism leave PD neurons in a compromised state. We are still uncertain as to the exact role of the mitochondria in PD. The next section will explore the biochemical aspects of mitochondrial dysfunction.

The Science behind Mitochondrial Dysfunction

The mitochondrion is the gate keeper for ROS production. Significant rises in oxidative damage can only occur through deficiencies in respiratory metabolism (Cookson, 2005). Since oxidant levels are raised in the SNpc due to dopamine metabolism, SNpc neurons are predisposed to increased oxidant damage. When neurons are incapable of reducing this oxidant-rich environment, the mitochondria is deleteriously impacted. The mitochondrion fuels its own destruction by rapidly producing even more oxidants. Nevertheless, what structures and pathways engender this response?

Normally during oxidative phosphorylation, electrons travel along the respiratory chain to complex I, II, III, and IV, along with cytochrome c and ubiquinone. The chain is commonly characterized as "leaky", which promotes the reduction of substrates, such as oxygen, thereby producing a superoxide molecule (O₂^{•-}). Iron-sulfur clusters within the complexes provide the donation of one electron to

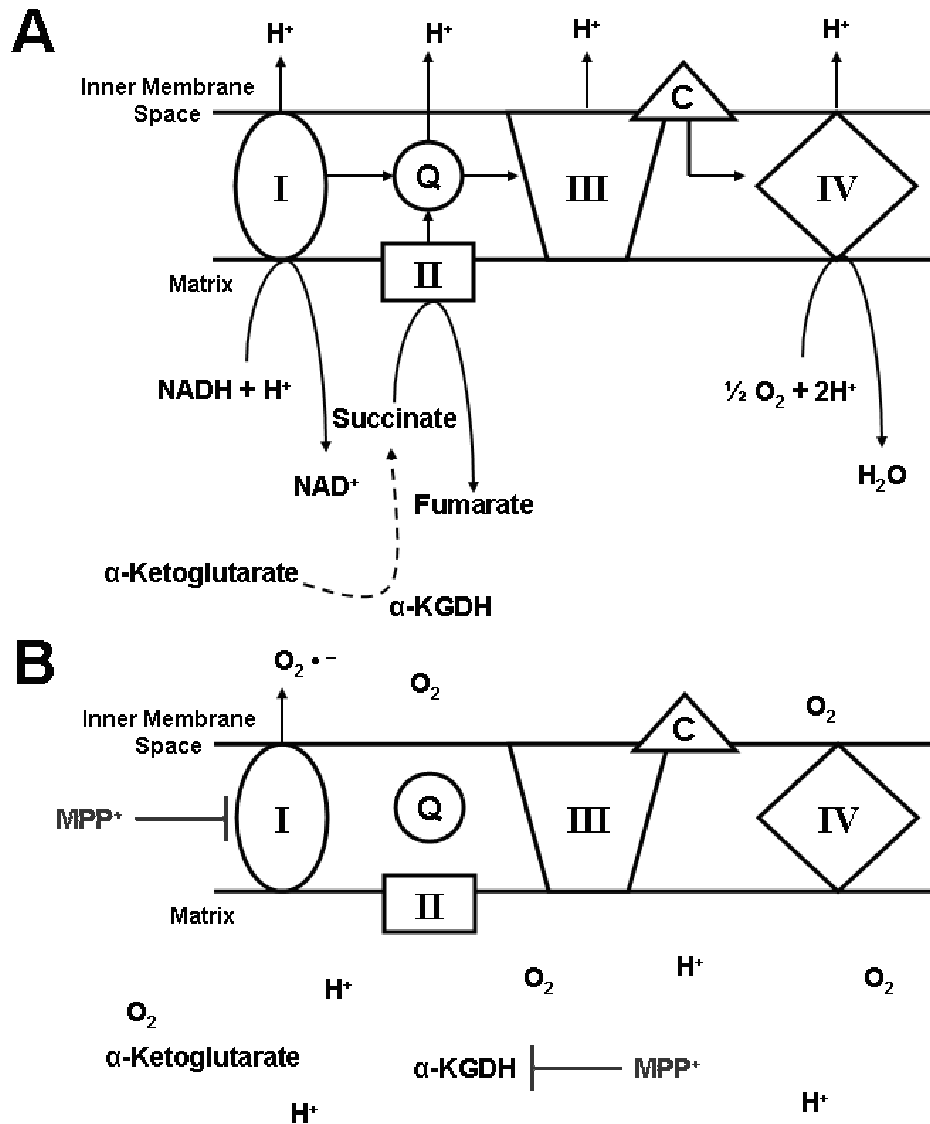


Figure 1. Effects of MPP⁺ Inhibition on Complex I and α -KGDH. **A.** Normal function of electron transport chain. **B.** MPP⁺ introduction into the mitochondria arrests ubiquinone (Q), thus electrons are not continuously transferred to complex III and IV. Oxygen is not reduced to water, so it is available for superoxide formation ($O_2^{\bullet -}$). This inactivity disrupts the proton gradient needed to make ATP in complex V (not shown).

make the toxic superoxide molecule. This donation increases in probability as the ETC becomes more and more inhibited. The inhibition of the respiratory chain leaves upstream components reduced for relatively long periods of time. The escape of electrons is most favorable during this time. Consequently, since the redox potential decreases, electrons are not shuttled to the next carrier. The release of electrons in this window of time produces ROS (Adam-Vizi, 2005).

Alternatively, superoxide production by complex I requires a pH gradient across the inner membrane space (Lambert et al., 2004), which can be achieved with ubiquinone inhibitors. Ubiquinone is produced at two places in the ETC, complex I and complex II. ROS production requires inhibition of both sites so that ubiquinone is unable to carry electrons to complex III and continue to complex IV. If electrons are not shuttled to complex IV, oxygen is not reduced to

water and remains saturated in the cell. Oxygen saturation is another prerequisite of ROS production (Boveris and Chance, 1973). Thus, inhibition of complex I only is not adequate to produce toxic oxidants.

Nonetheless, in the MPTP model of PD described above, MPP⁺ inhibits complex I. According to Lambert and colleagues (2004), complex I inhibition is not enough to produce superoxide radicals. The MPTP pathway must be developed further. Strikingly, MPP⁺ inhibits α -KGDH activity as well (McNaught et al. 1995). Reduction of α -KGDH thereby reduces succinate concentrations, the substrate for complex II. Succinate is then unable to carry electrons to complex II, in so doing inhibiting its activity. Thus, MPTP does, in effect, inhibit complex I and II (Figure 1).

Until recently, the respiratory chain, or electron transport chain, has been assumed as the

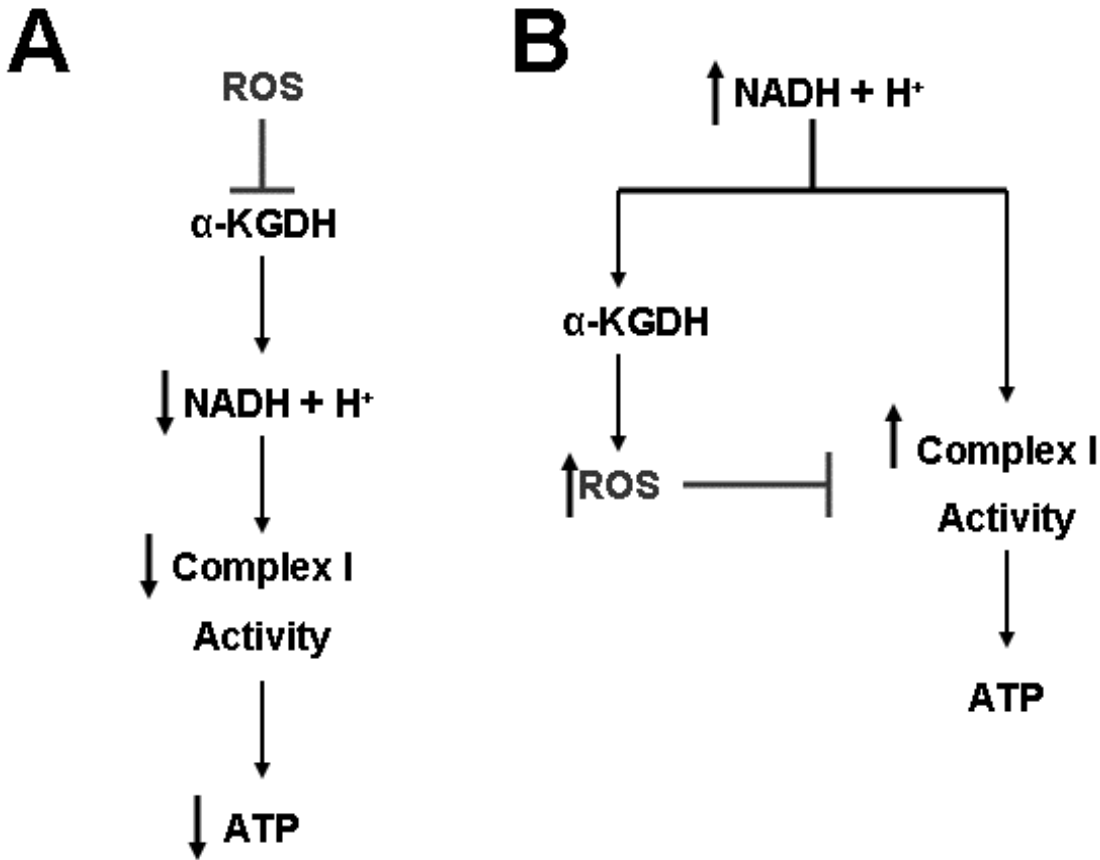


Figure 2. α -KGDH Regulation of Complex I Activity. **A.** ROS inhibits α -KGDH which decreases the production of NADH in the Krebs's cycle. The lack of NADH lowers complex I activity, thus ATP is not synthesized. **B.** A high concentration of NADH increases the activity of both α -KGDH and complex I. Yet, this dual activation is counterintuitive. α -KGDH produces ROS when NADH levels are high. This may, in turn, inhibit previously activated complex I.

principal generator or ROS (Starkov et. al., 2004). Previous studies (Chance et. al., 1979) have suggested the duality of α -KGDH and complex I as sources of ROS. Yet, complex I-dependent ROS production was always thought to surpass α -KGDH-related ROS production. New evidence suggests that α -KGDH regulates complex I ROS production. In the next section, the relationship between α -KGDH and complex I activity will be discussed further.

Non-Electron Transport Deficiencies: α -Ketoglutarate Dehydrogenase

The toxin MPTP serves as a convenient model for PD, but most cases are idiopathic. MPTP fails to replicate two other qualities of classical PD: Lewy body inclusions and progressive onset. MPTP-induced Parkinsonism occurs rapidly after exposure thus making it, at most, a model of PD. This begs the question, can complex I be inhibited by any other means? Extensive studies with the α -KGDH have revealed that possibility and more.

α -KGDH is regulated by the NADH/NAD⁺ ratio, Ca²⁺, and ADP (Adam-Vizi, 2005). Its loss-of-function would thus eliminate a key modulating location in glucose metabolism. Interestingly, the isolated enzyme produces hydrogen peroxide and superoxide

radicals (Tretter and Adam-Vizi, 2004). These results were found to hold true *in situ* as well (Starkov et. al., 2004). This generation of ROS was dependent on the NADH/NAD⁺ ratio, where increasing NADH levels account for this phenomenon. Increasing ROS levels actually inhibit α -KGDH (Tretter and Adam-Vizi, 1999). The inhibition of α -KGDH reduces NADH for the ETC, thus decreasing ATP production. Consequently, by these inverse pathways, α -KGDH is both a target and generator of oxidative stress

Markedly, α -KGDH may regulate complex I activity by these inverse pathways (Adam-Vizi, 2005). As stated before, inhibition of α -KGDH reduces NADH production which kinetically decreases complex I (NADH ubiquinone oxidoreductase) activity. The other inhibitory pathway is set in motion by a high NADH concentration. Thus, complex I is kinetically active under these conditions. The generation of ROS by α -KGDH in the mitochondrial matrix may disrupt complex I activity (Figure 2). Also, the ROS generated by α -KGDH would increase lipid peroxidation thereby disrupting calcium homeostasis. Lipid disruption would furthermore perturb the pH gradient across the inner membrane space. This loss of membrane potentiation is a step towards apoptosis. Loss of α -KGDH activity and inhibition of complex I is a similar to the MPTP-based model of PD.

Loss of α -KGDH also occurs in AD. Gibson and colleagues (1988) have demonstrated that α -KGDH activity is reduced by 40-75% in AD brains. Reduction in α -KGDH levels is negatively correlated with neurofibrillary tau tangle counts, suggesting this enzyme is involved in the neurodegenerative cascade. Even 10 to 15% reduction in available glucose or oxygen can reduce brain function, including decline in memory (Gibson et al., 2005). A current study shows how brain regions with low metabolic activity accumulate more plaques in conjunction with dementia (Shoghi-Jadid et al., 2002). The return of glucose to these brain regions reverses some behavioral deficits found in AD. This suggests that symptoms in AD are not always attributed to neurodegeneration. The question remains, how does α -KGDH activity decrease in AD and PD?

Genetic studies have attempted to answer this question. Two different groups have attempted to track the allele responsible for α -KGDH deficiencies in AD and PD. Of the three subunits of α -KGDH, the second has received most attention because of its noted ROS producing activity (Starkov et al., 2004). The other two subunits do not produce ROS. Polymorphisms on the second subunit have been paired with the apolipoprotein E4 gene in AD to cause dementia. Apolipoprotein E4 is not a factor on its own until it is paired with the α -KGDH polymorphism (Sheu et al., 1998). Similarly, a bi-allelic intragenic polymorphism of α -KGDH was found to constitute a genetic risk factor for PD (Kobayashi et al., 1998). The dysfunction of α -KGDH caused by the polymorphism suggests a causal genetic link to NDD. Whether α -KGDH activity is inhibited by a genetic link or ROS remains to be determined.

Since the identification of α -KGDH as a critical enzyme in AD and PD pathogenesis, several studies have sought to reverse the phenotypes in these NDD. In PD, acute lipoic acid, a key cofactor for α -KGDH, administration increases cerebral metabolism, thus reversing the α -KGDH deficit (Seaton et al., 1996). This was performed under the assumption that α -KGDH activity is decreased in PD brains. This study indirectly supports the α -KGDH-centric hypothesis of PD. Likewise, in AD, thiamine supplementation proved to have beneficial effects in patients (Mimori et al., 1996). Thiamine is necessary for α -KGDH activity, as well. Both of these studies support the hypothesis that α -KGDH is involved in AD and PD pathogenesis.

There are other mitochondrial connections to PD pathogenesis. Two recently discovered genes, when mutated, were found to cause LB negative Parkinsonism (Bonifati et al., 2003; Valente et al., 2004), similar to MPTP models. The genes DJ-1 and PINK1 will be discussed next.

Mitochondrial Links in PD Pathogenesis: DJ-1 and PINK1

Mutations in DJ-1 and PINK1 cause loss of dopaminergic function (Cookson, 2005). Both genes produce proteins with different functions yet have similar phenotypes.

DJ-1 may normally modulate gene expression in the cytoplasm during cell stress (Bonifati et al., 2003). Oxidation at C106 (Kinumi et al., 2004) of DJ-1 causes it to localize to the outer membrane of mitochondria under oxidative conditions. There, it protects cells against complex I inhibitors. Thus, mutations at C106 inhibit this protective mechanism.

DJ-1 is customarily oxidized in non cell death conditions. Accordingly, its localization to the outer membrane of mitochondria may suppress apoptosis (Canet-Aviles et al., 2004).

PINK1 is a serine/threonine kinase with an N-terminal mitochondrial localization signal (Valente et al., 2004). Valente and colleagues (2004) also observed that PINK1 protects cells against apoptosis induced by proteasome inhibitors. The PINK1 substrate is still unknown as well as its protection specificity. One feature is clear, however, both DJ-1 and PINK1 protect against loss of mitochondrial function.

Conclusion

The redox status of a cell is delicately modulated by several mechanisms. Mitochondria play an integral part in this regulation. The loss-of-function of mitochondrial proteins like complex I, α -KGDH, and PINK1 have implications for the viability of cells in NDD. In PD, dopaminergic neurons respond to stress in a unique way. Dopamine metabolism decreases the oxidative stress threshold required for apoptosis. Thus, mitochondrial impairments selectively target dopaminergic neurons. These impairments, in turn, further devastate mitochondrial function. The slippery slope of cell death in PD is characterized by the additively detrimental interactions between oxidative stress and mitochondrial dysfunction.

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