Role of Oxidative Stress and Mitochondria in Parkinson's Disease

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Abstract

Central to developing new treatment strategies for late onset sporadic Parkinson's disease (PD) and early onset familial PD is resolving the enigma of the specific vulnerability exhibited by substantia nigra dopamine (DA) neurons despite multiple risk factors. Neuropathological evidence from both human and experimental models of PD firmly supports a significant role for oxidative stress (OS) and mitochondrial dysfunction in the death of nigral DA neurons. Largely unknown are the genes underlying selective susceptibility of nigral DA neuron to OS and mitochondrial dysfunction and how they effect nigral DA cell death. To overcome the paucity of nigral DA neurons as well as the dilution effect of non-DA cells in brain tissues, we have developed wild type DA cell line model, SN4741 and mutant DJ-1 (-/-) DA cells, appropriate for microarray analysis and differential mitochondrial proteomics. Mutations in the DJ-1 gene (PARK7), localized in cytoplasm and mitochondria, cause autosomal recessive early onset PD.

Through microarray analysis using SN4741 cells followed by validation tests, we have identified a novel phylogenically conserved neuroprotective gene, *Oxi-a*, which is

specifically expressed in DA neurons. The knockdown of the gene dramatically increased vulnerability to OS. Importantly OS down-regulated the expression level of the gene and recovery of its expression via transient transfection exerted significant neuroprotection against OS insult. We also have identified altered expression of mitochondrial proteins and other familial PD genes in DJ-1 (-/-) mutant cells by differential mitochondrial proteomics. In DJ-1 (-/-) cells the knockdown of the other familial PD genes (Parkin and PINK1) dramatically increased susceptibility to OS. Thus, further functional characterization of the *Oxi-α* gene family and the mitochondrial alteration in the DJ-1 (-/-) cell model will provide the rationale for the neuroprotective therapy against both sporadic and familial PD.

Background

Neuropathological evidence from both human and experimental models of PD firmly supports a significant role for OS during the selective degeneration of nigral DA neurons. For instance, postmortem studies of the PD substantia nigra (SN) showed that levels of malondialdehyde (MDA), an intermediate in the lipid peroxidation, were increased compared to those in other PD brain regions and control tissues (1). The pathological role of OS in lipid peroxidation in the SN of PD was further confirmed by demonstrating the increased levels (10-fold) of lipid peroxides (2) and increased 4-

hydroxy-2-noneal (HNE)-modified protein adducts in nigral neurons of PD (3). Oxidative damage to DNA and proteins was also observed in SN of PD. For instance, the level of the DNA damage product 8-hydroxy-2'-deoxyguanosine (8-OHG) was selectively increased in SN of PD (4-5). The levels of a protein oxidation product, carbonyl protein, in PD were markedly increased in both the SN and other brain regions of PD (5). Studies of the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) animal model of PD showed significant pathological indications of OS in the SN region, such as increases in reactive oxygen species (ROS) and a lipid peroxidation indicator, MDA and a decrease in glutathione (GSH) content (6). Furthermore, MPTP-induced neurotoxicity was significantly attenuated by a free radical scavenger, pramipexole (7) and a free radical spin trap agent, MDL101002 (8). In another *in vivo* model of PD, 6-hydroxydopamine (6-OHDA) treatment also increased OS, manifested by increased MDA and decreased GSH, superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) (9-10). In the most recent cellular model of PD using rotenone, a selective inhibitor of mitochondrial complex I, oxidative damage was apparent (11). Rotenone-treated cells showed a loss of GSH and increased oxidative damage to DNA and protein (12).

Prior molecular analyses of OS-triggered DA cell death were largely based on a limited number of biochemical markers, such as ROS, oxidized protein and lipid, DNA fragmentation, activation of JNK, NF-κB and caspases, and morphological changes (13-16). Due to the rapid progress in functional genomics, the current focus of the biological analysis of cells and organisms is shifting from the investigation of individual events, to the complex and simultaneous evaluation of multiple systems and pathways (17-18). Analysis of gene expression profiles, particularly under various physiological conditions, can be performed on an unprecedented scale using cDNA microarrays and gene chips. These high throughput techniques can provide insights into underlying molecular pathology and begin to identify target genes for disease intervention. Until recently, the paucity of DA neurons and the dilution effect of non-DA cells present in both primary nigral cultures and midbrain tissues have hindered high throughput analysis of the pathological role of OS in nigral DA neurons. To overcome this technical hurdle, we have developed a DA cell line model, SN4741 (19), appropriate for cDNA microarray analysis (20-21). Our strategy for the identification of gene(s) underlying DA neuronal susceptibility to OS insult is as follows. First, temporal transcriptional regulation, especially down-regulation, by OS was determined using RNA slot blot analysis, employing the IMAGE clones as probes. Second, high

throughput *in situ* hybridization was performed to identify genes which were preferentially expressed in nigral DA neurons. Third, final confirmation studies were performed by either neuroprotection against OS via overexpression of full length cDNA or increased sensitivity test to OS by gene-specific knock-down. The present study will address these issues using the DA cell line model, SN4741.

Mitochondrial dysfunction was first implicated in PD because MPTP, whose metabolite MPP^+ inhibits mitochondrial complex I activity of the electron transport chain and is a substrate for the dopamine transporter, produced PD symptoms in drug abusers (22). Another highly selective complex I inhibitor rotenone caused a PD-like symptoms in rats (11). The inhibition of complex I appears to increase free radical production and oxidative stress and decrease ATP production. Thus, a biochemical link between the PD-related toxins and idiopathic PD was established when the decrease of complex I activity in SN and platelets of PD patients was discovered (23).

Mutations in DJ-1 are associated with autosomal recessive PD. DJ-1 is widely expressed in both brain and peripheral tisssues. Subcellular distribution of DJ-1 is primarily in cytoplasm with a small fraction associated with mitochondria (24). In particular the overexpressed DJ-1 is localized to mitochondria, suggesting its significant role in mitochondrial dysfunction. However, it is unknown how the functional loss of

DJ-1 results in the selective susceptibility of nigral DA neuron. To address this issue we have developed the mutant DJ-1 (-/-) DA cells, appropriate for a large scale preparation of mitochondria, genetic manipulation and proteomic analysis.

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