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Mitochondrial Dysfunction in Idiopathic Parkinson Disease

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Summary

Disordered mitochondrial metabolism may play an important role in a number of idiopathic neurodegenerative disorders. The question of mitochondrial dysfunction is particularly attractive in the case of idiopathic Parkinson disease (PD), since Vyas et al. recognized in the 1980s that the parkinsonism-inducing compound *N*-methyl-4-phenyl-1,2,3,6-tetrahydropyridine is a mitochondrial toxin. The unique genetic properties of mitochondria also make them worthy of consideration for a pathogenic role in PD, as well as in other late-onset, sporadic neurodegenerative disorders. Although affected persons occasionally do provide family histories that suggest Mendelian inheritance, the vast majority of the time these diseases appear sporadically. Because of unique features such as heteroplasmy, replicative segregation, and threshold effects, mitochondrial inheritance can allow for the apparent sporadic nature of these diseases.

Mitochondrial Electron-Transport–Chain Dysfunction in Parkinson Disease (PD)

Several investigators have described loss of electron-transport–chain activity in multiple tissues from individuals with PD. This biochemical defect has been seen in platelets (Parker et al. 1989*a;* Krige et al. 1992; Benecke et al. 1993; Haas et al. 1995), lymphocytes (Yoshino et al. 1992), brain (Schapira et al. 1989), muscle (Shoffner et al. 1991), and fibroblasts (Mytilineou et al. 1994). Immunoblot studies of brain tissues from individuals affected with PD have demonstrated disruption of NADH:ubiquinone oxidoreductase (complex I) subunits (Mizuno et al. 1989). Thus, PD is a systemic illness, but only certain cells, a group of neurons in the substantia nigra, are selectively vulnerable to its effects. The identification of this lesion in nontarget tissues, such as platelets, further suggests that it does not simply result from cell death consequent to PD. Also, although the magnitude of the complex I defect in PD is unclear (range 16%–71%, in published studies), even small perturbations of the electron-transport chain may carry pathogenic significance, since the effects of bioenergetic processes on cellular metabolism are protean.

Leber hereditary optic neuropathy (LHON) is a neurodegenerative disease with some parallels to PD. Various epidemiological, biochemical, and clinical similarities between these two diseases help validate the likely functional significance of the PD complex I defect. LHON, which may occur sporadically or in a matrilineal pattern typical of mitochondrial inheritance, results from point mutations in mitochondrial genes encoding complex I. The genetic defect in LHON and the resulting biochemical lesion are anatomically widespread, yet pathology is usually confined to the optic nerve and retina. Complex I catalytic dysfunction is demonstrable in LHON platelets and is comparable in magnitude to that seen in PD platelets (Parker et al. 1989*b*).

Studies of parkinsonism-inducing toxins, particularly *N*-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), are also relevant to the complex I defect in PD (Vyas et al. 1986). This compound is oxidized, by monoamine oxidase, to a lipophilic species, 1-methyl-4-phenyl pyridinium (MPP+), a complex I inhibitor that is concentrated in mitochondria. It is unclear whether the anatomic specificity seen in MPTP toxicity reflects specific concentration within nigral neurons with dopamine-uptake sites (Javitch et al. 1985) or whether this specificity reflects an intrinsic vulnerability of nigral neurons to this type of complex I inhibition.

Neuroleptic medications used to treat psychiatric and other disorders represent another class of agent that can induce parkinsonism in humans. Burkhardt et al. (1993) studied the effects of representative compounds from several classes of neuroleptic drugs and found that they tend to be very potent inhibitors of rat-brain complex I activity. Interestingly, the propensity of these agents to cause extrapyramidal symptoms generally correlates

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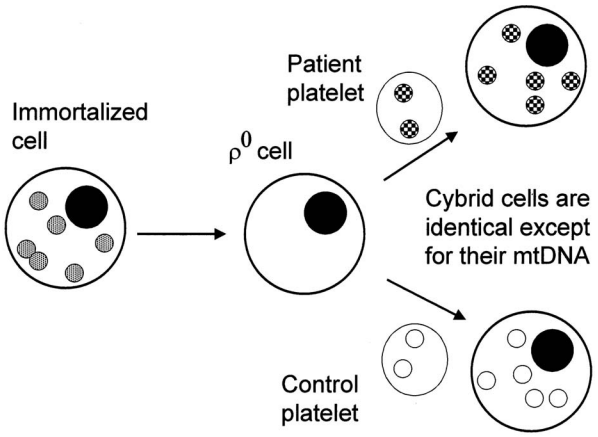

Figure 1 Cybrid technique. Immortalized cells are depleted of their endogenous mtDNA, via long-term ethidium bromide exposure, to form ρ^0 cells. In one variation of the technique, platelets (which have mitochondria and mtDNA but no nucleus or nuclear DNA) are fused with ρ^0 cells to form cytoplasmic hybrids ("cybrids"). This strategy allows for control of nuclear genetic and environmental input, which is the same in the cybrid lines thus generated. After many cycles of cell and mitochondrial replication, cybrid lines are assayed for phenotypic differences. Phenotypic differences between cell lines suggest that their mtDNA is not identical.

with their potency as complex I inhibitors. Studies of platelet complex I activity in patients taking clinically relevant doses of these agents demonstrated a loss of complex I activity comparable to that seen in PD platelet mitochondria. These factors provide a direct link between complex I dysfunction and PD.

Origin of Complex I Dysfunction in PD

Demonstrations of a loss of complex I activity in PD beg the question of etiology. Many attempts have been made to link PD to environmental factors, because of PD's generally non-Mendelian pattern of occurrence. The discovery of MPTP, an environmental agent capable of causing a PD-like syndrome, gave credence to the concept that environmental factors could represent a common etiologic cause for this disease. In a few instances—PD in manganese miners, for example—the illness can clearly be linked to an environmental toxin. However, identification of a specific environmental agent responsible for the majority of PD cases remains elusive. The demonstration of persistent complex I deficiency in many PD patients further suggests that, if an environmental toxin does indeed act to produce complex I dysfunction, either it is extraordinarily long-lasting or exposure is ongoing. The demonstration of a mitochondrial oxidative defect in cells replicating in culture also argues against a toxic etiology, since, in a rapidly expanding population of cultured cells, a toxin should be tremendously diluted and its effects lost (Mytilineou et al. 1994).

A genetic basis for the complex I–activity deficiency in PD is consistent both with the tissue-culture data and with the electron-transport phenotype in multiple tissues. Complex I contains >50 subunits and multiple prosthetic groups. The majority are encoded by nuclear genes, but typical Mendelian inheritance patterns are rarely observed, and there is a high degree of discordance among MZ twins (Ward et al. 1983; Marsden 1987; Marttila et al. 1988*a;* Tanner et al. 1997). Clinical experience therefore is inconsistent with causative mutations in any of the nuclear complex I genes. Seven of the subunits of complex I, however, are encoded by the mitochondrial genome. A complex I lesion resulting from mutation(s) in these genes might produce a more sporadic occurrence pattern, as is typical for this disease.

A direct approach to the question of mitochondrial gene involvement in the pathogenesis of PD was made through mitochondrial gene-transfer experiments. In these experiments, a culturable human cell line (SH-SY5Y neuroblastoma) was depleted of mtDNA by prolonged culture in the presence of ethidium bromide, which intercalates into mtDNA and interferes with mtDNA replication. After 3–4 mo, cells lose their mtDNA, and this is associated with loss of respiratory competence. The resulting cells, termed "ρ^0," become dependent on pyruvate and uridine for survival. mtDNA can be reintroduced via polyethylene glycol–mediated fusion of ρ^0 cells with either control or PD platelets that contain mtDNA, but no nuclear DNA, and that express the PD complex I defect (Parker et al. 1989*a*). The resulting cytoplasmic hybrid ("cybrid") thus contains mtDNA derived from either a control or a PD subject. Transformed cybrid cells propagate even when deprived of pyruvate and uridine. The effects of the exogenously derived mtDNA contained within a cybrid line can then be assessed via a variety of chemical and physical techniques (Miller et al. 1996). A schematic depicting cybrid methodology is shown in figure 1.

We used this strategy to investigate the origin of the PD-associated complex I defect. We compared PD cybrids to age-matched control cybrids and found a highly significant loss of complex I activity (Swerdlow et al. 1996). In these experiments, the decrement in complex I activity observed in PD cybrids relative to control cybrids was smaller than that seen in direct studies of PD-patient tissues. This probably reflects a tendency for cells that carry a lesser burden of defective mtDNA to grow faster and, thus, to be overrepresented in the culture; cybrid studies may therefore underestimate the magnitude of the defect in vivo. Alternatively, this finding could indicate that the complex I defect of PD arises from multiple origins that may include nuclear and/or environmental contributions. Similar studies were carried

out with cybrids for Alzheimer disease (AD), another sporadic neurodegenerative disorder, in which a different electron-transport–chain complex, cytochrome c oxidase (complex IV), is defective (Parker et al. 1990; Kish et al. 1992). These studies showed a loss of complex IV activity, as occurs in vivo in AD. They also confirm that the findings in PD cybrids are relatively specific and are not simply representative of a general neurodegenerative disease–associated phenomenon (Swerdlow et al. 1997*a*). We also found that PD cybrids were sensitized to the MPTP-derived toxic metabolite MPP^+ in that they demonstrated (relative to control cybrids) an increased tendency to undergo apoptotic cell death in the presence of low concentrations of the toxin. This finding again illustrates the functional relevance of the transferred, mtDNA-derived complex I lesion, and it illustrates how a genetic abnormality might interact synergistically with some agent in the environment to cause the disease phenotype.

Pathogenicity of Complex I Dysfunction in PD

Further studies of PD cybrids indicate that the mtDNA-transferable complex I lesion is sufficient to produce pathogenic changes at the cellular level. Multiple studies of PD tissues suggest that oxidative stress is present in this disease, but the origin of this finding is not clear (Beal 1995). Bioenergetic dysfunction could represent a source for reactive oxygen–species (ROS) generation; indeed, mitochondria are believed to constitute a major site of ROS production (Beal 1995). Excess production of ROS in PD cybrids is readily demonstrable when they are studied in the presence of the fluorescent ROS probe, dichlorofluorescein diacetate, which is trapped in cells after cleavage of ester moieties. The resulting dichlorofluorescein emits a characteristic fluorescence when attacked by ROS. The mtDNA-derived complex I defect may thus be the source of oxidative stress in PD. In further support of this possibility, Cassarino et al. (1997) found that several ROS-metabolizing enzymes are upregulated in PD cybrids, as they are in PD brain tissue (Marttila et al. 1988*b;* Saggu et al. 1989; Kalra et al. 1992; Damier et al. 1993).

Sheehan et al. (1997*b*) investigated calcium metabolism in PD cybrids and found that, after carbachol-induced generation of calcium transients, recovery of cytosolic calcium was prolonged in PD cybrids compared with control cybrids. Failure of calcium buffering after receptor-mediated stimulation has clear implications for in vivo excitotoxicity where elevated calcium levels are cytotoxic. This set of experiments also demonstrated that, although basal cytosolic calcium concentrations in PD and in control cybrids were similar, calcium release into the cytosolic compartment was decreased in PD cybrids after mitochondrial uncoupling, consistent with a loss of mitochondrial calcium sequestration in PD cybrids. These findings can be contrasted with similar studies in AD cybrids, which, again, indicates the relative specificity of the findings (Sheehan et al. 1997*a*).

Work from Schapira's laboratory, using an unrelated ρ^0 system, has recently confirmed the presence of a complex I lesion in PD cybrids (Gu et al. 1997). As a whole, these studies indicate that there is a functionally relevant abnormality in PD mtDNA. The phenotype of PD cybrids is also consistent with steadily mounting evidence that defective mitochondria are capable of initiating apoptosis (Kroemer et al. 1997). mtDNA-derived bioenergetic dysfunction may represent a relevant link in the pathogenesis of PD and other neurodegenerative disorders, including AD, that manifest mitochondrial dysfunction.

Support for Inheritable mtDNA Mutation in PD

A pathogenic role for mtDNA in PD raises the possibility that, in some families, PD may be transmitted in a typical matrilineal pattern such as that seen with LHON. Indeed, many kindreds with multiple PD-affected members and only maternal inheritance are described in the literature. Rarely, such kindreds are offered as evidence of mitochondrial inheritance (Wooten et al. 1997*a*), although it is often felt that such kindreds indicate autosomal dominance (Young et al. 1977; Morrison et al. 1996; Lazzarini et al. 1994). Unless a PD kindred is large enough to lend itself to Bayesian analysis, however, it is difficult to discern autosomal dominance from maternal inheritance when intergenerational transmission is solely matrilineal. Current evidence suggests that PD may arise from either Mendelian (Polymeropoulos et al. 1997) or mitochondrial genetic (Swerdlow et al. 1996; Gu et al. 1997) mechanisms. Only a few PD kindreds are extensive enough to suggest one or the other mode of inheritance. Most cases present sporadically, and no clear family history is discernible.

We suggest that mtDNA mutations are responsible for many of these sporadic cases of PD. We recently used cybrids to study the possibility of heritable mtDNA mutation in a multigenerational PD kindred in which intergenerational transmission of PD is exclusively maternal (Wooten et al. 1997*a*). Cybrids were made with mtDNA from 15 family members (Swerdlow et al. 1997*b*). Complex I activity was lower, and free-radical production was higher, in cybrids that contained mtDNA from persons descended through maternal (vs. paternal) lines. Low complex I activity and increased oxidative stress were apparent, even in young maternal descendants who were currently asymptomatic (but presumably at risk) for PD. These data suggest that heritable mtDNA mutation could contribute to PD in this family and that mitochondrial abnormalities are detectable well in advance of onset of symptoms.

Recent epidemiological data also support a role for

inherited mtDNA mutation in PD (Wooten et al. 1997*b*). One large, prospectively constructed PD patient database revealed that 32 of 265 PD probands had an affected parent. Although more mothers than fathers were affected, no statistically significant parental-gender predominance was demonstrated. However, of the 32 PD probands with an affected parent, 5 also had an affected sibling, and in each of these pedigrees, the affected parent was the mother; this finding provides statistically significant support for maternal inheritance. This resembles the pattern seen in studies of sporadic AD, performed with a similar strategy (Edland et al. 1996).

Attempts to examine PD mtDNA for the presence of specific disease-related sequence changes have been inconclusive. Initial screening for mtDNA deletions was mostly unrevealing, but RFLP analyses disclosed several mtDNA mutations that occurred more frequently in PD subjects than in unaffected controls (Shoffner et al. 1993; Kosel et al. 1996). In one study using RFLP techniques, screens for just two mtDNA mutations revealed that ~25% of PD patients carried mutated mtDNA (Kosel et al. 1996). In one PD mtDNA sequencing study, mutations were found in all ($n = 5$) subjects analyzed (Ikebe et al. 1995). Issues of heteroplasmy enormously complicate studies of mtDNA, however, and a definitive study of mtDNA in PD has yet to be performed.

Conclusions

The original neuropathological descriptions of PD emphasized the presence of eosinophilic cytoplasmic inclusions, called "Lewy bodies," in the pars compacta of the substantia nigra. Now, almost 2 centuries after the initial clinical description of this disease entity, by James Parkinson in 1817, we are still attempting to define their origin. Recent evidence now suggests that Lewy bodies may in fact represent degenerating mitochondria (Gai et al. 1997). Whether inherited or acquired, primary or secondary, mitochondrial dysfunction is likely to be found to be of great pathogenic significance in this disease.

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