Review

The mitochondrial impairment, oxidative stress and neurodegeneration connection: reality or just an attractive hypothesis?

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Aging is the most important risk factor for common neurodegenerative disorders such as Parkinson's and Alzheimer's diseases. Aging in the central nervous system has been associated with elevated mutation load in mitochondrial DNA, defects in mitochondrial respiration and increased oxidative damage. These observations support a 'vicious cycle' theory which states that there is a feedback mechanism connecting these events in aging and age-associated neurodegeneration. Despite being an extremely attractive hypothesis, the bulk of the evidence supporting the mitochondrial vicious cycle model comes from pharmacological experiments in which the modes of mitochondrial enzyme inhibition are far from those observed in real life. Furthermore, recent *in vivo* evidence does not support this model. In this review, we focus on the relationship among the components of the putative vicious cycle, with particular emphasis on the role of mitochondrial defects on oxidative stress.

Mitochondrial respiratory chain and reactive oxygen species production

Mitochondria, being the key players in ATP production and diverse cell signaling events, are essential organelles for the survival of eukaryotic cells. Unlike all other organelles in animals, the mitochondria have their own genome (mitochondrial DNA; mtDNA) that encodes components of the oxidative phosphorylation (OXPHOS) system. The mitochondrial OXPHOS machinery is composed of five multisubunit complexes (complex I–V). From Krebs cycle intermediates (NADH and $FADH_2$), electrons feed into complex I or II, and are transferred to complex III, then to complex IV, and finally to O_2. The redox energy released during the electron transfer process in complexes I, III and IV is utilized to actively pump out H^+ from the mitochondrial matrix to the intermembrane space, generating the electrochemical gradient of H^+ across the inner membrane which is ultimately utilized by complex V to produce ATP [1].

This elegant system for energy production, however, is not perfect. A small portion (up to 2%) of electrons passing through the electron transport chain, mostly at complex I and complex III, react with molecular oxygen and yield superoxide anion, which can be converted into other reactive oxygen species (ROS) such as hydrogen peroxide and the highly reactive hydroxyl radical through enzymatic and nonenzymatic reactions [2]. Cells are endowed with robust endogenous antioxidant systems to counteract excessive ROS. It is believed that ROS, in particular hydrogen peroxide, have physiological roles as signaling molecules [3,4]. However, when ROS production overwhelms the endogenous antioxidant systems, they can potentially damage various types of macromolecules, including proteins, lipids and nucleic acids. These damages are collectively referred to as 'oxidative stress,' and have been implicated in aging and various pathological processes. The mitochondrial 'vicious cycle' theory of aging states that ROS, generated from OXPHOS, induces mutations in the mtDNA, which in turn leads to OXPHOS dysfunction (Figure 1) [2,5]. The impaired OXPHOS function would lead to further production of ROS, which further exacerbates mtDNA mutations.

In this review, we discuss recent progress and some surprising new data that raise important questions regarding the mitochondrial vicious cycle and its contribution to aging and major neurodegenerative conditions, with emphasis on Alzheimer's disease (AD) and Parkinson's disease (PD). The main question we address here is whether OXPHOS defects are responsible for an increased oxidative stress *in vivo*.

Mitochondrial DNA mutations and oxidative stress in the aging brain and in neurodegenerative diseases

ROS-mediated formation of mutated mtDNA (large-scale deletions and point mutations) has been implicated in physiological senescence and age-related disorders such as sporadic neurodegenerative disorders, type II diabetes, cancer and cardiac diseases (reviewed in Refs [2,6]). Indeed, mtDNA with large deletions has been reported to accumulate with aging in various tissues of various mammalian species (reviewed by Kujoth *et al.* [7]). Although these observations do not address whether the accumulation of mutated mtDNA has a causal role in aging, they suggest that mutated mtDNA serves as a useful biomarker of aging regardless of the lifespan of

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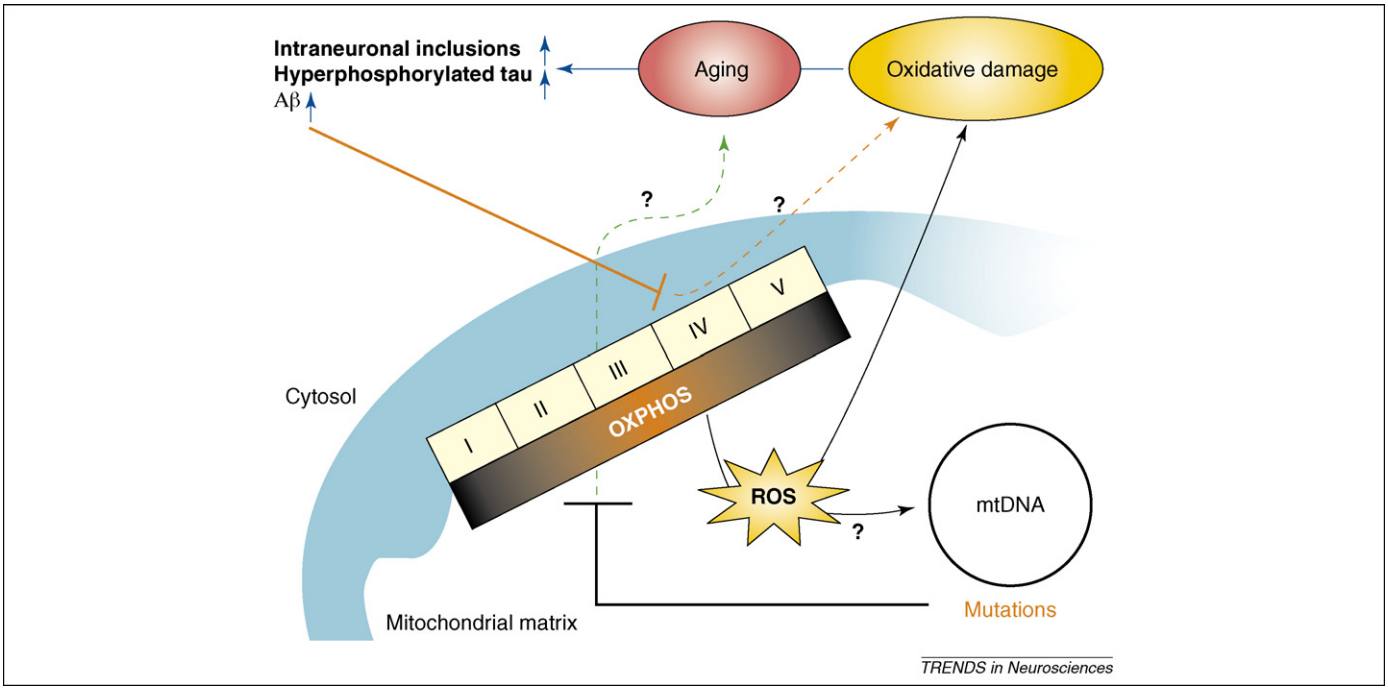

Figure 1. OXPHOS–oxidative stress–neurodegeneration connections. OXPHOS activity produces ROS, which could induce mutations in the mtDNA. In turn, mtDNA mutations can lead to the inhibition of OXPHOS, which can increase the production of ROS. The 'vicious cycle' theory of aging suggests that an exponential increase in ROS production and oxidative damage mediated by these interactions could be a strong contributor to age-associated neurodegenerative diseases. However, *in vivo* evidence for a causative relationship between these players has not been provided. Defects in OXPHOS can contribute to aging in an oxidative stress-independent manner, and the latter could be a marker of senescence. In addition, misfolded proteins, such as Aβ, were also shown to impair OXPHOS and possibly other metabolic systems.

specific organisms. The mammalian central nervous system (CNS) is not an exception. Several reports have presented clear age-dependent increases in the amount of deleted mtDNA in the brains of rodents and human [7]. Although in these reports the fraction of deleted mtDNA was estimated to be very low (less than 1%), recent studies that employed single-cell dissection in combination with quantitative real-time PCR showed that, in the human substantia nigra neurons of elderly subjects, deleted mutant mtDNA species accumulate to up to ~45% of total mtDNA [8,9]. This mutated:wild-type mtDNA ratio tended to be higher in PD patients [9], raising the intriguing possibility that stochastic age-associated elevation of mtDNA mutations could be responsible for sporadic neurodegeneration in nigral neurons. It appears that the extensive accumulation of deleted mtDNA is unique to the nigral neurons and not as pronounced in other regions or even in other cell types in the aging CNS. Kraytsberg *et al.* observed a heterogeneous staining pattern in aged substantia nigra neurons after *in situ* cytochrome oxidase (COX) activity staining, and noticed that COX-negative neurons contain a higher proportion of deleted mtDNA than COX-positive neurons [8]. The nature of mutations was apparently somatic (not inherited), because individual neurons contained unique types of deleted mtDNA. Although earlier studies were unable to demonstrate the correlation between the accumulation of deleted mtDNA and AD by analyzing human cortical tissues [10,11], there could be other 'regional hotspots' where deleted mtDNA preferentially accumulates and contributes to neuronal death in AD. It remains to be determined whether the COX deficiency correlates to oxidative stress in individual brain cells in any of these conditions.

Contribution of mutated mitochondrial DNA to aging and oxidative stress in mice

The 'mutator mice,' expressing a proofreading-deficient mitochondrial DNA polymerase (POLG), accumulate mtDNA mutations in an age-dependent manner and develop many features of premature aging [12–14]. Murine embryonic fibroblasts prepared from the mutator mice exhibited defects in OXPHOS function but, surprisingly, those cells as well as tissues from adult mice did not exhibit increased ROS production or oxidative damage. Although the generation of ROS depends on the specific defect in OXPHOS components and residual activities, the random nature of mutations in these mice would be expected to create such defects. The lack of oxidative stress raises questions of whether naturally accumulating mtDNA mutations are responsible for increased oxidative damage [15]. These results imply that mtDNA mutations could accelerate aging, even without the involvement of oxidative stress.

Contribution of ROS to mutations in mitochondrial DNA

Mounting evidence suggests that oxidative damage increases in the mammalian brain during aging [16]. It is generally assumed that mitochondria-driven ROS induces mutations in mtDNA. Recent evidence suggests that double-strand breaks might be the main mediators of mtDNA deletions. Work from our laboratory showed that double-strand breaks in mtDNA induce the formation of large deletions in mouse muscle [17] and brain (H.F. and C.T.M., unpublished), suggesting that this could be the main mechanism accounting for the generation of age-associated deletions in mtDNA. This mechanism has also