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Oxidative Stress, Mitochondria and mtDNA-mutator Mice

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Abstract

The oxidative stress theory of aging, an expansion of the mitochondrial theory of aging, is based around the idea of a vicious cycle, in which somatic mutations of mitochondrial DNA (mtDNA) provoke respiratory chain dysfunction leading to enhanced ROS production and in turn to the accumulation of further mtDNA mutations. Mitochondrial dysfunction and mtDNA mutations are amplified during the course of aging. Recently, results obtained from mtDNA-mutator mice further strengthen the role of mitochondria in the aging process. However, lack of increased oxidative stress in the mtDNA-mutator mice raises doubts in the direct connection of mtDNA mutations with increased ROS production, challenging the oxidative stress theory of aging. The purpose of this short review is to highlight several studies that provide direct evidence that accelerated aging is linked to mtDNA mutations, without an increase in oxidative damage.

The Oxidative Stress Theory of Aging

The ‘free radical theory’ of aging, formulated 50 years ago by Harman, proposes that aging and associated degenerative diseases can be attributed to deleterious effects of reactive oxygen species (Harman, 1956). This hypothesis has been extensively investigated and debated, but has not yet been clearly validated. Prior to 2004, most investigations took the approach of altering cellular antioxidant capacity and examining the effects on aging-related phenotypes. This approach produced positive results, suggesting that damage from free radicals play some role in aging (Landis & Tower, 2005). A current version of this theory is the ‘oxidative stress theory’ of aging. The ‘oxidative stress theory’ states that “A chronic state of oxidative stress exists in cells of aerobic organisms even under normal physiological conditions because of an imbalance of proxidants and antioxidants. This imbalance results in a steady-state accumulation of oxidative damage in a variety of macromolecules. Oxidative damage increases during aging, which results in a progressive loss in the functional efficiency of various cellular processes.” (Sohal & Weindruch, 1996)

Although reactive oxygen species production is a significant component of the proposed ‘oxidative stress theory’, this theory is consistent with and expands the ‘mitochondrial theory’ of aging. The main tenet of the ‘mitochondrial theory’ predicts that a ‘vicious cycle’ within the mitochondria contributes to the aging process. Briefly, components of the vicious cycle are: (1) normal metabolism causes reactive oxygen species production by the electron transport chain; (2) reactive oxygen species production induces damage to phospholipids, proteins, and nucleic acids in mitochondria; (3) reactive oxygen species-induced mitochondrial DNA

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(mtDNA) mutations lead to the synthesis of functionally impaired respiratory chain subunits, causing respiratory chain dysfunction and augmented reactive oxygen species production; and (4) the impact of the vicious cycle causes an exponential increase of mtDNA mutations over time, resulting in aging and associated degenerative diseases. A substantial amount of correlative data from morphological, bioenergetic, biochemical, and genetic studies of mammalian tissues supports this theory (Harper *et al.*, 2004; Balaban *et al.*, 2005). For example, the mitochondria are larger and fewer in number. The mitochondria have a greater frequency of abnormalities such as vacuoles, abnormal cristae, and paracrystalline inclusions.

As noted above, most of the available data are correlative and therefore do not exclude the possibility the mitochondrial damage and reactive oxygen species production are consequences rather than driving forces of aging. Recently, mouse models provide direct support for the involvement of mitochondria in the aging process, however, the contribution of the "vicious cycle" in the aging process is still debatable. In the past two years, two groups constructed mice containing a point mutation in the proof-reading domain of DNA polymerase-γ (POLG), the catalytic subunit of mtDNA polymerase (Trifunovic *et al.*, 2004; Kujoth *et al.*, 2005). The recombinant mtDNA polymerase holoenzyme has a significant reduction of exonuclease activity with no decrease in DNA polymerase activity. These "mtDNA-mutator" mice display a progressive and random accumulation of mtDNA point mutations. The mutations are uniform between tissues, suggesting that much of the mutation accumulation occurs during embryonic and /or fetal development. The mutation load is great at embryonic day 13.5 (7.8±0.4 mut/10 kb compared to 1.5±0.9 mut/10 kb in wildtype embryos) (Trifunovic *et al.*, 2005).

Phenotype of mtDNA-mutator Mice

These "mtDNA-mutator" mice display phenotypes resembling many characteristics of the aging process (accelerated aging). The life spans are reduced such that the median life span of the mtDNA-mutator mice is approximately 46–59 weeks and survive to the age of 61–66 weeks (Trifunovic *et al.*, 2004; Kujoth *et al.*, 2005). Although the mtDNA-mutator mice are born in normal Mendelian proportion and have a normal appearance at birth and during early adolescence (until the age of 25 weeks), a mild kyphosis (curvation of the spine, sign of osteoporosis) and hair loss occur. As the animals get older they show characteristics of aging such as weight loss, reduced subcutaneous fat, hair graying and alopecia, kyphosis, decreased bone density, anemia with progressive decrease in circulating red blood cells, reduced fertility, cardiac enlargement with functional alterations, muscle loss, accelerated thymic involution and presbycusis (age-related hearing loss).

MtDNA-mutator and the Oxidative Stress Theory of Aging

The mtDNA-mutator creates a random set of point mutations in genes for the respiratory chain subunits. The oxidative stress theory of aging predicts that levels of mtDNA mutations should increase exponentially as a consequence of a vicious cycle by accelerating oxidative stress. Surprisingly, point mutations in mtDNA-mutator mice accumulate in an approximately linear fashion, from midgestation to late adult life (Trifunovic *et al.*, 2005).

Interestingly, in spite of the widespread mtDNA mutations, these mice do not appear to show signs of increased oxidative damage to proteins, lipids, or DNA (Kujoth *et al.*, 2005; Trifunovic *et al.*, 2005). Several markers of oxidative stress have been determined in these mtDNA-mutator mice. Specifically, mitochondrial levels of hydrogen peroxide, produced when superoxide is enzymatically dismutated, are not elevated. Oxidative damage to mitochondrial proteins, determined by levels of protein carbonyls, is not significantly increased. Lipid peroxidation, determined by the amount of F2-isoprostanes, and oxidative damage to nuclei acids, determined by levels of 8-oxodeoxyguanosine (DNA) or 8-oxoguanosine (RNA), are not elevated. In addition, expression levels of antioxidant defense enzymes and aconitase

enzyme activity measurements indicate the absence of or only minor oxidative stress in tissues from mtDNA-mutator mice. Thus, increased levels of mtDNA mutations are not associated with increased ROS production or increased oxidative stress in mtDNA mutator mice. The profound aging phenotypes generated by accumulation of somatic mtDNA mutations are thus not mediated by dramatically increased ROS production. Collectively, these findings imply that there is *no vicious cycle* leading to increased oxidative damage.

Apoptosis and mtDNA-mutator Mice

Although accumulation of mtDNA mutations is not associated with increased markers of oxidative stress, there is a strong correlation with the induction of apoptosis in target tissues. Many tissues in the mtDNA-mutator mice contain increased levels of caspase-3, a downstream protease activated during many apoptotic pathways (Kujoth *et al.*, 2005). Foremost, an increase in caspase-3 activation is observed in tissues from old wild-type mice, demonstrating a phenomenon that occurs in aging rodents. In contrast, mitotic tissues of the mtDNA mutator mice (thymus, intestine, testis) have elevated levels of activated caspase-3 as early as 3 months of age. Postmitotic tissues, like skeletal muscle, also display increased activated caspase-3 but at a later stage, coinciding with tissue degeneration. Several tissues of the mtDNA-mutator mice (thymus, testes, intestine) also exhibit increased TUNEL staining, an indication of the DNA fragmentation that is a hallmark of apoptosis (Kujoth *et al.*, 2005). Thus, it is possible that tissue dysfunction in this model arises from increased apoptosis. In other words, the profound aging phenotype in these mice may be due to widespread induction of apoptosis, a phenomenon that could rapidly deplete dividing tissues of critical stem cells.

Summary

These findings indicate that the profound aging phenotypes in mtDNA-mutator mice are not generated by a “vicious cycle” of massively increased oxidative stress accompanied by exponential accumulation of mtDNA mutations. The results argue against any direct role of oxidative stress in the aging process. With that being said, mtDNA-mutator mice do exhibit respiratory chain deficiency. Respiratory chain dysfunction may accelerate aging by stimulating cellular processes such as bioenergy deficiency, alterations in signal threshold for cell death, and causing replicative senescence in stem cell. Thus the debate of oxidative stress and aging will continue.

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