Mitochondrial DNA Mutations and Apoptosis in Mammalian Aging

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Abstract

Mutations in mitochondrial DNA (mtDNA) accumulate during aging, but their significance to longevity and age-associated disease has been uncertain. Recently, in support of the hypothesis that mtDNA integrity is important, we have shown that age-associated diseases arise more rapidly in mice where mtDNA mutations and increased levels of apoptosis occur at higher rates than normal due to expression of an error-prone mtDNA polymerase. Further studies in this model may provide deeper insights into the relationship between mitochondria, aging, and susceptibility to age-associated diseases, such as cancer. (Cancer Res 2006; 66(15): 7386-9)

Mitochondrial DNA Mutations and Aging

Mitochondria contain their own ~16.5-kb circular DNA encoding essential subunits of the electron transport chain complexes as well as the tRNAs and rRNAs necessary for their translation. Because of the high gene density of the mitochondrial genome, mutations in mitochondrial DNA (mtDNA) have a high likelihood of affecting the expression or coding sequence of mitochondrial genes. This susceptibility to deleterious mutations is offset to an extent by the presence of multiple copies of the mitochondrial genome in every cell.

Point mutations and deletions in mtDNA accumulate with age in a wide variety of tissues in multiple species (1–7), and there is evidence to suggest that there can be functional consequences with respect to mitochondrial bioenergetics. Loss of cytochrome *c* oxidase activity has been correlated with the presence of mtDNA deletions in serial sections of aged muscle fibers (8), and clonal expansion of cytochrome *c* oxidase–deficient cells bearing pathogenic mtDNA point mutations in aged intestinal crypts has been reported (9). Until recently, however, the association between mtDNA mutations and aging has remained correlative. We (10) and others (11) have now addressed this issue by generating mice that display elevated mtDNA mutation frequencies throughout their tissues [3-11 times higher than wild-type (WT) mice] due to error-prone replication by an exonuclease-deficient derivative of the nuclear-encoded mtDNA polymerase γ. These mitochondrial mutator (*Polg*^{D257A}, herein called D257A) mice display a large array of phenotypes resembling aspects of accelerated aging; these include reduced life span, hair graying and alopecia, cardiac enlargement with functional alterations, muscle loss, reduced fertility, accelerated thymic involution, kyphosis, and decreased bone density, and age-related hearing loss (presbycusis). Additionally, these mice become anemic and display loss of intestinal crypts or disorganized villar structures. The accumulation of mtDNA mutations in D257A mice begins early in development (12), in contrast to observations in human tissues, where substantially increased frequencies of mtDNA mutations do not occur until after the 4th or 5th decade of life (2, 4, 7). In young adult WT mice, we have observed that levels of mtDNA mutations are quite high, averaging 3 to 10 mutations per mtDNA genome in tissues from 5- to 6-month-old mice. Thus, the accumulation of mtDNA damage in mice may begin at a relatively early age. Inducible and tissue-specific conditional alleles of *Polg*^{D257A} will be required to definitively address the effects of adult-onset accumulation of mtDNA mutations on the aging process and to distinguish primary defects from secondary indirect systemic phenotypes. Nevertheless, it is now clear that accumulation of mtDNA mutations can have direct phenotypic consequences, although the mechanism by which this occurs remains to be fully elucidated.

Oxidative Stress or Cell Death?

The free radical theory of aging postulates that reactive oxygen species (ROS) generated during mitochondrial respiration can lead to mtDNA mutations, among other targets, and impairment of normal electron transport. Disruption of electron transport could shuttle upstream electrons into superoxide production leading to elevated oxidative stress. We therefore examined adult D257A mice for markers of oxidative stress (10). Mitochondrial levels of hydrogen peroxide, produced when superoxide is enzymatically dismutated, were not elevated in D257A mice. Oxidative damage to mitochondrial proteins, as measured by levels of protein carbonyls, was not significantly different between WT and D257A mice. Similarly, lipid peroxidation, measured as the amount of F_2-isoprostanes, and oxidative damage to nucleic acids, determined by levels of 8-oxodeoxyguanosine (DNA) or 8-oxoguanosine (RNA), were also not elevated in the D257A mice. Thus, we found no evidence that high levels of mtDNA mutations necessarily increase generation of ROS. In fact, direct measurement of free radical leak at complexes I and III in D257A mice also shows that ROS production is not elevated at these sites in the mitochondria.[3] These observations are supported by similar recent data (12) and are also consistent with a lack of oxidative damage in a transgenic mouse model with heart-specific elevation of mtDNA mutations (13). Of course, the absence of increased steady-state levels of oxidative stress functioning downstream of a high mitochondrial mutational load does not preclude a role for oxidative damage in the generation of mtDNA mutations during natural aging. The mtDNA mutational spectrum in young adult WT mice is predominantly (75-100%) transitions (10, 11), however, as opposed to transversions typically associated with oxidatively damaged DNA. Furthermore, recent work suggests that polymerase-mediated replication errors may be the primary source of mtDNA mutations

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in human tissues (14). In any event, our studies have shown that persistent oxidative stress is apparently not required for the downstream effects of mitochondrial mutations.

Because many of the phenotypes of the D257A mice may be explained by loss of specific cell types in the affected tissues, we examined the extent of programmed cell death in this model (10). In cells committed to apoptosis, caspase-3 is activated by proteolytic cleavage. Comparison of heart, liver, muscle, and testes from young and old WT mice revealed increases in the amount of cleaved caspase-3 in all of the old tissues examined, indicating that activation of apoptosis is a normal component of aging. D257A mice showed a premature activation of apoptosis in many tissues, including liver, intestine, thymus, testes, and muscle. Elevated amounts of cleaved caspase-3 were present as early as 3 months of age in all of these tissues, except for skeletal muscle, which is postmitotic and where increases were observed after 9 months. Detection of DNA fragmentation during apoptosis via the terminal deoxynucleotidyl transferase–mediated dUTP nick end labeling assay confirmed the increase in apoptosis in thymus, testes, and intestine of 3-month-old D257A mice. These data indicate that the mitochondrial mutator mice bear the hallmarks of accelerated aging, at least in the context of apoptotic cell death. Importantly, in most of these tissues, apoptosis is occurring before the onset of histologic pathology, consistent with a key role for cell death in the phenotypes of the D257A mice.

An alternate model to explain the phenotypes of the D257A mice would involve a defect in cellular proliferation. Growth of mouse embryo fibroblasts isolated from D257A mice was no different from that of WT fibroblasts, regardless of whether cells were cultured under normoxic or hypoxic conditions (10). Thus, we feel that an intrinsic defect in cellular proliferation is not likely to be responsible for the aging phenotypes of the D257A mice.

Our data support a model in which loss of critical irreplaceable cells occurs through apoptosis, leading to tissue dysfunction (Fig. 1). We postulate that depletion of the stem cell compartment compromises tissue regeneration with age and that this process is greatly accelerated in D257A mice. How might mtDNA mutations trigger the apoptotic process? Mitochondrial bioenergetics seem to be compromised in the D257A mice (11, 12). We are currently investigating whether impaired electron transport and the resulting alteration of the electrochemical gradient could trigger the mitochondrial permeability transition, releasing apoptotic-promoting factors, such as cytochrome *c*, apoptosis-inducing factor, endonuclease G, etc. Indeed, cyclosporin A-mediated inhibition of permeability transition pore opening was successful in preventing cardiomyopathy in the heart-specific

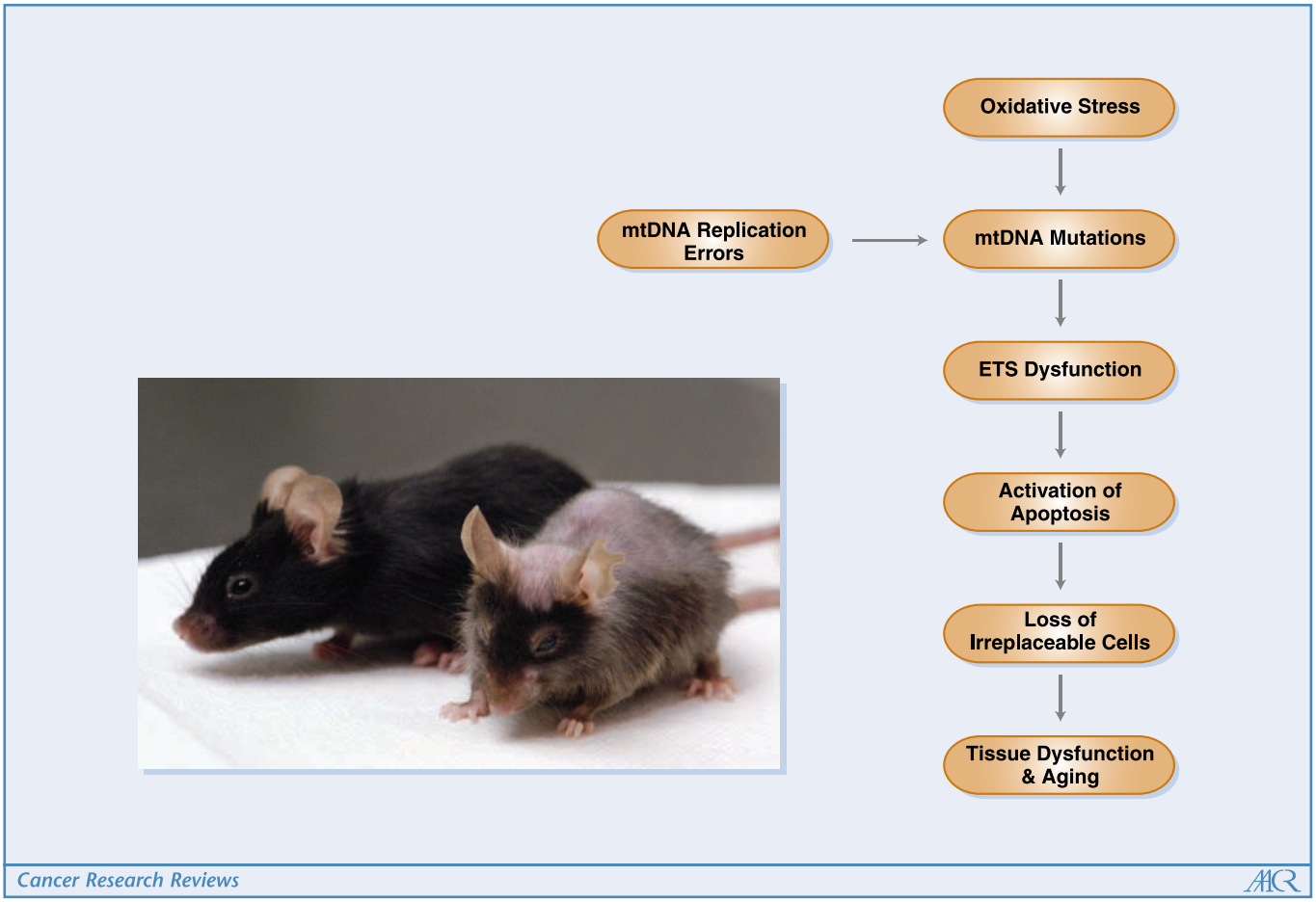

Figure 1. mtDNA mutations accumulate in natural aging potentially as a result of free radical-induced oxidative damage or nucleotide misincorporation during replication, the latter being particularly important in the D257A mice. Extensive mtDNA mutational loads result in electron transport (*ETS*) alterations. Such mitochondrial dysfunction is associated with increased activation of apoptosis, which we hypothesize depletes tissues of critical stem cell populations, hindering tissue regeneration and resulting in the accelerated aging phenotypes displayed by the D257A mice. *Inset,* similarly aged WT (*left*) and D257A (*right*) mice. Photo credit, Jeff Miller, University of Wisconsin-Madison, Madison, WI.

mitochondrial mutator model (15). Several genes are thought to regulate mitochondrial-mediated apoptosis, including *Bax* and *Bak*, proapoptotic members of the *Bcl-2* family, and $p66^{shc}$, a critical downstream effector of *p53*-dependent apoptosis (16). Examining the effects of these apoptotic modulators on the aging phenotypes of the D257A mice should help to establish whether apoptosis is required for the downstream effects of mitochondrial mutations.

If mtDNA mutations do not lead to increased ROS damage in D257A mice, how do we integrate these results into the field of oxidative stress in aging? The rate of mitochondrial ROS production, extent of mtDNA (but not nuclear DNA) oxidative damage, and degree of membrane fatty acid unsaturation (a determinant of vulnerability to lipid peroxidation) are all inversely correlated with longevity across species (17–20). Most of these variables, apart from fatty acid unsaturation, are reversed by caloric restriction (21). Mice expressing mitochondrially targeted catalase (MCAT) show reduced total DNA oxidative damage (in skeletal muscle), fewer mtDNA deletions, and extended mean and maximal life span by 17% to 21% (22), suggesting that mitochondrial accumulation of oxidative damage can limit rodent life span. However, mice with reduced levels of the mitochondrial MnSOD enzyme do not age any faster than their WT counterparts, despite harboring increased levels of oxidative damage to both nuclear and mtDNA (23). Thus, increased mitochondrial oxidative damage is not sufficient for accelerated aging. The D257A mice suggest that activation of apoptotic pathways is important; it will therefore be interesting to determine the extent of apoptotic activation in both MCAT and $MnSOD^{+/-}$ models. Intriguingly, mice that are both deficient in Werner's helicase and possess shortened telomeres display a phenotype strikingly similar to D257A mice and exhibit elevated levels of apoptosis (24). Fully reconciling the seemingly conflicting results from these mouse models remains a challenge but one that will presumably provide enormous insight into the role of mitochondrial function in aging.

mtDNA Mutations and Cancer

Although there is ample evidence in the literature for the accumulation of mtDNA mutations in a variety of tumor types (25), the mechanism by which such accumulation occurs and the relevance of these mutations to the transformed properties of the tumor cell have been a matter of debate. A 1998 study (26) screening a panel of human colorectal cancer cell lines found that the majority contained somatically acquired mtDNA mutations (that is, they were not present in nontumor tissues from the same patient), many of which were present in the homoplasmic state (i.e., most or all of the mtDNA molecules within a cell contain the given mutation). Most mutations, however, were not associated with mitochondrial defects. Subsequently, mtDNA mutations have been identified in multiple tumor types (25). Mathematical modeling suggests that homoplasmic mtDNA mutations need not accumulate through positive selection but can arise through genetic drift (27). Because cancer cells generally have incurred nuclear gene mutations that attenuate apoptotic signaling, they might better tolerate high mitochondrial mutational loads.

Recent work has provided support for the functional significance of mtDNA mutations on the transformed phenotype. These studies rely on the construction of cellular hybrids ("cybrids"), in which nuclei from donor cells are transplanted into enucleated cells whose mitochondria harbor mutations of interest. Transmitochondrial cybrids of HeLa cells carrying homoplasmic pathogenic mutations in the *MTATP6* gene displayed increased growth in culture and greater tumorigenicity in nude mice compared with HeLa cybrids containing nonpathogenic mtDNA (28). Transfection of a mitochondrially targeted WT nuclear version of the *MTATP6* gene into these cybrids slowed tumor growth. Conversely, WT cybrids transfected with a mutant nuclear *MTATP6* gene displayed increased tumor growth. Similarly, injection of PC3 prostate cancer cell–derived cybrids carrying one of these same MTATP6 mutations into nude mice generated tumors that were seven times larger than those produced by injection of WT cybrids (29). These studies indicate that pathogenic mtDNA mutations can indeed affect the progression of tumorigenicity.

The D257A mice provide a model with which to address the importance of mtDNA mutations in the development of neoplasms *in vivo*. It is conceivable that the early age of death in D257A mice and the increased levels of apoptosis in their tissues could act to limit malignancy. Nevertheless, to assess this question, we are crossing D257A mice with cancer-prone mouse strains (e.g., $p53^{-/-}$ or $Apc^{min/+}$) to learn whether mtDNA mutations affect tumor development.

Conclusion

The development of mitochondrial mutator mice has provided strong support for the importance of mtDNA mutations in the development of aging-related phenotypes. Although these mice may not be an exact phenocopy of normal human aging, they display many common aging features. Importantly, they provide a model with which to examine mechanistic features of the aging process that pertain to mitochondrial genome integrity and the response to mtDNA mutation. Determining the extent to which dietary or genetic interventions (e.g., altering apoptotic signaling pathways) can attenuate the aging phenotypes of the D257A mice should provide exciting insights into the mechanism by which mtDNA mutations can elicit their downstream effects.

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