Canaries in the Coal Mine: Mitochondrial DNA and Vascular Injury From Reactive Oxygen Species
R. Sanders Williams

Circ Res. 2000;86:915-916
doi: 10.1161/01.RES.86.9.915

Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7330. Online ISSN: 1524-4571

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Mitochondrial DNA (mtDNA), that curious and ancient genomic relic from the birth of the first eukaryotes, has surfaced once again in a context pertinent to cardiovascular biology and disease. In this issue of *Circulation Research*, Ballinger et al describe how human vascular endothelial and smooth muscle cells accumulate lesions in mtDNA when exposed to superoxide, hydrogen peroxide, nitric oxide, or peroxynitrite. In response to conditions that generate reactive oxygen species, damage to mtDNA is much more marked than damage to nuclear DNA, at least as assessed at a single transcriptionally inactive nuclear gene (β-globin). Ballinger et al also describe concomitant decreases in steady-state concentrations of mRNA transcribed from mitochondrial genes, in mitochondrial protein synthesis and membrane potential, and in total cellular ATP pools. They propose that these effects of reactive oxygen species contribute to vascular cell dysfunction so as to promote or accelerate the development of atherosclerosis.

The findings of Ballinger et al are reminiscent of previous observations in which increased frequency of mtDNA mutations was implicated in the pathobiology of cardiovascular disease. Mutated forms of mtDNA increase in number as a function of increasing age or ischemic heart disease in the human myocardium. Numerous reports describe mtDNA mutations in humans or animals with hypertrophic or dilated cardiomyopathy, and a similarly increased burden of mutated forms of mtDNA has been described in neural tissues of humans with neurodegenerative diseases. Although few studies have directly examined the mechanisms by which mtDNA damage arises in these conditions, reactive oxygen species have been proposed almost uniformly as the primary inciting agents.

What is the significance of these observations? Does damage to mtDNA acquired as a result of conditions that generate reactive oxygen species within vessels or within the myocardium indeed impair mitochondrial function and accelerate the course of atherosclerosis or cardiomyopathy? If so, what implications does this have for our understanding of disease mechanisms and for diagnosis or therapy of these disorders?

To consider these questions, let us first review a few facts about mitochondrial genetics in humans and other mammals. Human mtDNA is a closed circular molecule of double-stranded DNA that is only 16 kb in length, in contrast to nuclear DNA, which is 3 billion bp in length. The mitochondrial genome encodes only a dozen proteins, 2 ribosomal RNA subunits, and a complete set of transfer RNAs. Replication and transcription of mtDNA, as well as translation of mitochondrial mRNAs into protein, take place in the mitochondrial matrix space and are catalyzed by enzymes derived exclusively as products of nuclear genes and imported to mitochondria. All proteins encoded within mtDNA are essential constituents of hetero-oligomeric complexes residing in the mitochondrial inner membrane and comprising the electron transport chain for ATP generation via reduction of molecular oxygen. Each human cell contains many copies of mtDNA, ranging from a few hundred to a few thousand, compared with most nuclear genes, which have only 2 copies per cell. DNA repair mechanisms are poorly developed in mitochondria; therefore, the high copy number provides the primary defense against phenotypic abnormalities that otherwise would result from acquired mutations in individual mtDNA molecules within a given cell.

Mutated forms of mtDNA, without question, can cause cardiovascular disease. However, such a causal relationship is well established only in the small subset of individuals who inherit defective mitochondrial genomes (deletions involving several genes or point mutations involving a single gene) from their mothers at conception or those in whom spontaneous mutations arise during embryonic life. In these patients, mutated forms of mtDNA are distributed to the majority of cells within the heart and other tissues and accumulate in excess of the concentration of wild-type mtDNA molecules. From the most detailed and careful studies of cultured human cells, it seems that any single mutated form of mtDNA must approach 80% to 90% of the total pool of mitochondrial genomes before mitochondrial function and cellular energy metabolism are seriously impaired. In the human heart, such high levels of defective mitochondrial genomes certainly can produce conduction block and heart failure. However, the relative abundance of specific mutated forms of mtDNA in the myocardium of older individuals or in those with ischemic heart disease has not been observed to exceed 10% of the total pool of mtDNA. It can be argued, with reason, that a low level of damage to the mitochondrial genome associated with age or ischemia is a consequence, rather than a cause, of cellular injury or dysfunction and is unlikely to be of direct pathobiological significance. Arguments against this viewpoint rely on the unproven possibility that certain mutations may generate dominant-negative forms of mitochondrial gene products or that an aggregate burden of...
many different mtDNA mutations otherwise dysregulates mitochondrial function even when each specific gene defect is present only in low abundance. This debate is likely to remain unresolved until animal models are developed that permit direct experimental control over the abundance of mtDNA mutations without the confounding effects of cellular injury affecting other features of cells and tissues.17

Are mtDNA defects that arise in vascular cells as a result of reactive oxygen species the cause of impaired mitochondrial function? The study by Ballinger et al1 includes convincing data that mtDNA mutations appearing in their cells are accompanied by abnormalities of mitochondrial RNA and protein metabolism and by defective respiratory function, and the authors imply a causal relationship between these observations. However, unless one evokes dominant negative effects, the calculated average mutation frequency of 1 to 3 lesions per 10 kb of mtDNA is not of a magnitude likely to result in a significant deficiency of any specific mitochondrial gene product. Null mutations resulting from defects in 1 or 2 genes within a subset of mitochondrial genomes would be rescued by the abundance of normal copies of those genes still present within each cell. Therefore, quantitative deficiencies in mitochondrial RNA transcripts, mitochondrial protein synthesis, and respiratory function produced in their models are attributable more likely to the direct effects of reactive oxygen species on mitochondrial membranes and proteins (perhaps those involved in the import from the cytoplasm of proteins derived from nuclear genes) than to mtDNA mutations.

Do abnormalities in mitochondrial function caused by reactive oxygen species (irrespective of their relationship to mtDNA mutations) influence the course of atherosclerosis? Ballinger et al1 make some plausible speculations, but they present no direct evidence to support this contention. Accelerated atherosclerosis has not been described as a feature of any of the clinical syndromes resulting unequivocally from inherited mutations in mtDNA that are distributed in all tissues, but the question has not been addressed systematically, with appropriate measurements in a sufficient number of patients, to permit firm conclusions. Because patients with mtDNA defects are rare and often present in childhood, cleverly designed animal models will probably be required to resolve this issue.

Skepticism concerning the pathobiological significance of mtDNA mutations produced by oxidative stress in vascular cells should not be construed to mean that the observation is unimportant. To the contrary, mtDNA may serve as a sensitive indicator of oxidative damage in a manner that could be exploited for diagnostic purposes or to assess responses to antioxidant therapies. In earlier times, caged canaries were used as sentinels for toxic levels of gases that could contaminate the air of their tunnels and shafts. Mitochondrial DNA, caged within the double mitochondrial membrane in proximity to sites of generation of reactive oxygen species, perhaps can be viewed as serving an analogous role within the vessel wall or myocardium. A role for mtDNA as sentinel was recently proposed to be useful for early and noninvasive detection of certain forms of cancer.18 In addition to the observations of Ballinger et al,1 a recent study describes an increased frequency of deleted forms of mtDNA in human atherosclerotic lesions.19 Perhaps cardiovascular scientists can be sufficiently ingenious to devise ways by which detection of mutant mitochondrial genomes in cells of the vessel wall can improve methods for early diagnosis and more effective prevention of atherosclerotic vascular disease.

References


Key Words: atherosclerosis  mitochondria  reactive oxygen species