Mitochondrial DNA Damage, Oxidative Stress, and Atherosclerosis
Where There Is Smoke There Is Not Always Fire

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Atherosclerosis is an insidious disease that can remain undetected for decades before manifesting, sometimes lethally, in the form of a stroke or myocardial infarction. With >900,000 Americans experiencing a heart attack annually,1 there is a strong need to develop better treatments or preventative measures, but these will necessitate a better understanding of the underlying process. Most evidence suggests that atherosclerosis is initiated by chronic endothelial injury in response to the accumulation of low-density lipoprotein and oxidized low-density lipoprotein in the vessel wall, which stimulates an inflammatory response.2 Although the involvement of elevated circulating lipids and oxidative stress are well recognized in this process (Figure), critical questions remain. These include the question of what causes the atherosclerotic plaque to worsen progressively rather than to resolve, and what causes relatively innocuous, stable plaques to transform into unstable, vulnerable plaques, which are liable to rupture and lead to thrombus formation.

The major risk factors for atherosclerosis include systemic hypertension, elevated plasma lipids, smoking, diabetes mellitus, and aging. Many of these have been shown to damage mitochondria, leading to the proposal that mitochondrial injury is involved in plaque development.3 Mitochondria are important organelles that generate ATP, but they also produce superoxide, a highly reactive oxygen radical, as a by-product, which is rapidly dismutated to hydrogen peroxide. Such reactive oxygen species (ROS), can damage cellular proteins, membranes, and DNA. Whereas genomic DNA is relatively well insulated in the nucleus and is protected by histones, mitochondrial DNA (mtDNA), which encodes several essential proteins of the mitochondrial electron transport chain, is susceptible to oxidative damage. This suggests mitochondrial ROS may fuel a vicious circle in which ROS damages mtDNA, leading to more mitochondrial ROS, which further damages mtDNA, and so on until the cell is terminally damaged and undergoes apoptosis.2 The observation that mtDNA damage is associated with atherosclerosis in human atherosclerotic plaque material further supports this hypothesis.2 But where there is smoke, there is not always fire.

The Link Between mtDNA Damage and Atherosclerosis

In a comprehensive set of experiments, Yu et al2 have now carefully examined whether mitochondrial mutations are really causal in atherosclerosis plaque development. First, they examined the well-characterized, hyperlipidemic, apoE-deficient (ApoE−/−) mouse model of atherosclerosis. They found that the appearance of mtDNA lesions in the aortas of these mice preceded the development of aortic plaques. A high-fat diet increased the frequency of mtDNA lesions and also accelerated aortic plaque development. Interestingly, mtDNA adducts were also detected in circulating monocytes and in the liver, and mtDNA damage was shown to correlate with loss of mitochondrial complex I activity. Although still circumstantial, this initial evidence suggested that mtDNA damage leads to atherosclerosis by decreasing mitochondrial respiratory activity (Figure).

To obtain direct evidence, the authors made use of transgenic mice harboring a version of the mitochondrial DNA polymerase, polymerase gamma (polG), deficient in proof-reading activity. Over time, these mitochondrial mutator mice accumulate somatic point mutations in their mtDNA and, remarkably, suffer from accelerated aging, exhibiting weight loss, reduced subcutaneous fat, alopecia (hair loss), kyphosis (curvature of the spine), osteoporosis, anemia, reduced fertility, and heart enlargement.4 In their subsequent experiments the authors examined the effect of the polG mutation in the context of an ApoE−/− background to address the question of whether the mutator phenotype would aggravate atherosclerosis development in a susceptible background.

As expected, more mtDNA lesions were detected in the aortas of mice expressing mutant polG. Crucially, atherosclerotic plaque size was also significantly increased, finally providing direct evidence of a link between mtDNA damage and atherosclerosis. The next step was to examine the mechanism. The mitochondrial lesions caused a severe decrease in the activity of mitochondrial complex I and IV in the aortae. However, when oxygen radical production was measured, the results were more surprising. For this experiment, the authors made use of a recently developed, mitochondrially targeted, mass-spectrometric probe that is sensitive to hydrogen peroxide,5 and found that the mtDNA mutator phenotype had no significant effect on ROS production. This was confirmed by measurements of ROS production in vascular smooth muscle cells.
(VSMCs). Thus, although mtDNA damage can certainly exacerbate atherosclerosis, the mechanism appears not to involve increased ROS production.

The mice were also analyzed using an automated system for continuous analysis of the metabolic and locomotor activities of individual mice. This revealed that polG mutant mice were roughly half as active as their counterparts throughout a 24-h period. Despite their inactivity, polG mice gained less weight on a high-fat diet than their control siblings, with a dramatic reduction in percentage body fat. A continuous analysis of individual oxygen consumption revealed that the mice also consumed less oxygen. This indicated that their low weight and reduced cellular ATP content were not attributable to a general uncoupling of mitochondria, because this would be expected to increase oxygen consumption. Interestingly, polG mice had elevated levels of low-density lipoprotein and total plasma cholesterol, but no overt signs of diabetes mellitus.

In VSMCs isolated from polG mutator mice, ATP levels were severely depleted, cellular proliferation was curtailed, and they senesced early. Interestingly, these VSMCs were also more susceptible to oxidative stress, undergoing apoptosis. The development of atherosclerosis is a complex process involving interaction between numerous cell types in the vessel wall, but also with circulating leukocytes. Because the polG mice express mutant polG globally, it was therefore important to examine the effect of mtDNA damage in these cells. In the ApoE−/− background, the polG mutation increased mtDNA damage in circulating monocytes, which led to decreased ATP levels, an increase in apoptosis, and the secretion of proinflammatory cytokines (tumor necrosis factor α and interleukin 1β). Despite the induction of proinflammatory cytokines, inflammatory cell count was not elevated. To determine whether monocyte mtDNA damage contributed to plaque development, bone marrow transplant experiments were performed. These revealed that mutant polG in the blood cells alone had no effect on the size of the atherosclerotic plaque area, implying that it is mtDNA in the vascular smooth muscle cells that is the critical target.

However, in mice receiving mutant polG marrow, the size of the necrotic core within the plaque was increased and the area of the fibrotic cap was decreased, suggesting that mtDNA damage in monocytes can lead to an increase in plaque vulnerability. This is an important observation because, as mentioned, factors that are responsible for this increase in plaque vulnerability represent important potential therapeutic targets.

Rounding off this comprehensive study, the authors turned to a human population from their Virtual Histology in Vulnerable Atherosclerosis (VIVA) trial to examine whether leukocyte mtDNA correlated with atherosclerosis extent or plaque vulnerability. In this prospective study, intravascular ultrasound was used to evaluate plaque composition (fibrous, fatty, necrotic, calcified) of 1096 plaques in 170 patients.6 A particular type of plaque defined as thin-capped fibroatheroma was shown in this and a similar trial to confer the highest risk of subsequent cardiovascular events.6,7 Interestingly, when mtDNA lesions were quantified, they were uniquely associated with this type of atherosclerotic lesion.3 On the other hand, mtDNA adducts were not associated with any aspects of patient demographics, including smoking, age, sex, or drug therapy. Intriguingly, however, mtDNA adducts were positively associated with diabetes mellitus, and negatively with serum cholesterol.

Atherosclerosis and Aging

The concept that oxygen radicals produced from the mitochondria are responsible for aging and age-related pathologies such as atherosclerosis is as elegant as it is simple. This latest study contributes to the mounting evidence that, unfortunately, the theory does not conform to reality.3,8 Others have shown that, despite suffering from accelerated aging,4 polG mutant mice do not exhibit any signs of oxidative stress.9 Furthermore, in an extensive series of experiments in which the expression of 18 different antioxidant enzymes was manipulated, only 1 had any effect on lifespan, prompting the authors’ question: “Is the oxidative stress theory of aging dead?”10 If it is not ROS that connect mtDNA damage to atherosclerosis, then what does? Mitochondria have other roles apart from ATP generation, including heme synthesis, Ca2+ homeostasis,9,11 and the elimination of toxic aldehydes,12 which might be relevant. They also play a crucial role as arbiters of apoptotic and necrotic cell death. Notably, the same group has previously used an ingenious mouse model of inducible VSMC-specific apoptosis to show that VSMC apoptosis can accelerate plaque growth and induce features of plaque vulnerability in atherosclerosis.13,14 Thus, a likely scenario is that mtDNA damage leads to decreased ATP levels, causing the energy-starved cells within the plaque to undergo apoptosis. The lipodystrophy phenotype with lipidemia remains unexplained but could conceivably involve mtDNA mutations and apoptosis of adipocytes. Other cells such as endothelial cells contain mitochondria and may also be susceptible to mtDNA mutation and damage.15,16

The results of the current study might also prompt one to question whether ROS are even involved in the initial mtDNA damage. Intriguingly, recent evidence suggests that aging-associated mtDNA mutations accumulate, not as a result...
of de novo damage, but as a result of clonal expansion of mtDNA replication errors that occur during development.\textsuperscript{17} The field of aging research is evolving rapidly, but the actual mechanism leading to accumulation of mtDNA mutations has been suggested to involve progrowth signaling pathways such as insulin/insulin-like growth factor 1 signaling and the target of rapamycin pathways.\textsuperscript{17} How this relates to atherosclerosis development remains to be determined.

What do these findings tell us in terms of possible therapeutic angles? First, they evidently suggest that antioxidants may not have any effect on mtDNA-mediated pathways. Instead, novel approaches targeting mitochondrial health, such as the stimulation of mitophagy or mitochondrial fusion, should be investigated. Some of the benefits of PPAR agonists may be shown to be via promotion of mitochondrial biogenesis. On the other hand, sometimes the oldest remedies are the best—a recent intriguing study indicates that premature aging and death in poIG mice can be postponed for several months by improving mitochondrial function and decreasing apoptosis with endurance exercise.\textsuperscript{18} No doubt these mice will continue to provide important information in the future development of remedies targeting mtDNA in atherosclerosis.

In conclusion, where there is smoke there is not always fire; the concept of mtDNA damage and oxidative stress leading to atherosclerosis may need to be revised.

Disclosures

None.

References


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