Advanced atherosclerotic plaques demonstrate extensive DNA damage, seen in smooth muscle cells, endothelial cells, macrophages, and in circulating cells, and in both nuclei and mitochondria.1 DNA damage includes both single- or double-stranded breaks, deleted sections of DNA, nucleotide modifications, and extrusions of DNA from the nucleus (micronuclei). Reactive oxygen species (ROS) induce a variety of DNA damage, including oxidatively modified bases, apurinic/apyrimidinic sites, and strand breaks. Guanine is the most readily oxidized base, reacting with OH to generate a reducing neutral radical that reacts with O2, and via electron transfer, forms 8-oxo-7,8-dihydroguanine (8-oxo-G).2 8-oxo-G and its products are the most abundant DNA lesions on oxidative exposure, with 1 to 2 × 106 residues in nuclear DNA and 1 to 3 × 105 residues in mitochondrial DNA (mtDNA), and up to 107 8-oxo-G lesions are formed in the cell daily.3 Advanced plaques are characterized by extensive accumulation of 8-oxo-G, seen in both macrophages and smooth muscle cells.3,4 8-oxo-G is primarily repaired by base excision repair by several enzymes, including specific 8-oxo-G DNA glycosylases I and II (OGG1/2) and the Neu-like (NEIL) glycosylases; the excised DNA is repaired by AP endonucleases before gap filling by polymerases and ligation.

Although minor DNA damage is associated with transient growth arrest for DNA repair, more extensive DNA damage can lead to several sequelae, including cell senescence and apoptosis, which both promote inflammation. DNA damage, apoptosis, cell senescence, and inflammation are all present in atherosclerosis, suggesting that DNA damage may be a causal factor in these other processes. However, although DNA damage is present in atherosclerosis, it is unclear whether the endogenous levels actually found have any functional consequences. Indeed, mice seem to be able to tolerate high levels of oxidative DNA damage without obvious detrimental effects. For example, OGG1−/− mice are born and develop normally with a normal lifespan, despite a 7-fold increase in 8-oxo-G in nuclear DNA and a >20-fold increase in 8-oxo-G in mtDNA.3,4 Mitochondrial function in these mice is normal, including maximal respiration rates or mitochondrial ROS generation.5 Furthermore, chronic oxidative stress can increase 8-oxo-G levels >250-fold without apparent severe consequences.6

Against this background, the current article by Tumurkhuu et al7 shows that oxidative DNA damage directly results in the production of the archetypal proinflammatory cytokines interleukin (IL)1β and IL18 through the activation of NLRP3 (the NACHT, LRR, and PYD domains-containing protein 3) inflammasome, itself a major component of the innate immune system that functions as a pathogen recognition receptor that recognizes pathogen-associated molecular patterns. The NLRP3 inflammasome can also detect products of damaged cells such as extracellular ATP, crystalline uric acid, and cholesterol. These workers have previously shown that oxidized mtDNA can activate the NLRP3 inflammasome during apoptosis/pyroptosis.8 However, it was not known whether oxidized mtDNA could promote atherosclerosis, and if so, how this was mediated. Here, Tumurkhuu et al7 show that OGG1 is reduced in macrophages in atherosclerosis, and macrophages lacking OGG1 are more sensitive to oxidant stress, with increased release of cytochrome c, caspase 1 activation, NLRP3 activation, release of IL1β, and apoptosis. Low-density lipoprotein receptor (LDLR) null mice also lacking OGG1 in all tissues showed increased plaque and necrotic core areas, and reduced collagen content, with higher serum IL1β, MCP-1 (monocyte chemoattractant protein-1), and IL18. LDLR−/−OGG1−/− mice also had increased 8-oxo-G in their plaques, with most of the 8-oxo-G in the mitochondria, which was associated with caspase 1 activation. Thus, downregulation of OGG1 in atherosclerosis may directly promote inflammation and plaque development.

To prove that OGG1 and NLRP3 in hematopoietic cells were responsible for some of the observed effects, these workers transplanted LDLR−/− mice with bone marrow–lacking OGG1, OGG1 and NLRP3, or NLRP3 alone. Transplantation with OGG1−/− bone marrow led to larger atherosclerotic lesions and increased IL1β production, which was dependent on NLRP3. Finally, they identify a novel pathway involving miR33 that directly inhibits human OGG1 expression, and indirectly suppresses both mouse and human OGG1 via 5′ AMP-activated protein kinase (AMPK). In particular, AMPK can activate OGG1 transcription, and AMPK activity was reduced in atherosclerosis (Figure).

Previous studies have shown that endogenous levels of double-strand breaks13 and telomere damage14 can promote atherosclerosis, and lead to a more unstable plaque phenotype.

The opinions expressed in this article are not necessarily those of the editors or of the American Heart Association.

From the Division of Cardiovascular Medicine, Department of Medicine, University of Cambridge, Addenbrooke’s Centre for Clinical Investigation, Addenbrooke’s Hospital, United Kingdom.

Correspondence to Martin Bennett, MD, PhD. E-mail mrb@mole.bio.cam.ac.uk

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The finding that oxidized mtDNA can activate the NLRP3 inflammasome raised the possibility that oxidized mtDNA is directly proinflammatory in atherosclerosis, which the current study directly proves. Interestingly, despite mtDNA comprising only a small component of total cellular DNA, these workers found that most of the oxidized DNA in OGG1−/− mice was present in the mitochondria. Mitochondria are a major source of ROS, and mtDNA is more sensitive to oxidative DNA damage than nuclear DNA, possible because of the proximity to local sources of ROS, lack of protective histones, and reduced BER activity. Although mitochondrial function and ROS generation at baseline were reported as normal in OGG1−/− mice, mitochondrial overexpression of OGG1 can improve mitochondrial function and cell survival and reduce mtDNA deletions through increased repair of 8-OH-dG under oxidative stress conditions. Thus, although OGG1 activity and BER efficiency were not measured in the article by Tumurkhuu et al, OGG1 activity and BER may be more important under conditions of oxidant stress, such as those seen in atherosclerosis.

Like all excellent studies and important studies, the current article by Tumurkhuu et al raises some important questions and the prospect of tantalizing novel mechanisms to inhibit inflammation in atherosclerosis. However, further studies are required to both dissect the role of endogenous DNA damage and determine whether protection against damage or increased DNA repair are beneficial over and above the standard clinical approach of reducing risk factors for coronary artery disease that promote damage, including hypercholesterolemia, diabetes mellitus, and smoking. For example, the experimental system used by by Tumurkhuu et al uses loss of function of both OGG1 and NLRP3 in mice. While they show that atherosclerosis does show loss of macrophage OGG1 expression, rescue experiments overexpressing OGG1 would be required to demonstrate that the reduced OGG1 levels actually present in macrophages in vivo in atherosclerosis have the same functional consequences, and provide proof of principle for macrophage OGG1 being a therapeutic target. Similarly, it would be important to analyze BER activity in human macrophages in atherosclerosis in vivo. Finally, previous studies have also shown that while markers of DNA repair activity are reduced quickly in atherosclerotic plaques after withdrawal of the stimulus (eg, hypercholesteremia), the oxidative damage itself takes much longer to disappear, if at all. The reduced OGG1 expression in macrophages may be a causal factor underlying this observation, but there may also be other glycolases or BER components that are defective in atherosclerosis.

Similarly, the NLRP3 inflammasome is activated by multiple stimuli through both canonical and noncanonical pathways, many of them present in atherosclerotic plaques. It is unclear how important oxidized mtDNA is in comparison with these other stimuli, although many NLRP3 activators seem to act through mitochondria; indeed loss of OGG1 sensitized

![Diagram of macrophage OGG1 expression and downstream effects](http://circres.ahajournals.org/)

**Figure.** Schematic showing regulation of macrophage OGG1 expression and downstream effects. miR33, which targets multiple metabolic pathways including AMP-activated protein kinase, is upregulated in human atherosclerotic plaques, resulting in reduced AMP-activated protein kinase. OGG1 expression decreases over time in plaque macrophages, which results in increased oxidized mtDNA damage and greater amounts of cytosolic oxidized mtDNA. Oxidation of mtDNA is induced by reactive oxygen species derived from mitochondrial respiration, making mitochondria both the source and the target of oxidative stress. mtDNA damaged by oxidative stress activates the NLRP3 (the NACHT, LRR, and PYD domains-containing protein 3) inflammasome which causes the activation of caspase-1, which cleaves the precursor proforms of the proatherogenic cytokines IL-1β and IL-18 into their mature forms. Release of IL-1β and IL-18 results in chronic inflammation.
macrophages in the current study to cholesterol crystals, ke-
tocholesterol, and ATP. OGG1 regulates BER in both nuclear
and mitochondrial DNA and may have multiple effects on ath-
ersclerosis and in other cell types. However, they show that
while plaque size was reduced in OGG1−/−NLRP3−/− chimeras
compared with OGG1−/− alone, there was no significant dif-
ference between OGG1−/−/NLRP3−/−, and NLRP3−/− mice,
compared with OGG1−/− alone, there was no significant dif-
ference between OGG1−/−/NLRP3−/−, and NLRP3−/− mice,
highlighting the importance of the NLRP3 pathway in the ef-
fects mediated by OGG1 knockout. Finally, attempts to target
DNA damage and repair therapeutically are hampered by the
conserved nature of the DNA repair machinery. For example,
OGG1 activity is regulated by post-translational modifications,
and the enzymes responsible have multiple other substrates. In
this context, the regulation of macrophage OGG1 by miR33
may provide an important therapeutic possibility if it is found
to have selectivity.

However, a word of caution is also warranted. Although
miR33 has long been implicated in atherosclerosis, antagonism
of miR33 has variable effects on both serum lipids and ather-
ersclerosis at different time points,16 and rodents and humans
express different isoforms of miR33. As found by Tumurkhuu
et al,11 miR33 may have different targets in rodent models com-
pared with humans.

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None.

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Aarti Shah and Martin Bennett

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