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. 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 O$_2$ and via electron transfer, forms 8-oxo-7,8-dihydroguanine (8-oxo-G). 8-oxo-G and its products are the most abundant DNA lesions on oxidative exposure, with 1 to 2 x 10$^{05}$ residues in nuclear DNA and 1 to 3 x 10$^{05}$ residues in mitochondrial DNA (mtDNA), and up to 10$^{07}$ 8-oxo-G residues are formed in the cell daily. Advanced plaques are characterized by extensive accumulation of 8-oxo-G, seen in both macrophages and smooth muscle cells. 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.

Article, see p e76

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. Mitochondrial function in these mice is normal, including maximal respiration rates or mitochondrial ROS generation. Furthermore, chronic oxidative stress can increase 8-oxo-G levels >250-fold without apparent severe consequences.

Against this background, the current article by Tumurkhuu et al$^{11}$ shows that oxidative DNA damage directly results in the production of the archetypal proinflammatory cytokines interleukin (IL)-1$\beta$ and IL-18 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. However, it was not known whether oxidized mtDNA could promote atherosclerosis, and if so, how this was mediated. Here, Tumurkhuu et al$^{11}$ show that OGG1 is reduced in macrophages in atherosclerosis, and if so, how this was mediated. Here, Tumurkhuu et al$^{11}$ 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 IL-1$\beta$, 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 IL-1$\beta$, MCP-1 (monocyte chemoattractant protein-1), and IL-18. 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 hemopoietic 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 IL-1$\beta$ 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 breaks$^{13}$ and telomere damage$^{14}$ can promote atherosclerosis, and lead to a more unstable plaque phenotype.
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. 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
macrophages in the current study to cholesterol crystals, ketocolesterol, and ATP. OGG1 regulates BER in both nuclear and mitochondrial DNA and may have multiple effects on atherosclerosis 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 difference between OGG1−/−NLRP3−/−, and NLRP3−/− mice, compared with OGG1−/− alone, there was no significant difference between OGG1−/−NLRP3−/−, and NLRP3−/− mice, highlighting the importance of the NLRP3 pathway in the effects 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, conserved nature of the DNA repair machinery. For example, OGG1 activity is regulated by post-translational modifications, 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 atherosclerosis at different time points, and rodents and humans express different isoforms of miR33. As found by Tumurkhuu et al, miR33 may have different targets in rodent models compared with humans.

Sources of Funding
This work was funded through British Heart Foundation grant RG/13/14/30334 and the Cambridge National Institute of Health Research Biomedical Research Centre.

Disclosures
None.

References

Key Words: Editorials ■ atherosclerosis ■ DNA damage ■ microRNA ■ oxidative stress ■ reactive oxygen species
Controlling Inflammation Through DNA Damage and Repair

Aarti Shah and Martin Bennett

Circ Res. 2016;119:698-700
doi: 10.1161/CIRCRESAHA.116.309505

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circres.ahajournals.org/content/119/6/698

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation Research can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation Research is online at:
http://circres.ahajournals.org//subscriptions/