Controlling inflammation through oxidative DNA damage and repair

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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 (reviewed in [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 (AP) sites and strand breaks. Guanine is the most readily oxidized base, reacting with $^{\bullet}OH$ to generate a reducing neutral radical that reacts with O_2, and via electron transfer, forms 8-oxo-7,8-dihydroguanine (8-oxo-G)[2]. 8-oxo-G and its products are the most abundant DNA lesions on oxidative exposure, with 1-2/10^6 residues in nuclear DNA and 1-3/10^5 residues in mitochondrial DNA (mtDNA), and up to 10^5 8-oxo-G lesions are formed in the cell daily (reviewed in [1]). Advanced plaques are characterised 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 (BER) by a number of enzymes, including specific 8-oxo-G DNA glycosylases I[5] and II[6] (OGG1/2) and the Nei-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 a number of 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 appear 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[7,8]. Mitochondrial function in these mice is normal, including maximal respiration rates or mitochondrial ROS generation[9]. Furthermore, chronic oxidative stress can increase 8-oxo-G levels >250-fold without apparent severe consequences[10].

Against this background, the current paper by Tumurkhuu[11] shows that oxidative DNA damage directly results in production of the archetypal pro-inflammatory cytokines interleukin (IL)-1β and IL-18 through activation of the NLRP3 inflammasome, itself a major component of the innate immune system that functions as a pathogen recognition receptor that recognizes pathogen-associated molecular patterns (PAMPs). The NLRP3 inflammasome can also detect products of damaged cells such as extracellular ATP, and crystalline uric acid and cholesterol. These workers have previously shown that oxidized mtDNA can activate the NLRP3 inflammasome during apoptosis/pyroptosis[12]. 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 macrophages lacking OGG1 are more sensitive to oxidant stress, with increased release of cytochrome c, caspase-1 activation, NLRP3 activation, release of IL-1β, and apoptosis. LDLR$^{-/-}$ mice lacking OGG1$^{-/-}$ in all tissues showed increased plaque and necrotic core areas, and reduced collagen content, with higher serum IL-1β□□MCP□□ 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 hemopoeitic cells was 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-1β production, which was dependent upon NLRP3. Finally, they identify a novel pathway involving microRNA (miR)-33 that directly inhibits human OGG1 expression, and indirectly suppresses both mouse and human OGG1 via AMPK. In particular, AMPK can activate OGG1 transcription, and expression of AMPK was reduced in atherosclerosis **(Figure 1)**.

Previous studies have shown that endogenous levels of double strand breaks[13] and telomere damage[14] can promote atherosclerosis, and / or lead to a more unstable plaque phenotype. The finding that oxidized mtDNA can activate the NLRP3 inflammasome[12] raised the possibility that oxidized mtDNA is directly pro-inflammatory 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 due to the proximity to local sources of ROS, lack of protective histones, and reduced BER activity. Even though mitochondrial function and ROS generation at baseline were reported as normal in OGG1$^{-/-}$ mice[9], 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 paper by Tumurkhuu[11], OGG1 activity and BER may be more important under conditions of oxidant stress, such as those seen in atherosclerosis.

Like all excellent and important studies, the current paper by Tumurkhuu[11], raises some significant 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 and smoking. For example, the experimental system used by Tumurkhuu[11] uses loss of function of both OGG1 and NLRP3 in mice. While they show loss of macrophage OGG1 expression in atherosclerosis, 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 (for example hypercholesteremia), the oxidative damage itself takes much longer to disappear, if at all[15]. The reduced OGG1 expression in macrophages may be a causal factor underlying this observation, but there may also be other glycosylases or BER components that are defective in atherosclerosis.

Similarly, the NLRP3 inflammasome is activated by multiple stimuli through both canonical and non-canonical 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 appear to act through mitochondria; indeed loss of OGG1 sensitized macrophages in the current study to cholesterol crystals, ketocholesterol and ATP. OGG1 regulates BER in both nuclear and mtDNA, 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, 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, and the enzymes responsible have multiple other substrates. In this context, the regulation of macrophage OGG1 by miR133 may provide an important therapeutic possibility if it is found to have selectivity.

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Legend to Figure

Figure 1 Schematic overview of model for regulation of macrophage OGG1 expression in atherosclerosis and downstream effects
miR33, which targets multiple metabolic pathways including AMPK, is upregulated in human atherosclerotic plaques, resulting in reduced AMPK. miR33 downregulates OGG1 expression through AMPK and can also directly target human OGG1. OGG1 expression decreases over time in plaque macrophages, which results in increased oxidized mitochondrial DNA (mtDNA) damage and greater amounts of cytosolic oxidized mtDNA. Oxidation of mtDNA is induced by reactive oxygen species (ROS) derived from mitochondrial respiration, making mitochondria both the source and the target of oxidative stress. mtDNA damaged by oxidative stress activates the NLRP3 inflammasome which causes the activation of caspase-1, which cleaves the precursor proforms of the pro-atherogenic cytokines interleukin (IL)-1β and IL-18 into their mature forms. Release of IL-1β and IL-18 results in chronic inflammation.

Abbreviations: ASC, apoptosis-related speck-like protein containing a caspase recruitment domain; BER, base excision repair; IL, interleukin; mt, mitochondrial; ROS, reactive oxygen species