Can the DNA Damage Response Be Harnessed to Modulate Atherosclerotic Plaque Phenotype?

Babak Razani, Elaine W. Raines

Gray et al describe studies to examine smooth muscle cell (SMC)–selective effects of acceleration or inhibition of double-strand DNA break repair on atherosclerotic lesion phenotype in the apolipoprotein E–deficient (ApoE−/−) mouse model. Markers of the DNA damage response (DDR) and expression of DNA repair enzymes are both significantly elevated in human atherosclerotic plaques as compared with nonatherosclerotic mammary arteries and are also increased in experimental models of atherosclerosis, a process that can be reversed by dietary lipid lowering. The report by Gray et al provides important insights into the specific contribution of SMCs to plaque phenotype when the DDR is manipulated in vivo. Although complementary analyses of double-strand DNA break repair in other major cell types within atherosclerotic lesions are needed, data from the studies by Gray et al suggest the possibility of harnessing atheroprotective features of the ataxia telangiectasia mutated (ATM) kinase, a primary initiator of the DDR.

Global Impact of Cardiovascular Disease and the Influence of Lesion Phenotype Rather Than Lesion Size on Acute Cardiovascular Events

Cardiovascular disease is not only the leading cause of death in the Western World but has also surpassed infectious disease as the primary reason for mortality worldwide.

Obesity, poor nutrition, and conditions including diabetes mellitus, hypertension, lack of physical activity, smoking, and advancing age are all major risk factors for developing cardiovascular disease. Although the hallmark of disease progression is the insidious formation of the atherosclerotic plaque, it is the acute rupture of vulnerable plaques that is most commonly associated with cardiovascular mortality.

Autopsy studies are the basis for predicting the features of unstable lesions where it is evident that lesion phenotype and the distribution of macrophages and SMCs are particularly important. The presence of apoptotic SMC and macrophages, a large lipid-rich core, and a thin fibrous cap are notable features. Evidence derived from pathology specimens is also beginning to be supported by imaging studies correlating plaque phenotype with acute cardiovascular events. Thus, understanding the cellular mechanisms that lead to adverse remodeling of plaque architecture is critical for developing novel strategies to curb plaque instability and ensuing rupture.

Associations Between Oxidant Stress, DNA Damage, and the Progression of Atherosclerosis

Compromise of genome integrity, including the accumulation of DNA damage, is a consequence of cellular aging, ongoing cellular division, and exposure to environmental stressors. The pathogenesis of several diseases, particularly cancer, is defined by such genomic aberrations. Interestingly, associations between oxidant stress, DNA damage, and human atherosclerosis have been appreciated for the past 2 decades, but their causative versus associative role in disease progression is less clear. Recent studies demonstrated that mitochondrial DNA damage precedes lesion formation in the ApoE−/− mouse atherosclerosis model and promotes effects on both SMC and myeloid cells. Deletion of mitochondrial polymerase-γ proofreading activity, independent of reactive oxygen species, led to a modest increase in ApoE−/− lesion area that was associated with an elevation in the number of plaque cells undergoing apoptosis with concomitant reduction in cells positive for the proliferation marker Ki67. Deletion of polymerase-γ proofreading activity only in circulating cells did not alter lesion size but was linked with an increase in necrotic core area and a decrease in fibrous cap area. However, the specific effects of limiting targeted deletion of polymerase-γ proofreading activity to SMC or macrophages were not investigated. In the current study, the authors use polymerase chain reaction array analyses of cultured normal human aortic and plaque SMCs and detect upregulation of multiple mRNAs in plaque SMC associated with DNA repair pathways, particularly those involved in double-strand break sensing. Importantly, they localized several of the DNA repair pathway proteins to SMCs.

DNA Damage Repair Pathway Is a Tightly Regulated Pathway Readily Shifted by Environmental Changes

As a mechanism to protect genomic integrity, all cells undergo continuous surveillance and repair of DNA damage, particularly to highly deleterious double-strand DNA breaks.
This so-called DDR involves the induction of a signaling cascade that either triggers cell cycle arrest to activate DNA repair mechanisms or can induce apoptotic mechanisms when DNA damage approaches irreversibility. An initiating event in the DDR involves recruitment of the Mre11-Rad50-NBS1 ATPase-nuclease complex to double-strand breaks, followed by activation of the kinases ATM and ATR (ATM and Rad3 related). ATM and ATR phosphorylate >700 target proteins in human cells, including histone H2AX (used by Gray et al., along with ATM autophosphorylation, as markers of DDR), DNA repair factors and checkpoint mediators (such as NBS1 and the associated mediator of DNA damage checkpoint 1, MDC1), the stress response transcription factor p53, and many other cell cycle regulators. ATM can also be activated by oxidative stress and chromatin structure alterations, but the Mre11-Rad50-NBS1 complex is critical for double-stranded break-dependent activation of ATM. Thus by modulating functional levels of NBS1, 1 of 3 components of the Mre11-Rad50-NBS1 complex, Gray et al. alter phosphorylation of ATM targets and ATM-mediated signaling controlling cell cycle checkpoints and DNA repair (Figure). 

Transgenic Expression of NBS1 or Activation-Deficient NBS1 Accelerates or Impairs the DDR, Respectively, in SMC In Vivo

Two novel transgenic mouse models were generated expressing either wild-type NBS1 or one lacking the C-terminal domain necessary for ATM activation and downstream signaling. The 2 forms of NBS1 were expressed under the control of the SMC-selective promoter SM22 in the ApoE-/- mouse atherosclerosis model. Using a potent inducer of double-strand breaks, tert-butyl hydroperoxide (t-BHP), they show that SMCs derived from Sm22α-Nbs1/Apo−/− aortas enhance the recruitment of phosphorylated ATM and H2AX (P-ATM and γ-H2AX) to sites of DNA damage with resultant increases in several downstream ATM targets. In contrast, SMCs from Sm22α-ΔC Nbs1/Apo−/− aortas had blunted phosphorylation of ATM and its downstream targets. Interestingly, relatively late events in ATM signaling (ie, phosphorylation of p53, induction of p21, and the development of apoptosis) behaved oppositely, being suppressed in the setting of NBS1 overexpression and largely unchanged in the presence of ΔC)NBS1, except for apoptosis that was elevated. The transgenic approach used in this study does have the limitation that it was generated on a wild-type...
background, and therefore endogenous expression of wild-type NBS1 also occurs in both transgenics. However, the data overall provide evidence for a unique method to accelerate or inhibit the DDR in SMC in vivo.

Lesion plaque area in mice fed a high-fat diet from 6 to 20 weeks did not differ with selective SMC expression of wild-type NBS1 or mutated NBS1. However, comparison of the 2 transgenics relative to control ApoE−/− mice showed an increase in fibrous cap area and SMC content in Sm22α-Nbs1/Apoe−/− mice. In contrast, Sm22α-(ΔC)Nbs1/Apoe−/− mice demonstrated reductions in necrotic core area and fibrous cap/plaque area ratio. Although t-BHP-treated SMC derived from NBS1 transgenic mice showed enhanced proliferation and decreased apoptosis whereas those from (ΔC) NBS1 transgenics had increased apoptosis, no effects on proliferation or apoptosis were observed in either model in vivo (Figure, bottom right).

Based on the apparent suppression of phospho-p53 and p21 in cultured SMC from Sm22α-ΔNbs1/Apoe−/− mice, the authors suggest that increased repair may promote higher SMC content in vivo. However, increased repair in vivo was not established and cultured SMC may not mimic cells in vivo, as was also observed with the absence of effects on proliferation and apoptosis. This could be because of t-BHP being more injurious or the lack of extracellular matrix constituents normally present in vivo that modulate SMC proliferation and survival. In contrast, the significant effects of transgene expression on P-ATM in vivo are interesting potential candidates for regulation of the observed changes in the fibrous cap.

**Importance of Cell Selectivity in Modulating Atherosclerotic Plaque Phenotype**

This study is significant because it targeted the selective modulation of SMC DDR. Although DNA damage occurs in all cells and has been documented in a variety of cells contributing to plaque composition, it has not been selectively altered in distinct subtypes of plaque cells in vivo. The whole-body deficient mouse model of the classic DDR protein ATM leads to an increase in atherosclerotic plaque area, with both cultured macrophages and SMCs derived from these mice displaying increased levels of stress and inflammatory signaling (for example, activation of the c-Jun N-terminal kinase pathway)³. However, the predominant cell types whose DNA damage is contributing to plaque formation and complexity have not been elucidated. The report by Gray et al³ now links SMC DNA damage to altered SMC composition and potentially instability. However, it remains possible that DNA damage in macrophages and endothelium may also modulate SMC phenotype within lesions. Mouse models with cell-type-specific deficiency of the DNA repair machinery are needed to more clearly define the roles of the DNA repair system in atherogenesis. For instance, given the availability of ATM-flox mice,³ studies of SMC-, macrophage-, and even endothelial-specific ATM-deficient mice in the context of atherosclerosis would potentially help establish their relative contribution in plaque phenotype and progression. Furthermore, phenotyping of SMC-specific NBS1 transgenic of Gray et al³ in the absence of SMC-specific ATM deficiency would enable the dissection of ATM-dependent and ATM-independent DDR in atherosclerosis.

**Harnessing the DDR to Impede Atherosclerosis**

It has been recognized for some time that activation of the DDR, and specifically activation/phosphorylation of ATM, can be achieved by altering chromatin and chromosome topology even in the absence of DNA strand breaks.²¹ For example, the antimalarial chloroquine seems to also have the unique ability to unwind DNA without causing overt DNA damage and leads to activation of the ATM-p53 axis in vivo.²² This intriguing property has been exploited to demonstrate chloroquine’s salutary effects on the metabolic phenotype of mice, including reductions of atherosclerotic plaque burden and insulin resistance, observations that seem to be ATM dependent.²² More recently, enhanced ATM phosphorylation has been observed in macrophages from Apoe−/− mice deficient in wild-type p53-induced phosphatase 1 (Wip1), a negative regulator of ATM-dependent signaling.²³ Macrophage lesion area is also decreased at an early stage of lesion development (8 weeks of Western diet as in the chloroquine studies), an effect that was ATM but not p53 dependent. In vitro evaluation of macrophages from Wip1-deficient mice showed that they were defective in foam cell formation and had enhanced c-Jun N-terminal kinase signaling and autophagy-dependent cholesterol efflux through an ATM-mTOR (mammalian target of rapamycin)–dependent signaling pathway (Figure, bottom left). Although macrophages have largely been the focus of these reports evaluating early stages of lesion development, other aspects of plaque architecture including selective effects on specific cells in vivo have not been investigated. In this regard, it would be interesting to evaluate the effect of chloroquine on plaque composition in the wild-type-NBS1 and (ΔC)NBS1 transgenic mouse models used in the studies by Gray et al.³

**Conclusions and Future Directions**

The accumulation of genomic damage is an unfortunate consequence of living in a world abound with genotoxic stressors, spanning environmental toxins and ionizing radiation to byproducts of our own metabolism, reactive oxygen species. Although classically associated with cancer biology, accumulating data during the past 2 decades have shown ample evidence that DNA damage is also sine qua non of atherosclerotic plaques. From evaluation of mouse models, it seems that the absence of a robust DDR in cells of the plaque exacerbates atherosclerosis, but whether induction of DDR is atheroprotective or can affect salutary remodeling of the plaque is less clear. Clever use of the SMC-specific NBS1 transgenic mouse by Gray et al is not only the first cell-type–specific modulation of the DDR in atherosclerosis but also demonstrates that inducing the DDR can potentially be a novel method of altering plaque composition and stability. If we are to impact cardiovascular mortality, it is essential to understand and integrate the mechanisms by which an unstable, rupture-prone atherosclerotic plaque can be remodeled.

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References

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