Published in final edited form as:

Circ Res. 2015 February 27; 116(5): 770–773. doi:10.1161/CIRCRESAHA.115.305922.

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

Babak Razani and

Cardiovascular Division, Departments of Medicine and Pathology/Immunology, Washington University School of Medicine, St. Louis, MO

Elaine W. Raines

Department of Pathology, University of Washington School of Medicine, Seattle, WA

Abstract

*Gray et al*¹ 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 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 non-atherosclerotic mammary arteries,² and are also increased in experimental models of atherosclerosis, a process that can be reversed by dietary lipid lowering.³ The report by *Gray et al*¹ provides important insights into the specific contribution of SMCs to plaque phenotype when the DDR is manipulated *in vivo*. While 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*¹ suggest the possibility of harnessing atheroprotective features of the ataxia telangiectasia mutated (ATM) kinase,^{4, 5} a primary initiator of the DDR.

Keywords

Editorials; smooth muscle cells; DNA damage response (DDR); double-strand DNA breaks; lesion phenotype

The 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.⁶ Obesity, poor nutrition, and conditions including diabetes, hypertension, lack of physical activity, smoking and advancing age are all major risk factors for developing cardiovascular disease.⁷ Although the hallmark of disease progression is the insidious formation of the

Correspondence to Babak Razani, MD, Cardiovascular Division, Department of Medicine, Washington University School of Medicine, 660 S Euclid Blvd, Campus Box 8086, Saint Louis, MO 63110. brazani@im.wustl.edu; or Elaine W. Raines, Department of Pathology, University of Washington School of Medicine, Harborview Medical Center, Box 359675, 325 9th Ave., Seattle, WA, 98104, ewrainses@uw.edu.

Disclosures: None.

atherosclerotic plaque, it is the acute rupture of vulnerable plaques that is most commonly associated with cardiovascular mortality.⁸ 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/or macrophages, a large lipid-rich core and a thin fibrous cap are notable features.⁹⁻¹¹ Evidence derived from pathology specimens is also beginning to be supported by imaging studies correlating plaque phenotype with acute cardiovascular events.^{12, 13} 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 two decades, but their causative versus associative role in disease progression is less clear.¹⁴ 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.¹⁵ 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 PCR array analyses of cultured normal human aortic and plaque SMCs and detect up-regulation 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 in human atherosclerotic lesions.

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 DNA Damage Response (DDR) involves the induction of a signaling cascades that either triggers cell cycle arrest in order to activate DNA repair mechanisms, or can induce apoptotic mechanisms when DNA damage approaches irreversibility.¹⁶ An initiating event in the DDR involves recruitment of the Mre11-Rad50-NBS1 (MRN) ATPase-nuclease complex to double strand breaks, followed by activation of the kinases ATM (Ataxia telangiectasia mutated) and ATR (ATM and Rad3 related). ATM and ATR phosphorylate

more than 700 target proteins in human cells,¹⁷ including histone H2AX (used by *Gray et al.*,¹ along with ATM auto-phosphorylation, 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.¹⁸ ATM can also be activated by oxidative stress and chromatin structure alterations, but the MRN complex is critical for double-stranded break-dependent activation of ATM. Thus by modulating functional levels of NBS1, one of three components of the MRN complex, *Gray et al.*¹ alter phosphorylation of ATM targets and ATM-mediated signaling controlling cell cycle checkpoints and DNA repair (Figure 1).¹⁹

Transgenic expression of NBS1 or activation-deficient NBS1 accelerates or impairs the DNA damage response, respectively, in SMC *in vivo*

Two novel transgenic mouse models were generated expressing either wildtype NBS1 or one lacking the C-terminal domain necessary for ATM activation and downstream signaling. The two forms of NBS1 were expressed under the control of the SMC-selective promoter SM22 in the ApoE^{-/-} mouse atherosclerosis model. Utilizing a potent inducer of double-strand breaks tert-butyl hydroperoxide (t-BHP), they show that SMCs derived from *Sm22 α -Nbs1/ApoE^{-/-}* 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 *Sm22 α -(C)Nbs1/ApoE^{-/-}* aortas had blunted phosphorylation of ATM and its downstream targets. Interestingly, relatively late events in ATM signaling (i.e. phosphorylation of p53, induction of p21, and the development of apoptosis) behaved oppositely, being suppressed in the setting of NBS1 over-expression and largely unchanged in the presence of (C)NBS1, except for apoptosis that was elevated. The transgenic approach used in this study does have the limitation that it was generated on a wildtype background, and therefore endogenous expression of wildtype 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 6-20 weeks did not differ with selective SMC expression of wildtype NBS1 or mutated NBS1. However, comparison of the two transgenics relative to control ApoE^{-/-} mice showed an increase in fibrous cap area and SMC content in *Sm22 α -Nbs1/ApoE^{-/-}* mice. In contrast, *Sm22 α -(C)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 (C)NBS1 transgenics had increased apoptosis, no effects on proliferation or apoptosis were observed in either model *in vivo* (Figure 1, bottom right).

Based on the apparent suppression of phospho-p53 and p21 in cultured SMC from *Sm22 α -Nbs1/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 due to t-BHP being more injurious and/or the lack of extracellular matrix constituents normally present *in vivo* that modulate SMC

proliferation and survival.²⁰ In contrast, the significant effects of transgene expression on P-ATM *in vivo* is an interesting potential candidate for regulation of the observed changes in the fibrous cap.

The 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,² 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 JNK pathway).⁵ However, the predominant cell types whose DNA damage are contributing to plaque formation and complexity have not been elucidated. The report by Gray *et al*¹ now links SMC DNA damage to altered SMC composition and potentially instability. However, it remains possible that DNA damage in macrophages and/or 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,²¹ studies of SMC-, macrophage-, and even endothelial-specific ATM deficient mice in the context of atherosclerosis would definitely help establish their relative contribution in plaque phenotype and progression. Furthermore, phenotyping of Gray *et al*'s¹ SMC-specific NBS1 transgenic in the absence of SMC-specific ATM deficiency would enable the dissection of ATM-dependent and -independent DDR in atherosclerosis.

Harnessing the DNA Damage Response 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 anti-malarial chloroquine appears 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*.^{21, 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 appear to be ATM-dependent.^{4, 5} More recently, enhanced ATM phosphorylation has been observed in macrophages from *Apoe*^{-/-} mice deficient in wildtype 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 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 JNK signaling and autophagy-dependent cholesterol efflux through an ATM-mTOR-dependent signaling pathway (Figure 1, 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 wildtype-NBS1 and (C)NBS1 transgenic mouse models used in the studies by *Gray et al.*¹

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 over the past two decades have shown ample evidence that DNA damage is also sine qua non of atherosclerotic plaques. From evaluation of mouse models, it appears 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. *Gray et al.*'s¹ clever use of the SMC-specific NBS1 transgenic mouse 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.

Acknowledgments

Sources of Funding: B. Razani is supported by the National Heart, Lung, and Blood Institute grants K08 HL098559 and R01 HL125838; and funding for E.W. Raines is from the National Heart, Lung, and Blood Institute R01 grant HL067267.

References

1. Gray KL, Kumar SV, Figg N, Harrison J, Baker L, Mercer JR, Littlewood TD, Bennett MR. Effects of DNA Damage in Smooth Muscle Cells in Atherosclerosis. *Circ Res*. 2015;114. Epub ahead of print.
2. Martinet W, Knaapen MWM, De Meyer GRY, Herman AG, Kockx MM. Elevated levels of oxidative DNA damage and DNA repair enzymes in human atherosclerotic plaques. *Circulation*. 2002; 106:927–932. [PubMed: 12186795]
3. Martinet W, Knaapen MWM, De Meyer GRY, Herman AG, Kockx MM. Oxidative DNA damage and repair in experimental atherosclerosis are reversed by dietary lipid lowering. *Circ Res*. 2001; 88:733–739. [PubMed: 11304497]
4. Razani B, Feng C, Semenkovich CF. p53 is required for chloroquine-induced atheroprotection but not insulin sensitization. *J Lipid Res*. 2010; 51:1738–1746. [PubMed: 20208057]
5. Schneider JG, Finck BN, Ren J, Standley RN, Takagi M, Maclean KH, Bernal-Mizrachi C, Muslin AJ, Kastan MB, Semenkovich CF. ATM-dependent suppression of stress signaling reduces vascular disease in metabolic syndrome. *Cell Metab*. 2006; 4:377–389. [PubMed: 17084711]
6. WHO. Global status report on noncommunicable diseases 2010. WHO; 2011. Noncommunicable diseases and mental health. online
7. Fuster V. Top 10 cardiovascular therapies and interventions for the next decade. *Nat Rev Cardiol*. 2014; 11:671–683. [PubMed: 25267423]
8. Burke AP, Farb A, Malcom GT, Liang YH, Smialek J, Virmani R. Coronary risk factors and plaque morphology in men with coronary disease who died suddenly. *N Engl J Med*. 1997; 336:1276–1282. [PubMed: 9113930]
9. Davies MJ, Thomas A. Thrombosis and acute coronary-artery lesion in sudden cardiac ischemic death. *New Engl J Med*. 1984; 310:1137–1140. [PubMed: 6709008]

10. Davies MJ, Richardson PD, Woolf N, DKatz DR, Mann J. Risk of thrombosis in human atherosclerotic plaques: role of extracellular lipid, macrophage and smooth muscle cell content. *Br Heart J*. 1993; 69:377–381. [PubMed: 8518056]
11. Thim T, Hagensen MK, Bentzon JF, Falk E. From vulnerable plaque to atherothrombosis. *J Intern Med*. 2008; 263:506–516. [PubMed: 18410594]
12. Takaya N, Yuan C, Chu B, Saam T, Underhill H, Cai J, Tran N, Polissar NL, Isaac C, Ferguson MS, Garden GA, Cramer SC, Maravilla KR, Hashimoto B, Hatsukami TS. Association between carotid plaque characteristics and subsequent ischemic cerebrovascular events: a prospective assessment with MRI: initial results. *Stroke*. 2006; 37:818–823. [PubMed: 16469957]
13. Motoyama S, Sarai M, Harigaya H, Anno H, Inoue K, Hara T, Naruse H, Ishi J, Hishida H, Wong ND, Virmani R, Kondo TYO, Narula J. Computed tomographic angiography characteristics of atherosclerotic plaques subsequently resulting in acute coronary syndrome. *J Am Coll Cardiol*. 2009; 54:49–57. [PubMed: 19555840]
14. Gray K, Bennett M. Role of DNA damage in atherosclerosis-bystander or participant? *Biochem Pharmacol*. 2011; 82:693–700. [PubMed: 21726542]
15. Yu E, Calvert PA, Mercer JR, Harrison J, Baker L, Figg NL, Kumar SV, Wang JC, Hurst LA, Obaid DR, Logan A, West NE, Clarke MC, Vidal-Puig A, Murphy MP, Bennett M. Mitochondrial DNA damage can promote atherosclerosis independently of reactive oxygen species through effects on smooth muscle cells and monocytes and correlates with higher-risk plaques in humans. *Circulation*. 2013; 128:702–712. [PubMed: 23841983]
16. Derheimer FA, Kastan MB. Multiple roles of ATM in monitoring and maintaining DNA integrity. *FEBS Lett*. 2010; 584:3675–3681. [PubMed: 20580718]
17. Matsuoaka S, Ballif BA, Smogorzewska A, McDonald ERr, Hurov KE, Luo J, Bakalarski CE, Zhao Z, Solimini N, Lerenthal Y, Shiloh Y, Gygi SP, Elledge SJ. ATM and ATR substrate analysis reveals extensive protein networks responsive to DNA damage. *Science*. 2007; 316:1160–1166. [PubMed: 17525332]
18. Paull TT. Mechanisms of ATM activation. *Annu Rev Biochem*. 2015; 84:12.11–12.28.
19. Falck J, Coates J, Jackson SP. Conserved modes of recruitment of ATM, ATR and DNA-PKcs to sites of DNA damage. *Nature*. 2005; 434:605–611. [PubMed: 15758953]
20. Raines EW, Koyama H, Carragher NO. The extracellular matrix dynamically regulates smooth muscle cell responsiveness to PDGF. *Ann N Y Acad Sci*. 2000; 902:39–51. [PubMed: 10865824]
21. Callén E, Jankovic M, Wong N, Zha S, Chen HT, Difilippantonio S, Di Virgilio M, Heidkamp G, Alt FW, Nussenzweig A, Nussenzweig M. Essential role for DNA-PKcs in DNA double-strand break repair and apoptosis in ATM-deficient lymphocytes. *Mol Cell*. 2009; 34:285–297. [PubMed: 19450527]
22. Loehberg CR, Thompson T, Kastan MB, Maclean KH, Edwards DG, Kittrell FS, Medina D, Conneely OM, O'Malley BW. Ataxia telangiectasia-mutated and p53 are potential mediators of chloroquine-induced resistance to mammary carcinogenesis. *Cancer Res*. 2007; 67:12026–12033. [PubMed: 18089834]
23. Le Guezennec X, Brichkina A, Hunag YF, Kostromina E, Han W, Bulavin DV. Wip1-dependent regulation of autophagy, obesity and atherosclerosis. *Cel Metab*. 2012; 16:68–80.

Figure 1. Smooth muscle- and potential macrophage-selective effects of enhancing the DNA damage response on the phenotype of atherosclerotic lesions

The composition of an advanced lesion of atherosclerosis is depicted in which features associated with a vulnerable plaque are shown (top). The arrow indicates a shoulder region particularly at risk for plaque rupture with a thin fibrous cap and underlying apoptotic macrophages and a lipid-rich core. The SMC (bottom right) shows the DNA damage response pathway that was enhanced by Gray et al¹ through SMC over-expression of a wildtype form of an essential MRN protein NSB1. ATM is recruited to the MRN complex that senses double-strand DNA breaks and leads to enhanced signaling, and consequently to increased ATM auto-phosphorylation and ATM-dependent phosphorylation of numerous targets including the histone H2AX (γ H2AX) and others which control cell cycle checkpoints and DNA repair, such as p53. In turn, activation of ATM also modulates central mediators of the response, such as c-Jun N-terminal kinase, JNK. Observed SMC effects in atherosclerotic plaques in vivo are summarized. Although there is much more limited information on modulation of the DNA damage response in macrophages (bottom, left), a summary of data from in vitro studies is shown (indicated by brackets on the figure). These include the observation of enhanced ATM activation following stimulation with either chloroquine⁵ or with deficiency of the ATM inhibitor WIP1.²³ Increases (up arrow),

decreases (down arrow), or no change (flat double-arrow) have been indicated below the cells.

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript