Redox Biology 22 (2019) 101081

Contents lists available at ScienceDirect

Redox Biology

journal homepage: www.elsevier.com/locate/redox

Cardiovascular disease is obNOXious: New insights into NoxA1 in smooth muscle phenotype

A. Wayne Orr[a,b,c,*], Matthew D. Woolard[d,**]

[a] Departments of Pathology and Translational Pathobiology, LSU Health – Shreveport, Shreveport, LA 71130, United States
[b] Departments of Cell Biology and Anatomy, LSU Health – Shreveport, Shreveport, LA 71130, United States
[c] Departments of Cellular and Molecular Physiology, LSU Health – Shreveport, Shreveport, LA 71130, United States
[d] Departments of Microbiology and Immunology, LSU Health – Shreveport, Shreveport, LA 71130, United States

Smooth muscle cells play an important role in the pathophysiology of atherosclerotic cardiovascular disease. Following the accumulation of lipid-laden macrophages within arterial intima, smooth muscle cells undergo a phenotypic transition and migrate from the vessel media into the growing neointima [1]. While these cells contribute to plaque size, the smooth muscle cells in the plaque proliferate and deposit extensive extracellular matrix to form a protective fibrous cap that limits plaque rupture and subsequent clinical thrombotic events. However, smooth muscle migration and proliferation can drive the development of restenotic vascular remodeling following vascular injury, such as post-balloon angioplasty and stent placement, suggesting both positive and negative consequences for smooth muscle fibroproliferative remodeling in cardiovascular disease [2]. In addition to growth and migration, smooth muscle cells can also take on proinflammatory and macrophage-like phenotypes that may contribute to plaque development [1,3]. While these phenotypes are thought to contribute to leukocyte recruitment and lipid handling, respectively, their relative contribution to atherosclerosis remains largely unknown [1,3].

NADPH oxidases play a critical role in multiple aspects of cardiovascular disease. In smooth muscle cells, the primary NADPH oxidase isoforms include NOX1 and NOX4, although NOX2 has been detected at low levels [4]. NOX1 shows enhanced expression following carotid injury [5], and both NOX1 and NOX4 show enhanced expression in response to mitogenic stimuli, such as PDGF-BB and Ang II [4,6]. However, these enzymes differ considerably in their localization and enzymatic activity. NOX1 is primarily found in the plasma membrane, caveoli, and endosomes, whereas NOX4 localizes to focal adhesions, the endoplasmic reticulum, and mitochondria [7–9]. Additionally, NOX1 interacts with multiple regulatory proteins to drive inducible superoxide production, while NOX4 is constitutively active and generates primarily hydrogen peroxide [10,11]. Consistent with this discrepancy, NOX1 knockdown blunts superoxide production in response to Ang II and PDGF-BB, and NOX1 deletion reduces Ang II-induced superoxide production and increases in blood pressure in vivo [12,13]. Similarly, smooth muscle-specific NOX1 overexpression enhances superoxide production, smooth muscle proliferation, and neointimal area following carotid injury, whereas NOX1 knockout mice show decreased superoxide production, reduced proliferation, and diminished neointima [12,14]. However, the role of NOX1 in atherosclerosis has been controversial with both enhanced plaque formation and reduced plaque formation described following NOX1 deletion [15–17]. NOX1 deletion shows a strong reduction in diabetic atherosclerosis [18], which could be due to the enhanced expression of NOX1 in diabetic atherosclerosis or due to the enhanced activation of PKCβ in diabetic atherosclerosis which stimulates NOX1 activation [19–21].

In this issue of *Redox Biology*, Vendrov et al. utilized a combination of genetic mouse models and cell culture studies to provide strong evidence that the NOX1 coactivator protein NoxA1 critically regulates smooth muscle growth, migration, and phenotypic modulation in stenotic and atherosclerotic vascular remodeling. In a previous study by this group, NoxA1 expression was shown to be elevated during stenotic and atherosclerotic vascular remodeling, and NoxA1 overexpression worsened stenosis following carotid wire injury [22]. The current study utilized global NoxA1 deletion and smooth muscle-specific NoxA1 deletion to definitively demonstrate a critical role for NoxA1 in pathogenic vascular remodeling (Vendrov et al., Redox Biol., 2018). NoxA1 knockout mice showed reduced stenotic vascular remodeling following wire injury associated with reduced smooth muscle proliferation and migration in cell culture models. Furthermore, NoxA1 global deletion in LDL receptor knockout mice and ApoE knockout mice showed diminished superoxide production with reduced plaque formation, and smooth muscle-specific NoxA1 deletion in ApoE knockout mice similarly showed reduced superoxide levels and atherosclerotic plaque formation (Vendrov et al., Redox Biol., 2018). These data are consistent with an important role for smooth muscle NOX1 in promoting atherosclerotic plaque formation, adding further evidence to a pathological

* Correspondence to: Department of Pathology and Translational Pathobiology, Biomedical Research Institute, LSU Health Sciences Center – Shreveport, 1501 Kings Hwy, Rm. 6-21, Shreveport, LA 71130, United States.
** Correspondence to: Department of Microbiology and Immunology, Biomedical Research Institute, LSU Health Sciences Center – Shreveport, 1501 Kings Hwy, Rm. 3-36, Shreveport, LA 71130, United States.
E-mail addresses: aorr@lsuhsc.edu (A.W. Orr), mwoola@lsuhsc.edu (M.D. Woolard).

https://doi.org/10.1016/j.redox.2018.101081
Received 12 December 2018; Accepted 14 December 2018
Available online 19 December 2018

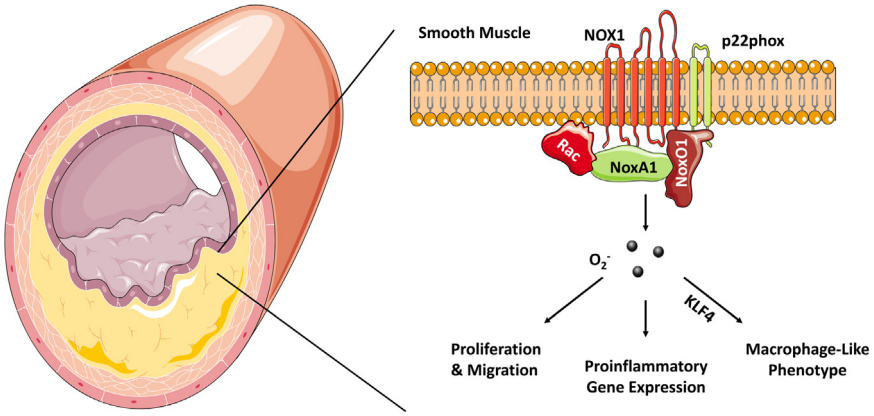

Fig. 1. Smooth muscle NOX1 signaling in atherosclerosis. NoxA1 critically regulates NOX1-dependent superoxide production in atherosclerotic smooth muscle cells to drive plaque formation through smooth muscle proliferation, migration, and transition to a proinflammatory, macrophage-like phenotype.

role for NOX1 signaling in atherosclerotic vascular remodeling.

The conversion of smooth muscle cells to a proinflammatory, macrophage-like phenotype is thought to promote atherosclerosis plaque progression [1]. This smooth muscle phenotypic transition is dependent upon the induction of reprogramming transcription factors, including KLF4 and Oct4 [23,24]. In the current study, the authors show that the loss of NOXA1 in smooth muscle cells reduces nuclear KLF4 expression within atherosclerotic plaques and reduces oxidized phospholipid elicited expression of KLF4 and the macrophage marker CD68 (Vendrov et al., Redox Biol., 2018). The loss of KLF4 from smooth muscle cells reduces the expression of multiple proinflammatory genes (e.g. MCP-1) [23], and the loss of NOXA1 in smooth muscle cells similarly results in diminished proinflammatory gene expression (MCP-1, VCAM-1, MMP2). Therefore, these studies provide strong evidence implicating NOXA1 as a key regulator of smooth muscle cell dedifferentiation into the proinflammatory, macrophage-like phenotype.

The work of Vendrov et al. highlights the importance of smooth muscle-derived, NOX1-dependent superoxide production in atherosclerotic plaque formation and identifies NoxA1/NOX1 as a potential new therapeutic target to treat cardiovascular disease. However, several important questions remain. While NoxA1/NOX1 clearly regulates plaque formation, the effect of NoxA1/NOX1 inhibition in models of atherosclerotic plaque regression, which could be accomplished using the NoxA1ds inhibitor [25], would provide better insight into its potential as a therapeutic target. The reduction in smooth muscle proliferation and migration observed with NoxA1/NOX1 inhibition could result in a vulnerable plaque phenotype, and the role of NOX1 in plaque stability or matrix deposition has not been addressed. As such, NOX1 inhibitors may be better suited to limit restenotic vascular remodeling that is driven primarily by smooth muscle proliferation and migration. Finally, smooth muscle phenotypic transition occurs bidirectionally, and it remains unknown whether NOXA1/NOX1 inhibition would reverse the phenotypic transition in cells that have already attained the smooth muscle macrophage-like phenotype.

In summary, NOX1 signaling plays a critical role in vascular remodeling through the regulation of smooth muscle proliferation and migration. The study by Vendrov et al. definitively demonstrates a critical role for NoxA1/NOX1 signaling in atherosclerotic plaque formation by driving both smooth muscle proliferation and migration and by promoting the KLF4-dependent transition to a proinflammatory, macrophage-like phenotype (Fig. 1).

References

[1] M.R. Bennett, S. Sinha, G.K. Owens, Vascular smooth muscle cells in atherosclerosis, Circ. Res. 118 (4) (2016) 692–702.
[2] A.V. Finn, et al., Concept of vulnerable/unstable plaque, Arterioscler. Thromb. Vasc. Biol. 30 (7) (2010) 1282–1292.
[3] A.W. Orr, et al., Complex regulation and function of the inflammatory smooth muscle cell phenotype in atherosclerosis, J. Vasc. Res. 47 (2) (2010) 168–180.
[4] B. Lassegue, et al., Novel gp91(phox) homologues in vascular smooth muscle cells: nox1 mediates angiotensin II-induced superoxide formation and redox-sensitive signaling pathways, Circ. Res. 88 (9) (2001) 888–gp94.
[5] K. Szocs, et al., Upregulation of Nox-based NAD(P)H oxidases in restenosis after carotid injury, Arterioscler. Thromb. Vasc. Biol. 22 (1) (2002) 21–27.
[6] K. Wingler, et al., Upregulation of the vascular NAD(P)H-oxidase isoforms Nox1 and Nox4 by the renin-angiotensin system in vitro and in vivo, Free Radic. Biol. Med 31 (11) (2001) 1456–1464.
[7] L.L. Hilenski, et al., Distinct subcellular localizations of Nox1 and Nox4 in vascular smooth muscle cells, Arterioscler. Thromb. Vasc. Biol. 24 (4) (2004) 677–683.
[8] L.L. Camargo, et al., Vascular Nox (NADPH oxidase) compartmentalization, protein hyperoxidation, and endoplasmic reticulum stress response in hypertension, Hypertension 72 (1) (2018) 235–246.
[9] A.E. Vendrov, et al., NOX4 NADPH oxidase-dependent mitochondrial oxidative stress in aging-associated cardiovascular disease, Antioxid. Redox Signal. 23 (18) (2015) 1389–1409.
[10] R.K. Ambasta, et al., Noxa1 is a central component of the smooth muscle NADPH oxidase in mice, Free Radic. Biol. Med. 41 (2) (2006) 193–201.
[11] K. von Lohneysen, et al., Structural insights into Nox4 and Nox2: motifs involved in function and cellular localization, Mol. Cell Biol. 30 (4) (2010) 961–975.
[12] A. Dikalova, et al., Nox1 overexpression potentiates angiotensin II-induced hypertension and vascular smooth muscle hypertrophy in transgenic mice, Circulation 112 (17) (2005) 2668–2676.
[13] K. Matsuno, et al., Nox1 is involved in angiotensin II-mediated hypertension: a study in Nox1-deficient mice, Circulation 112 (17) (2005) 2677–2685.
[14] M.Y. Lee, et al., Mechanisms of vascular smooth muscle NADPH oxidase 1 (Nox1) contribution to injury-induced neointimal formation, Arterioscler. Thromb. Vasc. Biol. 29 (4) (2009) 480–487.
[15] C.G. Sobey, et al., NOX1 deficiency in apolipoprotein E-knockout mice is associated with elevated plasma lipids and enhanced atherosclerosis, Free Radic. Res. 49 (2) (2015) 186–198.
[16] A.L. Sheehan, et al., Role for Nox1 NADPH oxidase in atherosclerosis, Atherosclerosis 216 (2) (2011) 321–326.
[17] S. Kumar, et al., Accelerated atherosclerosis development in C57Bl6 mice by overexpressing AAV-mediated PCSK9 and partial carotid ligation, Lab. Invest. 97 (8) (2017) 935–945.
[18] S.P. Gray, et al., NADPH oxidase 1 plays a key role in diabetes mellitus-accelerated atherosclerosis, Circulation 127 (18) (2013) 1888–1902.
[19] M.C. Wendt, et al., Differential effects of diabetes on the expression of the gp91phox homologues nox1 and nox4, Free Radic. Biol. Med. 39 (3) (2005) 381–391.
[20] J. Streeter, et al., Phosphorylation of Nox1 regulates association with NoxA1 activation domain, Circ. Res. 115 (11) (2014) 911–918.
[21] L. Kong, et al., PKCbeta promotes vascular inflammation and acceleration of atherosclerosis in diabetic ApoE null mice, Arterioscler. Thromb. Vasc. Biol. 33 (8) (2013) 1779–1787.
[22] X.L. Niu, et al., Nox activator 1: a potential target for modulation of vascular reactive oxygen species in atherosclerotic arteries, Circulation 121 (4) (2010) 549–559.
[23] L.S. Shankman, et al., KLF4-dependent phenotypic modulation of smooth muscle cells has a key role in atherosclerotic plaque pathogenesis, Nat. Med. 21 (6) (2015) 628–637.
[24] O.A. Cherepanova, et al., Activation of the pluripotency factor OCT4 in smooth muscle cells is atheroprotective, Nat. Med. 22 (6) (2016) 657–665.
[25] D.J. Ranayhossaini, et al., Selective recapitulation of conserved and nonconserved regions of putative NOXA1 protein activation domain confers isoform-specific inhibition of Nox1 oxidase and attenuation of endothelial cell migration, J. Biol. Chem. 288 (51) (2013) 36437–36450.