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

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 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 isofoms 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 endoplasmatic 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 knockout 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...
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, NOXA1-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).

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