

Clarity on the Isoform-Specific Roles of NADPH Oxidases and NADPH Oxidase-4 in Atherosclerosis

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Atherosclerosis is a complex disease that remains a leading cause of death and disability. The formation of vascular plaques arises from the interaction of dyslipidemia with the altered function of blood vessels and immune cells. The exact cause of atherosclerosis is not known, but from the 1950s onward, oxidative modifications of lipids and proteins were detected in vascular lesions, and the degree of oxidation was found to correlate with the severity of disease. Based on these findings, a logical hypothesis emerged that suppression of these oxidative modifications might prevent atherosclerosis. Based on promising preclinical data, antioxidant therapies, which use a broad spectrum approach to suppress the actions of many oxidants, were introduced with much promise for the treatment of atherosclerosis, as well as cancer and aging. The results of numerous clinical trials have been clear, and broad spectrum antioxidant therapies do not provide protection against atherosclerosis.

There are many sources of reactive oxygen species within atherosclerotic lesions, and one of the most prominent reasons cited for the failure of antioxidant therapies is a lack of specificity. A highly specialized and abundant source of reactive oxygen species is the family of transmembrane NADPH-dependent oxidoreductases (Nox enzymes) that synthesize superoxide \( \text{O}_2^- \). There are 7 related isoforms, and 4 (Nox1, Nox2, Nox4, and Nox5) are expressed in vascular and immune cells. Much evidence exists to connect increased expression and activity of the Nox1 and 2 isoforms with the development of atherosclerosis in mouse and primate models, as well as in humans. Reactive oxygen species production from Nox2 is stimulated by interaction with the subunits p47phox and p67phox, whereas Nox1 is activated by complex formation with NOXO1 and NOXA1, as well as with p47phox. Genetic deletion of p47phox protects against lesion formation in the aorta of mouse models which suggests that either Nox1 or Nox2 or both are important for the development of atherosclerosis.

Important no role of this isoform in the development of atherosclerosis. In contrast, the deletion of Nox1 does not impact atherosclerosis in the aorta at baseline, but it does in the presence of diabetes mellitus, which induces the expression of Nox1 and its subunits. In humans, the loss of Nox2 activity in granulomatous disease is associated with reduced carotid, but not coronary atherosclerosis, suggesting that Nox enzymes might have different roles depending on the location of the lesion. This concept is supported to some extent in animal studies where deletion of p47phox and Nox2 have no effect on atherosclerosis in the aortic sinus, but reduce atherosclerosis in the descending aorta. Nox5 is upregulated in atherosclerotic lesions of humans, but as this gene is absent from the mouse genome, its impact on the development of lesions remains unknown. A role for Nox4 in atherosclerosis was comparatively obscure until recently.

In the February 2016 issue of ATVB, a study by Gray et al sheds new light on the role of Nox4 in atherosclerosis. Nox4 is the black sheep of the Nox family. Unlike the other Nox isoforms, it is constitutively active, primarily regulated by changes in gene expression (inducible Nox), and emits hydrogen peroxide instead of \( \text{O}_2^- \). This latter property of Nox4 has been controversial because all of the Nox enzymes synthesize \( \text{O}_2^- \) initially, but Nox4 is able to rapidly convert \( \text{O}_2^- \) to hydrogen peroxide. This is an important property because hydrogen peroxide does not interact with and degrade NO signaling, and the loss of endothelial NO is a well-accepted pathway to increased atherosclerosis. Previously, Gray et al had shown that Nox4 deletion was without effect on atherosclerosis at 10 weeks in apolipoprotein E knockout mice rendered diabetic with streptozotocin. The current study highlights the temporal complexity of atherosclerotic lesions and shows that at 20 weeks, Nox4 expression is reduced in the aorta of diabetic apolipoprotein E knockout mice, which is in agreement with the reduced levels of Nox4 observed in advanced human lesions. Furthermore, at 20 weeks, an increased plaque burden was observed in Nox4 knockout mice, which reveals a previously unappreciated protective role of Nox4 in models of atherosclerosis. The strength of any scientific finding is enhanced by independent verification and particularly so with complex models, such as atherosclerosis. Using different strategies, including the global knockout of Nox4, and endothelial overexpression of Nox4, inducible deletion of Nox4, and knockout of Nox4 in a distinct model of atherosclerosis, four studies have shown, almost contemporaneously, that plaque burden is universally reduced when Nox4 is present. Thus, the evidence seems clear that Nox4 has a protective role in the setting of atherosclerosis.

A beneficial role for Nox4 in the vasculature has previously been reported, and Nox4 has been shown to preserve eNOS.
expression, promote angiogenesis, and reduce inflammation. These actions are consistent with the protective role of Nox4 in atherosclerosis described by Gray et al and others where it reduces inflammation and fibrosis and improves endothelial function without affecting dyslipidemia. However, the consequences of Nox4 expression are not always benevolent, and it has been shown to promote lung fibrosis and pulmonary hypertension. These Jekyll and Hyde effects of Nox4 most likely arise from cell type-specific effects (fibroblast and smooth muscle versus endothelial), and the positive effects of supporting eNOS function in the endothelium may not be beneficial when eNOS is uncoupled and producing O_2^−.

Collectively, there is compelling evidence for a contributory role of Nox1 and Nox2 in the development of atherosclerotic lesions in mice under various conditions and a protective role for Nox4. These findings also serve to highlight the limitations of antioxidant strategies that nonspecifically reduce reactive oxygen species and, therefore, would inhibit the effects of both deleterious and protective Nox enzymes. A more effective strategy might be isoform-specific targeting, such as recently described for Nox2, but this would leave Nox1 unopposed and the potential consequences of individual isoforms regulating the development of atherosclerosis in different vascular beds and under different conditions, that is, diabetes mellitus. There are no available therapeutics that activate Nox4, and this strategy might be constrained by possible untoward actions of antioxidant strategies that nonspecifically reduce reactive oxygen species in the artery wall.

Disclosures

None.

References


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