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Clarity on the Isoform Specific Roles of NADPH-oxidases (Nox) and Nox4 in Atherosclerosis

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Keywords

Atherosclerosis; diabetes; ROS; Nox; Nox1; Nox2; Nox4

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[1]. 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 employ 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, broad spectrum antioxidant therapies do not provide protection against atherosclerosis [2, 3].

There are many sources of reactive oxygen species (ROS) within atherosclerotic lesions and one of the most prominent reasons cited for the failure of antioxidant therapies is a lack of specificity[1]. A highly specialized and abundant source of ROS is the family of transmembrane NADPH-dependent oxidoreductases (Nox enzymes) that synthesize superoxide (O_2^-). There are 7 related isoforms, and 4 (Nox1, Nox2, Nox4 and Nox5) are expressed in vascular and immune cells[4]. 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 [5, 6]. ROS 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 [7]. 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 [8, 9]. Studies with Nox2 knockout mice point to an important role of this isoform in the development of atherosclerosis in the aorta [10]. In contrast, the deletion of Nox1 does not impact atherosclerosis in the aorta at baseline[11, 12], but it does in the presence of diabetes which induces the expression of Nox1 and its subunits [11, 13]. In humans, the loss of Nox2 activity in granulomatous disease is associated with reduced carotid, but not

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coronary atherosclerosis, suggesting that Nox enzymes might have different roles depending on the location of the lesion[14]. This concept is supported to some extent in animal studies where deletion of p47phox [15] and Nox2[16] have no effect on atherosclerosis in the aortic sinus, but reduce atherosclerosis in the descending aorta [8, 10]. Nox5 is upregulated in atherosclerotic lesions of humans[17] 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 this issue, a study by Gray et al [18] 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 (iNOX) and emits hydrogen peroxide (H_2O_2) instead of O_2^-. This latter property of Nox4 has been controversial as all of the Nox enzymes synthesize O_2^- initially, but Nox4 is able to rapidly convert O_2^- to H_2O_2. This is an important property as H_2O_2 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 ApoE knockout mice rendered diabetic with streptozotocin[11]. 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 ApoE 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[18]. 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[18] endothelial overexpression of Nox4[19], inducible deletion of Nox4[20] and knockout of Nox4 in a distinct model of atherosclerosis[21], four studies have shown, almost contemporaneously, that plaque burden is universally reduced when Nox4 is present. Thus, the evidence seems very 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[22–24]. These actions are consistent with the protective role of Nox4 in atherosclerosis described by Gray et al and others where it reduces inflammation, fibrosis and improves endothelial function[18–21] 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[25]. 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 non-specifically reduce ROS 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[26], 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 i.e. diabetes. There are no available therapeutics that activate Nox4 and this strategy might be constrained by possible untoward actions of Nox4 in other cell types and organ systems. Alternatively, strategies that can target multiple Nox enzymes (Nox1–2 and 5), while preserving Nox4 activity, might be more effective[27].

References

1. Stocker R, Keaney JF Jr. Role of oxidative modifications in atherosclerosis. Physiol Rev. 2004; 84:1381–1478. [PubMed: 15383655]
2. Vivekananthan DP, Penn MS, Sapp SK, Hsu A, Topol EJ. Use of antioxidant vitamins for the prevention of cardiovascular disease: Meta-analysis of randomised trials. Lancet. 2003; 361:2017–2023. [PubMed: 12814711]
3. Waters DD, Alderman EL, Hsia J, Howard BV, Cobb FR, Rogers WJ, Ouyang P, Thompson P, Tardif JC, Higginson L, Bittner V, Steffes M, Gordon DJ, Proschan M, Younes N, Verter JI. Effects of hormone replacement therapy and antioxidant vitamin supplements on coronary atherosclerosis in postmenopausal women: A randomized controlled trial. JAMA. 2002; 288:2432–2440. [PubMed: 12435256]
4. Bedard K, Krause KH. The nox family of ros-generating nadph oxidases: Physiology and pathophysiology. Physiol Rev. 2007; 87:245–313. [PubMed: 17237347]
5. Stanic B, Pandey D, Fulton DJ, Miller FJ Jr. Increased epidermal growth factor-like ligands are associated with elevated vascular nicotinamide adenine dinucleotide phosphate oxidase in a primate model of atherosclerosis. Arterioscler Thromb Vasc Biol. 2012; 32:2452–2460. [PubMed: 22879585]
6. Sorescu D, Weiss D, Lassegue B, Clempus RE, Szocs K, Sorescu GP, Valppu L, Quinn MT, Lambeth JD, Vega JD, Taylor WR, Griendling KK. Superoxide production and expression of nox family proteins in human atherosclerosis. Circulation. 2002; 105:1429–1435. [PubMed: 11914250]
7. Banfi B, Clark RA, Steger K, Krause KH. Two novel proteins activate superoxide generation by the nadph oxidase nox1. J Biol Chem. 2003; 278:3510–3513. [PubMed: 12473664]
8. Barry-Lane PA, Patterson C, van der Merwe M, Hu Z, Holland SM, Yeh ET, Runge MS. P47phox is required for atherosclerotic lesion progression in apoe(−/−) mice. J Clin Invest. 2001; 108:1513–1522. [PubMed: 11714743]
9. Vendrov AE, Hakim ZS, Madamanchi NR, Rojas M, Madamanchi C, Runge MS. Atherosclerosis is attenuated by limiting superoxide generation in both macrophages and vessel wall cells. Arterioscler Thromb Vasc Biol. 2007; 27:2714–2721. [PubMed: 17823367]
10. Judkins CP, Diep H, Broughton BR, Mast AE, Hooker EU, Miller AA, Selemidis S, Dusting GJ, Sobey CG, Drummond GR. Direct evidence of a role for nox2 in superoxide production, reduced nitric oxide bioavailability, and early atherosclerotic plaque formation in apoe−/− mice. Am J Physiol Heart Circ Physiol. 2010; 298:H24–32. [PubMed: 19837950]
11. Gray SP, Di Marco E, Okabe J, et al. Nadph oxidase 1 plays a key role in diabetes mellitus-accelerated atherosclerosis. Circulation. 2013; 127:1888–1902. [PubMed: 23564668]
12. Sobey CG, Judkins CP, Rivera J, Lewis CV, Diep H, Lee HW, Kemp-Harper BK, Broughton BR, Selemidis S, Gaspari TA, Samuel CS, Drummond GR. Nox1 deficiency in apolipoprotein e-knockout mice is associated with elevated plasma lipids and enhanced atherosclerosis. Free Radic Res. 2015; 49:186–198. [PubMed: 25496431]
13. Ali MI, Ketsawatsomkron P, Belin de Chantemele EJ, Mintz JD, Muta K, Salet C, Black SM, Tremblay ML, Fulton DJ, Marrero MB, Stepp DW. Deletion of protein tyrosine phosphatase 1b improves peripheral insulin resistance and vascular function in obese, leptin-resistant mice via reduced oxidant tone. Circ Res. 2009; 105:1013–1022. [PubMed: 19797171]
14. Sibley CT, Estwick T, Zavodni A, et al. Assessment of atherosclerosis in chronic granulomatous disease. Circulation. 2014; 130:2031–2039. [PubMed: 25239440]

15. Hsich E, Segal BH, Pagano PJ, Rey FE, Paigen B, Deleonardis J, Hoyt RF, Holland SM, Finkel T. Vascular effects following homozygous disruption of p47(phox) : An essential component of nadph oxidase. Circulation. 2000; 101:1234–1236. [PubMed: 10725280]
16. Kirk EA, Dinauer MC, Rosen H, Chait A, Heinecke JW, LeBoeuf RC. Impaired superoxide production due to a deficiency in phagocyte nadph oxidase fails to inhibit atherosclerosis in mice. Arterioscler Thromb Vasc Biol. 2000; 20:1529–1535. [PubMed: 10845868]
17. Guzik TJ, Chen W, Gongora MC, Guzik B, Lob HE, Mangalat D, Hoch N, Dikalov S, Rudzinski P, Kapelak B, Sadowski J, Harrison DG. Calcium-dependent nox5 nicotinamide adenine dinucleotide phosphate oxidase contributes to vascular oxidative stress in human coronary artery disease. J Am Coll Cardiol. 2008; 52:1803–1809. [PubMed: 19022160]
18. Gray SP, Di Marco E, Kennedy K, Chew P, Okabe J, El-Osta A, Calkin AC, Biessen EA, Touyz RM, Cooper ME, Schmidt HH, Jandeleit-Dahm KA. Reactive oxygen species can provide atheroprotection via nox4-dependent inhibition of inflammation and vascular remodeling. Arterioscler Thromb Vasc Biol. 2016; 36:295–307. [PubMed: 26715682]
19. Craige SM, Kant S, Reif M, Chen K, Pei Y, Angoff R, Sugamura K, Fitzgibbons T, Keaney JF Jr. Endothelial nadph oxidase 4 protects apoe−/− mice from atherosclerotic lesions. Free Radic Biol Med. 2015; 89:1–7. [PubMed: 26169727]
20. Schurmann C, Rezende F, Kruse C, Yasar Y, Lowe O, Fork C, van de Sluis B, Bremer R, Weissmann N, Shah AM, Jo H, Brandes RP, Schroder K. The nadph oxidase nox4 has anti-atherosclerotic functions. Eur Heart J. 2015; 36:3447–3456. [PubMed: 26385958]
21. Langbein H, Brunssen C, Hofmann A, Cimalla P, Brux M, Bornstein SR, Deussen A, Koch E, Morawietz H. Nadph oxidase 4 protects against development of endothelial dysfunction and atherosclerosis in ldl receptor deficient mice. Eur Heart J. 2015
22. Craige SM, Chen K, Pei Y, Li C, Huang X, Chen C, Shibata R, Sato K, Walsh K, Keaney JF Jr. Nadph oxidase 4 promotes endothelial angiogenesis through endothelial nitric oxide synthase activation. Circulation. 2011; 124:731–740. [PubMed: 21788590]
23. Schroder K, Zhang M, Benkhoff S, Mieth A, Pliquett R, Kosowski J, Kruse C, Luedike P, Michaelis UR, Weissmann N, Dimmeler S, Shah AM, Brandes RP. Nox4 is a protective reactive oxygen species generating vascular nadph oxidase. Circ Res. 2012; 110:1217–1225. [PubMed: 22456182]
24. Zhang M, Brewer AC, Schroder K, Santos CX, Grieve DJ, Wang M, Anilkumar N, Yu B, Dong X, Walker SJ, Brandes RP, Shah AM. Nadph oxidase-4 mediates protection against chronic load-induced stress in mouse hearts by enhancing angiogenesis. Proc Natl Acad Sci U S A. 2010; 107:18121–18126. [PubMed: 20921387]
25. Hecker L, Vittal R, Jones T, Jagirdar R, Luckhardt TR, Horowitz JC, Pennathur S, Martinez FJ, Thannickal VJ. Nadph oxidase-4 mediates myofibroblast activation and fibrogenic responses to lung injury. Nat Med. 2009; 15:1077–1081. [PubMed: 19701206]
26. Quesada IM, Lucero A, Amaya C, Meijles DN, Cifuentes ME, Pagano PJ, Castro C. Selective inactivation of nadph oxidase 2 causes regression of vascularization and the size and stability of atherosclerotic plaques. Atherosclerosis. 2015; 242:469–475. [PubMed: 26298737]
27. Chen F, Yu Y, Qian J, Wang Y, Cheng B, Dimitropoulou C, Patel V, Chadli A, Rudic RD, Stepp DW, Catravas JD, Fulton DJ. Opposing actions of heat shock protein 90 and 70 regulate nicotinamide adenine dinucleotide phosphate oxidase stability and reactive oxygen species production. Arterioscler Thromb Vasc Biol. 2012; 32:2989–2999. [PubMed: 23023377]