The NOX on Pulmonary Hypertension

Karl A. Sanders, John R. Hoidal

Chronic pulmonary arterial hypertension (PAH) is a devastating clinical disorder that contributes to the morbidity and mortality of adult and pediatric patients with a wide range of lung and heart diseases. Diseases leading to pulmonary hypertension are frequently associated with hypoxia within discrete areas of the lung. Acutely, the regional response to hypoxia is a reversible contraction of pulmonary artery smooth muscle cells (PASMC), which is a protective physiologic response that serves to redirect blood to better-ventilated areas of the lung. This constrictor response of PASMC contrasts with that of systemic arterial smooth muscle cells, which usually relax in response to hypoxia, indicating that oxygen (O_2) sensing mechanisms in vascular smooth muscle are adapted to the environment from which they are derived. Importantly, chronic hypoxia induces irreversible changes of profound vascular remodeling characterized by medial and adventitial thickening of the muscular and elastic vessels and muscularization of previously nonmuscularized more distal small vessels. This is the basis for debilitating persistent PAH.

Reactive oxygen species (ROS) are important regulators of vascular tone and function. In the lung, ROS are implicated in acute hypoxic vasoconstriction. Administration of superoxide dismutase significantly attenuates pulmonary vasoconstriction because of hypoxia. Moreover, several studies have now shown that agents promoting ROS generation stimulate both systemic arterial smooth muscle cells and PASMC proliferation implicating ROS in the vascular remodeling associated with chronic hypoxia. Again, suppression of endogenous ROS production inhibits smooth muscle cell (SMC) proliferation and promotes apoptosis. In animal models, ROS production has been directly linked to the vascular remodeling associated with chronic hypoxia-induced PAH. Furthermore, chronic hypoxia-associated increases in ROS generation may interact with and modulate agonist-mediated PA vasoconstrictor responses.

This idea that there is a paradoxical increase in ROS generation during hypoxia, although still somewhat controversial, is gaining support. Observations using a variety of experimental techniques and in many cells and tissue types support this phenomena, and the related concept that hypoxia-induced ROS may be both a physiological and pathophysiologic response to environmental stress. Of note, with the exception of xanthine oxidase, most oxidases and electron transfer systems have K_m's for O_2 low enough to support ROS generation at very low intracellular O_2 concentrations, supporting the feasibility of this apparent paradox. The ability to detect ROS production has been historically challenging, and one must address such work with informed skepticism. However, verification of the results by other methods and the ability to block the signal with appropriate antioxidants supports the credibility of the observations.

In this issue of Circulation Research in the article "Hypoxia-dependent regulation of nonphagocytic NADPH oxidase subunit NOX4 in the pulmonary vasculature", Mittal et al publish their findings suggesting that the NADPH oxidase homologue NOX4 may have a key role in vascular remodeling associated with the development of hypoxia-induced PAH. Mice maintained for 21 days under hypoxic (10%) conditions had substantially increased NOX4 expression in medial PASMC. The expression of other NOX homologues or NADPH oxidase subunits previously reported in pulmonary or systemic vascular smooth muscle was not increased. Importantly, pulmonary arteries from subjects with idiopathic pulmonary arterial hypertension (IPAH) also had increased expression of NOX4. The importance of NOX4 was further suggested by studies demonstrating that reducing NOX4 expression by RNA interference attenuated PASMC proliferation in culture.

NOX4 was first described in 2000 as an enzyme highly expressed in the kidney tubular system. For a long time, superoxide generation by a NADPH oxidase was considered to occur solely in phagocytes by the gp91phox component of the membrane-associated cytochrome subunit for the purpose of host defense. Like the other five mammalian NOX oxidases with homology to gp91phox (now referred to as NOX2), NOX4 has the capacity to transfer electrons across biologic membranes and generate superoxide and other downstream ROS. The physiological functions of these NOX homologues likely include post-translational processing of proteins, cellular signaling, regulation of gene expression, and cell differentiation. NOX enzymes are widely distributed in nonphagocytic cells and appear to contribute to a variety of pathological processes. NOX deficiency may lead to immunosuppression, lack of otoconogenesis, or hypothyroidism. Increased NOX activity is also postulated to contribute to a large number of pathologies.

Mittal and colleagues findings of perinuclear and punctate staining of NOX4 on immunohistochemistry is consistent with other recent reports of endoplasmic reticulum (ER) localization. Such localization may represent an accumulation at its site of synthesis. However, this subcellular location is consistent with the suggested functions of the

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(Circ Res. 2007;101:224-226.)

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Circulation Research is available at http://circres.ahajournals.org

DOI: 10.1161/CIRCRESAHA.107.158246

224
Schematic representation of the possible interplay of TGF-β1, HIF-1α and NOX4 in the pathogenesis of pulmonary smooth muscle hypertrophy and hyperplasia because of chronic hypoxia. Chronic hypoxia stabilizes HIF-1α, enabling increased transcription of HIF-1α-responsive genes (ex. furin). TGF-β1 levels increase because of hypoxia-mediated expression and/or activation by furin. TGF-β1 increases NOX4 expression and subsequent ROS production. ROS mediate PASMC hyperplasia and hypertrophy, as well as contribute to increased gene expression and/or stabilization of HIF-1α. TGF-β1 also contributes to HIF-1α stabilization by reducing levels of HIF-1α prolyl hydroxylase. Hypoxia may also regulate NOX4 by mechanisms apart from those mediated by HIF-1 and/or TGF-β1.

In conclusion, the report by Mittal et al adds to accumulating information that the NOX enzymes, and in particular NOX4, play an important role in chronic responses of the pulmonary vasculature to changes in O2 tension. The finding of increased expression of NOX4 in IPAH, while needing validation, is also noteworthy. These exciting findings may facilitate our understanding of the pathophysiology of PAH because of chronic hypoxia, as well as other causes.

**Sources of Funding**

This work was supported by National Heart, Lung, and Blood Institute grant HL-67281 (to J.R. Hoidal) and VA salary support for K.A. Sanders and J.R. Hoidal.

**Disclosures**

None.

**References**


enzyme. For example, the localization of NOX4 to the perinuclear region is consistent with the recent observation that NOX4 participates in phosphorylation of the retinoblastoma protein with subsequent cellular proliferation.20 Likewise, localization of NOX4 to the ER might facilitate translation of gene products relevant to cellular differentiation or hypertrophy. Support of this is provided by the observation that NOX4 mediates phosphorylation of the eukaryotic translation initiation factor 4E binding protein-1 and cellular hypertrophy in TGF-β1–stimulated human airway smooth muscle cells.20 Thus, the locations that NOX4 has thus far immunolocalized to in PASMC suggest a variety of functions relevant to the pathology of chronic hypoxia-induced PAH.

The findings reported by Mittal and colleagues are somewhat hard to fully reconcile with results reported by Liu et al demonstrating that disruption of the murine NOX2 gene completely abolishes chronic hypoxia-induced PASMC and vascular remodeling.9 Perhaps attenuated ROS production because of disrupted endothelial, adventitial, and phagocytic NOX2 allows unchecked production of endothelial nitric oxide which overwhelms the actions of PASMC NOX4. Absence of ROS generated by NOX2 within the pulmonary arterial vessel wall might also alter NOX4 expression.27 Finally, expression of NOX components may vary among species and in vessels from different sites of the pulmonary circulation. Regardless, both studies suggest that disruption of NOX enzymes might have therapeutic potential in chronic hypoxia-induced PAH.


**Key Words:** NOX4 □ NADPH oxidase □ hypoxia □ pulmonary hypertension
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Circ Res. 2007;101:224-226
doi: 10.1161/CIRCRESAHA.107.158246

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Print ISSN: 0009-7330. Online ISSN: 1524-4571

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World Wide Web at:
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