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 (SMC) proliferation and promotes apoptosis.6–8 In animal models, ROS production has been directly linked to the vascular remodeling associated with chronic hypoxia-induced PAH.14 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.15,16 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.17 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 otocogenesis, or hypothyroidism. Increased NOX activity is also postulated to contribute to a large number of pathologies.17

Mittal and colleagues findings of perinuclear and punctate staining of NOX4 on immunohistochemistry is consistent with other recent reports of endoplasmic reticulum (ER) localization.18–21 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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Circulation Research is available at http://circres.ahajournals.org DOI: 10.1161/CIRCRESAHA.107.158246
enzyme. For example, the localization of NOX4 to the perinuclear region is consistent with the recent observation that NOX4 participates in phosphorylation of the retinoblas-toma 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 studies by Mittal et al address important gaps in our understanding of the pathobiochemistry of PAH, but many important questions remain. By what mechanism does hypoxia induce NOX4 expression and proliferation? Hypoxia increases TGF-β1 expression as well as production of the TGF-β1 activating protein furin.22,23 TGF-β1 promotes HPASMC proliferation through a signaling pathway involving NOX4 induction.19 NOX4-generated ROS might facilitate increased stabilization and/or expression of the hypoxia-inducible factor-1 (HIF-1α) component HIF-1α.24,25 Furin is increased during hypoxia by a HIF-1α-mediated mechanism.23 TGF-β1 also promotes HIF-1α stability by reducing levels of HIF-1α prolyl hydroxylase.26 Thus, numerous mechanisms exist whereby TGF-β1, NOX4, and HIF may contribute to the increased presence of each other during hypoxia, with resulting PASMC hypertrophy and proliferation (Figure).

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 PAH 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.

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.

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*Circ Res.* 2007;101:224-226
doi: 10.1161/CIRCRESAHA.107.158246

*Circulation Research* is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the World Wide Web at:
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