Things We “kNOw” and Do Not “kNOw” about Pulmonary Hypertension

Pulmonary hypertension (PH) is a devastating disease affecting >100 million people worldwide and defined by increased pulmonary artery pressure (>25 mm Hg) and pulmonary vascular resistance, which often results in right ventricular failure and premature death (1, 2). The World Health Organization classifies patients with PH into five groups on the basis of etiology (3). Multiple phenotypes such as vasoconstriction, dysregulated pulmonary vascular cell proliferation and remodeling, and inflammation contribute to the PH disease process (4–6) making therapeutic design complicated (7, 8).

Nitric oxide (NO) regulates pulmonary vascular tone and remodeling, inflammation, and coagulation. Three different nitric oxide synthase (NOS) isoforms control enzymatic formation of NO that differ in catalytic activity, location, and regulation. These are neuronal NOS (nNOS; NOS I), inducible NOS (iNOS; NOS II), and endothelial NOS (eNOS; NOS III) (9, 10). Several studies have shown that loss of eNOS-derived NO contributes to PH. This can occur by lower eNOS levels or lower NO bioavailability as a result of eNOS uncoupling despite no changes, or even increased eNOS expression (11, 12). Mice genetically deficient in eNOS spontaneously develop PH and right ventricular failure (13, 14), and NO metabolite levels are lower in patients with pulmonary arterial hypertension (PAH) compared with control patients (15, 16), with the levels inversely correlating with the severity of disease. Moreover, therapies that increase NO in the pulmonary vasculature (e.g., inhaled NO or nitrite), or those that enhance downstream cyclic guanosine monophosphate-dependent signaling, are among the few available therapies to counter vasoconstriction in group 1 PAH (1, 17–20). Treatment options for the other PH groups, however, are currently limited or lacking.

In this issue of the *Journal*, Ogoshi and colleagues (pp. 232–244) confirmed a critical role for eNOS in PH, using a mouse model of hypoxia-induced PH (21). Indices of PH including elevated right systolic pressure and hypertrophy, pulmonary vascular remodeling, and decreased survival were more prominent in eNOS-deficient mice compared with wild-type mice or mice lacking either nNOS or iNOS. These data further support a functional role for eNOS in protection against PH (13, 14, 22). The most intriguing findings by Ogoshi and colleagues were that mice devoid of all three NOS isoforms (triple NOS knockouts) had a greater degree of PH compared with mice lacking only eNOS; mortality was approximately twofold higher in triple NOS knockouts. This result reveals an unexpected interaction between NO produced by eNOS and either nNOS and/or iNOS. Although this aspect was not investigated in detail in the current study, it is notable that iNOS expression was increased in hypoxia-exposed eNOS-deficient mice, suggesting iNOS may offer compensatory

protection when eNOS is lacking. The obvious hypothesis that hypoxia-induced PH will be more severe when both eNOS and iNOS are absent remains to be tested using double NOS knockout mice. Furthermore, additional studies including the potential for nNOS or iNOS-derived nitrite, in maintaining NO signaling in the absence of eNOS, would be interesting and shed new mechanistic insights underlying the interplay between different NOS isoforms and PH.

The authors also showed that the NOS triple knockouts have significantly elevated circulating bone marrow (BM)-derived vascular smooth muscle progenitor cells compared with wild-type mice. BM transplantation studies confirmed a role of myeloid cells in mediating pulmonary vascular remodeling. Moreover, transplantation of BM-derived myeloid cells from wild-type mice into eNOS-deficient mice prevented prolonged hypoxia-induced PH and was associated with higher plasma NO-metabolite levels. These data suggest that one of the NOS isoforms, in myeloid cells, and most likely iNOS, provides protection when eNOS is lacking and underscores the potential for crosstalk between NOS isoforms in modulating inflammatory diseases (23). The present study also shows that repletion of NO using nitrate or isosorbide dinitrate protects against hypoxia-induced PH in triple NOS-deficient mice. Although inhaled NO or inhaled nitrite have shown efficacy in normalize PA pressures in PAH (20, 24), relatively few studies have tested nitrate, which is metabolized to nitrite and other nitroso species (25). Interestingly, a previous study also showed that dietary nitrate prevented hypoxia-induced PH, but in this case, protection was dependent on eNOS (26). However, the effects of nitrate on hypoxia-induced PH in mice only lacking eNOS were not tested in the current study, and therefore preclude a direct comparison. Irrespective of the mechanism or mechanisms, both studies showed that nitrate was an effective and preventive therapeutic and suggested that individuals that consume diets rich in nitrate-containing foods (e.g., green leafy vegetables [25]) may be less susceptible to PH.

Finally, the authors performed extensive RNA profiling measurements to gain insight into the mechanism or mechanisms whereby triple NOS deficiency predisposed mice to more severe PH. We limit comment on these data and focus on implications regarding therapeutic options. In this regard, it is important to address the model used and implications for clinical PH. Ogoshi et al modeled group 3 PH or hypoxemic PH, which is PH associated with chronic lung diseases (chronic obstructive pulmonary disease, interstitial lung disease, sleep breathing disorders). PH in this group is generally mild to moderate, with mean pulmonary artery pressure on right heart catheterization between 25 and 34 mm Hg. At this time, there are no PH-specific drugs that are approved for use in group 3 PH. Common treatment strategies used for PAH group 1, including therapies that improve pulmonary NO-dependent signaling, have not been effective for the treatment of group 3 PH and are not advised because of their potential worsening of vasoconstriction and gas exchange (5). The data presented by Ogoshi et al suggest that NO deficiency exacerbates group 3 PH and that systemic NO supplementation may provide protection (26).

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The potential distinction between pulmonary versus systemic NO therapy may be important. For example, the latter may mitigate any deficit in NO metabolism regulated by BM-derived myeloid cells. Further supporting the concept of NO deficiency in group 3 PH, the authors demonstrated that NOS-knockout mice had more severe PH in a bleomycin-induced fibrosis model. Furthermore, lower levels of NO metabolites in BAL fluid from patients with idiopathic pulmonary fibrosis were observed providing clinical relevance. There are several questions that arise from the current study, including: Do lower NO levels in the setting of chronic lung diseases increase the risk for developing secondary PH in group 3? If NO deficiency is an underlying feature of group 3 PH, then can NO-repletion therapies (nitrate or nitrite) be administered to slow or reverse PH without potentially worsening the disease? Is there a role for BM-derived cells in the progression of group 3 patients with PH, is it applicable to all PH groups, and could systemic BM/myeloid targeting of NO replacement therapy be beneficial or harmful to these patients? And how does eNOS-derived NO regulate NO metabolism in BM-derived myeloid cells? Addressing these questions may have implications for the precise treatment of pulmonary vascular disease, as well as other diseases with altered NO metabolism. ■

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