Emerging Pathogenetic Mechanisms of Pulmonary Arterial Hypertension: Nitric Oxide and More

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Pulmonary arterial hypertension (PAH) is a rare but serious clinical condition characterized by a progressive increase of pulmonary arterial pressure and resistance leading to right ventricular and premature death. Although PAH clinically includes 9 different subgroups, it has a common final pathology, which is obstructive thickening/hypertrophy in the vascular wall components, mainly affecting distal pulmonary arteries. Vascular proliferation and remodeling, in comparison with vasoconstriction or thrombosis, is now recognized as a principal contributor to increased pulmonary resistance. The exact processes that initiate the pathological changes seen in PAH are largely unknown. Nonetheless, recent advances in cellular and molecular biology have improved our understanding of some of key mechanisms responsible for pathobiology of PAH. The pathobiology of PAH is multifactorial, and involves various cellular mediators and pathways.

Endothelial cells are major regulators of vascular function, and pulmonary arterial endothelial cells (PAEC) have been perceived as the most likely cell type in which dysfunction initiates PAH. Endothelial dysfunction leads to reduced production of vasodilators and growth inhibitors such as nitric oxide (NO) and prostacyclin, and increased production of vasoconstrictors and promitogens such as thromboxane A2 and endothelin-1. Vascular NO production is catalyzed by endothelial NO synthase (eNOS) which is expressed constitutively in most endothelial cells. Due to the wide availability and versatility of NO in many vascular beds, its role in PAH has been pursued in many studies. In the present issue of Korean Circulation Journal, Koo et al. added to these studies by exploring the expression of NOS in the rat model of pulmonary hypertension induced by monocrotaline (MCT) administration, which is a commonly used technique to simulate PAH in animals.

Koo et al. reported a significant increase in eNOS expression on day 28, and an increase in matrix metalloproteinase-2 (MMP-2) on day 5 and 28 in the lung tissue of MCT-injected rats, all of which were abrogated by bosentan treatment. Levels of eNOS expression during the development of pulmonary hypertension have been reported at variable levels. Pulmonary eNOS expression was observed as unchanged, decreased or increased in experimental or human PAH. Nevertheless, there is growing consensus that pulmonary arterial wall in PAH has reduced levels of NO. Thus, an inconsistency appears between expression of eNOS and tissue level of NO in the model of PAH. However, recent research has elucidated a number of cellular and molecular processes which might account for the underlying disturbances observed in PAH, which could reconcile the conflicting data.

The discovery of the association of PAH with a mutation of bone morphogenetic protein receptor-2 (BMPR2) has increased knowledge on the pathobiology of PAH. Mutations in the BMPR2 gene have been found in nearly 70% of familial PAH, and up to 25% of idiopathic PAH. Bone morphogenetic protein (BMP), a member of the TGF-β superfamily, regulates cell growth, differentiation, and apoptosis. Mutation or downregulation of BMPR2 increases susceptibility to apoptosis in endothelial cells, and promotes proliferation of pulmonary arterial smooth muscle cells (PASMCs). Apart from genetic alteration, ultrastructural changes in the PAH model suggest that another mechanism plays a role. These chang-
es, featured by increased endoplasmic reticulum (ER), increased Golgi stacks, vacuolization, and accumulation of Weibel-Palade bodies (exocytic vesicles), point to disruption of cytoplasmic membrane trafficking within cellular elements in the arterial lesion in PAH. Indeed, recent experiments have shown that MCT treatment induces similar enlargement of Golgi and ER along with loss of cell surface raft/carveolar protein caveolin-1 (cav-1) in PAEC.\textsuperscript{13} Cell fractionation and immunofluorescence techniques revealed the marked trapping of cav-1 and eNOS in Golgi and showed the trapping of BMPR2 and diverse Golgi tethers, SNAREs, and SNAPs.\textsuperscript{13} This suggests that molecular machinery of vesicular trafficking was disrupted, particularly at the stage of disassembly.\textsuperscript{17} Interestingly, it has been demonstrated that not only are mutant BMPR2 proteins sequestrated in the Golgi, but they can also bind to wild type BMPR1 receptor exerting a dominant-negative functional effect.\textsuperscript{18} The sequestration of eNOS in an intracellular compartment away from cell-surface caveolae would result in reduced NO in the pulmonary artery despite sustained or even increased protein levels of eNOS. Moreover, intracellular generation of NO may further exacerbate troubled trafficking by increasing S-nitrosylation of N-ethylmaleimide sensitive factor (NSF), an ATPase required for disassembly of cis-SNARE complexes.\textsuperscript{19} Thus, the “Golgi blockade hypothesis”\textsuperscript{17} may in part account for the discrepancy between NO level and eNOS expression, observed in PAH models of Koo et al.\textsuperscript{5} and others.\textsuperscript{8}

As mentioned, pathobiology of PAH is multifactorial, involving multiple mediators and pathways. Reduced NO bioavailability in PAH can also be induced by other mechanisms including competition for substrate L-arginine and presence of endogenous inhibitors of eNOS.\textsuperscript{20,21} In addition, pathways other than NO-related or BMPR-mediated have been postulated as pathogenic elements which can contribute to the development of PAH.\textsuperscript{22} These include RhoA GTPase signaling, angiopteen-TIE2 signaling, serotonin, K\textsubscript{v} 1.5 channel expression, mitochondrial metabolism, and adventitial regulation of extracellular matrix/fibrosis/inflammation.\textsuperscript{23,24} An intriguing feature of PAH is a metabolic shift from oxidative phosphorylation to glycolysis even in the presence of adequate oxygen, a behavior originally observed in cancers (“Warburg phenotype”).\textsuperscript{25} As in cancers, there is O\textsubscript{2-}independent perpetuation of the metabolic/redox shift that normally occur in response to hypoxia, creating “pseudohypoxic environment” with normoxic hypoxia-inducible factor 1\textgamma (HIF-1\textgamma) activation in PAH.\textsuperscript{20} Activated HIF-1\textgamma turns on glycolytic genes and suppresses oxidative metabolism by increasing pyruvate dehydrogenase kinase (PDK) transcription. The consequence of this metabolic shift includes decreased K\textsubscript{v} 1.5 expression leading to membrane depolarization and elevation of cytosolic K\textsuperscript{+} and Ca\textsuperscript{2+}. The resulting Ca\textsuperscript{2+} overload, later reinforced by activation of transient receptor potential (trp) channels,\textsuperscript{26} leads to Ca\textsuperscript{2+}-calcineurin-dependent activation of proliferation transcription factor NFAT.\textsuperscript{27} In both PAH PASMCs and cancer cell lines, this generates a proliferative, apoptosis-resistant phenotype.\textsuperscript{27}

Koo et al.\textsuperscript{5} have demonstrated the increased expression of both eNOS and MMP-2 in the MCT-induced PAH model, and suggested the causal role of eNOS for the activation of MMP-2. However, the causality may be debatable because eNOS upregulation (day 28, after MCT injection) occurs later than MMP-2 (day 5). In another experimental model, the earliest change in the subcellular structure could be found just 18-24 hours after single exposure of PAECs to MCT.\textsuperscript{28} Actually, the authors later mentioned a compensation mechanism as a possible explanation for the temporal sequence of gene expression.\textsuperscript{29} To date, data which could firmly support or negate these hypotheses have been lacking. For a disease as multifaceted as PAH, it would be inconceivable that one factor, for example, aberrant NO production or BMPR2 mutation, would represent a “universal” cause. To obtain comprehensive insight into this condition, further investigations are needed. A better understanding of the underlying mechanisms comprising pathobiology of PAH will provide a paradigm shift, from which future therapeutic strategy can be formulated.

REFERENCES


