EDITORIAL
DOI 10.4070/kcj.2011.41.2.58

Print ISSN 1738-5520 / On-line ISSN 1738-5555

Open Access

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

Young Dae Kim, MD

Department of Internal Medicine, Dong-A University College of Medicine, Busan, Korea

Refer to the page 83-90

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.[1] 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.[2] Vascular proliferation and remodeling, in comparison with vasoconstriction or thrombosis, is now recognized as a principal contributor to increased pulmonary resistance.[3] 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.[4] 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 A_2 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.[5] 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.[5] 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.[6-8] Nevertheless, there is growing consensus that pulmonary arterial wall in PAH has reduced levels of NO.[9] 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,[10)17)] 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.[11] Bone morphogenetic protein (BMP), a member of the TGF-¥á 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 vascular smooth muscle cells (PASMCs).[12)13)] Apart from genetic alteration, ultrastructural changes in the PAH model suggest that another mechanism plays a role. These chang-

Correspondence: Young Dae Kim, MD, Department of Internal Medicine, Dong-A University College of Medicine, 1 Dongdaesin-dong 3-ga, Seo-gu, Busan 602-715, Korea
Tel: 82-51-240-2959, Fax: 82-51-255-8174
E-mail: kimdy@mail.donga.ac.kr

• The author has no financial conflicts of interest.

es, featured by increased endoplasmic reticulum (ER), increased Golgi stacks, vacuolization, and accumulation of Weibel-Palade bodies (exocytic vesicles),[14] 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.[15] 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.[15] This suggests that molecular machinery of vesicular trafficking was disrupted, particularly at the stage of disassembly.[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.[18] The sequestration of eNOS in an intracellular compartment away from cell-surface carveolae 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.[19] Thus, the "Golgi blockade hypothesis"[17] may in part account for the discrepancy between NO level and eNOS expression, observed in PAH models of Koo et al.[5] and others.[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.[20][21] In addition, pathways other than NO-related or BMPR-mediated have been postulated as pathogenetic elements which can contribute to the development of PAH.[10][22] These include RhoA GTPase signaling, angiopeptin-TIE2 signaling, serotonin, K_V 1.5 channel expression, mitochondrial metabolism, and adventitial regulation of extracellular matrix/fibrosis/inflammation.[10][22] 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").[23] As in cancers, there is O_2-independent perpetuation of the metabolic/redox shift that normally occur in response to hypoxia, creating "pseudohypoxic environment" with normoxic hypoxia-inducible factor 1¥á (HIF-1¥á) activation in PAH.[24] Activated HIF-1¥á turns on glycolytic genes and suppresses oxidative metabolism by increasing pyruvate dehydrogenase kinase (PDK) transcription. The consequence of this metabolic shift includes decreased K_V 1.5 expression leading to membrane depolarization and elevation of cytosolic K^+ and Ca^{2+}. The resulting Ca^{2+} overload, later reinforced by activation of transient receptor potential (trp) channels,[25] leads to Ca^{2+}-calcineurin-dependent activation of proliferation transcription factor NFAT.[26] In both PAH PASMCs and cancer cell lines, this generates a proliferative, apoptosis-resistant phenotype.[27]

Koo et al.[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.[28] Actually, the authors later mentioned a compensation mechanism as a possible explanation for the temporal sequence of gene expression.[5] 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

1) Galiè N, Hoeper MM, Humbert M, et al. *Guidelines on diagnosis and treatment of pulmonary hypertension: the task force on diagnosis and treatment of pulmonary hypertension of the European society of cardiology and of the European respiratory society. Eur Heart J 2009; 30:2493-537.*
2) Pietra GG, Capron F, Stewart S, et al. *Pathologic assessment of vasculopathies in pulmonary hypertension. J Am Coll Cardiol 2004;43(12 Suppl S):25S-32S.*
3) Jeffery TK, Morrell NW. *Molecular and cellular basis of pulmonary vascular remodeling in pulmonary hypertension. Prog Cardiovasc Dis 2002;45:173-202.*
4) Cool CD, Groshong SD, Oakey J, Voelkel NF. *Pulmonary hypertension: cellular and molecular mechanisms. Chest 2005;128(6 Suppl):565S-71S.*
5) Koo HS, Kim KC, Hong YM. *Gene expression of nitric oxide synthase and matrix metalloproteinase-2 in monocrotaline-induced pulmonary hypertension in rats. Korean Circ J 2011;41:83-90.*
6) Mukhopadhyay S, Xu F, Sehgal PB. *Aberrant cytoplasmic sequestration of eNOS in endothelial cells after monocrotaline, hypoxia, and senescence: live-cell caveolar and cytoplasmic NO imaging. Am J Physiol Heart Circ Physiol 2006;292:H1373-89.*
7) Giaid A, Saleh D. *Reduced expression of endothelial nitric oxide synthase in the lungs of patients with pulmonary hypertension. N Engl J Med 1995;333:214-21.*
8) Resta TC, Gonzales RJ, Dail WG, Sanders TC, Walker BR. *Selective upregulation of arterial endothelial nitric oxide synthase in pulmonary hypertension. Am J Physiol 1997;272:H806-13.*
9) McLaughlin VV, McGoon MD. *Pulmonary arterial hypertension. Circulation 2006;114:1417-31.*
10) Morrell NW, Adnot S, Archer SL, et al. *Cellular and molecular basis of pulmonary arterial hypertension. J Am Coll Cardiol 2009;54(1 Suppl):S20-31.*
11) Thomson JR, Machado RD, Pauciulo MW, et al. *Sporadic primary*

pulmonary hypertension is associated with germline mutations of the gene encoding BMPR-II, a receptor member of the TGF-beta family. J Med Genet 2000;37:741-5.

12) Teichert-Kuliszewska K, Kutryk MJ, Kuliszewski MA, et al. *Bone morphogenetic protein receptor-2 signaling promotes pulmonary arterial endothelial cell survival: implications for loss-of-function mutations in the pathogenesis of pulmonary hypertension. Circ Res 2006; 98:209-17.*
13) Yang X, Long L, Southwood M, et al. *Dysfunctional Smad signaling contributes to abnormal smooth muscle cell proliferation in familial pulmonary arterial hypertension. Circ Res 2005;96:1053-63.*
14) Smith P, Heath D, Yacoub M, Madden B, Caslin A, Gosney J. *The ultrastructure of plexogenic pulmonary arteriopathy. J Pathol 1990; 160:111-21.*
15) Mathew R, Huang J, Shah M, Patel K, Gewitz M, Sehgal PB. *Disruption of endothelial-cell caveolin-1alpha/raft scaffolding during development of monocrotaline-induced pulmonary hypertension. Circulation 2004;110:1499-506.*
16) Mukhopadhyay S, Xu F, Sehgal PB. *Aberrant cytoplasmic sequestration of eNOS in endothelial cells after monocrotaline, hypoxia, and senescence: live-cell caveolar and cytoplasmic NO imaging. Am J Physiol Heart Circ Physiol 2006;292:H1373-89.*
17) Sehgal PB, Mukhopadhyay S. *Dysfunctional intracellular trafficking in the pathobiology of pulmonary arterial hypertension. Am J Respir Cell Mol Biol 2007;37:31-7.*
18) Nishihara A, Watabe T, Imamura T, Miyazono K. *Functional heterogeneity of bone morphogenetic protein receptor-II mutants found in patients with primary pulmonary hypertension. Mol Biol Cell 2002; 13:3055-63.*
19) Iwakiri Y, Satoh A, Chatterjee S, et al. *Nitric oxide synthase generates nitric oxide locally to regulate compartmentalized protein S-nitrosylation and protein trafficking. Proc Natl Acad Sci U S A 2006;103: 19777-82.*
20) Xu W, Kaneko FT, Zheng S, et al. *Increased arginase II and decreased NO synthesis in endothelial cells of patients with pulmonary arterial hypertension. FASEB J 2004;18:1746-8.*
21) Pullamsetti S, Kiss L, Ghofrani HA, et al. *Increased levels and reduced catabolism of asymmetric and symmetric dimethylarginines in pulmonary hypertension. FASEB J 2005;19:1175-7.*
22) Archer SL, Weir EK, Wilkins MR. *Basic science of pulmonary arterial hypertension for clinicians: new concepts and experimental therapies. Circulation 2010;121:2045-66.*
23) Archer SL, Gomberg-Maitland M, Maitland ML, Rich S, Garcia JG, Weir EK. *Mitochondrial metabolism, redox signaling, and fusion: a mitochondria-ROS-HIF1alpha-KV1.5 O2-sensing pathway at the intersection of pulmonary hypertension and cancer. Am J Physiol Heart Circ Physiol 2008;294:H570-8.*
24) Bonnet S, Michelakis ED, Porter CJ, et al. *An abnormal mitochondrial-hypoxia inducible factor-1alpha-Kv channel pathway disrupts oxygen sensing and triggers pulmonary arterial hypertension in fawn hooded rats: similarities to human pulmonary arterial hypertension. Circulation 2006;113:2630-41.*
25) Landsberg JW, Yuan JX. *Calcium and TRP channels in pulmonary vascular smooth muscle cell proliferation. News Physiol Sci 2004;19: 44-50.*
26) Bonnet S, Rochefort G, Sutendra G, et al. *The nuclear factor of activated T cells in pulmonary arterial hypertension can be therapeutically targeted. Proc Natl Acad Sci U S A 2007;104:11418-23.*
27) Bonnet S, Archer SL, Allalunis-Turner J, et al. *A mitochondria-K^+ channel axis is suppressed in cancer and its normalization promotes apoptosis and inhibits cancer growth. Cancer Cell 2007;11:37-51.*
28) Reindel JF, Roth RA. *The effects of monocrotaline pyrrole on cultured bovine pulmonary artery endothelial and smooth muscle cells. Am J Pathol 1991;138:707-19.*