Pathogenesis of Pulmonary Arterial Hypertension
The Need for Multiple Hits

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Pulmonary hypertension can be classified into 4 categories: pulmonary arterial hypertension (PAH), pulmonary venous hypertension, pulmonary hypertension associated with hypoxemia, and pulmonary hypertension due to chronic thrombotic or embolic disease. PAH is a progressive and often fatal condition that predominantly affects women. Approximately 10% of patients diagnosed with PAH without a demonstrable cause have a family history of the disease and are referred to as having familial PAH (FPAH), whereas the remainder are classified as having idiopathic PAH (IPAH).

Regardless of the initial pathogenic trigger, the elevated pulmonary arterial pressure and vascular resistance in patients with FPAH and IPAH are primarily caused by sustained pulmonary vasoconstriction, lumen obliteration of small- and medium-sized arteries and arterioles in association with the formation of plexiform lesions and in situ thrombosis, and concentric thickening of pulmonary arteries resulting from intimal fibrosis, and medial hypertrophy resulting from excessive proliferation of smooth muscle cells. The plexiform lesion, a histological hallmark of FPAH and IPAH, has been demonstrated to result from monoclonal proliferation of endothelial cells, migration and proliferation of smooth muscle cells, and accumulation of circulating cells (eg, macrophages, endothelial progenitor cells). These observations suggest that the pulmonary vasculature in FPAH and IPAH patients is phenotypically different from that in normal subjects because of inheritable or acquired mutations (or polymorphisms) of certain genes that are specifically involved in regulating proliferation, apoptosis, and differentiation in pulmonary arterial smooth muscle cells (PASMCs) and pulmonary arterial endothelial cells (PAECs).

The pathogenesis of PAH remains incompletely understood; however, heterozygous mutations of the bone morphogenetic protein (BMP) receptor type II (BMP-RII) gene (BMPR2) have been implicated in the development of both FPAH and IPAH. BMPR2 mutations, which are distributed in the 13 coding exons of the gene and its flanking intronic sequences, have been identified in ≈40% of FPAH patients and ≈15% of IPAH patients. Because the mutant alleles are typically of low penetrance, loss of the wild-type allele as a result of somatic mutations in cells responsible for the plexiform lesion, monoclonal endothelial cell proliferation, and concentric medial hypertrophy may contribute to the onset and progression of IPAH. In other words, as indicated by Machado et al in this issue of Circulation, “FPAH and BMPR2 mutation–positive IPAH might follow the classic ‘2-hit’ model of tumorigenesis and... inactivation of the remaining wild-type BMPR2 allele might be one of the somatic mutations necessary to precipitate disease.”

Using polymerase chain reaction analysis, Yeager et al demonstrated microsatellite instability within the transforming growth factor-β (TGF-β) receptor type II (TGF-β-RII) gene, hMSH2 mismatch repair gene, and Bax (a proapoptotic protein) gene in PAECs isolated from plexiform lesions of severe IPAH patients but not in cells of FPAH patients or lung tissues of normal subjects. The study by Machado et al in 7 white patients diagnosed with FPAH (ie, at least 1 family member has the same disease in addition to the patient) provides compelling evidence that microsatellite instability is not common in FPAH and that somatic loss of the remaining wild-type BMPR2 allele in heterozygous mutation carriers (or loss of heterozygosity) does not contribute to the onset and progression of FPAH. Therefore, mutations (or polymorphisms) in other genes and altered functions and activities of other gene products may be required as a “trigger” for or “contributor” to the development of the disease. In other words, the onset and progression of PAH, or the histological and functional phenotype of plexogenic pulmonary arteriopathy, require multiple “hits” in BMPR2 mutation carriers and in individuals without BMPR2 mutations but with mutations and abnormalities in other genes and gene products. These hypotheses are further supported by the following recent observations: (1) Transgenic mice expressing a dominant-negative BMPR2 gene in smooth muscle and the heterozygous BMPR2+/− and BMPR1a+/− knockout mice develop only mild pulmonary hypertension, whereas most FPAH and IPAH patients have severe pulmonary hypertension; (2) mutations or polymorphisms in other genes have been identified and linked to IPAH, such as serotonin receptor (HTR2B) and transporter (SLC6A4), K+ (KCNA5) and Ca2+ (TRPC6) channels, and angiotensin-1 (ANGPT1); and (3) the development of PAH is linked to exogenous factors such as viral and bacterial infection and anorexigen intake. Therefore, the
triggers for the onset and the contributors to the progression of PAH in individuals with genetic predispositions (eg, germline mutations or polymorphisms in BMPR2 and other genes) may be genetic (eg, combination of mutations in various genes such as HTR2B, SLC6A4, KCNA5, TRPC6, and ANGPT1), environmental, or acquired factors (eg, viral and bacterial infection, anorexigen intake), or all 3 (Figure).

BMPs, which belong to the TGF-β superfamily, are synthesized and secreted from a variety of cell types, including PASMCs and PAECs. Signal transduction of BMP-mediated effects in the pulmonary vasculature involves homomeric or heteromeric dimerization of the BMP receptors (BMP-Rla, -Rlb, and -RII) and activation (or phosphorylation) of the downstream signaling proteins, Smads (mothers against decapentaplegic proteins). In the absence of BMP ligands, both homomeric and heterogenous BMP receptors (BMP-Rla, BMP-Rlb, and BMP-RII) are found in the surface membrane of living cells. Binding of BMP ligands to either of the BMP receptors (BMP-Rla, -Rlb, or -RII) induces or augments heterodimerization of BMP-RI and BMP-RII. The ligand/receptor complex is required for activating the BMP-RI kinase.18

The activated cytoplasmic kinase of BMP-RI then phosphorylates the receptor-activated Smad (R-Smad) proteins (eg, Smad-3, -5, and -8), which then dimerize with co-Smad (eg, Smad-4). The R-Smad and co-Smad complex then translocates into the nucleus to regulate transcription of the target genes that contain the Smad binding sequence (5’-CAGAC-3’ and 5’-GTCTG-3’) in their promoter. In addition to the R-Smads and co-Smads, humans also express the antagonistic Smads, including Smad-6 and -7, which mediate negative feedback within TGF-β/BMP signaling pathways and regulatory inputs from other pathways. The antagonistic Smads serve as an R-Smad decoy to compete for the activated tyrosine kinase and therefore to inhibit activation of R-Smads. Smad-6 is an antagonistic Smad that preferentially inhibits BMP signaling (ie, activation of Smad-1/-5/-8). Among the Smad-responsive genes (ie, the genes containing the Smad binding sequences in their promoters), many encode proteins that are required for arresting cell growth and inducing apoptosis (Figure).18

In addition to activating gene transcription by binding onto the Smad binding sequence in the promoter, increased Smads in the nucleus can also form heterogenous polymers with corepressors, such as the homeodomain protein TGIF and the 2 related proteins c-Ski and SnoN, to induce repression of the transcription of target genes. An additional inhibitor of Smads is the Smad-interacting protein 1 (SIP1), a zinc finger/homeodomain protein that interacts with Smad-1 and -5 in mammalian cells and inhibits BMP-mediated effects.18 Therefore, BMP-mediated activation and translocation of Smads can exert both augmenting and inhibitory effects on gene transcription, depending on cell types and other transcription factors involved in the signal transduction cascade.

Increased Smads in the nucleus can also form heterogenous polymers with other transcription factors (eg, AP-1) to mediate antiproliferative and proapoptotic effects on a variety of cell types. Furthermore, Smad proteins have been reported to interact with calmodulin, a Ca2+-sensitive protein in the cytosol. Overexpression of calmodulin inhibits Smad activation and attenuates the response of BMP signal transduction, suggesting that a rise in [Ca2+]cyt may exert an inhibitory effect on the BMP signaling pathway by activating calmodulin and attenuating BMP-mediated antiproliferative or proapoptotic effects on human PASMCs.

In cells isolated from normal subjects, BMPs inhibit proliferation in PASMCs,19 induce apoptosis in PASMCs,20 and enhance survival in PAECs and endothelial progenitor cells.21 The BMP-mediated apoptosis in normal PASMCs appears to be at least partially due to downregulation of Bcl-2, an antiapoptotic protein.20 The antiproliferative and proapoptotic effects of BMPs in normal PASMCs would lead to a well-maintained balance of the proliferation/apoptosis ratio and to maintenance of the thin pulmonary vascular wall. The antiproliferative effect of BMPs in normal PAECs would protect endothelial integrity, prevent the “leakage” of circulating growth factors to the media (for stimulating PASMC growth), and enhance endothelium-dependent vasodilation. In PASMCs from IPAH patients, the antiproliferative effect of BMPs is converted to proliferative19 and the proapoptotic effect is significantly attenuated,20 whereas in PAECs from IPAH patients, the antiapoptotic or survival effect of BMPs is converted to proapoptotic.21 The reversed effects of BMPs on PASMCs and PAECs in IPAH patients, because of unknown phenotypical changes, may play an important pathogenic role in pulmonary vascular remodeling and ultimately in pulmonary hypertension.

As mentioned earlier, increased PASMC contraction (resulting from elevated [Ca2+]cyt or enhanced Ca2+ sensitivity of contractile proteins), increased PASMC proliferation and inhibited PASMC apoptosis, monoclonal PAEC proliferation, and endothelial injury all are involved in the development of sustained pulmonary vasoconstriction, lummen obliteration of small pulmonary arteries with plexiform lesions, and pulmonary vascular wall thickening due to medial hypertrophy. In addition to BMP ligands and the Smad signaling pathway, many other ligands, receptors, and signal transduction cascades are involved in regulating contraction, migration, proliferation, differentiation, and apoptosis of PASMCs and PAECs. Indeed, it was recently demonstrated that upregulated 5-HT receptors and transporters11,12 and increased circulating levels of 5-HT (serotonin), upregulated canonical transient receptor potential channels and downregulated Kv channels,13–15 upregulated angiotensin-1 and downregulated BMPR-Ia,16 and imbalanced prostacyclin/thromboxane A2 or nitric oxide/endothelin-1 ratio22,23 are involved in the development of sustained pulmonary vasoconstriction and vascular remodeling in IPAH patients (Figure). Furthermore, both loss-of-function (eg, KCNA5) and gain-of-function (eg, SERT) mutations24,25 in these genes also have been identified in patients with IPAH and linked to the development of the disease. In addition, PAH occurs in the settings of scleroderma, viral hepatitis and other forms of chronic liver disease, and HIV infection, which suggests that triggers for these diseases also may serve as causes for the onset and progression of PAH.
Schematic depicting the potential “hits” involved in the development of PAH. A rise in [Ca$_{\text{cyt}}$] in PASMCs (due to decreased Kv channel activity (1) and membrane depolarization, which opens voltage-dependent Ca$^{2+}$ channels [VDCC]; upregulated TRPC channels that participate in forming receptor-operated [ROC] and store-operated [SOC] Ca$^{2+}$ channels (2); and upregulated membrane receptors [eg, serotonin, endothelin, or leukotriene receptors] (3) and their downstream signaling cascades) causes pulmonary vasoconstriction, stimulates PASMC proliferation, and inhibits the BMP-signaling pathway that leads to antiproliferative and pro-apoptotic effects on PASMCs. Dysfunction of BMP signaling due to BMPR2 mutation and BMP-RII/BMP-RI downregulation (4) and inhibition of Kv channel function and expression (1) attenuate PASMC apoptosis and promote PASMC proliferation. Increased angiopoietin-1 (Ang-1) synthesis and release (5) from PASMCs enhance 5-HT production and downregulate BMP-RIa in PAEC and further enhance PASMC contraction and proliferation, whereas inhibited nitric oxide and prostacyclin (PGI$_2$) synthesis (6) in PAECs would attenuate the endothelium-derived relaxing effect on pulmonary arteries and promote sustained vasoconstriction and PASMC proliferation. Increased activity and expression of the 5-HT transporter (5-HTT) (7) would serve as an additional pathway to stimulate PASMC growth via the mitogen-activated protein kinase (MAPK) pathway. Furthermore, exogenous viral and bacterial infection and inflammation (8) may contribute to vasoconstriction and vascular medial hypertrophy in patients with mutations in multiple genes or with “susceptible predispositions” in these pathways. In addition, a variety of splicing factors, transcription factors, protein kinases, extracellular metalloproteinasases, and circulating growth factors would serve as the so-called “hits” to mediate the phenotypical transition of normal cells to contractile or hypertrophied cells and to maintain the progression of PAH. SR indicates sarcoplasmic reticulum; IP$_3$, inositol 1,4,5-trisphosphate; DAG, diacylglycerol; PLC, phospholipase C; PKC, protein kinase C; GPCR, G protein coupled receptor; RTK, receptor tyrosine kinase, PDGF, platelet-derived growth factor; ROS, reactive oxygen species; and AVD, apoptotic volume decrease.
Extensive in vivo and in vitro experiments suggest that the onset of PAH is caused by mutations of multiple genes (either loss-of-function or gain-of-function mutations) and by abnormalities in multiple proteins and signal transduction cascades that are important in maintaining a normal phenotype of PASMCs and PAECs. After the onset of PAH or the initial stage of "pathogenic" phenotypical changes in PASMCs and PAECs, the progression of PAH would be initiated and maintained by the distinct response of the phenotypically changed PASMCs/PAECs to mitogenic agonists and apoptotic inducers. In other words, the PASMCs and PAECs from IPAH patients undergo significant phenotypical changes after the initial transition from a normal phenotype to a "mis-guided" phenotype, which would then alter their "normal" responses to circulating or locally secreted agonists, growth factors, and apoptosis inducers. The phenotypical changes of PASMCs and PAECs, for example, convert the antiproliferative effects of BMPs (and other proteins and agents) on PASMCs to proliferative effects. Via cDNA microarray experiments, we recently found that in vitro treatment of PASMCs with BMP-2 oppositely regulated mRNA expression in >1000 genes.

In summary, mutations of BMPR2 may serve as a predisposition that increases the susceptibility of the mutant BMPR2 carrier to develop PAH in the presence of various endogenous abnormalities of other genes and gene products and in the presence of exogenous stimuli (eg, viral and bacterial infection, anorexigen intake, chronic exposure to hypoxia). The loss-of-function mutations in BMPR2 or dysfunction of BMP receptors and their downstream signal transduction by Smad proteins appear to be insufficient alone to initiate the disease process. A combination of multiple genetic defects and multiple signal transduction abnormalities in PASMCs and PAECs is required for the pathogenesis of FPAH and IPAH. Furthermore, the onset and progression of PAH may be caused by distinct mechanisms. The onset is likely a transition (eg, dedifferentiation or transdifferentiation) of PASMCs and PAECs from a normal to a "pathogenic" or "mis-guided" phenotype, whereas the progression is sustained by one of the following abnormalities: cellular factors that create a proliferative, antiapoptotic, and vasostrictive milieu; circulating factors that promote a proliferative, antiapoptotic, and vasoconstrictive milieu; and genetic and molecular signaling factors that promote gene transcription and a cellular synthetic cycle, thereby promoting a proliferative, antiapoptotic, and vasoconstrictive milieu. Although it is clear that platelets, fibroblasts, and circulating cells are involved in the progression of PAH, the phenotypical change of PASMCs and PAECs resulting from multiple genetic and acquired defects is probably the major cause for the onset of the disease. Accordingly, future efforts directed at developing effective therapeutic strategies for PAH should target multiple genes, gene products, and signal transduction pathways.

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Expression of human herpesvirus 8 in primary pulmonary hypertension. 


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