Cellular and Molecular Pathobiology of Pulmonary Arterial Hypertension

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Pulmonary arterial hypertension (PAH) has a multifactorial pathobiology. Vasoconstriction, remodeling of the pulmonary vessel wall, and thrombosis contribute to increased pulmonary vascular resistance in PAH. The process of pulmonary vascular remodeling involves all layers of the vessel wall and is complicated by cellular heterogeneity within each compartment of the pulmonary arterial wall. Indeed, each cell type (endothelial, smooth muscle, and fibroblast), as well as inflammatory cells and platelets, may play a significant role in PAH. Pulmonary vasoconstriction is believed to be an early component of the pulmonary hypertensive process. Excessive vasoconstriction has been related to abnormal function or expression of potassium channels and to endothelial dysfunction. Endothelial dysfunction leads to chronically impaired production of vasodilators such as nitric oxide and prostacyclin along with overexpression of vasoconstrictors such as endothelin (ET)-1. Many of these abnormalities not only elevate vascular tone and promote vascular remodeling but also represent logical pharmacologic targets. Recent genetic and pathophysiologic studies have emphasized the relevance of several mediators in this condition, including prostacyclin, nitric oxide, ET-1, angiopeptin-1, serotonin, cytokines, chemokines, and members of the transforming-growth-factor-beta superfamily. Disordered proteolysis of the extracellular matrix is also evident in PAH. Future studies are required to find which if any of these abnormalities initiates PAH and which ones are best targeted to cure the disease. (J Am Coll Cardiol 2004;43:13S–24S)

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The pulmonary circulation is a low-pressure, high-flow system with a great capacity for recruitment of normally unperfused vessels. As a consequence, the walls of pulmonary arteries are thin, in keeping with their low transmural pressure. Pulmonary arterial hypertension (PAH) is a disease of the small pulmonary arteries, characterized by vascular narrowing leading to a progressive increase in pulmonary vascular resistance. The consequence of this increased right ventricle afterload is the failure of the afterload-intolerant right ventricle.

Vasoconstriction, remodeling of the pulmonary vessel wall, and thrombosis contribute to the increased pulmonary vascular resistance in PAH (1). However, it is now recognized that pulmonary arterial obstruction by vascular proliferation and remodeling is the hallmark of PAH pathogenesis (2). The process of pulmonary vascular remodeling involves all layers of the vessel wall and is complicated by the finding that cellular heterogeneity exists within the compartment of the pulmonary arterial wall (2).

CELLULAR CHANGES

Smooth muscle cells and fibroblasts. Each cell type (endothelial, smooth muscle, and fibroblast) in the pulmonary vascular wall plays a specific role in the response to injury (2). A feature common to all forms of PAH remodeling is the distal extension of smooth muscle into small peripheral, normally nonmuscular, pulmonary arteries within the respiratory acinus. The cellular processes underlying musclelogenesis of this distal part of the pulmonary arterial tree are incompletely understood. In addition, a hallmark of severe pulmonary hypertension is the formation of a layer of myofibroblasts and extracellular matrix between the endothelium and the internal elastic lamina, termed the neointima. In some model systems, particularly in hypoxia models, the adventitial fibroblast appears to be the first cell activated to proliferate and to synthesize matrix proteins in response to the pulmonary hypertensive stimulus (3). The mechanisms that enable the adventitial fibroblast to migrate into the media (and ultimately the intima) are currently unclear, but there is good evidence to suggest that upregu-
loration of matrix metalloproteinases (MMP2 and MMP9) occurs and that these molecules are involved in migration.

In many forms of pulmonary hypertension, as the vessel wall thickens, a concomitant increase occurs in neovascularization of the vasa vasaor. This neovascularization occurs primarily in the adventitia, although it extends into the outer parts of the media. This adventitial vessel formation could provide a conduit for circulating progenitor cells to access the vessel wall from the adventitial side. It is unknown whether circulating progenitor cells derived from the bone marrow contribute directly to the adventitial thickening and perhaps medial thickening, or whether bone marrow-derived progenitor cells simply enhance the proliferative and migratory activity of the local adventitial fibroblasts. Significant attention in the future will have to be focused on the role of circulating precursor cells to vascular remodeling (4). In addition, evidence shows that PAH may be associated with alterations of both rates of proliferation and apoptosis, which, in balance, result in thickened, obstructive pulmonary arteries.

**Endothelial cells.** Disorganized endothelial cell proliferation leading to formation of plexiform lesions is described in many cases of PAH (1,5). The initiating stimulus or injury that results in abnormal endothelial proliferation is unknown, but may include hypoxia, shear stress, inflammation, or response to drugs or toxins on a background of genetic susceptibility.

However, although many animal models of pulmonary hypertension exist, none recapitulate the histology of PAH, particularly the presence of plexiform lesions. Endothelial cells may respond to injury in various ways affecting the process of vascular remodeling. Injury can alter not only cell proliferation and apoptosis but also homeostatic functions of the endothelium (including coagulation pathways, and production of growth factors and vasoactive agents). The cells comprising plexiform lesions are endothelial channels supported by a stroma containing matrix proteins and myofibroblasts. Endothelial cells express markers of angiogenesis, such as vascular endothelial growth factor (VEGF) and its receptors (5). In addition, cells comprising plexiform lesions of idiopathic PAH are monoclonal in origin (6). Therefore, although the lesions themselves are probably hemodynamically irrelevant, they may represent more than simply the result of severe elevation of intravascular pressures.

It has been suggested that the endothelial proliferation seen in these lesions may be a marker of a fundamental endothelial abnormality in idiopathic PAH, possibly playing a key role in the pathogenesis of the condition. Intriguing defects in growth suppressive genes have been reported in plexiform lesions of patients with idiopathic PAH, including transforming growth factor-beta (TGF-β) receptor-2 and the apoptosis-related gene, Bax (7). Thus, in approximately 30% of plexiform lesions there is a somatic frameshift mutation in the transforming growth factor-beta type-2 receptor (TGF-βR2) gene encoding a premature stop codon. Furthermore, in 90% of plexiform lesions the TGF-βR2 protein is not expressed, in contrast to the abundant expression in endothelial cells outside these lesions. Thus, it has been proposed that somatic mutations in growth regulatory genes allow clonal expansion of endothelial cells, that contribute to the formation of plexiform lesions and vascular obliteration (7). Human herpesvirus-8 infection may also contribute to the growth of monoclonal endothelial cells in plexiform lesions from patients with idiopathic PAH (8). These findings suggest that triggers, including vasculotropic viruses, can encourage the growth of endothelial cells by dysregulating cell growth or growth factor signaling.

**Inflammatory cells.** Inflammatory mechanisms appear to play a significant role in some types of pulmonary hypertension including monocrotaline-induced cases in rats and PAH of various origins in humans including connective tissue diseases and human immunodeficiency virus infection (9). Interestingly, some patients with severe PAH associated with systemic lupus erythematosus have improved with immunosuppressive therapy, emphasizing the relevance of inflammation in this subset of patients (9). Patients with idiopathic PAH also have some immunological disturbances speaking in favor of a possible role for inflammation in the pathophysiology of this disease. Indeed, a subset of PAH patients have circulating autoantibodies including antinuclear antibodies, as well as elevated circulating levels of proinflammatory cytokines IL-1 and IL-6. Lung histology also revealed inflammatory infiltrates (macrophages and lymphocytes) in the range of plexiform lesions in severe PAH as well as an increased expression of chemokines RANTES and fractalkine (10). Further analysis of the role of inflammatory mechanisms is necessary to understand whether this component of the disease is relevant to its pathophysiology.

**Platelets and thrombosis.** Thrombotic lesions and platelet dysfunction are potentially important processes in PAH (11). In situ pulmonary artery thrombosis may be initiated or aggravated by abnormalities in the clotting cascade, such as platelet abnormalities or deficiency in coagulation factors. In the vasa vasaor, platelet thrombi can be observed in severe cases, possibly related to increased shear stress or hypoxia. The role of platelets and their interactions with the endothelium in the development of plexiform lesions is an area of ongoing investigation.
activity and fibrinolytic function of the pulmonary endothelium are altered in PAH. This dysfunction is reflected by the demonstration of elevated plasma levels of von Willebrand factor and plasminogen activator inhibitor type-1. At the present time, it is widely accepted that the shear stress itself or injury of the lung vessels generates a thrombogenic surface with subsequent thrombotic lesions. Thus, it appears this prothrombotic diathesis is shared by many forms of pulmonary hypertension, and is not unique to PAH.

Moreover, an increasing body of evidence also suggests that enhanced interactions between platelets and the pulmonary artery wall may contribute to the functional and structural alterations of pulmonary vessels. Vascular abnormalities in PAH may lead to release by platelets of various procoagulant, vasoactive, and mitogenic mediators. Indeed, in addition to its role in coagulation, the platelet stores and releases important contributors to pulmonary vasoconstriction and remodeling such as thromboxane A2, platelet-activating factor, serotonin (5-hydroxytryptamine [5-HT]), platelet-derived growth factor (PDGF), TGF-\(\beta\), and VEGF. In most cases, however, it remains unclear whether thrombosis and platelet dysfunction are causes or consequences of the disease (11).

**Molecular Mechanisms**

Pulmonary vasoconstriction is believed to be an early component of the pulmonary hypertensive process. Excessive vasoconstriction has been related to abnormal function or expression of potassium channels, as well as to endothelial dysfunction (1,2). Endothelial dysfunction leads to chronically impaired production of vasodilators such as nitric oxide (NO) and prostacyclin along with prolonged overexpression of vasoconstrictors such as endothelin (ET)-1, which not only affect vascular tone, but also promote vascular remodeling and, therefore, represent logical pharmacological targets (Fig. 1). It appears that most stimuli that acutely enhance vasoconstriction ultimately also cause cell proliferation (e.g., K+ channel inhibition, ET-1).

**Prostacyclin, vasoactive intestinal peptide, and NO.** Prostacyclin (prostaglandin I2) is an important endogenous pulmonary vasodilator acting through activation of the cyclic adenosine monophosphate (cAMP)-dependent pathways. Prostacyclin also inhibits the proliferation of vascular smooth muscle cells and decreases platelet aggregation. Prostacyclin synthesis is decreased in endothelial cells from PAH patients. Analysis of urinary metabolites of prostacyclin showed a decrease in the amount of excreted

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*Figure 1.* Consequences of pulmonary artery endothelial cell dysfunction on pulmonary artery smooth muscle cell tone and proliferation. Dysfunctional pulmonary artery endothelial cells (blue) have a decreased production of prostacyclin and nitric oxide, with an increased production of endothelin-1-promoting vasoconstriction and proliferation of pulmonary artery smooth muscle cells (red). cAMP = cyclic adenosine monophosphate; cGMP = cyclic guanosine monophosphate; ET = endothelin; ET\(A\) = endothelin receptor A; ET\(B\) = endothelin receptor B; PDE5 = phosphodiesterase type 5.
6-ketoprostaglandin F1α, a stable metabolite of prostacyclin, in patients with idiopathic PAH (12). Moreover, pulmonary endothelial cells of PAH patients are characterized by reduced expression of prostacyclin synthase (13), and prostacyclin therapy has been shown to improve hemodynamics, clinical status, and survival of patients displaying severe PAH.

Impaired endothelium-derived vasodilation is further supported by the demonstration of reduced NO synthase expression in pulmonary endothelial cells from PAH patients (14). A novel therapeutic strategy in PAH aims at increasing NO-dependent, cyclic guanosine monophosphate-mediated pulmonary vasodilation by inhibition of the breakdown of cyclic guanosine monophosphate by phosphodiesterase type-5. In a small group of PAH patients, sildenafil has been shown to be safe and effective on a chronic basis (15).

Vasoactive intestinal peptide (VIP), a neuropeptide primarily functioning as a neurotransmitter, acts as a potent systemic and pulmonary vasodilator. It also inhibits the proliferation of vascular smooth muscle cells and decreases platelet aggregation; VIP acts through two receptor subtypes (VPAC-1 and -2), which are coupled to adenylate cyclase and expressed in the lung vasculature (16). Stimulation of VPAC receptors leads to the activation of the cAMP and cyclic guanosine monophosphate (cGMP) systems. Low serum concentrations and decreased VIP immunoactivity were shown in pulmonary arteries from patients with idiopathic PAH. In addition, higher expression of VIP receptors and elevated specific receptor binding activity in pulmonary artery smooth muscle cells from idiopathic PAH was demonstrated, which presumably reflects VIP deficiency. Acute and chronic responses to inhaled VIP have been recently demonstrated in a small number of PAH patients (16).

**ET-1.** Through its action on the endothelin receptor A (ET\(_{\text{A}}\)) in pulmonary artery smooth muscle cells, ET-1 leads to a rapid increase in intracellular calcium and sustained activation of protein kinase C. Early activation of the p42/p44 isoforms of mitogen-activated protein kinase and induction of the early growth response genes c-fos and c-jun are also observed (2). The mitogenic action of ET-1 on pulmonary artery smooth muscle cells occurs through the ET\(_{\text{A}}\) or endothelin receptor B (ET\(_{\text{B}}\)) subtype, depending on the anatomical location of cells. For instance, ET\(_{\text{A}}\) mediate mitogenesis in cells derived from the main pulmonary artery, whereas in cells from resistance arteries both receptor subtypes may contribute. There is strong evidence that endothelium-derived ET-1 is a major player in the vasodilator/vasoconstrictor imbalance characteristic of PAH. Levels of lung and circulating ET-1 are increased in animals and patients with pulmonary hypertension of various etiologies (17). These observations indicate that ET-1 is likely to contribute to the vasoactive component of PAH, as well as to the abnormal pulmonary vascular remodeling characteristic of the condition. Results of chronic ET receptor antagonist therapy support the relevance of this pathway in PAH.

**Potassium channels.** Lessons relevant to PAH can be learned from understanding the mechanism of hypoxic pulmonary vasoconstriction, although PAH also involves cell proliferation and abnormalities of apoptosis (18). Hypoxic pulmonary vasoconstriction is elicited when hypoxia inhibits one or more voltage-gated potassium channels (Kv) in the pulmonary artery smooth muscle cells of resistance pulmonary arteries (Fig. 2). The resulting membrane depolarization increases the opening of voltage-gated calcium channels, raising cytosolic calcium and initiating constriction. The Kv1.5 is downregulated in pulmonary artery smooth muscle cells in humans with PAH (19), and both Kv1.5 and Kv2.1 are downregulated in rats with chronic hypoxia-induced pulmonary hypertension (20).

Furthermore, deoxyribonucleic acid microarray studies have shown downregulation Kv channel genes in PAH lungs (21). The selective loss of these Kv channels leads to pulmonary artery smooth muscle cell depolarization, an increase in the intracellular calcium, and both vasoconstriction and cell proliferation. It is not clear whether these Kv channel abnormalities are genetically determined or acquired. However, it is clear that the appetite suppressants dexfenfluramine and aminorex directly inhibit Kv1.5 and Kv2.1 (22). Augmenting Kv pathways should cause pulmonary vasodilation and promote regression of pulmonary remodeling. Drugs including dichloroacetate and sildenafil may enhance the expression and function of these potassium channels. Most of the hemodynamic effects of NO are mediated by cGMP, which causes vasodilation by activating protein kinase G, which phosphorylates and activates BK\(_{\text{CA}}\) channels, as one of several mechanisms by which it lowers cytosolic calcium.

**Serotonin.** In PAH, circulating serotonin levels are elevated, whereas the level in platelets, the major repository of serotonin (5-hydroxytryptamine [5-HT]), is low (23).
5-Hydroxytryptamine is produced by the gastrointestinal tract enterochromaffin cells and pulmonary neuroepithelial bodies and stored in platelets. A role for 5-HT has been suggested in PAH (23,24). First, a correlation between high plasma 5-HT levels and PAH was observed in a patient with congenital thrombocytopathy characterized by a defect in the platelet 5-HT storage capacities. Subsequently, elevated plasma 5-HT levels were demonstrated in a series of PAH patients (23). This could not be corrected by lung transplantation or epoprostenol therapy, indicating that raised plasma 5-HT cannot be the mere consequence of elevated pulmonary pressure (23). In the 1960s, an association between PAH and the anorexigen aminorex was identified. Aminorex induces platelet 5-HT release and inhibits monoamine oxidase, potentially inhibiting its metabolism, thus increasing plasma 5-HT levels. More recently, it was shown that exposure to fenfluramine derivatives increased the risk of developing PAH. By interacting with the 5-HT transporter (5-HTT), these anorexigen release 5-HT from platelets and inhibit its reuptake and raise circulating free 5-HT. Additionally, treatment of rats with 5-HT potentiates the effects of hypoxia on pulmonary arterial pressure and remodeling. The mechanism by which 5-HT affects the pulmonary vasculature is still a matter of debate (Fig. 3).

The 5-HTT expression, activity, or both in pulmonary artery smooth muscle cells contribute to the pulmonary vascular remodeling occurring in both clinical and experimental PAH (24); 5-HTT is encoded by a single gene on chromosome 17q11.2, and a variant in the upstream promoter region of the 5-HTT gene has been described. This polymorphism with long (L) and short (S) forms affects 5-HTT expression and function with the L-allele inducing a greater rate of 5-HTT gene transcription than the S-allele. The L-allelic variant was found to be present in homozygous forms in 65% of idiopathic PAH patients but only in 27% of controls (25). Moreover the 5-HTT gene polymorphism contributes to interindividual differences in hypoxia-induced 5-HTT expression and potentially affects susceptibility to hypoxic pulmonary hypertension (26). Mice overexpressing the 5-HTT gene exhibit spontaneous pulmonary hypertension in absence of hypoxia (and exaggerated pulmonary hypertension after hypoxic exposure) (27). Finally, recent studies have shown that selective serotonin reuptake inhibitors protect against hypoxic pulmonary hypertension in mice (28).

In human large pulmonary arteries, the 5-HT1 receptor mediates 5-HT-induced contraction. Further investigation identified the 5-HT1B as that mediating contraction in human small muscular pulmonary arteries (29). In addition,
there is an increase in the expression of the 5-HT\textsubscript{1B} receptor in PAH. Contractile responses to 5-HT in the rat pulmonary circulation are mediated by the 5-HT\textsubscript{2A} receptor in control rats, but in chronic hypoxic pulmonary hypertensive rats the response is increased, and this is mediated by the 5-HT\textsubscript{1B} receptor. Molecular studies confirmed that messenger ribonucleic acid (mRNA) for the 5-HT\textsubscript{1B} receptor is increased in these vessels. Converging evidence that the 5-HT\textsubscript{1B} receptor may be involved in the development of hypoxia-induced PAH comes from studies using the 5-HT\textsubscript{1B}/1D antagonist and studies in the 5-HT\textsubscript{1B} receptor knockout mouse (30). Development of right ventricular hypertrophy, and enhanced vasoconstriction to 5-HT\textsubscript{1B}-receptor stimulation, is absent in chronic hypoxic pulmonary hypertensive 5-HT\textsubscript{1B} knockout mice compared with their wild-type controls, and pulmonary vascular remodeling is markedly reduced.

A role for other 5-HT receptors such as 5-HT\textsubscript{2B} has been suggested. The 5-HT\textsubscript{2B} receptor is activated by nordexfenfluramine, the active dexfenfluramine metabolite. Interestingly, development of chronic hypoxic pulmonary hypertension is ablated in 5-HT\textsubscript{2B} receptor knockout mice, and the 5-HT\textsubscript{2B} receptor transcript is increased in idiopathic PAH patients (31). An interesting link between the K\textsuperscript{+} channel hypothesis and the role of serotonin is the finding that K\textsuperscript{+} channel inhibitors cause serotonin release and inhibit K\textsuperscript{+} currents in megakaryocytes (32). Moreover, the anorexigen, which inhibit serotonin reuptake and cause serotonin release, are K\textsuperscript{+} channel blockers (22). This led to the hypothesis that chronic depolarization of platelets and pulmonary artery smooth muscle cells could lead to a vasoconstricted, pro-proliferative, serotoninemic phenotype.

**TGF-β superfamily.** The TGF-β superfamily is composed of multifunctional mediators, including the TGF-β isoforms (TGF-β1–3), the bone morphogenetic proteins (BMPs), activins, and growth and differentiation factors (33,34). The TGF-β superfamily has diverse roles in a wide variety of physiological processes (Fig. 4). Germline mutations in the gene coding for BMP type-II receptor (BMPR2) have been identified in 60% of familial PAH and 10% to 30% of idiopathic PAH (35–37). The absence of BMPR2 mutations in some families and in the majority of sporadic and associated cases suggests that there may be further genes, possibly related to the BMP/TGF-β pathway, to be identified. Indeed, mutations in the TGF-β receptors, ALK-1 and endoglin, have been identified in PAH patients with a personal or family history of hereditary hemorrhagic telangiectasia (38,39).
The BMPR-II is a constitutively active serine/threonine kinase receptor, signaling via formation of heterocomplexes with one of three type-I receptors (ALK-3/BMPR-IA, ALK-6/BMPR-IB or ALK-2) in response to ligand. The main ligands identified for BMPR-II are BMP2, BMP4, BMP7, GDF5, and GDF6. The BMPR-II phosphorylates a glycine-serine-rich domain on the proximal intracellular portion of the associated type-I receptor. Activation of the type-I receptor kinase domain initiates phosphorylation of cytoplasmic signaling via the Smad family of proteins. The BMPs signal via a specific set of Smad proteins (Smad1, Smad5, and Smad8) termed “Receptor activated” or R-Smads, which complex with the common partner Smad or Co-Smad, Smad4, to allow translocation of this signaling complex to the nucleus where they can regulate gene transcription. However, Smads bind only weakly to DNA and require the presence of transcriptional co-activators or co-repressors.

There are multiple levels at which BMP signaling is regulated, including the presence of endogenous inhibitors of BMP-receptor interactions (chordin, noggin, and BAMBI), the formation of specific type-II/type-I receptor heterocomplexes, the activation of inhibitory Smads (I-Smads, Smad6, and Smad7), and the cell-specific expression of transcription factors. Such diverse levels of regulation may be responsible for the tissue specificity of BMP signaling and may, for example, underlie the lung specificity of PAH. Human pulmonary artery smooth muscle cells and endothelial cells express a wide range of TGF-β superfamily receptors, including BMPR-II and BMPR-IB, and bind 125I-TGF-β and 125I-BMP4.

Furthermore, activation of these receptors by BMPs leads to phosphorylation of Smad1 and induction of mRNAs for Smad6 and Smad7. Although signaling via Smads is well characterized, there is increasing evidence that MAP kinases, including ERK, p38MAPK, and JNK kinases, are activated in specific cell types by TGF-β and BMPs (33). It appears that in some cases the p38 kinase pathway can bypass the Smad pathway and mediate some of the BMP2 pathway effects on nuclear transcription and apoptosis. Recent evidence suggests that abnormal activation of alternative signaling pathways may be critical to the pathogenesis of PAH (40).

The critical role of the BMP pathway in vascular development is evident from studies in knockout mice. Homozygosity for a null mutation in BMPR2 is lethal during early embryogenesis (41), and mice deficient in Smad5, one of the BMP-restricted Smads, die owing to defects in angiogenesis, with failure to recruit vascular smooth muscle to endothelial structures. The net result of TGF-β signaling on vascular growth and structure is complex. Whether the TGF-β superfamily inhibits or promotes cell proliferation is highly context-specific.

In situ hybridization and immunohistochemical studies have demonstrated that both BMPR-II mRNA and protein are present predominantly on the pulmonary vascular endothelium, macrophages, and to a lesser extent on medial smooth muscle cells (42). Lung BMPR-II protein expression is dramatically reduced in patients harboring an underlying BMPR2 mutation predicted to cause truncation of the protein (42). In addition, BMPR-II expression is markedly reduced in PAH cases in which no BMPR2 mutation was identified (41). A small but significant reduction was also observed in cases of secondary pulmonary hypertension. Reduced BMPR-II expression was specific for this receptor because no change was observed in the level of expression of other endothelial markers, including CD31. These findings stress the importance of understanding how other environmental and genetic factors regulate the expression of BMPR-II in lung cells. Thus, further characterization of the regulation of BMPR-II expression is likely to add to our understanding of exogenous factors influencing BMPR-II transcription and may provide important clues as to why the vascular abnormality is restricted to the lung, particularly as BMPR-II is widely expressed in normal adult tissues.

A recently published study has provided further support for the hypothesis that intact BMP signaling is important for the maintenance of the normal pulmonary vasculature (43). In this study the type-I receptor BMPR-IA was downregulated in the lung tissue of a heterogeneous group of patients with pulmonary hypertension. Furthermore, investigators showed a reciprocal relationship between BMPR-IA expression and that of angiopoietin-1, and they demonstrated that angiopoietin-1 downregulates BMPR-IA expression in human pulmonary artery endothelial cells (43).

Additionally, BMP2, -4, and -7 inhibit the proliferation of smooth muscle cells derived from normal pulmonary arteries and from PAH patients with congenital heart diseases, but they fail to suppress proliferation of cells from patients with idiopathic or familial PAH (43). An attractive hypothesis is that a failure of the growth inhibitory effects of BMPs in idiopathic or familial PAH cells could contribute to the vascular obliteration and remodeling that characterize the condition. The failure to suppress growth of idiopathic or familial PAH cells was observed in all cases, whether or not specific BMPR2 mutations were identified, suggesting that defective BMP-mediated signaling may be a common factor in idiopathic or familial PAH. The mechanism by which BMPR2 mutations disrupt BMPR-II signaling has begun to be elucidated (Fig. 5) (40,44,45). Interestingly, a feature common to all mutants is a gain of function involving p38MAPK activation.

In pulmonary artery smooth muscle cells from patients with idiopathic PAH, TGF-β1 causes enhanced cell proliferation in contrast to the growth inhibitory effect observed in normal cells (43). This is not due to alterations in TGF-β1 receptor ratios or downregulation of TGF-β1 type-II receptor (44). Transforming growth factor-beta is also known to increase production of extracellular matrix. In human lung fibroblasts, TGF-β increases elastin expression by stabilization of elastin mRNA, and thus it is possible that increased elastin expression observed in PAH may be due to
alterations in this pathway. Although studies from other cell types have found TGF-β to induce collagen production, no correlation has been found between procollagen and TGF-β staining in lungs of PAH patients (46). The TGF-β superfamily may regulate the activity of other factors implicated in vascular remodeling. The TGF-β1 induces ET-1 in human pulmonary artery cells probably via activation of protein kinase A (47). Connective tissue growth factor production can also be stimulated by TGF-β in pulmonary fibroblasts (48). Clearly much remains to be learned of the interaction of the TGF-β/BMP pathway with other factors already demonstrated to play important roles in the control of vascular tone and growth.

Angiogenesis and apoptosis. Vascular endothelial growth factor is an endothelial-cell-specific-angiogenic mitogen acting via two high-affinity tyrosine kinase receptors (VEGFR-1 and VEGFR-2). Although the physiological role of the abundantly expressed VEGF in the lung is unknown, it has been proposed that VEGF supports pulmonary endothelial cell maintenance and survival. In PAH, the VEGF expression is increased within the pulmonary vasculature, including the plexiform lesions (5,49). Although the isoform VEGF-A has been most extensively studied in the context of pulmonary hypertension and has been proposed to play a protective role, a recent study identified a pathogenic role for VEGF-B.

In contrast to VEGF-A, the VEGF-B appears to exacerbate remodeling as VEGF-B knockout mice (VEGF-B−/−) exposed to chronic hypoxia exhibit significantly less pulmonary vascular remodeling compared with wild-type mice (VEGF-B+/−) (50). Recent animal studies have emphasized the positive effects of VEGF in models of pulmonary hypertension (51). Indeed, cell-based VEGF gene transfer has proven an effective method of preventing the development and progression of pulmonary hypertension in the monocrotaline model (51). Vascular endothelial growth factor would minimize...
progression of the disease by preventing loss of existing vessels or by inducing the development of new blood vessels within the lung (51).

In idiopathic PAH, VEGFR-1 expression is increased, whereas within the plexiform lesions it is VEGFR-2 that is expressed (52). In rats, it has been shown that the combination of chronic blockade of VEGFR-2 and chronic hypoxia could cause pulmonary endothelial cell dysfunction and cell death, allowing the selection of an apoptosis-resistant proliferating endothelial cell phenotype and the subsequent development of severe pulmonary hypertension (53). Because endothelial cell death, cell proliferation, and the development of severe pulmonary hypertension could be blocked by a broad-spectrum caspase inhibitor, it appeared that the selection of an apoptosis-resistant endothelial cell phenotype might be the critical event responsible for pulmonary artery endothelial cell proliferation (53). Therefore, apoptosis of endothelial cells may underlie the propensity to vascular disease (53).

Various other growth factors including PDGF, basic fibroblast growth factor, insulin-like growth factor-1, and epidermal growth factor have also been implicated in the development of remodeling and all have been reported to be increased in the pulmonary hypertensive lung. The mechanism that leads to induction of these growth factors in the pulmonary vasculature is unclear, though reactive oxygen species have been implicated because hydrogen peroxide induces PDGF expression in human pulmonary endothelial cells, as does hypoxia and mechanical stretch and shear stress.

Angiopoietin-1 is an angiogenic factor essential for lung vascular development (43). Produced by smooth-muscle cells and precursor pericytes, angiopoietin-1 stabilizes the development of blood vessels by recruiting muscle cells, through migration and division, to endothelial tubes, creating mature arterial structures. The receptor for angiopoietin-1, TIE2, is present only on vascular endothelium. The ligand-receptor interaction between angiopoietin-1 secreted by smooth-muscle cells and endothelium-specific TIE2 during organ development induces the proliferation of muscle cells around the endothelium vascular network.

After development is completed, angiopoietin-1 is expressed at a minimally detectable level in the human lung. Recent studies attempted to analyze the putative role of angiopoietin-1 in pulmonary hypertension, but they reached entirely antithetical conclusions (43,54,55). The findings by Du and colleagues (43) suggest that all forms of nonfamilial pulmonary hypertension are characterized by upregulation of angiopoietin-1 and phosphorylated TIE2, correlating directly with the severity of the disease. A mechanistic link
between familial PAH and acquired pulmonary hypertension was supported by the finding that angiopoietin-1 shuts off the expression of BMPR1A, a transmembrane protein required for BMPR2 signaling, in pulmonary arteriolar endothelial cells (43). Interestingly, rodents engineered to express angiopoietin-1 in the lung develop pulmonary hypertension (54). These animals manifest diffuse medial thickening in small pulmonary vessels, resulting from smooth muscle cell hyperplasia.

In addition, angiopoietin-1 stimulates pulmonary arteriolar endothelial cells through a TIE2 pathway to produce and secrete 5-HT (54). These revelations suggest that pulmonary hypertension vasculopathy occurs through an angiopoietin-1/TIE2/5-HT paracrine pathway and imply that these signaling molecules may be targets for strategies to treat this disease.

By contrast, Zhao et al. (55) have demonstrated that angiopoietin-1 may have a protective role in at least some forms of pulmonary hypertension. In their study, cell-based gene transfer with angiopoietin-1 improved survival and pulmonary hemodynamics in monocrotaline-exposed rats by a mechanism involving the inhibition of apoptosis and protection of the pulmonary microvasculature (55). These two approaches of the role of angiopoietin-1 in PAH reflect distinct views on the mechanisms leading to the disease. On the one hand, the investigators assume that the primary cellular defect contributing to the disease is smooth muscle cell hyperplasia, and that this is mediated by excess angiopoietin-1. On the other hand, it is hypothesized that endothelial apoptosis underlies disease progression, and that this can be prevented by administration of angiopoietin-1. At the present time one cannot conclude whether angiopoietin-1 is the cause or the cure of PAH, but more data are required to evaluate the angiopoietin-1/TIE2 pathway in this condition (56).

Proteolysis. Evidence that proteolysis of the extracellular matrix may be important in the pathobiology of pulmonary vascular disease came from observations of degradation of elastin in pulmonary arteries from patients with a congenital heart defect and pulmonary vascular disease (57). Interestingly, rodents engineered to express angiopoietin-1 in the lung develop pulmonary hypertension (54). These animals manifest diffuse medial thickening in small pulmonary vessels, resulting from smooth muscle cell hyperplasia.

Moreover, repression of nuclear partitioning of AML1 as well as repression of phosphorylation of Erk (a member of the MAP kinase family) was achieved with NO donors, and this also repressed elastase activity (61). Suppression of 5-HT receptors repressed elastase activity and TGF-β in chronic hypoxia. Also, to be further investigated is the relationship between BMPR-II and the induction of elastase activity. Hypothetically, it has been proposed that BMPR-II induces Smad1/5 interaction with AML1, thus preventing its interaction with Smad4 and repressing its ability to partner with other transcription factors and to induce elastase activity. Conversely, a mutation in BMPR-II would result in derepression of AML1 and induction of elastase and other AML1-dependent genes.

Evidence from the published data indicates that serine elastases activate MMPs and also repress tissue inhibitors of MMPs. In other injury models, elastase activity has been shown to precede MMP activity and is responsible for its induction. In the monocrotaline model, an elevation in elastase activity is seen on day 2 after injection of the toxin, whereas MMP activity is not increased until day 21. Both MMPs and elastases can degrade most components of the matrix in addition to elastin and collagen. Degradation of collagen leads to ligation of β3 integrins activation of the MAP kinase pathway and transcription of tenascin C. This glycoprotein cooperatively interacts with growth factors such as epidermal growth factor in inducing smooth muscle cell proliferation. Repression of this pathway by elastase inhibitors has been shown in cell and organ culture and in whole animals to induce apoptosis of smooth muscle cells, and regression of severe vascular disease (58,59).

Conclusions. It is clear that PAH has a multifactorial pathobiology, and it is unlikely that one factor or gene mutation will explain all forms and cases of PAH (Fig. 6). However, the current understanding of the mechanisms underlying PAH has allowed the rapid development of drugs including prostacyclin, endothelin receptor antagonists, and phosphodiesterase inhibitors. Our improved understanding of additional pathways in this condition will presumably lead to the development of novel therapeutic strategies in the near future, such as ion channel replacement therapy or cell-based therapies, using bone marrow precursor cells.

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