



Molecular pathogenesis of pulmonary arterial hypertension

Marlene Rabinovitch

Stanford University School of Medicine, Stanford, California, USA.



Recent clinical and experimental studies are redefining the cellular and molecular bases of pulmonary arterial hypertension (PAH). The genetic abnormalities first identified in association with the idiopathic form of PAH – together with a vast increase in our understanding of cell signaling, cell transformation, and cell-cell interactions; gene expression; microRNA processing; and mitochondrial and ion channel function – have helped explain the abnormal response of vascular cells to injury. Experimental and clinical studies now converge on the intersection and interactions between a genetic predisposition involving the BMPR2 signaling pathway and an impaired metabolic and chronic inflammatory state in the vessel wall. These deranged processes culminate in an exuberant proliferative response that occludes the pulmonary arterial (PA) lumen and obliterates the most distal intraacinar vessels. Here, we describe emerging therapies based on preclinical studies that address these converging pathways.

Pathological features of PAH

Pulmonary arterial hypertension (PAH) is diagnosed by an elevation in mean pulmonary arterial (PA) pressure above 25 mmHg at rest or 30 mmHg with exercise. Patients usually present with much higher levels of PA pressure, but only vague and insidious symptoms of increasing fatigue and dyspnea; some patients are diagnosed only after syncopal episodes, which can reflect suprasystemic levels of PA pressure and low cardiac output. The causes of PAH were reclassified according to a consensus at the Fourth World Symposium of Pulmonary Hypertension in Dana Point, California, USA, in 2008 and published by Simonneau and colleagues in 2009 (see *Dana Point classification of pulmonary hypertension* and ref. 1). While this review focuses on pulmonary hypertension category 1 (i.e., PAH), pathophysiologic insights can also be gained from understanding other causes of pulmonary hypertension. Hence, experimental studies in hypoxic animals are also discussed.

In neonates and in infants, PAH likely results from failure of the neonatal pulmonary vasculature to dilate at birth, and pathological changes in the blood vessels are evident in the first few days of life. Most prominent is abnormal muscularization of distal PAs at the alveolar duct and wall levels and a striking reduction in their number. In older infants and adults, there is also progressive intimal hyperplasia leading to occlusive changes in the PAs and plexiform lesions (Figure 1 and see below).

PA EC alterations in the clinical (2) and experimental (3) setting precede muscularization of distal PAs. In PA ECs from patients with idiopathic PAH (IPAH), an increase in Tie2 receptor expression in ECs releases serotonin, mediating SMC proliferation (4). Dysfunctional ECs can either release factors that stimulate SMC proliferation, such as FGF-2 (5), or fail to produce agents that normally suppress proliferation of SMCs in response to growth factors, such as apelin (encoded by *Apln*) (6).

Muscularization of distal, normally nonmuscular, PAs at the alveolar duct and wall level is associated with differentiation of pericytes into SMCs that subsequently proliferate. The progressive thickening of the wall of more proximal intraacinar and preacinar

muscular arteries and the obliteration associated with neointimal formation are attributed to increased proliferation and migration of cells considered to be SMCs because they are α -SMA positive (7). The origin of these cells is unclear, and the mechanism of their dysregulation is the subject of intense study. They may represent a specialized subpopulation of SMCs; they may originate as stem cells (8) or fibrocytes (9) or transform from ECs (10, 11). The loss of distal PAs could be caused by alterations in ECs and/or pericytes resulting in apoptosis (12).

Later in disease, there is dysregulated EC proliferation, producing aberrant channels in the otherwise obliterated lumen of the vessel and in the adventitia (i.e., the plexiform lesion). These channels may reflect clonal expansion of apoptosis-resistant ECs (13), or they may be derived from circulating endothelial progenitor cells (EPCs) that accumulate at sites of endothelial denudation or injury and expand locally (14). PA ECs from patients with IPAH produce decreased amounts of NO. Synthesized largely by eNOS in ECs of the pulmonary vasculature, NO is a vasodilator and suppressor of SMC proliferation. The reduction in NO may be related to high arginase levels (15), because L-arginine, the substrate of eNOS, is required to produce NO. ECs from patients with PAH may be highly proliferative in response to growth factors (14) and exhibit high rates of glycolysis (16) that reflect impaired mitochondrial metabolism. The dysfunction of these cells is revealed in their inability to form normal tubes in culture, consistent with the fact that they fail to restore occluded or lost precapillary vessels.

Strategies to improve NO delivery to tissues have been successful experimentally, and provision of nitrite can block both the sequelae of hypoxia and inflammation in PAH patients (see *Emerging therapeutic strategies for pulmonary hypertension* and ref. 17). Similarly, inhibiting asymmetric dimethyl arginine (ADMA) by activating dimethylarginine dimethyl hydrolase (DDAH) may be useful in allowing greater production of NO (18). Current therapies for PAH include targeting reduced prostacyclin and increased endothelin levels. A more complete discussion can be found in the supplemental material (available online with this article; doi:10.1172/JCI60658DS1).

Additional features of PAH include thickening of the pulmonary adventitia and venous hypertrophy (19) and increased expression of TGF- β ; matrix proteins such as elastin, fibronectin, and tenascin-C;

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Dana Point classification of pulmonary hypertension

Category 1 (PAH)

- IPAH
- Heritable PAH
- Drugs and toxins
- Portal hypertension
- HIV
- Schistosomiasis
- Congenital heart disease
- Connective tissue diseases
- Chronic hemolytic anemia
- Pulmonary capillary hemangiomatosis
- Pulmonary veno-occlusive disease
- Persistent pulmonary hypertension of the newborn

Category 2

- Related to left heart disease

Category 3

- Related to chronic lung disease

Category 4

- Related to thromboembolic disease

Category 5

- Related to sarcoid and other rare disorders

and glycosaminoglycans (20). In addition to macrophages, B and T cells (21) abound in the perivascular space and are often seen invading the vessel wall, and there is heightened expression of inflammatory mediators such as S100A4 (22) and fractalkine (23).

Genetics of PAH: BMPR2 and other TGF- β family members

Genetic studies have demonstrated that 70% or more of patients with hereditary PAH (24, 25), and 10%–20% of patients with sporadic IPAH, are heterozygous for a mutation in bone morphogenetic protein (BMP) receptor type 2 (BMPR2). BMPR2 is a member of the TGF- β superfamily of growth factor receptors. Mutations can affect different functions of BMPR2, namely the ligand-binding domain, the signaling mechanism, and the interaction of the receptor with the cytoskeleton. BMPR2 is expressed ubiquitously and, in association with a coreceptor (usually BMPR1A), can signal through many different pathways, including pSmad1/5 (26), p-p38 (27), pERK, JNK, and Akt/PI3K (28, 29).

The penetrance of heritable PAH is low: 80% of family members carrying BMPR2 mutations will never develop PAH (30). The presence of a BMPR2 mutation is much lower (i.e., 6%–8%) in patients with PAH related to a congenital left-to-right shunt (31) and is rare in patients with PAH associated with appetite suppressants (32). The functional link between mutations in BMPR2 and PAH is reinforced by the fact that independent of a mutation in BMPR2, most IPAH patients have reduced BMPR2 protein expression, as do, to some extent, patients with PAH associated with other conditions (33). In addition, mutations in the effectors of the signaling pathway and in the TGF- β superfamily of receptors have also been described in patients with PAH and IPAH (34). For example, activin-like kinase type 1 (ALK1) and endoglin are mutated in patients with hereditary hemorrhagic telangiectasia and with PAH (35, 36). In fact, the ALK1-deficient mouse develops spontaneous pulmonary

hypertension, whereas the BMPR2-heterozygous mouse model requires additional perturbations, such as both hypoxia and serotonin or inflammation, to elicit an exaggerated pulmonary hypertensive phenotype (37). In addition, somatic mutations in ECs from patients with pulmonary hypertension have been described (38).

New links have been established among BMPR2 signaling, Smad activation, and the processing of microRNAs (39), particularly miR-21, which can control functions critical to SMC contractility (40). Indeed, altered microRNA processing has been described in heritable PAH (41), and altered microRNA profiles are seen in experimental and clinical PAH (42).

Various strategies have been undertaken to rescue BMPR2 loss of function directly by gene therapy (43) and by improving trafficking of mutant receptors (44). Since loss of BMPR2 leads to an adverse response to TGF- β signaling (45), therapies that inhibit the TGF- β receptor ALK5 may also be efficacious in PAH (46).

BMPR2 and vascular cell dysfunction

When loss of BMPR2 is induced by RNA interference in PA ECs, the cells become susceptible to apoptosis (47). This vulnerability of ECs with loss of BMPR2 can explain the reduced number of peripheral alveolar duct and wall PAs. Our group has shown that BMPs, via BMPR2, activate the canonical Wnt signaling pathway to induce PA EC survival and proliferation and activate the noncanonical pathway to induce migration, critical features in angiogenesis and regeneration of damaged blood vessels (48). Further work by our group showed that a complex between β -catenin and PPAR γ is essential in directing these functions and that a key downstream gene transcribed by this complex is apelin (6). Apelin has autocrine effects in promoting PA EC survival and migration and paracrine effects in suppressing aberrant PA SMC growth. Mice with PPAR γ deleted in ECs develop spontaneous PAH (49) that is reversed by treatment with apelin (6). Our studies also showed the efficacy of using naturally occurring PPAR γ adducts, such as nitro-fatty acids (NO₂-FAs), to rescue BMPR2 dysfunction. Both NO₂-FA and similarly reactive electrophilic keto-FAs display nanomolar affinities for PPAR γ (50) and mediate distinctive patterns of PPAR γ -dependent gene expression. This is in addition to their antioxidant properties, related to activation of Nrf2, and antiinflammatory properties, related to inhibition of NF- κ B. Another transcription factor that has a beneficial effect in rescuing BMPR2 dysfunction in PA ECs appears to be Id1 (51).

Loss of BMPR2 causes proliferation of PA SMCs in response to TGF- β 1 and BMP2, in contrast to the inhibition of SMC proliferation and increased susceptibility to apoptosis normally observed with these morphogens. Normal BMPR2 signaling negatively regulates PDGF (52) and likely other growth-promoting factors implicated in the pathobiology of PAH, such as EGF (53). Since BMP4 induces differentiation of fetal lung fibroblasts into SMCs and inhibits their proliferation (54), lack of BMP4/BMPR2 interaction might expand the myofibroblast population of cells, accounting for the adventitial and intimal thickening of the PAs in PAH.

In cultured PA SMCs, BMP2 regulates PPAR γ transcriptional activity (52), and *APOE* is a key target gene. Mice with deletion of *ApoE* develop pulmonary hypertension on a high-fat diet (55), as do mice with deletion of *Pparg* in SMCs (52), even when fed a normal diet. In systemic arterial SMCs, apoE can repress proliferation by phosphorylating and internalizing the coreceptor of PDGF, LDL receptor-related protein 1 (LRP1) (56). Repression of the PDGF receptor by imatinib (Gleevec) can reverse experimental pulmonary hypertension in rats (57) and may improve outcome in patients with end-stage PAH

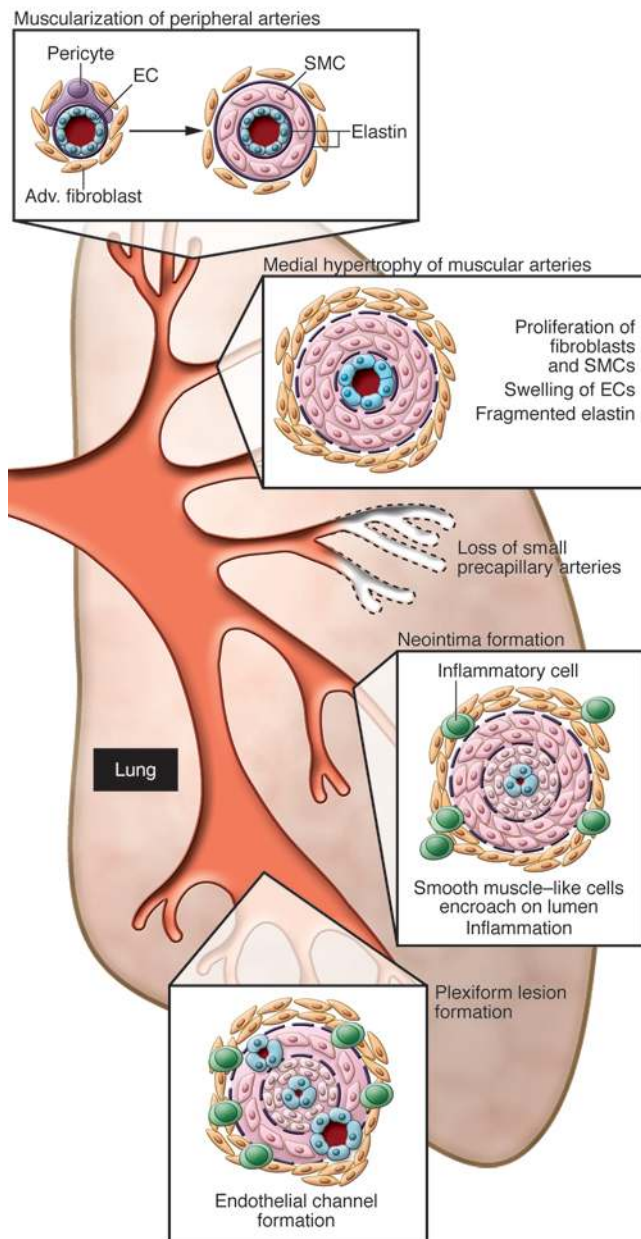


Figure 1

Vascular abnormalities associated with PAH. This schema depicts the abnormalities throughout the pulmonary circulation: abnormal muscularization of distal and medial precapillary arteries, loss of precapillary arteries, thickening of large PAs, and neointimal formation that is particularly occlusive in vessels less than 500–100 μ M and in plexiform lesions therein.

lating osteopontin is also increased in PAH and indicates patients with more severe symptomatology (64). Given the functional significance of BMPR2 signaling, it is not surprising that penetrance of the mutation appears to depend on the level of production of BMPR2 from the normal allele (30). Conversely, haploinsufficiency of BMPR2 (65), or even expression of dominant-negative BMPR2 in SMCs (66), in mice requires addition of other stimuli to bring out a more severe PAH phenotype (67).

Drugs and toxins

The high incidence of PAH in patients taking anorexigens implicated serotonin-like compounds in the pathobiology of the disease (68). There are additive effects of serotonin and the anorexigen dexfenfluramine in causing PAH (69). There is also evidence in female PAH patients of an additive effect between serotonin and 17 β -estradiol levels (70). Serotonin has structural and functional similarities to compounds such as cocaine and amphetamine. Abuse of these compounds is also associated with PAH (71). This suggests a relationship between heightened signaling via G protein-coupled serotonin and serotonin-like receptors and PAH. There is also evidence of interplay among HIV, cocaine abuse, and heightened activity of PDGF (72).

Serotonin receptors and transporters

Elevated serotonin levels and serotonin transport have been implicated in the pathology of experimental and clinical PAH. One study identified a gain-of-function polymorphism in the serotonin transporter in patients with PAH (73), but this modifier was not observed in different populations of PAH patients. Serotonin exaggerates vasoreactivity in the fawn-hooded rat (74), and there is attenuated severity of pulmonary vascular disease in mice lacking the serotonin transporter gene (75). In contrast, mice overexpressing the serotonin transporter globally (76) or specifically in SMCs (77) develop worsened hypoxia-induced PAH. PDGF-mediated proliferation of SMCs can be compounded by increased activity of the serotonin transporter, since this enhances PDGF receptor β -mediated signaling (78). Other studies have shown that serotonin-mediated stimulation of the serotonin transporter and the serotonin receptors induces expression of cyclins and c-fos (79), which are critical to the PA SMC proliferative response.

Serotonin stimulates an increase in S100A4, a member of the S100 family of calcium-binding proteins (80) that induces proliferation and migration of PA SMCs (80) and is increased in neointimal lesions from patients with IPAH and PAH associated with other conditions. Moreover, a mouse that overexpresses S100A4 can spontaneously (albeit rarely) develop pulmonary vascular pathology (22), and consistently does so when concomitantly infected with murine gamma herpes (MHV68, the murine homolog of HHV8; ref. 81). The latter study, coupled with the observation of increased incidence of PAH in patients with HIV, underscores the importance of immune mechanisms and inflammation in PAH pathobiology as well as the above-described interplay among serotonergic compounds, PDGF, and HIV (72).

(58). Moreover, dasatinib, a very potent inhibitor of the BCR/ABL tyrosine kinase, also appears promising as a future therapy (59).

In addition to apoE, another key target of PPAR γ is adiponectin, which can sequester PDGF-BB (60) and repress PA SMC proliferation. Treatment of *ApoE*^{-/-} mice with the PPAR γ agonist rosiglitazone reverses PAH and raises adiponectin levels (55). However, rosiglitazone adversely affects PA EC survival in association with disruption of the PPAR γ / β -catenin transcription factor complex (6). This side effect was not observed with naturally occurring adducts, such as NO₂-FAs. Other sequelae of BMPR2 dysfunction implicated in proliferation of vascular SMCs include heightened production of osteopontin (61) and tenascin-C (62). Osteopontin is increased in pulmonary vascular lesions and in serum of patients with PAH and can increase PA SMC proliferation and migration. Tenascin-C can cluster integrins and activate growth factor receptors in SMC and induce their proliferation (63). Circu-

**Emerging therapeutic strategies for pulmonary hypertension****Vasodilation**

Fasudil (rho kinase inhibitor)
VIP
Adrenomedullin
Guanylate cyclase activator

Inflammation

Elastase inhibitor
B cell antagonist
HDAC1 inhibition
Immunosuppressants
NFATc inhibition

Metabolism

PPAR γ agonists (e.g., NO₂-FAs)
Nitrites
DCA (PDK inhibitor)
Antioxidants
Protection against ER stress
Antiglycolytic (stimulate FA oxidation)
Serotonin antagonist

Induction of apoptosis of SM-like cells

Tyrosine kinase inhibitor
Elastase inhibitor

Promotion of vascular regeneration

Apelin
EC-based therapy
BMP2 replacement

Preservation of RV function**Inflammation and immune mechanisms**

Increasing attention is being focused on the proinflammatory state of the vessel wall in the progression of PAH. The development of PAH in a subset of patients with HIV infection may be a function of the patient's HLA class II alleles (82). Moreover, a link was made between expression of HHV8 (associated with Kaposi sarcoma) and IPAH (83). It also appears that the Kaposi sarcoma virus can stimulate lysosome-mediated degradation of BMP2 (84). Recent studies suggested a link between the HIV-nef gene and plexogenic pulmonary vascular lesions in PAH in HIV-infected patients and SIV-infected nonhuman primates (85).

There is a high incidence of PAH in areas of the world endemic for schistosomiasis. About 10% of patients with schistosomiasis will develop portal hypertension, and 10% of those will have PAH. Chronic infection of mice with high-dose cercariae results in severe but spotty lung vascular remodeling (86, 87), albeit with a relatively modest pulmonary hypertensive response. Treatment of the schistosomiasis in these mice induces regression of the pathology (86). Allergic responses to ovalbumin or to *Aspergillus* species in mice can also cause extensive spotty pulmonary vascular remodeling, without pulmonary hypertension (88). In this model, an IL-13-mediated increase in α -resistin is associated with SMC proliferation, but the functional significance of this molecule in scleroderma and PAH (89) and in experimental schistosomiasis (87) is not known. In mice lacking prostaglandin synthase, induction of allergic inflammation with the house dust mite induces intense pulmonary vascular remodeling, changes that are reversed by administration of prostaglandin E₂ (90).

In the experimental setting, haploinsufficiency of BMP2 is associated with an exaggerated pulmonary hypertensive response to an inflammatory stimulus (91). Other experimental models of chronic inflammation, such as repeated injections of endotoxin (92) or TNF- α (93), also cause pulmonary vascular changes. In the rodent model of pulmonary hypertension, depletion of T cell subsets worsens the pathology (94). This has been attributed to unbalanced B cell activity resulting from impaired Tregs (95). In the athymic rat given the VEGF receptor blocker, reconstitution with Tregs was sufficient to rescue PAH in association with elevated BMP2 levels. In inflammatory PAH, complement appears to play an essential role (96).

Heightened circulating levels of cytokines and their receptors have been demonstrated in IPAH patients; these include fractalkine, stromal derived factor-1 (SDF-1), monocyte chemoattractant protein-1 (MCP-1), and granulocyte-monocyte colony-stimulating factor (reviewed in ref. 97). Loss of BMP2 induces IL-6 (98), a cytokine that can cause severe pulmonary vascular disease in rodents (99) in association with SMC proliferation (100) mediated by the transcription factor Kruppel-like factor 5 (101). Regulation of epigenetic factors may reverse inflammatory processes. In hypoxia-associated PAH, activation of fibroblasts expressing elevated levels of cytokines was linked to increased histone deacetylase 1 (HDAC1), inhibition of which reversed both the fibroblast phenotype and PAH in experimental animals (102).

An increase in perivascular macrophages is essential to the development of hypoxia-induced pulmonary hypertension in experimental animals, and this phenomenon is observed in lung tissue from patients with IPAH (103). Mononuclear fibrocytes, cells that have characteristics of both fibroblasts and leukocytes (104), have been identified as key contributors to the remodeling of the pulmonary vasculature. These cells may migrate into the vessel wall through the angiomas located in the expanding adventitia (9). At least some of the cells in the neointimal lesions may have originated as invading fibrocytes; indeed, high levels of circulating fibrocytes are found in adults and children with pulmonary hypertension (105). Tertiary lymphoid tissue was described in patients with IPAH as evidence of altered immune regulation (106). Similarly, circulating autoantibodies are observed in patients with both autoimmune and idiopathic and other associated forms of pulmonary hypertension, but the role of these antibodies in the pathology is the subject of considerable investigation (107).

Previous studies suggested that heightened expression of the transcription factor nuclear factor of activated T cells c2 (NFATc2) (108), which is associated with activated inflammatory cells, may underlie PAH. Increased nuclear NFATc2 is observed in T cells from IPAH patients and in pulmonary vascular lesions, and this can lead to repression of voltage-gated K⁺ (Kv) 1.5 channel expression and influx of intracellular Ca⁺, causing contraction and proliferation of SMCs. NFATc2 nuclear translocation can be inhibited by cyclosporine as well as by tacrolimus (also known as FK-506). Recently, it was shown that miR-204 is reduced in PAH, and low miR-204 increases the level of phosphatase Shp2, which in turn activates NFATc2 (109).

Elastase activity

Ultrastructural studies of PAs from children with congenital heart defects and associated PAH suggested that elastolytic activity may be an early feature of this complication (2). Elevated serine elastase activity was subsequently documented in rodent and murine models of PAH (110), which led to the successful experimental use of elastase inhibitors to prevent pulmonary vascular pathology (Figure 2 and

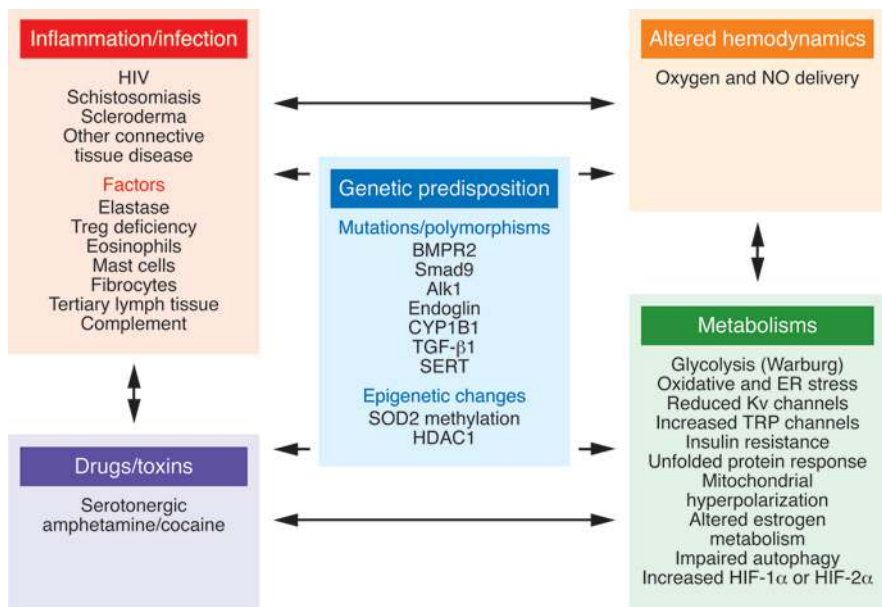


Figure 2 Factors that converge in the molecular pathogenesis of PAH. This schema focuses on the interactions among inflammation, altered cellular metabolism, and genetic/epigenetic abnormalities in the pathogenesis of PAH.

refs. 111, 112). The mechanism relating elastase activity to PAH is based on studies in cultured PA SMCs showing that serum and EC factors can mediate the cells' production of serine elastase. This enzyme can release growth factors from the extracellular matrix (5) and induce production of matrix metalloproteinases and tenascin-C, a glycoprotein associated with activation of SMC growth factor receptors and survival pathways (Figure 2 and ref. 63). These studies led to work showing that elastase inhibitors not only prevent, but also reverse, experimental pulmonary hypertension by inducing apoptosis of SMCs (113). Regression of experimental pulmonary hypertension was also achieved by blocking a downstream effector of elastase, the EGF receptor (53). Blockade of the PDGF tyrosine kinase receptor by imatinib had a similar effect (57). Similarly, transfection of a dominant-negative survivin construct (114) blocked a downstream effector of growth factor signaling and was also effective in reversing PAH through SMC apoptosis. Recently, we found that the relevant PA SMC elastase in PAH is neutrophil elastase (115). PA SMCs from mice that overexpress S100A4 have elevated levels of neutrophil elastase mRNA and protein, as do PA SMCs from patients with IPAH. Inhibition of elastase with the naturally occurring serine elastase inhibitor elafin attenuated the development of neointimal lesions in the S100A4 mice that are infected with virus.

An interesting link has been made between metabolism and inflammation by showing that the reduced adiponectin seen in insulin resistance (116) leads to recruitment of eosinophils that can play a profound role in the development of pulmonary vascular disease. Another study linked endothelin with the unfolded protein response, mitochondrial dysfunction, and inflammation (117).

Glycolytic metabolism and the Warburg effect

The fawn-hooded rat, which has defective serotonin metabolism and develops PAH in response to relative alveolar hypoxia at mile-high altitude, also has abnormal oxygen sensing in the mitochondria

dria of SMCs, leading to reduced Kv channel function (118). Hyperpolarized mitochondria cause normoxic stimulation of HIF-1α (a phenomenon known as the Warburg effect), leading to reduced cytochrome c oxidase and SOD levels and impaired Kv channel expression and function (Figure 2). In the fawn-hooded rat, in association with hyperpolarized mitochondria, there is hypermethylation of the SOD2 promoter in PAs, but not aortic SMCs. Demethylating agents can reverse the apoptosis-resistant phenotype of the SMCs and the pulmonary hypertension in these rats (119).

Interestingly, BMP2-mediated BMPR2 signaling has been directly related to expression of Kv channels (120); conversely, serotonin can directly inhibit rat PA Kv channels (121). Moreover, transfer of Kv channels has been used as an experimental strategy in animal models to prevent and reverse PAH (122). Reversal of the mitochondrial abnormality and Kv channel dysfunction can be achieved through the pyruvate dehydrogenase kinase (PDK) inhibitor dichloroacetate (DCA) (118), in association with regression of pulmonary vascular remodeling. The link

between metabolism and inflammation is reinforced by studies showing that the cytokine TNF-α can cause mitochondrial hyperpolarization that is also reversible by DCA (123).

Manipulation of the glycolytic pathway can also be achieved by suppression of malonyl coenzyme decarboxylase. This reduces FA oxidation and reverses chronic pulmonary hypertension (124). Interestingly, ER stress increases the distance between the ER and the mitochondria in PA, but not systemic SMCs, and this occurs through activation of ATF6 and transcription of Nogo-B. The increased distance results in inhibition of calcium-sensitive mitochondrial enzymes and decreases mitochondrial-dependent apoptosis (123), thus facilitating SMC proliferation.

It appears that the inability of cells to undergo autophagy (125) is linked to heightened oxidative stress, HIF-1α stabilization, increased intracellular Ca⁺, suppression of Kv channel function, and worsening of hypoxia-induced pulmonary hypertension (126). An increase in HIF-2α also promotes PAH, as is evident in von Hippel Lindau disease (127).

Recent reports of elevated transient receptor potential (TRP) calcium channels TRP3 and TRP6 in PA SMCs from IPAH patients indicated that inhibition of these channels can repress the heightened proliferation observed in SMCs (128). Inhibition of PKA or activation of cAMP seem to have a similar effect (129).

Gender and estrogen metabolism

Reduced expression of BMPR2 from the normal allele can distinguish unaffected from PAH-affected family members with a BMPR2 mutation (30), and this appears to be estrogen dependent. The female predisposition to IPAH may also be related to aberrant expression of a cytochrome, CYP1B1, that leads to a mitogenic estrogen metabolite (130). Experimental studies attribute the manifestation of pulmonary vascular pathology in S100A4-overexpressing female mice to 17β-estradiol induction of receptor for advanced



glycosylation end-products (RAGE), the S100A4 receptor (131). In contrast, both estrogen and activation of the estrogen receptor β (ER β) is protective against the development of experimental pulmonary hypertension (132, 133), so preferential engagement of the ER α may dictate the development of PAH in females. Also, the lack of insulin resistance and of PAH in *ApoE*^{-/-} female mice fed a Western diet has been attributed to heightened levels of adiponectin (55); the low level of adiponectin in males is related to testosterone-mediated degradation. Metabolic syndrome, however, is increased in females with IPAH compared with the general population (134), so low adiponectin may place females at heightened risk for PAH.

Stem and progenitor cells

A major effort is being directed at understanding the mechanisms underlying recruitment of circulating EPCs (135) and endothelial-derived autocrine signals (136), with the view toward applying this knowledge to regenerating normal microvessels. EPCs engineered to express eNOS were successfully applied first to experimental PAH (137), and are currently being tested in the clinical setting. Mesenchymal cells also attenuate experimentally induced PAH and improve RV performance (138, 139).

Although the focus in understanding the mechanism of PAH has been on the small PAs (less than 500 μ M), changes in impedance (140) resulting from stiffening of the more proximal as well as the distal PAs may also be a critical determinant of RV function (141). BMPR2 mutations associated with PAH may also influence the remodeling pathology of proximal PAs and of cardiac myocytes and fibroblasts. For example, a mosaic deletion of BMPR1A improves distal PA remodeling, but creates stiffer proximal PAs (142). Basic questions remain as to why the RV might fail under

conditions such as scleroderma (143), when PA pressure and resistance are not highly elevated. However, a discussion of RV and large PA function in the context of PAH merits its own review.

New directions

This review attempts to show that the myriad molecules and pathways implicated in PAH pathobiology are all interconnected. There is a genetic predisposition that is linked either directly or indirectly to the BMPR2 signaling pathway in vascular and in inflammatory/immune cells. This affects the response of the tissue to an infectious or inflammatory stimulus. Inflammation and oxidative stress also have profound effects on mitochondrial metabolism and gene regulation (Figure 2).

Thus far, in clinical or in experimental studies, little attention has been given to the microbiome in pulmonary hypertension and to the induction of inflammasomes and their potential role in the remodeling associated with PAH (144). Next-generation sequencing combined with proteomics and metabolomics will further identify gene variants and epigenetic changes that underlie the pathobiology of PAH. The use of induced pluripotent stem cells differentiated into vascular cells could be useful (145) in better stratifying and personalizing newly emerging PAH therapies (see *Emerging therapeutic strategies for pulmonary hypertension*), if these cells reflect the genetic and epigenetic features causing the cellular dysfunction associated with PAH.

Address correspondence to: Marlene Rabinovitch, Stanford University School of Medicine, CCSR 2245B, Stanford, California 94305-5162, USA. Phone: 650.723.6928; Fax: 650.723.6700; E-mail: marlener@stanford.edu.

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