Basic Science of Pulmonary Arterial Hypertension for Clinicians

New Concepts and Experimental Therapies

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Pulmonary arterial hypertension (PAH) is a syndrome in which pulmonary arterial obstruction increases pulmonary vascular resistance, which leads to right ventricular (RV) failure and a 15% annual mortality rate. The present review highlights recent advances in the basic science of PAH. New concepts clarify the nature of PAH and provide molecular blueprints that explain how PAH is initiated and maintained. Five basic science concepts provide a framework to understand and treat PAH: (1) Endothelial dysfunction creates an imbalance that favors vasoconstriction, thrombosis, and mitogenesis. Restoration of this balance by inhibition of endothelin and thromboxane or augmentation of nitric oxide (NO) and prostacyclin is the paradigm on which most current therapy is based. (2) PAH has a genetic component. Mutations (bone morphogenetic protein receptor-2 [BMPR2]) and single-nucleotide polymorphisms (SNPs; ion channels and transporter genes) predispose to PAH. (3) Excess proliferation, impaired apoptosis, and glycolytic metabolism in pulmonary artery smooth muscle, fibroblasts, and endothelial cells suggest analogies to cancer. Many experimental therapies reduce PAH by decreasing the proliferation/apoptosis ratio; these include inhibitors of pyruvate dehydrogenase kinase (PDK), serotonin transporters (SERT), survivin, 3-hydroxy-3-methylglutaryl coenzyme A reductase, transcription factors (hypoxia-inducible factor [HIF]-1α and nuclear factor of activated T lymphocytes [NFAT]), and tyrosine kinases. Augmentation of voltage-gated K^+ channels (Kv1.5) and BMPR2 signaling also addresses this imbalance. Tyrosine kinase inhibitors used to treat cancer are currently in phase 1 PAH trials. (4) Refractory vasoconstriction may occur due to rho kinase activation. Fewer than 20% of PAH patients respond to conventional vasodilators; however, refractory vasoconstriction may respond to rho kinase inhibitors. (5) The RV can be targeted therapeutically. Although increased afterload initiates RV failure, which is the major cause of death/dysfunction in PAH, the RV may be amenable to cardiac-targeted therapies. The RV in PAH has features of ischemic, hibernating myocardium.

Guided by these new concepts and armed with a better understanding of disease mechanisms, we are poised to identify new therapeutic targets. To achieve balance in a rapidly evolving field, we invited colleagues to contribute Figures and legends illustrating pathways in their area of expertise that are important to the pathogenesis and treatment of PAH. These contributors are acknowledged in the Acknowledgments section.

Epidemiology

There are 5 categories of pulmonary hypertension (PH) in the latest World Health Organization classification[1]: (1) PAH; (2) PH associated with left-sided heart disease; (3) PH associated with lung disease/hypoxia; (4) thromboembolic PH; and (5) miscellaneous. The present review focuses on category 1 (PAH), which includes idiopathic and familial PAH, as well as PAH associated with a variety of conditions (including connective tissue diseases and congenital heart disease), pulmonary venoocclusive disease, pulmonary capillary hemangiomatosis, and persistent pulmonary hypertension of the newborn. The incidence and prevalence of PAH, respectively, are estimated at 2.4 cases/million annually and 15 cases/million in France[2] and 7.6 cases/million annually and 26 cases/million in Scotland.[3] The global prevalence of PAH is hard to estimate because accurate diagnosis of PAH is difficult, and access to care is limited in many countries. Because diseases that are risk factors for PAH, such as HIV, schistosomiasis, and sickle cell disease, are more prevalent in the developing world, the global burden of PAH is likely greater than is recognized currently.[4] In developed countries, prevalence will also likely increase as newer associations with PAH emerge, including dialysis[5] and the metabolic syndrome,[6] and as widespread access to echocardiography identifies PAH earlier and in more individuals.

Definition

PAH is a small subset of pulmonary hypertensive syndromes (World Health Organization categories 2 to 5). PAH is defined by a resting mean pulmonary artery pressure (PAP) >25 mm Hg, pulmonary vascular resistance (PVR) >3

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Wood units, and pulmonary capillary wedge pressure <15 mm Hg (in the absence of other causes of PH). Unlike PAH, PH is ubiquitous, the sole diagnostic criterion being a resting mean PAP >25 mm Hg. This larger PH group often does not have intrinsic pulmonary vascular disease. Their PH is due to high flow, elevated left ventricular end-diastolic pressure, lung disease/hypoxia, or valve disease. There is no randomized clinical trial evidence that World Health Organization category 2 to 5 patients benefit from PAH-specific therapies and research to study these patients is critically required.

Prognosis

The 1-year incident mortality rate of PAH remains high (15%) despite treatment with prostacyclin, endothelin antagonists, and phosphodiesterase (PDE)-5 inhibitors.[7] Moreover, the mortality rate is much higher in cohorts of incident (new) rather than prevalent (preexisting) cases. Because PAH is a syndrome, it is not surprising that the prognosis varies depending on the associated comorbid conditions. Prognosis in PAH associated with congenital heart disease tends to be better than in idiopathic PAH (iPAH; 3-year survival rate 77% versus 35%).[8] In another cohort of PAH patients treated with Flolan, survival in iPAH patients was better (65% at 3 years).[9] Prognosis was worse in older patients and was also worse in PAH associated with scleroderma versus iPAH.[9] PAH associated with scleroderma has a 3-year survival rate of only 34% to 47%.[9,10]

Current Therapies

Treatment of PAH involves the use of prostanoids (given intravenously, by inhalation, subcutaneously, or orally), endothelin receptor blockers, and/or PDE5 inhibitors. L-type calcium channel blockers (eg, nifedipine) can be effective but are only safe for use in patients who respond to a 1-time vasodilator challenge with a >20% fall in mean PAP and no decline in cardiac output (a subset representing 12% to 20% of PAH patients).[11] Most patients empirically receive anticoagulation to prevent thrombosis in situ and diuretics to limit edema. PAH treatments remain expensive and/or difficult to deliver and are more palliative than curative. A year of sildenafil is estimated to cost $13 000 versus approximately $56 000 for bosentan, whereas costs for inhaled iloprost and intravenous prostacyclin exceed $90 000 per year. The only randomized PAH study that has shown a survival benefit used intravenous Flolan (GlaxoSmithKline, Brentford, United Kingdom), which, compared with conventional therapy, decreased mortality in 81 World Health Organization class IV patients.[12] Thus, there is a pressing need for less expensive and more effective therapies.

Most current treatments (prostacyclin, endothelin antagonists, and warfarin) address endothelial dysfunction by augmenting vasodilator and antiproliferative mediators and inhibiting vasoconstrictor, prothrombotic, and mitogenic pathways. Our increasing knowledge of the cellular and molecular basis of PAH suggests many potential new therapeutic agents.

Histology

The histological findings in PAH include intimal hyperplasia, medial hypertrophy, adventitial proliferation/fibrosis, occlusion of small arteries, thrombosis in situ, and infiltration of inflammatory/progenitor cells. Angioproliferative "plexiform" lesions are found in PAH but not in other PH categories (Figure 1). Plexiform lesions (and other complex lesions) are often located downstream from occluded arteries and express the transcription and growth factors typically seen in angiogenesis, including vascular endothelial growth factor (VEGF) and HIF-1α (Figure 2).[13] PAH typically spares the airway, veins, bronchial circulation, capillaries, and systemic vasculature (Figure 1). The various histological abnormalities of PAH are heterogeneous in their distribution and prevalence within the lungs. The natural progression of lesion severity (presumably from medial hypertrophy to plexiform arteriopathy) and the functional relevance of plexiform lesions remain uncertain, although regression of histologically proven PAH has been documented after single lung transplantation.[14] Human lung tissue is invaluable, offering cells for culture, histological sections for immunohistological assessment of pathogenetic pathways, and tissue to be mined by laser capture microdissection for biomarkers. It remains the "gold standard" against which to judge animal models.

Animal Models

The evaluation of these novel targets occasionally involves the off-label use of drugs approved for another indication (eg, Gleevec, Novartis Oncology, East Hanover, NJ) in humans, but is largely based on studies in cellular and animal models. Cautious interpretation of preclinical studies is mandatory, and one must recognize the strengths and weaknesses of various animal models and the risks of extrapolation to humans with PAH. Notably, no animal model completely recapitulates human PAH. Promising rodent models include monocrotaline-treated rats with or without pneumonectomy[15] or abdominal aortocaval shunt,[16] fawn-hooded rats (FHR, which spontaneously develop PAH and are also hypoxia sensitive),[17,18] and rats treated with a single dose of VEGF-receptor antagonist (SU5416) plus hypoxia.[19] Models that combine multiple insults yield more severe PAH with better hemodynamic and histological fidelity to human PAH. This may be relevant to the pathogenesis of human PAH, which also appears to require multiple "hits." Murine models of PAH offer mechanistic insight on the relevance of single genes. Mice that transgenically overexpress SERT,[20] BMPR2 dominant-negative mutations,[21] or S100A4/Mts1 (metastasin 1),[22] an accepted marker of a tumor's metastatic potential, develop PH.

New Paradigms

PAH was once regarded largely as a disease of excess vasoconstriction. This view was incomplete, and new concepts help us understand the fundamental causes of this syndrome.

PAH Is a Panvasculopathy

Let's take a tour of the molecular pathology of PAH, beginning at the lumen of a small pulmonary artery (Figure

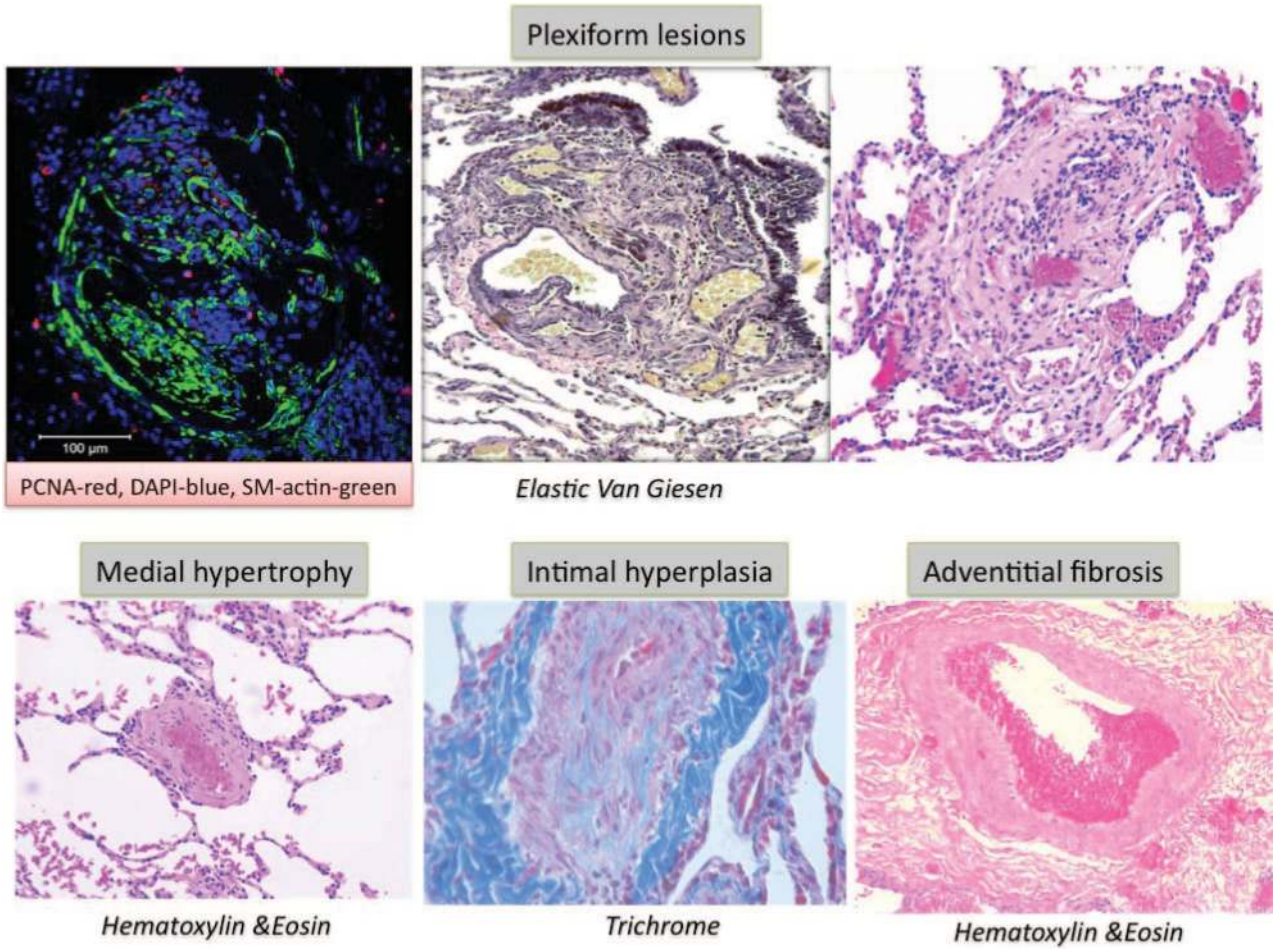

Figure 1. Histology of PAH. Top, Plexiform lesions. Upper Left, Evidence of cell proliferation (red is proliferating cell nuclear antigen [PCNA], green is smooth muscle [SM] actin, and blue is DAPI). Bottom, Medial hypertrophy, intimal fibrosis, and adventitial proliferation.

3). In the blood, levels of serotonin, a proliferative, fibrogenic vasoconstrictor, are elevated (Figure 4).[23] In the endothelium, the vasodilator/vasoconstrictor ratio is decreased (Figure 5),[24–26] whereas prothrombotic factors, including tissue factor,[27] are increased. It is hypothesized that widespread endothelial apoptosis early in PAH culminates in selection of apoptosis-resistant endothelial precursor cells that proliferate and eventually form plexiform lesions (Figure 2).[19] In the media, pulmonary artery smooth muscle cell (PASMC) apoptosis is suppressed, and proliferation is enhanced. Many factors drive PASMC proliferation, including mutation[28] or downregulation[29] of BMPR2 (Figure 6), mitochondrial metabolic abnormalities (Figure 7), de novo expression of the antiapoptotic protein survivin,[19,30] increased expression/activity of SERT,[20,31] increased expression/activity of platelet-derived growth factor (PDGF) receptor,[32] tyrosine kinase activation (Figure 8),[33] and decreased expression of Kv1.5, a voltage-gated, O_2-sensitive potassium channel. Kv1.5 downregulation occurs in human PAH,[34] rat PAH models (whether induced by chronic hypoxia[35,36] or monocrotaline[30] or in FHR[18]), and transgenic mice with PAH due to SERT overexpression[20] or BMPR2 mutation.[37] Loss of Kv1.5, the same channel that is inhibited by hypoxia to initiate hypoxic pulmonary vasoconstriction,[38] depolarizes the membrane and elevates cytosolic K^+ and Ca^{2+} (Figure 9). The resulting calcium overload, later reinforced by activation of transient receptor potential (trp) channels,[39] leads to Ca^{2+}-calcineurin–dependent activation of the proliferative transcription factor NFAT.[40] Normoxic activation of HIF-1$\propto$ occurs in FHR[18] and human PAH.[13,18] In the adventitia, metalloprotease activation causes architectural disruption, which permits cell migration and generates mitogenic peptides (tenascin; Figure 10).[41] Adventitial fibroblasts are also hyperproliferative in PH, displaying increased sensitivity to serotonin.[42] Circulating autoantibodies[4] and lung infiltration by inflammatory cells are common, particularly in PAH associated with connective tissue disease and schistosomiasis (Figure 11).[43] Finally, there are increased endothelial precursor cells and mesenchymal and bone marrow–derived stem cells,[44] although it is uncertain whether this is harmful or beneficial (Figure 12).

PAH Has a Genetic Component

The bone morphogenetic proteins (BMPs) are part of the transforming growth factor-β superfamily. Most patients ($>$80%) with familial PAH have loss-of-function mutations in BMPR2[45–47] that promote cell proliferation. BMPR2 is a constitutively active serine-threonine kinase receptor, which, in response to ligand (BMPs 2, 4, 6, 7, 9, and 10), forms

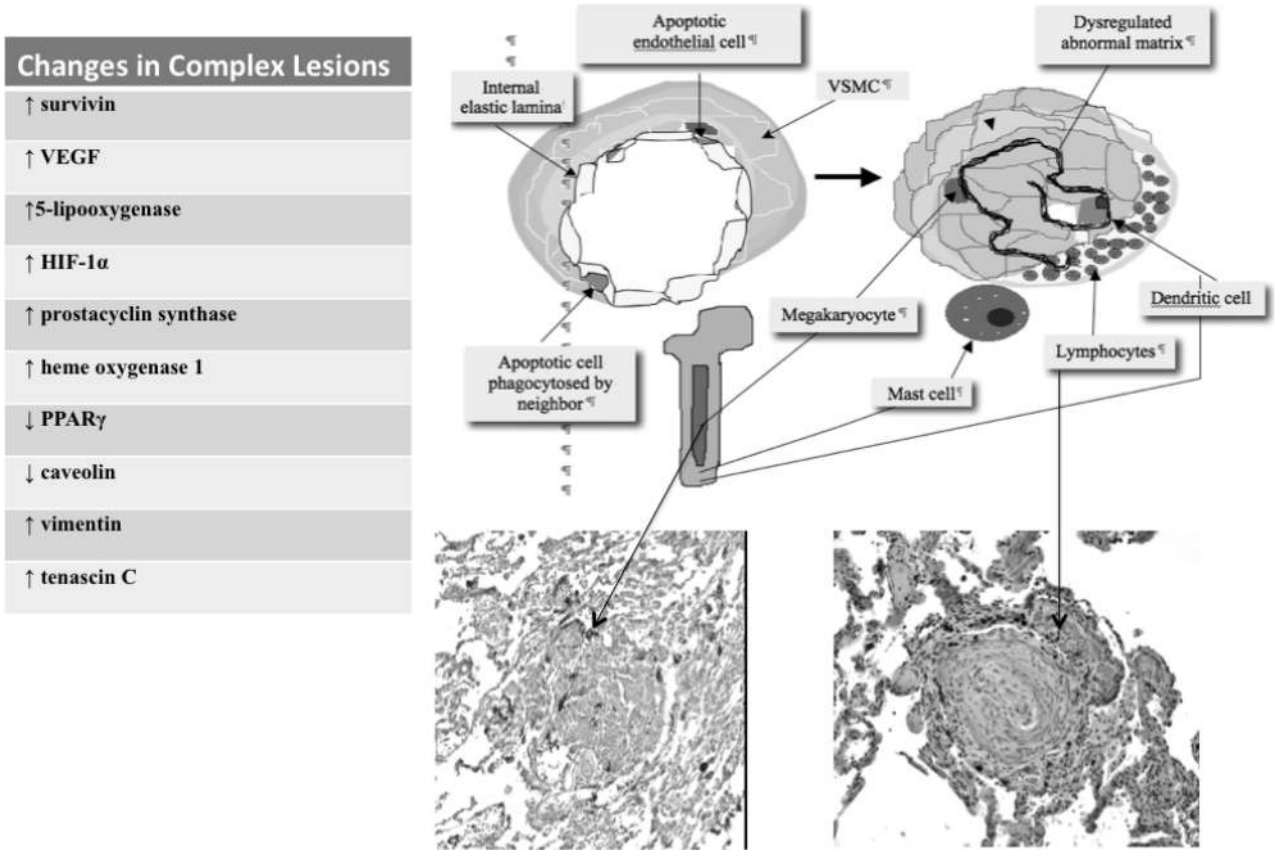

Figure 2. Formation of complex and plexiform lesions in PAH. Transformation of an arteriole into a complex vascular lesion with near-total or total lumen obliteration usually occurs at a vessel bifurcation. The concept depicted is one of initial apoptosis of cells forming the endothelial monolayer (upper panel, left). Disorganized endovascular angiogenesis results from proliferation of phenotypically abnormal cells due to (1) phagocytosis of apoptotic monolayer endothelial cells by neighboring endothelial cells, (2) activation of stem cell–like endothelial cells, or (3) attachment of bone marrow–derived "repair cells" to the injured endothelium. Bone marrow participation in the formation of these lesions is postulated because megakaryocytes, mast cells, and dendritic cells can be released and attach to the injured vessel. Perivascular lymphocytes may cluster in the lymphatics adjacent to the adventitia. The lesion also shows a dysregulated matrix. Growth factors released by megakaryocytes and mast cells may contribute to angiogenic growth, and T and B lymphocytes may reflect a local immune response. The table insert lists the phenotypic changes seen in plexiform lesions.

heterodimers with any of 4 type 1 receptors (BMPR1A, BMPR1B, Alk1, and Alk2), which results in phosphorylation of the intracellular portion of the type 1 receptor by BMPR2. Receptor activation initiates a cytosolic Smad protein–signaling cascade. Receptor-activated Smads complex with common partner SMAD (Smad4), and the complex translocates to the nucleus, where it regulates gene transcription (Figure 6). The inhibitors of DNA binding (Id) genes are major targets of BMP/Smad signaling.[48] The Smad-DNA interaction is weak and requires co-repressors or activators. BMPs can also act via an alternative BMPR2-independent pathway that involves mitogen-activated protein kinases (eg, p38MAPK, extracellular signal-regulated kinase 1 and 2).

Most heterozygous BMPR2 mutations in PAH result in defective Smad signaling, although p38MAPK signaling is retained.[49] The loss of normal BMPR2-Smad activity may exaggerate the susceptibility of vascular cells to proliferate and suppress apoptosis. BMPs 2, 4, and 7 suppress PASMC proliferation in normal individuals and patients with secondary PH but are ineffective in PAH.[28] The BMPR2-Smad pathway may display tissue heterogeneity, because it can be regulated by endogenous Smad inhibitors (eg, chordin and noggin) and by inhibitory Smads (6 and 7), and also because of variable heterodimer receptor composition.[50] This also may explain the restriction of the vascular disease to the pulmonary circulation.

Mice with conditional, endothelial BMPR2 deletions are predisposed to PAH, although PH occurs in only a subset, reminiscent of the incomplete penetrance seen in familial PAH.[51] Mice with a smooth muscle cell (SMC)–specific overexpression of a BMPR2 dominant-negative mutant develop a vasospastic form of PH that lacks vascular remodeling but is associated with downregulation of Kv1.5 expression. PH in these mice is reversed by nifedipine.[37] Perhaps disordered BMP signaling, by reducing Kv1.5 transcription, creates an early vasospastic form of PAH that in time becomes fixed by vascular remodeling.

Initial enthusiasm that BMPR2 mutations might represent a "universal" cause of PAH has been tempered. BMPR2 mutation is uncommon (prevalence 10% to 20%) in the nonfamilial category 1 PAH population. Moreover, in familial PAH, penetrance is low (ie, only ≈25% of carriers in affected families develop PAH).[52] Although modifier genes, such as SERT and transforming growth factor-β, may explain

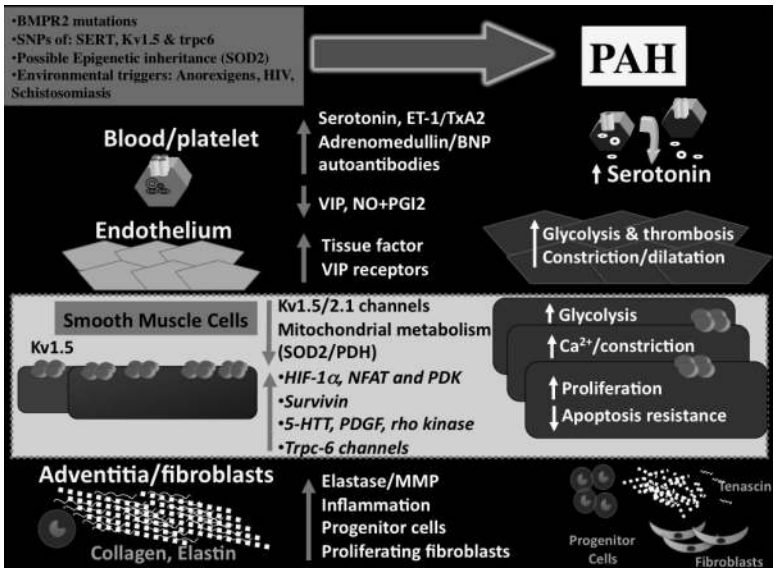

Figure 3. PAH is a panvasculopathy. Abnormalities can be seen at each level of the small pulmonary arteries, beginning in the blood and traveling outward to the adventitia. Although most of these abnormalities are likely secondary (rather than being the initiating cause of PAH), they nonetheless offer interesting therapeutic targets. Contributed by Dr Archer. SOD2 indicates superoxide dismutase 2; ET-1, endothelin-1; TxA2, thromboxane A_2; BNP, brain natriuretic peptide; PGI_2, prostacyclin; 5-HTT, 5-hydroxy-tryptamine; and MMP, matrix metalloproteinase.

the variable penetrance, aberrant BMPR2 function alone is neither a necessary nor a sufficient precondition for most cases of PAH.[53] Moreover, BMPR2 heterozygous mice do not develop PAH and are not predisposed to hypoxic PH; however, they do have an exaggerated hypertensive response to serotonin.[54]

In genetically normal animals, BMPR2 expression decreases as PH develops.[29] One posttranscriptional mechanism that accounts for downregulation of BMPR2 protein is activation of microRNAs. microRNAs regulate gene expression by inhibiting translation. A computational algorithm on the BMPR2 gene predicted that microRNAs encoded by microRNA cluster 17/92 (miR-17/92) might regulate BMPR2. Overexpression of miR-17/92 did reduce BMPR2 protein, and it appears that BMPR2 is targeted directly by miR-17-5p and miR-20a.[55]

However, results of BMPR2 rescue therapy have been mixed. Intravascular BMPR2 gene therapy, which uses an endothelium-targeted vector, reduced chronic hypoxic PH in rats[56]; however, nebulized BMPR2 adenovirus (with a promiscuous promoter) did not regress monocrotaline-induced PAH.[29] Further study is required and may be productive in identifying BMPR2-related targets for pharmacological manipulation in PAH. For example, inhibition of transforming growth factor-β signaling prevents PAH in the monocrotaline model via inhibition of activin receptor-like kinase-5.[57]

Alternative Genetic Mechanisms

Work is currently under way to search for modifier genes and for possible epigenetic mechanisms (gene methylation) of inheriting PAH or enhancing disease susceptibility. SNPs are genes that differ from normal by a single alternative nucleotide. SNPs can change the function/location of the encoded protein. SNPs occur in a significant proportion of the population and may explain susceptibility to PAH. SNP variants for PAH-relevant genes (including SERT,[58] Kv1.5,[59] and TRPC6 [trp cation channel, subfamily C, member 6][60]) may predispose to PAH. The consequences of SNPs can be complex; for example, the TRPC6 SNP not only increases TRPC6 expression but also creates a binding sequence and

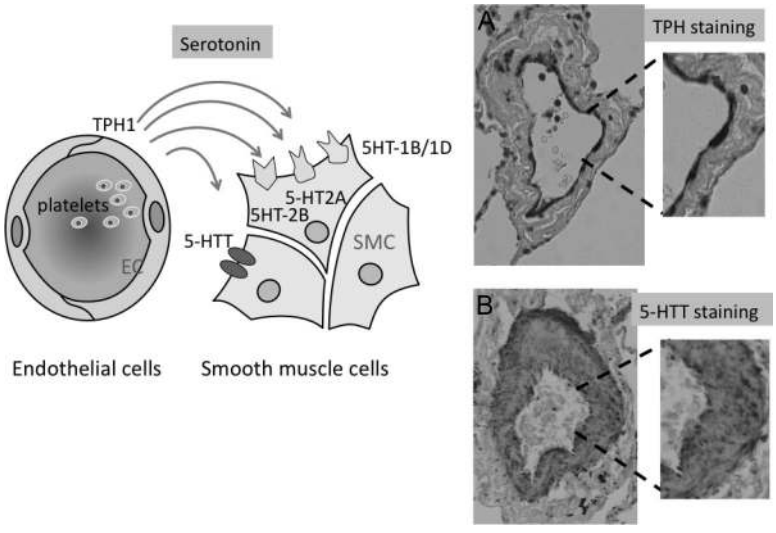

Figure 4. Serotonin (5-HT) abnormalities in PAH. Increased bioavailability of serotonin during progression of PAH results from an increased release of serotonin from platelets and from an increased synthesis of serotonin by endothelial cells that produce serotonin and express tryptophan hydroxylase-1 (TPH1), the key enzyme that controls 5-HT synthesis. Overexpression of 5-HTT (SERT) by PASMCs is responsible for the increased mitogenic effect of serotonin on these cells. 5-HT receptors, including 5-HT1B/1D and 5-HT2A receptors, mediate 5-HT–induced pulmonary artery contraction of pulmonary vessels. 5-HT2A receptors located on platelets potentiate the aggregation response to various platelet activators. 5-HT2B receptors expressed by PASMCs are also involved in the pulmonary vascular remodeling process.

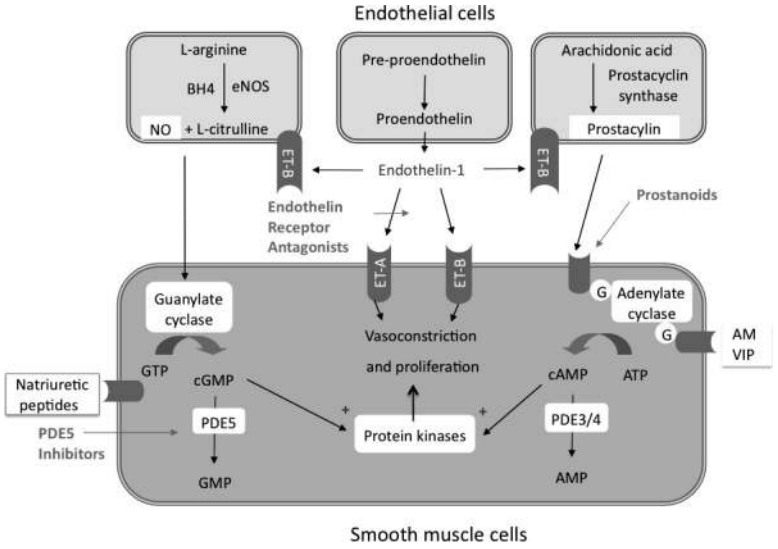

Figure 5. The endothelium and vasodilator/antiproliferative pathways: NO, generated from L-arginine, and natriuretic peptides stimulate production of cGMP. cGMP causes vasorelaxation and inhibits proliferation of vascular SMCs. PDE5 inhibitors (eg, sildenafil) enhance this vasodilatory mechanism by preventing cGMP degradation. Prostacyclin from endothelial cells also promotes relaxation and inhibits cell proliferation via a cAMP-dependent mechanism. Endothelin is a potent vasoconstrictor and stimulates proliferation via ET_A receptors on SMCs, while stimulating NO and prostacyclin release via endothelial ET_B receptors. Adrenomedullin (AM) and VIP are additional endothelium-derived, cAMP-dependent vasodilators that are dysregulated in PAH.

activates the inflammatory transcription factor nuclear factor-κB.[60]

Excess Proliferation and Impaired Apoptosis Suggest Similarities to Cancer in PAH

Otto Warburg, 1931 Nobel laureate, proposed that a shift in glucose metabolism from oxidative phosphorylation to glycolysis (despite adequate oxygen supply) was central to the cause and maintenance of cancers. Several observations indicate that PAH shares this "Warburg phenotype."[18,61] As highlighted by Voelkel et al,[62] both cancer and PAH manifest excessive cell proliferation and impaired apoptosis. Although PAH does not metastasize or disrupt tissue boundaries, emerging data show it shares a mitochondrial-metabolic abnormality with cancer (Figure 7). PDK is pathologically activated in both conditions.[18,63] This enzyme phosphorylates and inhibits pyruvate dehydrogenase (PDH).[64] PDH catalyzes the irreversible oxidation of pyruvate, yielding acetyl-coenzyme A and CO_2, and is a key enzyme in controlling the rate of oxidative metabolism. PDK activation thus impairs the Krebs cycle and creates a glycolytic shift in glucose metabolism. Subversion of the mitochondrial O_2-sensing mechanism, normally used to sense and respond to decreases in po_2,[65] appears to cause the sensor to signal hypoxia despite adequate po_2. These acquired (and reversible) mitochondrial abnormalities of fusion/fission and metabolism[61] are postulated to cause the observed normoxic activation of HIF-1∝ in PAH[18] and cancer.[63] Once active, HIF-1α turns on glycolytic genes and suppresses oxidative metabolism by increasing PDK transcription. The downstream consequences of this mitochondrial-metabolic abnormality include mitochondrial hyperpolarization, reduced production of reactive oxygen species, and decreased Kv1.5 expression.

These metabolic abnormalities, which enhance cell proliferation and impair apoptosis, can be partially corrected by a simple, mitochondria-targeted strategy. Dichloroacetate, a PDK inhibitor, restores PDH activity, increases glucose oxidation, restores mitochondrial membrane potential, and reverses normoxic HIF-1α activation.[18] Dichloroacetate, which inactivates PDK by causing conformational changes in its nucleotide- and lipoyl-binding pockets,[60] regresses experimental PAH.[7,33]

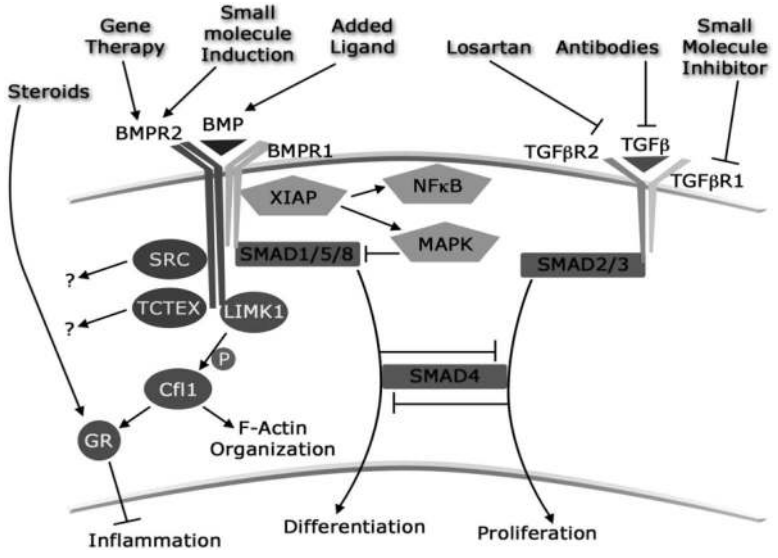

Figure 6. BMPR2 mutations, a genetic basis for familial PAH. BMPR2 mutations are found throughout the gene, and a universal functional consequence of these mutations has not been identified. Best studied is BMPR1 signaling through SMAD transcription factors. Mutations that lead to loss of SMAD signaling decrease cell differentiation, enhance vascular tone, increase transforming growth factor (TGF)-β signaling, and likely increase proliferation. Signaling through XIAP (X-linked inhibitor of apoptosis), which also requires BMPR1, can impact both the nuclear factor-κB (NFκB) and mitogen-activated protein kinase (MAPK) pathways, leading to increased MAPK phosphorylation and presumably proinflammatory signaling. BMPR2 has a long, evolutionarily conserved cytoplasmic tail domain unique in the TGF-β superfamily, that binds SRC, RACK1 (receptor for activated C-kinase 1), and LIMK1 (LIM domain kinase 1). BMPR2 mutation in vivo leads to decreased cofilin (Cfl1) phosphorylation by LIMK1, with the effect both of alterations in F-actin organization and defects in glucocorticoid receptor (GR) nuclear translocation.

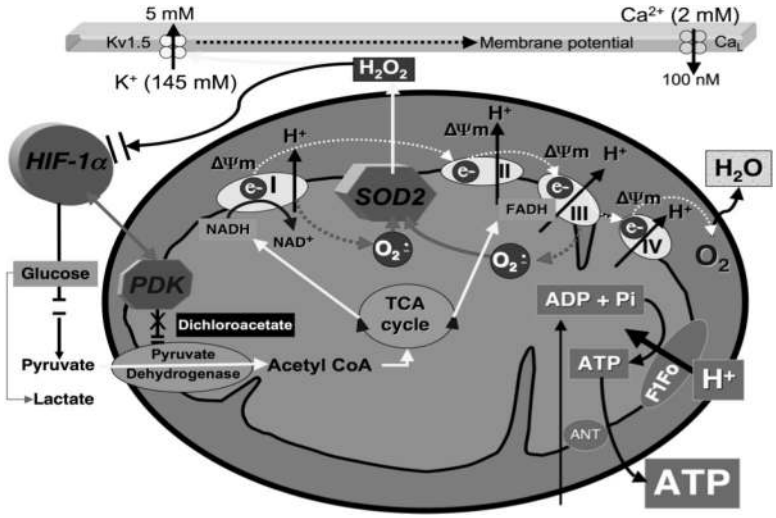

Figure 7. Mitochondrial metabolism in PAH. In aerobic metabolism, PDK is inactive, PDH is active, and electron donors (mitochondrial NADH and FADH) produced by the tricarboxylic acid cycle (TCA or Krebs cycle) pass electrons down a redox-potential gradient in the electron transport chain to molecular O_2. This electron flux powers H^+ ion extrusion, which creates the proton-motive force responsible for creating the negative membrane potential ($\Delta\Psi m$) of mitochondria and powering F1Fo ATP synthase. Side reactions between semiquinones and molecular O_2, which account for ≈3% of net electron flux, create superoxide anion in proportion to pO_2. Superoxide dismutase (SOD2) rapidly converts superoxide anion (produced at complexes I and III) to H_2O_2, which serves as a redox messenger signaling "normoxia." In hypoxia (and PAH and cancer), there is activation of HIF-1α and PDK, which inhibits PDH, shifting metabolism toward glycolysis.Acetyl CoA indicates acetyl-coenzyme A.

Dichloroacetate inhibits proliferation, enhances apoptosis, and can regress both human cancer, in a xenotransplantation model,[63] and experimental PAH (chronic hypoxic PH, monocrotaline PAH, and FHR PAH).[18,36,66] Dichloroacetate also regresses spontaneous PH in transgenic mice that overexpress SERT in PASMCs.[67] Dichloroacetate has been used safely in long-term treatment of patients with inherited lactic acidosis due to mitochondrial diseases, which suggests the potential for translation to the clinic.

The RV in PAH

The fetal/neonatal RV ejects blood at relatively high pressure into the pulmonary circulation. With maturation, the pulmonary circulation develops into a low-pressure circuit, and the RV involutes, becoming thin-walled. Chronic pressure overload, as occurs in PAH, stimulates RV hypertrophy. Surprisingly little is known about the specific mechanisms underlying RV hypertrophy (RVH) and RV dysfunction in the setting of PAH. Although the obvious approach to reducing RVH and RV failure is to treat the underlying pulmonary arterial disease, recent experimental evidence suggests that the RV can be targeted therapeutically in PAH.[36] In RVH, PDE5, which was expressed in the fetal RV, is selectively reexpressed. Inhibition of this enzyme (ie, by sildenafil) enhances RV contractility without affecting the left ventricle,[68] which lacks PDE5.

In contrast to the normal RV, which can vary its substrate utilization from fatty acids to glucose as needed, metabolism in RVH is reliant on glucose metabolism.[69] In hypoxia-induced PH, expression of the glucose transporter GLUT4 is significantly increased in the RV, which suggests a metabolic switch to glycolysis. AMP-activated protein kinase, which has a key role in the control and regulation of energy metabolism, stimulates fatty acid metabolism and glycolysis, preserving ATP production.[70,71] AMP-activated protein kinase activation in ventricular hypertrophy[72–74] preserves ATP levels by increasing glucose transport and accelerating glycolysis and by inhibiting acetyl-coenzyme A carboxylase.[71] In RVH, there is a systolic flow impediment in the right coronary artery that is proportional to RV pressure and mass.[75] New evidence shows the RV in PAH is glycolytic, in part owing to activation of PDK, and it behaves as hibernating myocardium, demonstrating enhanced glucose oxidation and improved contractility in response to dichloroacetate.[76] Future PAH therapies should consider the effects of agents on both the RV and the pulmonary vasculature.

Therapeutic Pathways in PAH

Prostanoids and Prostanoid Receptors

One of the most successful therapeutic strategies for PAH has been to augment endogenous prostacyclin production with exogenous prostanoids (Table; Figure 5). Fatty acid cyclooxygenase converts arachidonic acid to prostaglandin H_2, a substrate for both prostaglandin I_2 (prostacyclin) synthase and thromboxane synthase. Prostaglandin I_2 synthase is expressed in pulmonary vascular endothelium and generates prostacyclin, which relaxes PASMCs and inhibits platelet aggregation through interaction with prostacyclin receptors and stimulation of cAMP. Thromboxane synthase, in platelets and endothelium, produces thromboxane A_2. Thromboxane A_2 stimulates vasoconstriction and platelet aggregation through thromboxane/prostaglandin receptors. Endothelial dysfunction and platelet activation in PAH reduce prostacyclin levels and increase thromboxane A_2 production.

Continuous intravascular infusion of epoprostenol (Flolan) decreases PVR, increases cardiac output, and improves life expectancy.[12] Its poor stability, expense, and requirement for continuous intravenous infusion have fostered development of more stable analogs and alternative routes of administration: Iloprost (inhalation), treprostinil (subcutaneous), and beraprost (oral). New prostacyclin agonists and thromboxane antagonists are in clinical trials. The combination of a prostanoid (such as iloprost) and a PDE5 inhibitor enhances pulmonary hemodynamic effects and improves exercise capacity in PAH.[103,104]

Nitric Oxide and cGMP

NO is a radical, synthesized from L-arginine by 3 NO synthases (NOS). Endothelial NOS (eNOS) is the principal mediator of endothelium-dependent vasodilation in the pulmonary circulation. Endothelium-derived NO diffuses into

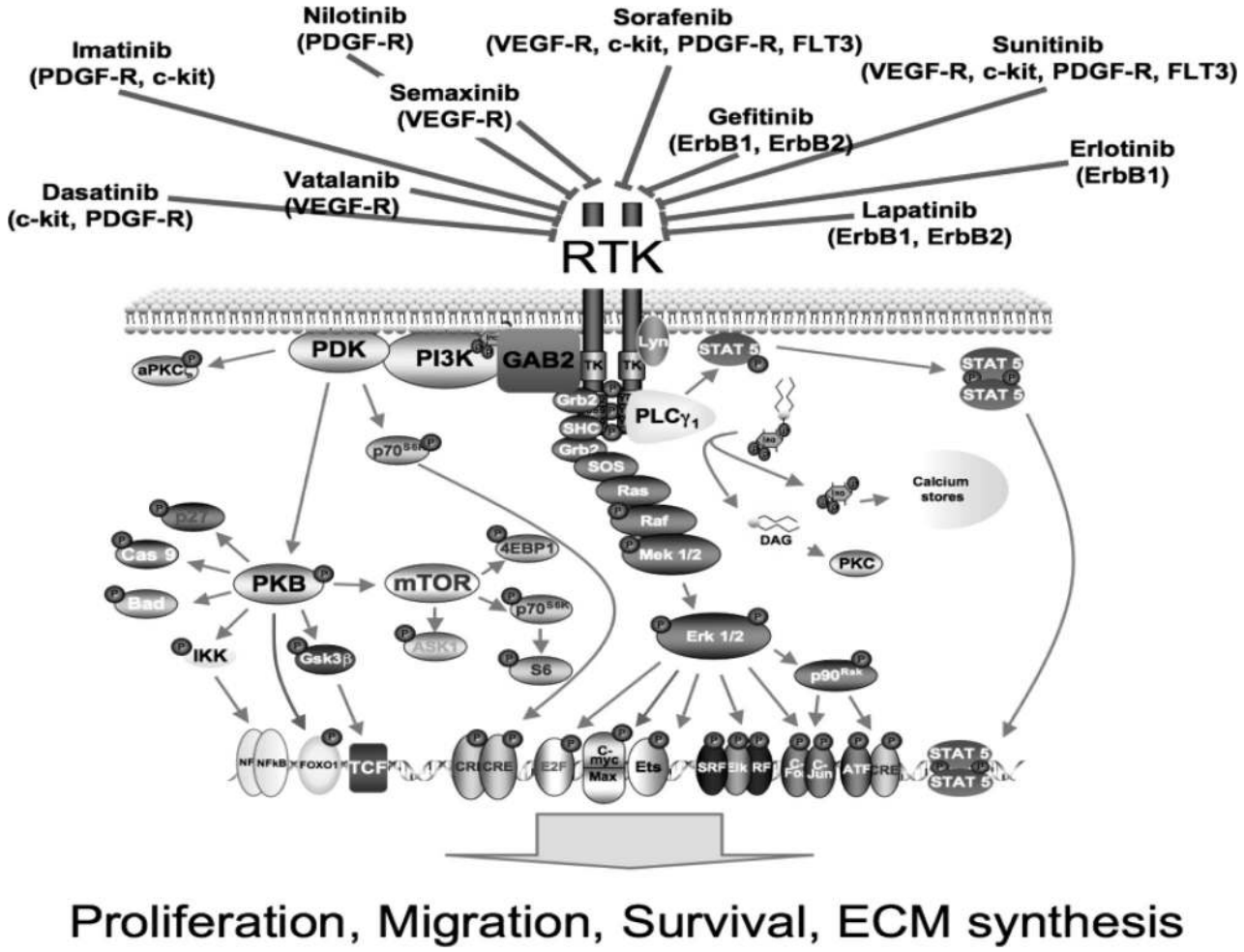

Figure 8. Receptor tyrosine kinases (RTK) and their inhibitors. This complex kinase cascade offers many therapeutic targets to treat PAH. ATF indicates activating transcription factor; BAD, BCL-XL/BCL-2–associated death promoter; c-kit, CD117; DAG, diacylglycerol; 4EBP1, 4E-binding protein 1; ECM, extracellular matrix; EGF-R, epidermal growth factor receptor; ErbB1 and ErbB2, epidermal growth factor receptors; ERK, extracellular signal-regulated kinase; flt3, fms-like tyrosine kinase receptor-3; GAB2, GRB2-associated binding protein; GSK, glycogen synthase kinase; IKK, IκB kinase; JAK, Janus kinase; JNK, Jun N-terminal kinase; MEF, myocyte-specific enhancer-binding nuclear factor; MEK, mitogen-activated protein kinase/ERK kinase; MERM, ezrin/radixin/moesin family of cytoskeletal linkers; mTOR, mammalian target of rapamycin; NFκB, nuclear factor-κB; NHERF, sodium-hydrogen exchange regulatory factor; P, phosphotyrosine; p70S6K, p70 ribosomal S6 kinase; PDGF-R, PDGF receptor: PDK, phosphoinositide-dependent kinase; PI3K, phosphoinositide-3 kinase; PIP2, phosphatidylinositol-4,5-bisphosphate; PIP3, phosphatidylinositol-3,4,5-trisphosphate; PKB, protein kinase B; PKC, protein kinase C; PLC, phospholipase C; SOS, Son of Sevenless; STAT, signal transducer and activator of transcription; SHP, Src homology 2-containing protein tyrosine phosphatase; TK, tyrosine kinase; and VEGF-R, VEGF receptor.

PASMCs, where it stimulates soluble guanylate cyclase (sGC) to produce cGMP (Table; Figure 5). The cardiovascular effects of cGMP are mediated by interaction with at least 3 groups of proteins: cGMP-dependent protein kinases, cGMP-regulated PDE, and cyclic nucleotide–gated ion channels. PDE5, the molecular target of sildenafil, decreases intracellular cGMP levels and opposes cGMP-dependent protein kinase–dependent signaling elicited by NO and natriuretic peptides.

The NO pathway is impaired in several ways in PAH. NOS expression[105] and NO bioavailability[106] are depressed. Moreover, PDE5 is induced both in PASMCs[107] and the RV,[68] which hastens inactivation of cGMP. Finally, production of endogenous NOS inhibitors, asymmetrical and symmetrical dimethylarginines (ADMA and SDMA), is enhanced in PH.[108,109]

Pharmacological or genetic perturbations of the NO pathway demonstrate the pivotal role of the cGMP pathway in regulating PVR. Mice develop PH if they are rendered deficient in eNOS, GTP cyclohydrase-1 (GTP-CH1, the rate-limiting enzyme in synthesis of the NOS cofactor tetrahydrobiopterin [BH_4]), or dimethylarginine dimethylaminohydrolase (DDAH, the enzyme responsible for eliminating endogenous NOS inhibitors).[110–112] Inhibition of NO production in humans by use of a competitive NOS antagonist (N^G-monomethyl-L-arginine) increases PVR.[113,114] Sustained pharmacological NOS inhibition causes PH in rats, although there is disproportionate systemic hypertension.[115]

NONOates

Inhalation of exogenous NO gas (0.1 to 100 parts per million) decreases PAP and improves oxygenation and hemodynamics in children and adults with diverse forms of PH.[116] Although

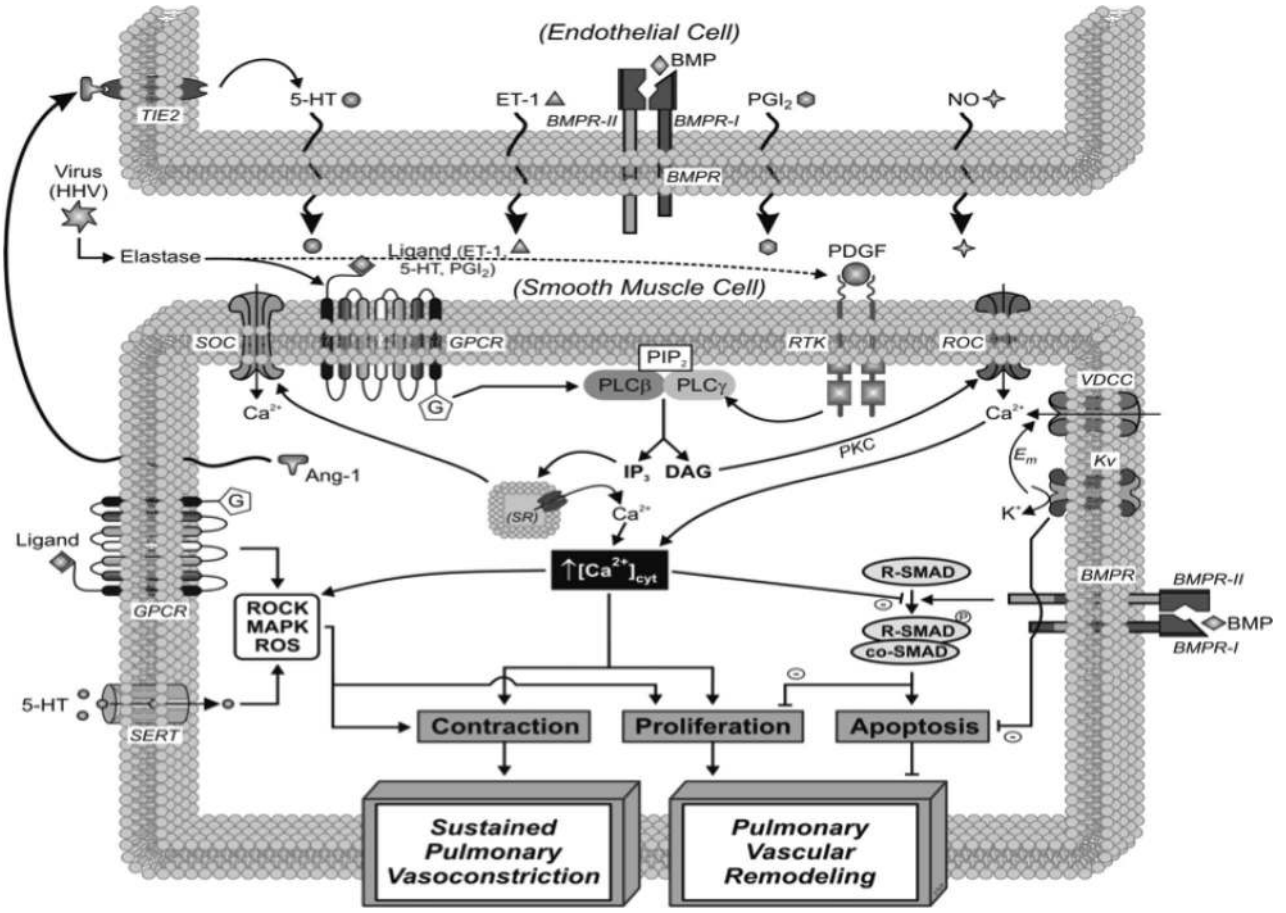

Figure 9. Ion channels in PAH. Schematic depiction of the cellular mechanisms linked with vasoconstriction and remodeling in pulmonary endothelial cells (PAECs) and PASMCs in PAH. Central themes of interest for the development of PAH include the following: (1) Impaired ion channel expression and function in PASMCs (Kv, VDCC, SOC, ROC); (2) increased cytosolic calcium ($[Ca^{2+}]$cyt) in PASMCs (mediated by ion channel function and receptor stimulation); (3) altered signaling via membrane receptors (GPCR, TIE-2, BMPR, RTK) and transporters (ie, SERT) in endothelial cells and PASMCs; (4) changes in redox status; (5) enhanced production of vasoconstrictor or mitogenic factors; and (6) viral signaling via GPCR and RTK. Paracrine interactions between PAECs and PASMCs are noteworthy. Ang-1 indicates angiopoietin-1; DAG, diacylglycerol; ET-1, endothelin-1; GPCR, G protein–coupled receptor; HHV, human herpes virus; 5-HT, serotonin; MAPK, mitogen-activated protein kinase; NO, nitric oxide; PGI2, prostaglandin I2; PIP_2, phosphatidylinositol-4,5-bisphosphate; PKC, protein kinase C; PLC, phospholipase C; ROC, receptor-operated Ca^{2+} channels; ROCK, Rho-associated kinase; ROS, reactive oxygen species; RTK, receptor tyrosine kinase; SERT, 5-HT transporter; SOC, store-operated Ca^{2+} channels; SR, sarcoplasmic reticulum; and VDCC, voltage-dependent Ca^{2+} channels.

long-term therapy with inhaled NO for PAH is feasible,[84] delivery is complicated by the instability of NO, which mandates continuous inhalation. In addition, higher concentrations of NO and especially its oxidation products are toxic. Consequently, inhaled NO dosing must be monitored carefully to prevent exposure to toxic nitrogen oxides and methemoglobin. Alternative strategies that exploit the specificity of inhaled NO but utilize more stable NO sources are appealing. One such strategy uses NO/nucleophile adducts, such as diethylenetriamine/NO. NONOates spontaneously release predictable amounts of NO when exposed to physiological pH. Daily nebulization of diethylenetriamine/NO (half-time of NO release >20 hours) for ≈1 week reduces PH in monocrotaline-induced PAH without causing systemic hypotension.[117] Diethylenetriamine/NO has been used effectively to improve pulmonary hemodynamics in intubated patients with adult respiratory distress syndrome.[118] It may be valuable to investigate the many NONOates for long-term ambulatory use in PAH.

PDE Inhibitors

Eleven PDE families are known; however, they vary in substrate affinity, selectivity, and regulatory mechanisms.[119] In the pulmonary circulation, PDE5 and PDE1 are highly relevant (Figure 5). PDE1 has 3 isoforms that are regulated by calcium-calmodulin and can hydrolyze both cAMP and cGMP. Both PDE1A and PDE1C are upregulated in pulmonary arteries from patients with iPAH.[120] Infusion of the PDE1 inhibitor 8-methoxymethyl-isobutyl-1-methylxanthine reduces PVR and RVH in rodent PH models.[120]

PDE5 expression, normally absent in cardiac myocytes, is upregulated in the RV in PAH.[68] PDE5 inhibition in PAH models increases RV contractility through a cGMP-mediated inhibition of PDE3.[68] Thus, in PAH, sildenafil has an effect on the RV similar to the PDE3 inhibitor milrinone. A single dose of sildenafil (75 mg) reduces PVR without lowering systemic vascular resistance in PAH patients and simultaneously lowers wedge pressure and increases cardiac output.[121] Sildenafil causes sustained improvement in hemody-

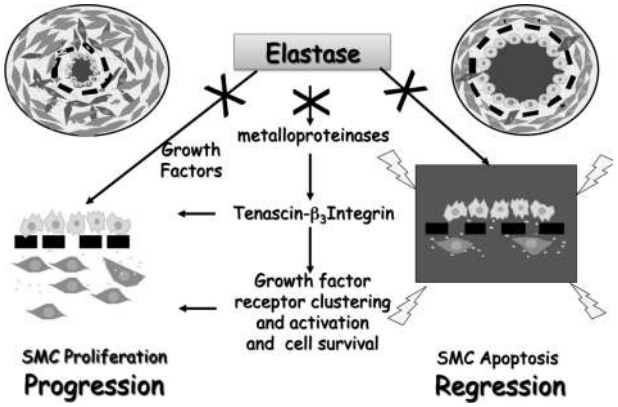

Figure 10. Disordered elastin metabolism and deposition in PAH. Elastase degrades elastin and other components of the extracellular matrix, thereby releasing bound growth factors that are both mitogenic and motogenic for PASMCs. Heightened elastase activity also activates matrix metalloproteinases, which upregulate the glycoprotein tenascin-C. When tenascin-C binds cell-surface integrins, such as α-v β3 on PASMCs, these integrins cluster, and cell shape changes in a way that clusters and activates growth factor receptors and increases cell-survival signals. Thus, pathway activation causes both release of growth factors and activation of their receptors. Transmission of cell-survival signals occurs even in the absence of ligand (growth factor) binding. Blocking elastase activity or growth factor receptors can therefore arrest progression of PASMCs by blocking proliferation and induce regression by enhancing apoptosis.

namics and functional capacity in PAH, and it has been approved as a first-line oral treatment for PAH (reviewed in Archer and Michelakis[122]). The combination of a PDE inhibitor (PDE3/4 or PDE5), even at subtherapeutic doses, with a prostanoid augments the hemodynamic/functional benefit of the prostanoid.[104,123,124]

sGC Activators

sGC is a heterodimer that consists of α- and β-subunits. sGC expression is upregulated as a compensatory mechanism in human PAH.[125] Experimental hypoxic PH is exacerbated in mice that lack sGCα1.[126] Thus, augmentation of sGC activation is an attractive therapeutic strategy. There are NO-independent, heme-dependent sGC stimulators (eg, BAY 41-2272) and NO- and heme-independent sGC activators (eg, BAY 58-2667 and HMR-1766).[127] BAY 41-2272 stimulates sGC directly but also sensitizes the enzyme to NO, which results in synergism. It improves pulmonary hemodynamics in models of persistent PH of the newborn.[128,129] NO-independent sGC activators, such as BAY 58-2667, HMR-1766, and S-3448, provide an additive rather than synergistic effect when combined with NO donors. Both BAY 41-2272 and BAY 58-2667 reverse established PH in rodent models, although this benefit is partially dependent on endogenous NOS activity.[130] BAY 63-2521 (Riociguat), an sGC stimulator, is available orally, has a favorable safety profile, and has entered phase III trials in PAH.[91]

Enhancing NOS Activity: BH_4 and Transcription Enhancers

BH_4 is an important NOS cofactor, essential for dimerization and for oxygenation of L-arginine to create NO and L-citrulline. Without BH_4, NOS becomes uncoupled and produces superoxide anion, which rapidly reacts with NO, producing peroxynitrite, further attenuating NO bioavailability.[131]

The rate-determining step for the de novo production of BH_4 is catalyzed by GTP-CH1. Mice with impaired GTP-CH1 activity exhibit reduced lung BH_4 and spontaneously develop PH with vascular remodeling.[111] Conversely, congenital overexpression of GTP-CH1 in vascular endothelium protects mice from hypoxic PH.[111] In a porcine model of persistent PH of the newborn, combined therapy with BH_4 and a superoxide dismutase mimetic (which enhances survival of endogenous NO) restores endothelial function.[132]

Although there is no evidence for GTP-CH1 deficiency in PAH, GTP-CH1 polymorphisms are associated with variations in NO bioavailability and systemic hypertension. Some PAH patients show increased markers of oxidative stress,[133] and this may result in conversion of BH_4 to dihydrobiopterin. BH_4 is a cofactor for several enzymes and is well tolerated when administered in its synthetic form, sapropterin, to patients with phenylketonuria.[134] This observation paves the way for studies in PAH patients.

eNOS transcription enhancers, such as AVE9488 and AVE3085, similarly aim to increase NO signaling. Theoretically, an increase in eNOS without corresponding increases in cofactors such as BH_4 could lead to uncoupling and the formation of superoxide ions. However, AVE9488 treatment also increases BH_4 levels and improves eNOS coupling in apolipoprotein E–knockout mice.[135] To the best of our knowledge, this agent has not been used in vivo in humans.

Vasoactive Peptides and Endopeptidase Inhibitors

Endothelin Receptor Antagonists

Endothelin-1 is a vasoconstrictor that acts via 2 receptors, ET_A and ET_B, to regulate vascular tone and cell proliferation (Figure 5). Both receptor subtypes are found on PASMCs and mediate vasoconstriction, whereas the ET_B receptor on endothelial cells mediates NO and prostacyclin release, causing vasodilation. Lung and circulating endothelin-1 levels are increased in PAH patients.[136] Endothelin receptor antagonists such as bosentan, ambrisentan, and sitaxsentan cause a significant but modest improvement in pulmonary hemodynamics, exercise capacity (6-minute walk distance), and symptoms and are approved for management of PAH. There are no trial data to indicate whether selective ET_A antagonism offers advantages over combined ET_A and ET_B antagonism (bosentan), nor is the relative efficacy compared with PDE5 inhibitors known (although a small trial suggests some benefits of sildenafil).[137] Liver toxicity and teratogenicity are class effects. Although comparisons with historical control data suggest that bosentan monotherapy increases survival,[138] there are no robust survival data from appropriately designed clinical trials.

The endothelins are produced from big endothelin by endothelin-converting enzyme. Endothelin-converting enzyme inhibitors are an alternative approach to reducing endothelin levels. Although studies with this drug class (eg, daglutril) have been conducted in patients with systemic hypertension and heart failure, data for PH are limited.

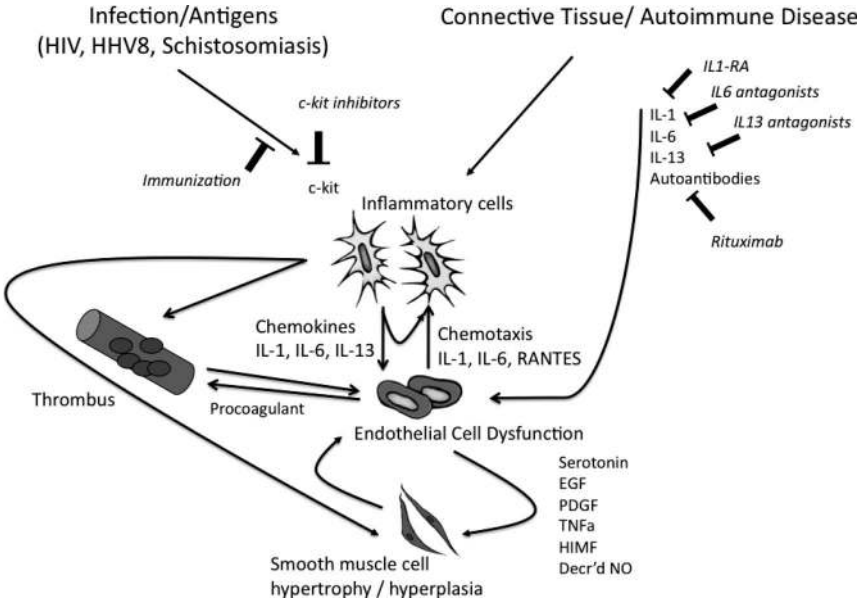

Figure 11. The role of inflammation in the pathogenesis of PAH. Initial inflammatory stimuli can occur in the form of infectious or foreign antigens or autoimmune disease, leading to an appropriate but potentially excessive immune response. The host immune response to these varied stimuli results in the release of proinflammatory cytokines (which can recruit bone marrow–derived cells), stimulation of resident inflammatory cells, and endothelial cell dysfunction. Endothelial cell injury and the cellular response can increase endovascular thrombosis. A network of cytokines released by the inflammatory and endothelial cells can also cause aberrant PASMC proliferation. The triad of endothelial cell proliferation, PASMC proliferation, and thrombus formation contributes to PAH. Proinflammatory cytokines and cell-cell interactions can potentially be targeted therapeutically. Decr'd NO indicates decreased NO; EGF, epidermal growth factor; HHV8, human herpes virus 8; HIMF, hypoxia-induced mitogenic factor, also called RELMa and FIZZ1; IL, interleukin; RANTES, regulated on activation, normal T cell expressed and secreted; and TNFa, tumor necrosis factor-α.

Natriuretic Peptides

The natriuretic peptides (atrial natriuretic peptide and brain natriuretic peptide) are synthesized in and released from myocardial tissue in response to stretch, and their elevation in the blood in PAH indicates the extent of RV dysfunction (Figure 5).[139] C-type natriuretic peptide is produced in vascular tissue. These peptides interact with the extracellular domain of the natriuretic peptide receptors NPR-A and NPR-B, which are transmembrane guanylate cyclases. On binding, the intracellular domain hydrolyzes GTP to cGMP.[140] Genetic inactivation of NPR-A is associated with PH, whereas sustained administration of atrial natriuretic peptide attenuates PAH in animal models.[141] Short-lived natriuretic peptides are not feasible agents for long-term therapy. An alternative approach is to inhibit metabolism of endogenous natriuretic peptides with neutral endopeptidase inhibitors. Neutral endopeptidase inhibitors have demonstrated efficacy in animal models both as monotherapy and in combination with PDE5 inhibition,[142] but this combination is untested in patients.

Adrenomedullin

This vasodilator peptide activates several signaling pathways, such as cAMP, NO-cGMP, and PI3K (phosphatidylinositol 3-kinase)/Akt. It decreases mean PAP and RVH in hypoxic rats and exhibits antiproliferative properties.[143] Adrenomedullin-2, a novel peptide, acts by the same receptors as adrenomedullin, and its levels are also elevated in the RV of rats with hypoxic PH. When aerosolized, adrenomedullin-2 reduces monocrotaline-induced PAH in rats and improves survival.[144] In humans with PAH, inhaled adrenomedullin causes a modest reduction in PVR and increases peak O_2 consumption during exercise without exerting significant effects on the systemic vasculature.[98]

Vasoactive Intestinal Polypeptide

Vasoactive intestinal polypeptide (VIP) is a 28–amino acid peptide that increases cardiac output, scavenges oxygen free radical species, inhibits platelet activation, and is a potent vasodilator. Its effects are mediated by the G protein–coupled receptors VPAC1 and VPAC2. Receptor activation stimulates both adenylate- and guanylate cyclase–signaling pathways. VIP-knockout mice spontaneously develop PH.[145] They overexpress proinflammatory genes and genes involved in pulmonary vascular remodeling and underexpress antiproliferative genes,[146] including eNOS/NOS3, prostacyclin synthase, GTP-CH1, and BMP-2. Thus, VIP is also a key regulator of multiple genes that control the process of vascular remodeling.

VIP receptor expression (particularly of the VPAC2 subtype) and receptor-binding affinity are increased in PASMCs from PAH patients; conversely, serum and lung VIP levels are low in PAH. VIP inhibits the proliferation of PASMCs from PAH patients.[97] Nebulized VIP (200 μg daily) improves pulmonary hemodynamics in PAH patients and, when continued for 3 months, reduces PVR and improves 6-minute walk distance, with little effect on the systemic circulation. The medical use of peptides in general and VIP specifically is complicated by their rapid degradation by endogenous proteases. A sustained-release liposomal VIP preparation has extended pharmacological effects and may facilitate the development of VIP as a PAH treatment.

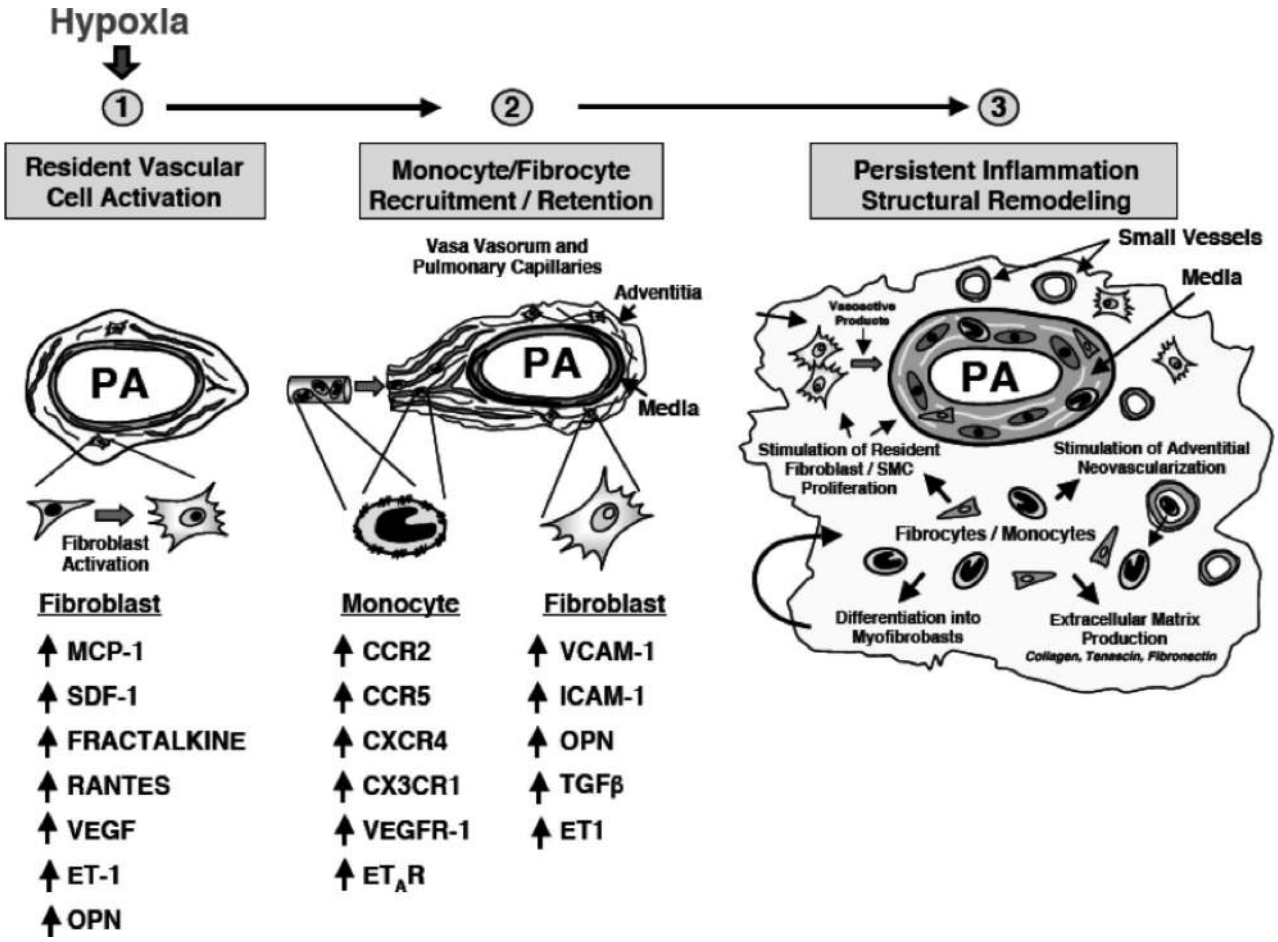

Figure 12. Cellular basis for pulmonary vascular remodeling: Lessons from hypoxia. Fibroblasts, monocytes, and fibrocytes play critical roles in orchestrating hypoxia-induced pulmonary vascular remodeling. Hypoxia or hypoxia-associated stimuli increase production by resident fibroblasts (and probably PASMCs) of chemokines/cytokines, including monocyte chemoattractant protein (MCP)-1, stromal cell–derived factor (SDF)-1, fractalkine (CX3CL1), RANTES (regulated on activation, normal T cell expressed and secreted), VEGF, osteopontin (OPN), and endothelin (ET-1). These and other factors stimulate recruitment of monocytes and monocyte-derived mesenchymal precursors (fibrocytes) to the vessel wall. Upregulation of monocyte receptors for these ligands (CCR2, CXCR4, CX3CR1, VEGFR-1, and ETR-A) occurs. Monocytes are retained in the vessel wall by the upregulation of adhesion molecules on fibroblasts, including vascular cell adhesion molecule (VCAM), intracellular adhesion molecule (ICAM), and OPN. As monocytes and fibrocytes accumulate in the vessel wall, they exert potent effects on the proliferative, migratory, matrix-producing, and contractile capabilities of resident fibroblasts and PASMCs through the secretion of transforming growth factor (TGF)-β, PDGF-A and -B, epidermal growth factor, interleukin-6, insulin-like growth factor-1, matrix metalloproteinase-9, and others. In addition, these cells produce potent proangiogenic molecules such as VEGF, S100A4, and fibroblast growth factor-β that likely play roles in stimulating further angiogenesis in the vessel wall. PA indicates pulmonary artery.

BMPR2-Targeted Treatment Strategies

Loss of BMPR2 function after germ-line mutation has been linked strongly to the development and progression of familial and sporadic forms of iPAH. This has directed attention to strategies targeted at repairing BMPR2 signaling in patients with proven mutations. Gene mutations can directly inactivate BMPR2 (rescue strategies using viral vectors discussed above) or can suppress function by impairing its trafficking to the cell surface. Substitution of cysteine residues in the ligand-binding domain prevents BMPR2 trafficking to the cell membrane, and this can be rescued (in a cell model).[147] In cystic fibrosis, in which impaired protein trafficking also occurs, sodium 4-phenylbutyrate can improve membrane trafficking of the chloride channel.[148] Mutant BMPR2 protein that is trapped intracellularly can be rescued by use of chemical chaperones (thapsigargin, glycerol, or sodium 4-phenylbutyrate), which increases membrane expression.[147] It remains uncertain how much mutant BMPR2 must reach the cell membrane to induce a clinically relevant effect.

An alternative to restoring BMPR2 function is to inhibit proproliferative pathways that are unchecked by BMPR2 dysfunction. PASMCs in familial PAH demonstrate increased sensitivity to transforming growth factor-β/activin receptor–like kinase 5 signaling, which suggests transforming growth factor-β blockade as a therapeutic strategy. The activin receptor–like kinase 5 inhibitor, SB525334, reverses PAH and RVH in a rodent model, which indicates that strategies that inhibit activin receptor–like kinase 5 signaling may have therapeutic benefit.[149]

Inhibitors of Serotonin and SERT

Plasma serotonin is increased in iPAH patients, even after lung transplantation,[23] which suggests that serotonin is either a causative factor in iPAH or is associated with such a factor.

Table. Therapeutic Pathways in PAH

Target	Goal	Drug	Used in Humans	Reference
Current therapies				
L-type Ca^{2+} channels	Decrease SMC Ca^{2+}	L-type Ca^{2+} channel blockers	Yes	77
Coagulation cascade	Decrease thrombosis	Warfarin	Yes	78, 79
Prostacyclin receptors	Increase cAMP	Epoprostenol	Yes	80
Endothelin receptors A and B	Inhibit constriction and proliferation	Bosentan	Yes	81
Endothelin receptor A	Inhibit constriction and proliferation	Sitaxsentan	Yes	82
PDE5 inhibitors	Increase cGMP	Sildenafil	Yes	83
Guanylate cyclase activators	Increase cGMP	iNO	Yes	84
Novel targets				
Rho kinase	Decrease Ca^{2+} sensitivity in SMCs	Rho kinase inhibitors: fasudil	Yes, acute trial	85, 86
Rho A prenylation	Decrease Ca^{2+} sensitivity in SMCs	Statins	Yes	15
Serine elastases	Decrease MMP activation	Elastase inhibitors	No	41
Kinase-associated receptors	Inhibit PDGF or EGF activity	Tyrosine kinase inhibitors	Imatinib, sorafenib	87, 88
PDH kinase	Normalize mitochondrial function	Dichloroacetate	Yes*	36
NFAT	Decrease antiapoptotic bcl-2; slow proliferation	Cyclosporine A	Yes*	40
Immune system	Immunosuppression	Mycophenolate mofetil	Yes*	89
Survivin	Inhibits antiapoptotic effect of survivin	Transfection of dominant negative	Yes*	30
Guanylate cyclase	Increase cGMP	· Direct sGC activators, eg, Riociguat (BAY 63-2521) · DHEA	Phase III trial	90, 91
Cyclooxygenase	Inhibit thromboxane A_2	Aspirin	Yes*	27
Ornithine decarboxylase	Inhibit polyamine synthesis	α-Difluoromethylornithine	No	92
Cyclin-dependent–kinase inhibitor γ27	Inhibit SMC proliferation	Heparin	Yes*	93
PPAR-Y	Increase PPARγ activity	Rosiglitazone	Yes*	94
Angiopoietin and TIE2	Inhibit SMC proliferation	Adenoviral gene transfection	No, and conflicting results	95
Serotonin transporter	Inhibit SMC proliferation	SSRI	Yes†	96
VPAC 1 and 2 receptors	Inhibit SMC proliferation	Vasoactive intestinal peptide, inhaled	Yes	97
Adrenomedullin receptors	Inhibit SMC proliferation, vasodilation	Adrenomedullin	Yes‡	98, 99
BMPR2	Enhance BMPR2 signaling	Adenoviral transfection and/or enhancing receptor trafficking to membrane	No conflicting results	29, 56
eNOS	Increase cGMP and NO signaling	eNOS-transfected EPCs	Phase I trial in progress	100–102
eNOS	Increase cGMP and NO signaling	VEGF-transfected fibroblasts	No	102

iNO indicates inhaled NO; MMPs, matrix metalloproteinases; EGF, epidermal growth factor; DHEA, dehydroepiandrosterone; and SSRI, selective serotonin reuptake inhibitors.

Human-use qualifiers: *Used in humans but in a disease other than PAH; †used in humans with PAH but retrospective data; and ‡used in humans with PAH but only in an short-term hemodynamic study.

In addition, PAH endothelial cells do generate more serotonin than controls. SERT expression is increased in PASMCs from iPAH patients, and these cells proliferate more rapidly in response to serotonin than control cells.[150] In some patients with severe iPAH, the LL SERT polymorphism is associated with greater SERT expression and higher mean PAP than the LS or SS genotypes[150]; however, in 2 separate iPAH cohorts, this relationship was not detected.[151,152] The proliferation of bovine and iPAH PASMCs in response to serotonin depends on serotonin internalization via SERT and is blocked by selective serotonin reuptake inhibitors such as fluoxetine.[31] Fluoxetine reduces hypoxic PH in rats.[153] In a retrospective cohort study of PAH patients, the use of selective serotonin reuptake inhibitors was associated with a trend toward a reduced risk of death.[96] The time is right for a randomized clinical trial of fluoxetine versus placebo on a background of conventional PAH therapy.

Another target is tryptophan hydroxylase, the enzyme that synthesizes serotonin. Deletion of tryptophan hydroxylase 1 reduces pulmonary vascular remodeling and hypoxic PH.[154] The 5-hydroxytryptamine 2A ($5\text{-}HT_{2A}$) receptor mediates serotonin-induced proliferation in rat pulmonary artery fibroblasts.[42] Genetic deficiency of the $5\text{-}HT_{2B}$ serotonin receptor reduces hypoxic PH in mice.[155] Terguride, a potent antagonist of $5\text{-}HT_{2B}$ and $5\text{-}HT_{2A}$ receptors and a partial dopamine agonist, is currently in a phase II study in PAH patients and has received orphan drug status from the European Medicines Agency. PRX-08066, a selective $5\text{-}HT_{2B}$ antagonist, is in a

phase II trial (ClinicalTrials.gov identifier NCT00345774), having demonstrated evidence of efficacy in inhibiting hypoxia-induced rises in PAP in humans. SERT and the 5-HT receptors may act in concert to mediate the proliferative effects of serotonin on PASMCs, which suggests simultaneous inhibition of the receptor and transporter as a strategy (Figure 4).

Much has been written about the potential role of serotonin in the origin of PAH associated with anorexigens such as dexfenfluramine. Transgenic mice lacking tryptophan hydroxylase are protected from dexfenfluramine-induced PAH.[156] Given that PAH was still uncommon even among those who consumed anorexigens,[157] it seems likely that a combination of factors is required to cause disease. For example, the effects of serotonin on the pulmonary vasculature are modified by interaction between the serotonin pathway and BMPR2 signaling. Sustained serotonin infusion causes exaggerated PAH and pulmonary vascular remodeling in BMPR2-haploinsufficient mice compared with wild-type mice.[54] There is also a link to mitochondrial metabolism and Kv1.5 channel downregulation and the serotonin pathway. Specifically, SERT-overexpressing mice have decreased Kv1.5 expression respond favorably to therapy with the PDK inhibitor dichloroacetate.[67]

Rho Kinase Inhibitors

In response to calcium/calmodulin, MLC kinase phosphorylates myosin light chain (MLC), which causes PASMC contraction; conversely, MLC phosphatase dephosphorylates MLC, which causes relaxation. Rho kinase inhibits MLC phosphatase, which leads to prolonged, refractory vasoconstriction. Rho kinase participates in the vasoconstriction elicited by many vasoactive agents involved in PAH, such as serotonin, endothelin-1, and thromboxane A_2. Rho kinase inhibitors (Y-27632, fasudil) also markedly reduce PH in PAH models such as the FHR, the chronic hypoxia/SUGEN 5416 model, and the monocrotaline model, which illustrates the critical role of refractory vasoconstriction in these models.[85] In humans with PAH, fasudil, a rho kinase inhibitor, causes modest, immediate reductions in PVR.[86] The challenge with the use of rho kinase inhibitors is avoidance of systemic vasodilatation. Airway nebulization offers a potential means of selectively inhibiting rho kinase in the lung. Rho kinase also participates in vascular SMC proliferation. There is a rho kinase–dependent mechanism by which serotonin transactivates the PASMC BMPR1A receptor and downstream-signaling Smads 1/5/8.[158] In SERT-overexpressing mice, Rho kinase inhibition reduces PAH and vascular remodeling, and this is associated with suppression of extracellular signal-regulated kinase phosphorylation in pulmonary artery fibroblasts.[159]

Restoration of Potassium Channels

Downregulation of the expression and activity of voltage-gated K^+ channels, notably Kv1.5, is a finding common to human PAH and all rodent PAH models. Kv channels not only regulate the resting membrane potential (E_M) but are also involved in survival signaling, which suggests that K^+ channel activation or augmentation therapy could be beneficial in PAH (Figures 7 and 9).[160]

Potassium channels are tetrameric, membrane-spanning proteins that selectively conduct K^+. K^+ leaks from PASMCs down its intracellular/extracellular concentration gradient (145/5 mmol/L), which helps to establish E_M at approximately −60 mV. E_M controls vascular tone by regulating the gating of large-conductance, voltage-gated calcium channels (the target of nifedipine, a clinically important PAH treatment[11]). Depolarization, in response to K^+ channel inhibition/downregulation, activates these channels, elevating cytosolic calcium and causing constriction. By regulating intracellular K^+ and calcium, K^+ channels also regulate cell proliferation and apoptosis and thus vascular remodeling.

PASMCs express a diverse array of K^+ channels (including voltage-gated [Kv] channels). Several channels are germane to PAH, most notably Kv1.5. Acute inhibition of Kv1.5 by hypoxia initiates hypoxic pulmonary vasoconstriction.[65] Interestingly, anorexigens such as dexfenfluramine, which promote PAH, also acutely inhibit PASMC K^+ current and block Kv1.5. Expression of Kv1.5 increases longitudinally in the pulmonary circulation and is maximal in resistance arteries, the major site of pathology in PAH. Selective loss of Kv channel expression (and membrane depolarization) is a hallmark of human[161] and experimental[35,160,162,163] PAH. Restoration of Kv1.5 expression reduces hypoxic PH.[160]

K^+ channel downregulation increases PASMC proliferation and reduces apoptosis, which contributes to obstructive vascular remodeling.[30,36,164,165] Increased cell proliferation reflects, in part, activation of the Ca^{2+}-calcineurin–dependent proliferative transcription factor NFAT.[40] There are several theories for how Kv downregulation impairs apoptosis (notably by preventing cell shrinkage and/or by elevating cytosolic K^+, which inhibits caspases). Kv channel downregulation also occurs in cancer, the prototypic proliferative, antiapoptotic disease.[63]

Inhibition of Transcription Factors

A variety of transcription factors (HIF-1α, NFAT, and c-Jun[166]) govern the expression of Kv1.5 in PASMCs and regulate other factors important to the pathogenesis of PAH. HIF-1α is activated even during normoxia in the PASMCs of patients and FHR with PAH.[18] HIF-1α activation promotes cell survival, and inhibition of HIF-1α may be beneficial. Inhibition of HIF-1α restores Kv1.5 expression and Kv current in experimental PAH.[18] The high cytosolic calcium in PAH PASMCs results in nuclear translocation (activation) of NFAT. NFAT promotes PASMC proliferation and decreases Kv1.5 expression.[40] NFAT inhibition, with either cyclosporine or the more specific peptide inhibitor VIVIT, regresses experimental PAH.[40] NFAT activation also likely contributes to the hyperpolarized mitochondria seen in PAH PASMCs. The antiapoptotic protein bcl-2, which promotes mitochondrial hyperpolarization, is upregulated in iPAH.[167] Inhibitors of NFAT increase Kv1.5 expression[40] and inhibit bcl-2 expression in monocrotaline-induced PAH.[40] NFAT inhibition also decreases hypoxic PH.[40] Moreover, NFATc3 knockout mice do not show pulmonary artery remodeling after

chronic hypoxia.[40] HIF-1α and NFAT inhibition are promising therapeutic strategies.

Inhibition of Transient Receptor Potential Channels

Upregulation of TRPC6, a nonselective cation channel, occurs in PAH and is another mechanism by which excess amounts of extracellular calcium enter the cells in PAH, independent of L-type calcium channel.[39,168–171] Chronic increases in calcium, in part via trp channels and in part via calcineurin-dependent pathways involving NFAT activation,[40] drive PASMC proliferation, which makes trp channel inhibition an interesting therapeutic strategy (Figure 9).

Mitochondria-Metabolic Dysfunction in PAH

PASMCs from FHR and PASMCs[18] and endothelial cells[172] from human PAH exhibit dysmorphic and hyperpolarized mitochondria and a glycolytic shift in metabolism. Such a shift to glycolysis, which occurs independent of po_2, was first described in cancer cells (the Warburg phenotype) and is thought to confer resistance to apoptosis. Key molecular contributors to this metabolic phenotype include activation of HIF-1α, which in turn activates transcription of PDK (Figure 7).

Increased expression of HIF-1α activates a panel of glycolytic genes (such as the glucose transporter, glut 1). HIF-1α simultaneously suppresses the activity of the mitochondrial electron transport chain by transactivating the PDK gene, which phosphorylates and inhibits the PDH complex.[64] PDH catalyzes the irreversible oxidation of pyruvate, yielding acetyl-coenzyme A and CO_2. Phosphorylation of any of the 3 regulatory serines of PDH by PDK completely inhibits PDH.[173]

Dichloroacetate inhibits all 4 PDK isoforms, thereby activating PDH and promoting glucose oxidation. In PAH PASMCs (but not normal PASMCs), dichloroacetate depolarizes the mitochondria, which increases hydrogen peroxide production and restores Kv1.5 expression. The net effect of inhibiting PDK is an induction of apoptosis and a decrease in proliferation. Interestingly, there is little effect of dichloroacetate on normal cells, because PDK is normally relatively inactive. Dichloroacetate regresses many forms of experimental PAH (chronic hypoxic PH, monocrotaline PAH, and FHR PAH).[18,63,90] An advantage in translating the use of dichloroacetate from rats to humans is that it has been used safely as a treatment for lactic acidosis in children and has been tested acutely in adults with heart failure. New isoform-selective PDK inhibitors are in development for diseases such as diabetes mellitus.

Tyrosine Kinase Inhibition

Excessive expression or activity of a variety of growth factors, including PDGF, basic fibroblast growth factor, epidermal growth factor, and vascular endothelial growth factor (VEGF), contributes to obstructive pulmonary vascular remodeling in PAH. Most growth factor receptors are transmembrane receptor tyrosine kinases, and they activate diverse signaling pathways (Figure 8).[174,175] Inhibition of epidermal growth factor and PDGF receptors has beneficial effects on hemodynamics, remodeling, and survival in experimental PAH.[32,176] In humans, there are case reports of a beneficial effect of adding imatinib to baseline therapy.[87] The mechanisms for the potential beneficial effect of imatinib are unclear, because it inhibits the tyrosine kinases, PDGF receptors, BCR-ABL, and c-kit.

In addition to receptor tyrosine kinases, serine/threonine kinases, such as the Raf family and its downstream pathways, offer targets for intervention in PAH (Figure 8). Sorafenib is a "multikinase inhibitor," blocking the serine/threonine kinases Raf-1 and b-Raf, tyrosine kinases, PDGF and VEGF receptors, c-kit, and Flt-3, with IC_{50} values between 6 and 70 nmol/L. Sorafenib is approved for the treatment of renal and hepatocellular carcinoma. Sorafenib prevents and reverses PAH and cardiac remodeling in monocrotaline-treated rats and may have more pronounced effects on RV function than imatinib.[33] Phase 1 clinical trials with both imatinib and sorafenib (ClinicalTrials.gov identifier NCT00452218) have been conducted. The sorafenib trial was a 16-week, phase Ib, single-center, open-label trial of the safety and tolerability of sorafenib in patients with PAH already receiving therapy with prostacyclin, treprostinil, or iloprost, alone or with sildenafil. Sorafenib was well tolerated at 200 mg twice daily in 12 patients.[88] The most common adverse events were moderate skin reactions on the hands and feet and alopecia. The results of the imatinib trial had not been published at the time of the present review.

Elastase and Matrix Metalloproteinases

Increased elastolytic activity may be an early feature of PH, and serum elastase levels are elevated in experimental PAH.[177] Endogenous elastases may contribute to the development of PAH by liberating mitogens (eg, tenascin c[41]) and growth factors from the matrix and activating growth factor receptors in a ligand-independent manner (Figure 10). Elastase inhibitors can attenuate or reverse experimental PAH,[178] but synthetic inhibitors with acceptable toxicity in humans have yet to be developed. Augmentation of the expression of endogenous elastin inhibitors, such as elafin,[179] may prove to be a better strategy.

Peroxisome Proliferator–Activated Receptor Activation

Peroxisome proliferator–activated receptors (PPARs) are ligand-activated transcription factors that belong to the nuclear receptor superfamily. On ligand activation, PPARs heterodimerize with the retinoid X receptor and bind to PPAR response elements in regulatory promoter regions of their target genes. A series of recent observations suggests that PPARγ could be a drug target in PAH.[95,180] PPARγ is a downstream target of BMP2 in human PASMCs.[95] PPARγ is important for BMP2-mediated inhibition of PDGF-induced vascular SMC proliferation.[180] Mice lacking SMC PPARγ develop PAH.[180] PPARγ activation stimulates apolipoprotein E expression. Recombinant apolipoprotein E inhibits PDGFR-β–mediated SMC proliferation and migration.[181] PPARγ targets, independent of apolipoprotein E, may also be important in the suppression of pulmonary vascular remodeling, because male apolipoprotein $E^{-/-}$ mice fed a high-fat diet develop PAH that is reversed by rosiglitazone, a PPARγ

agonist.[95] PPARγ agonists have direct antiinflammatory and proapoptotic effects. PPARs can also interact with signaling molecules to regulate gene expression, independent of DNA binding. PPARγ can impair the phosphorylation of extracellular signal-regulated protein kinase,[182] which is implicated in PASMC proliferation and migration. iPAH patients have reduced lung expression of PPARγ and apolipoprotein E mRNA. Because the thiazolidinedione rosiglitazone is widely used in the treatment of type II diabetes mellitus, a trial in PAH would be feasible. Despite this promise, rosiglitazone failed to ameliorate PH in hypoxic-PH rats, although it did reduce RVH and pulmonary vascular remodeling.[183]

Inflammation

Aside from the association of PAH with several collagen vascular autoimmune disorders (eg, scleroderma, systemic lupus erythematosus, and mixed connective disease) and schistosomiasis, several observations argue for a role of inflammation in the pathogenesis of PAH. These include the presence of T cells, B cells, and macrophages in plexiform lesions; the detection of autoantibodies to endothelial cells and fibroblasts; raised blood cytokine and chemokine levels; and the association of PAH with certain infections such as human herpes virus 8. Mice that overexpress S100A4/Mts1 develop extensive and severe neointimal lesions after injection of the γ-murine herpes virus-68 (the murine homolog of human herpes virus 8).[184] PAH also develops in a subset of patients with HIV disease. The HIV *nef* gene was also implicated recently in plexogenic pulmonary vascular lesions associated with PAH in HIV-infected patients and simian immunodeficiency virus–infected nonhuman primates.[185]

Athymic nude rats, which lack T cells, appear more sensitive than normal rats to the development of PAH when challenged with the VEGF-receptor antagonist SU-5416.[186] A protective role for T cells was established by the administration of splenocytes from euthymic rats. In iPAH, regulatory T cells (T_{reg} cells) are increased, whereas $CD8^+$ cytotoxic T cells are decreased.[187] T_{reg} cells maintain immunotolerance and are potent inhibitors of antitumor and possibly antiviral immune responses. The increase in T_{reg} cells may be a normal counterregulation or compensation for an initial inflammatory response.

Can the immune system be targeted therapeutically in PAH? Mycophenolate mofetil, a potent immunosuppressant used in humans, prevents monocrotaline-induced PAH in rats[188]; however, regression trials (a more clinically relevant standard for experimental PAH therapies) are needed.

Endothelial Progenitor Cells

Endothelial progenitor cells (EPCs) arise from mesodermal stem cells or hemangioblasts in the bone marrow. Circulating in plasma, they home to sites of ischemia or endothelial injury and differentiate into mature endothelial cells in situ, contributing to revascularization and vascular homeostasis. EPCs can be considered a potential therapeutic target, a predictive biomarker,[189] or a vector for cell-based therapy. Circulating EPC numbers (defined by $CD34^+/KDR^+$-positive and $CD34^+/CD133^+/KDR^+$-positive cells) are significantly lower in patients with Eisenmenger syndrome than in normal control subjects.[190] Some but not all investigators have reported reduced levels of EPCs in iPAH patients. Differences in the markers used to identify and quantify EPCs complicate interpretation of the data.

The in vitro functions of endothelial-like mononuclear cells (eg, colony-forming capacity, adherence, migration, and sensitivity to apoptosis) isolated from the blood of iPAH patients differ from those of healthy controls.[190–193] Whether these difference are beneficial, promoting revascularization in the hypertensive lung,[102] or contribute to the pathology, by augmenting pulmonary vascular remodeling,[192] is unclear. This distinction is important given that some treatments (eg, sildenafil) are associated with a dose-dependent increase in the abundance of circulating EPCs,[190] and potential new therapies for PAH, such as statins and PPARγ agonists, also induce the mobilization and differentiation of EPCs.

Administration of EPCs has produced improvements in pulmonary hemodynamics, vascular remodeling, and survival in monocrotaline-induced PAH.[102] Cell therapy has been less effective in hypoxia-induced PAH and may contribute to the pathological vascular remodeling (Figure 12). The benefits of cell therapy may be enhanced by the expression of genes that inhibit SMC proliferation or stimulate angiogenesis (eg, *eNOS*).[100–102] Even fibroblasts can be made somewhat therapeutic when they are transfected with VEGF. These modified fibroblasts prevent worsening of monocrotaline-induced PAH.[102]

Two small pilot studies in which adults and children with iPAH were given a single intravenous infusion of autologous mononuclear cells provide support for the therapeutic potential of cell-based therapy in patients.[194,195] A therapeutic trial (PHACeT [Pulmonary Hypertension: Assessment of Cell Therapy], ClinicalTrials.gov identifier NCT00469027) to assesses the safety of administering autologous, cultured, eNOS-transduced mononuclear cells in iPAH patients has commenced.

Much remains to be done in the field of cell-based therapies for PAH, particularly because it remains uncertain whether influx of progenitor cells into the lung in PAH is beneficial or harmful. Moreover, it appears increasingly likely that any beneficial effects of progenitor cells relates to substances they secrete (paracrine effects) rather than to actual engraftment and transdifferentiation into healthy lung cells. In Figure 12, lessons learned from remodeling in hypoxia are reviewed. In hypoxia, inflammatory and progenitor cells appear to contribute to pathological remodeling; however, it is not certain whether this applies to PAH.

Miscellaneous Pathways With Therapeutic Implications

Statins, heparins, dehydroepiandrosterone, and inhibitors of angiopoietin 1, STAT3, polyamines, survivin, and the cell cycle offer potential treatments for PAH and are discussed, owing to page limits, in the online-only Data Supplement.

Conclusions

In this review of the basic science of PAH, we have assessed emerging concepts of the molecular mechanisms of PAH and identified the novel therapeutic targets suggested by this

science. New therapeutic strategies include enhancing endothelial function/vasodilation by use of guanylate cyclase activators or vasodilator peptides, such as adrenomedullin and VIP; augmenting the BMPR2/SMAD pathway; inhibiting serotonin and SERT; modulating expression/activity of ion channels (Kv1.5 and TRPC6); inhibiting transcription factors (NFAT and HIF-1∝); increasing apoptosis (survivin inhibitors); inhibiting tyrosine kinases; inhibiting the contractile apparatus (rho kinase inhibitors); preserving elastin; and modulating the influx of inflammatory and progenitor cells. Opportunity also exists to accelerate drug development with the testing of molecules that are already approved for the management of cancer, vascular dysfunction, and metabolic disorders. These conditions share the pathophysiological abnormalities of PAH (endothelial dysfunction, excessive cell proliferation, disordered apoptosis, and inflammation). Repurposed drugs that have potential in PAH include PDE5 inhibitors (for erectile dysfunction), imatinib (for chronic myelogenous leukemia), sorafenib (for renal carcinoma), and dichloroacetate (for mitochondrial diseases). We do not endorse the off-label application of these agents in clinical practice; however, there is a compelling need to study these potentially curative agents in preclinical and, when appropriate, clinical trials. This is an exciting time in the search for a cure for PAH, and it is time for physicians, armed with a basic science playbook, to take the field.

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Disclosures

None.

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KEY WORDS: mitochondria ■ endothelin ■ pulmonary heart disease ■ pulmonary arteries ■ therapeutics ■ heart ventricles ■ rare diseases