

## Basic Science of Pulmonary Arterial Hypertension for Clinicians : New Concepts and Experimental Therapies

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## Basic Science of Pulmonary Arterial Hypertension for Clinicians

### New Concepts and Experimental Therapies

Stephen L. Archer, MD; E. Kenneth Weir, MD; Martin R. Wilkins, MD

**P**ulmonary 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]-1 $\alpha$  and nuclear factor of activated T lymphocytes [NFAT]), and tyrosine kinases. Augmentation of voltage-gated K<sup>+</sup> 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<sup>1</sup>: (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<sup>2</sup> and 7.6 cases/million annually and 26 cases/million in Scotland.<sup>3</sup> 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.<sup>4</sup> In developed countries, prevalence will also likely increase as newer associations with PAH emerge, including dialysis<sup>5</sup> and the metabolic syndrome,<sup>6</sup> 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.<sup>7</sup> 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%).<sup>8</sup> In another cohort of PAH patients treated with Flolan, survival in iPAH patients was better (65% at 3 years).<sup>9</sup> Prognosis was worse in older patients and was also worse in PAH associated with scleroderma versus iPAH.<sup>9</sup> PAH associated with scleroderma has a 3-year survival rate of only 34% to 47%.<sup>9,10</sup>

### 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).<sup>11</sup> 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.<sup>12</sup> 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-1 $\alpha$  (Figure 2).<sup>13</sup> 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.<sup>14</sup> 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

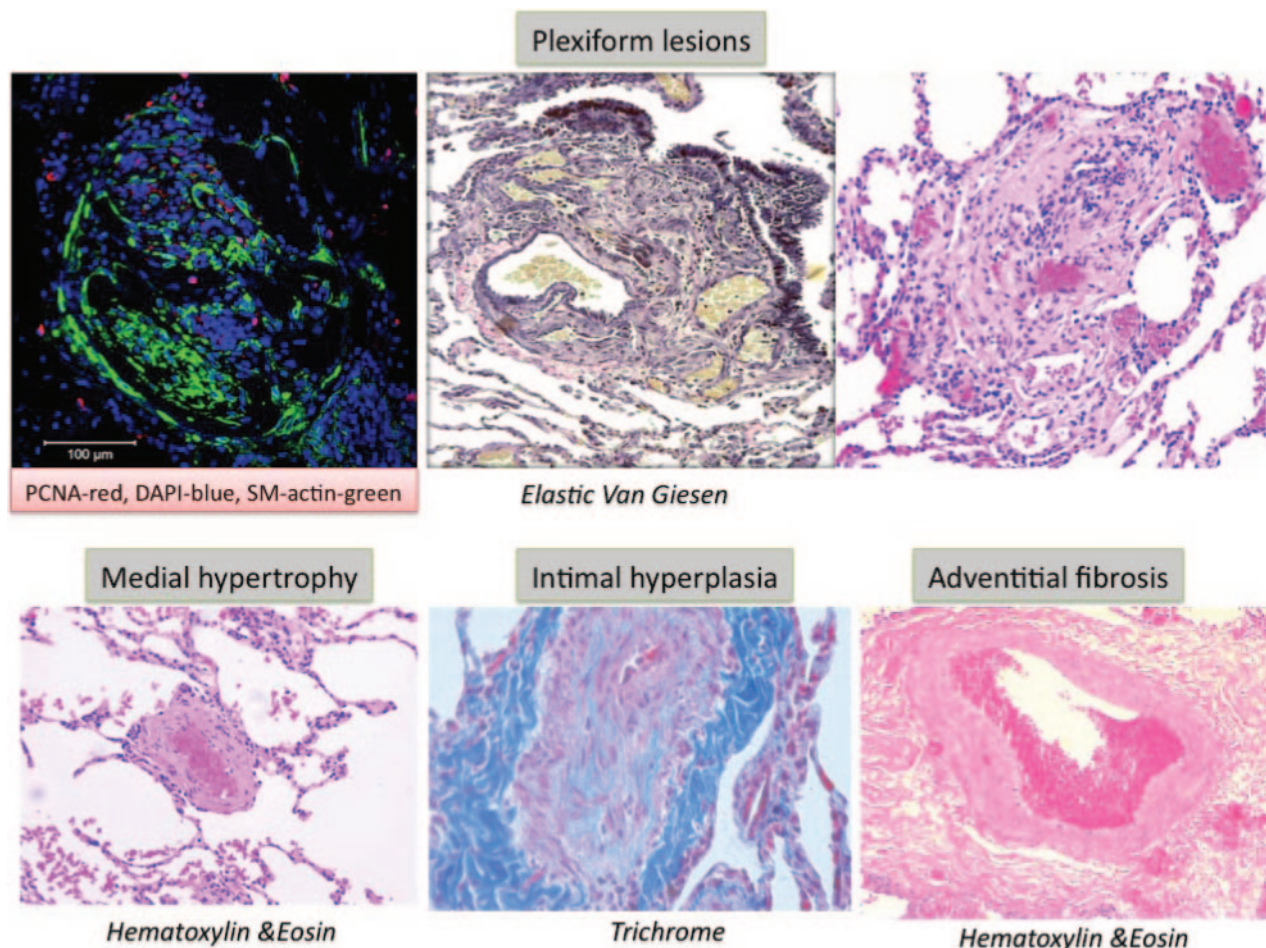
The evaluation of these novel targets occasionally involves the off-label use of drugs approved for another indication (eg, Gleevec, Novartis Oncology, East Hanover, NJ) in humans, but is largely based on studies in cellular and animal models. Cautious interpretation of preclinical studies is mandatory, 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<sup>15</sup> or abdominal aortocaval shunt,<sup>16</sup> fawn-hooded rats (FHR, which spontaneously develop PAH and are also hypoxia sensitive),<sup>17,18</sup> and rats treated with a single dose of VEGF-receptor antagonist (SU5416) plus hypoxia.<sup>19</sup> 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,<sup>20</sup> BMPR2 dominant-negative mutations,<sup>21</sup> or S100A4/Mts1 (metastasin 1),<sup>22</sup> 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



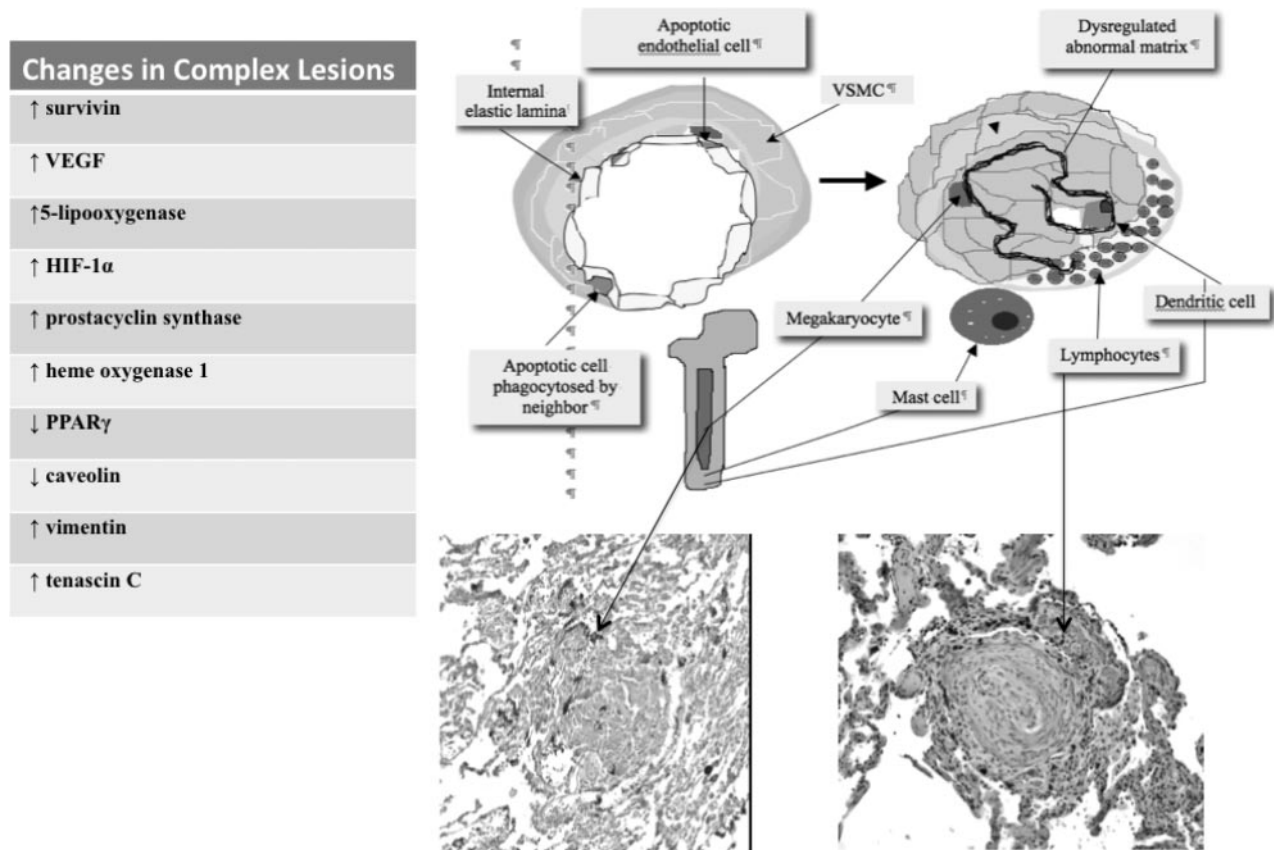
**Figure 1.** Histology of PAH. Top, Plexiform lesions. Upper Left, Evidence of cell proliferation (red is proliferating cell nuclear antigen [PCNA], green is smooth muscle [SM] actin, and blue is DAPI). Bottom, Medial hypertrophy, intimal fibrosis, and adventitial proliferation.

3). In the blood, levels of serotonin, a proliferative, fibrogenic vasoconstrictor, are elevated (Figure 4).<sup>23</sup> In the endothelium, the vasodilator/vasoconstrictor ratio is decreased (Figure 5),<sup>24–26</sup> whereas prothrombotic factors, including tissue factor,<sup>27</sup> 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).<sup>19</sup> In the media, pulmonary artery smooth muscle cell (PASMC) apoptosis is suppressed, and proliferation is enhanced. Many factors drive PASMC proliferation, including mutation<sup>28</sup> or downregulation<sup>29</sup> of BMPR2 (Figure 6), mitochondrial metabolic abnormalities (Figure 7), de novo expression of the antiapoptotic protein survivin,<sup>19,30</sup> increased expression/activity of SERT,<sup>20,31</sup> increased expression/activity of platelet-derived growth factor (PDGF) receptor,<sup>32</sup> tyrosine kinase activation (Figure 8),<sup>33</sup> and decreased expression of Kv1.5, a voltage-gated, O<sub>2</sub>-sensitive potassium channel. Kv1.5 downregulation occurs in human PAH,<sup>34</sup> rat PAH models (whether induced by chronic hypoxia<sup>35,36</sup> or monocrotaline<sup>30</sup> or in FHR<sup>18</sup>), and transgenic mice with PAH due to SERT overexpression<sup>20</sup> or BMPR2 mutation.<sup>37</sup> Loss of Kv1.5, the same channel that is inhibited by hypoxia to initiate hypoxic pulmonary vasoconstriction,<sup>38</sup> depolarizes the membrane and

elevates cytosolic K<sup>+</sup> and Ca<sup>2+</sup> (Figure 9). The resulting calcium overload, later reinforced by activation of transient receptor potential (trp) channels,<sup>39</sup> leads to Ca<sup>2+</sup>-calcineurin-dependent activation of the proliferative transcription factor NFAT.<sup>40</sup> Normoxic activation of HIF-1 $\alpha$  occurs in FHR<sup>18</sup> and human PAH.<sup>13,18</sup> In the adventitia, metalloprotease activation causes architectural disruption, which permits cell migration and generates mitogenic peptides (tenascin; Figure 10).<sup>41</sup> Adventitial fibroblasts are also hyperproliferative in PH, displaying increased sensitivity to serotonin.<sup>42</sup> Circulating autoantibodies<sup>4</sup> and lung infiltration by inflammatory cells are common, particularly in PAH associated with connective tissue disease and schistosomiasis (Figure 11).<sup>43</sup> Finally, there are increased endothelial precursor cells and mesenchymal and bone marrow-derived stem cells,<sup>44</sup> 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- $\beta$  superfamily. Most patients (>80%) with familial PAH have loss-of-function mutations in BMPR2<sup>45–47</sup> 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.

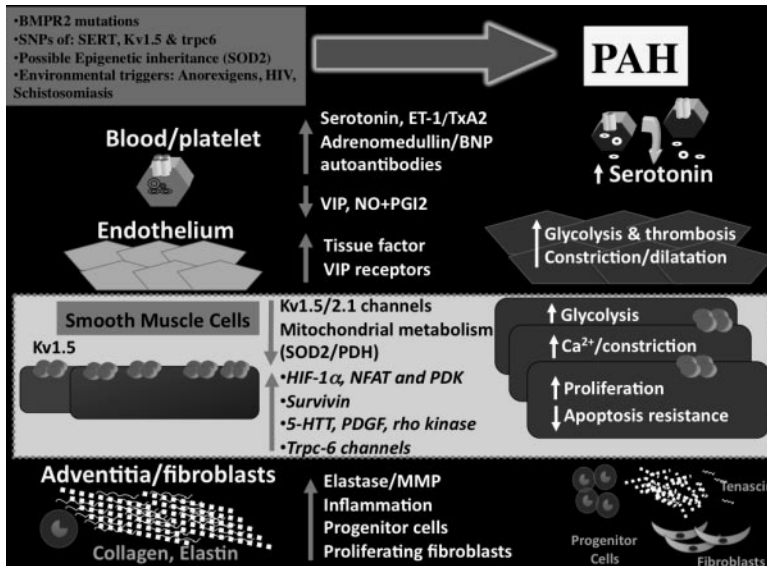
heterodimers with any of 4 type 1 receptors (BMPR1A, BMPR1B, Alk1, and Alk2), which results in phosphorylation of the intracellular portion of the type 1 receptor by BMPR2. Receptor activation initiates a cytosolic Smad protein–signaling cascade. Receptor-activated Smads complex with common partner SMAD (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.<sup>48</sup> 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.<sup>49</sup> 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.<sup>28</sup> 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.<sup>50</sup> 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.<sup>51</sup> 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.<sup>37</sup> 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  $\approx$ 25% of carriers in affected families develop PAH).<sup>52</sup> Although modifier genes, such as SERT and transforming growth factor- $\beta$ , 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<sub>2</sub>; BNP, brain natriuretic peptide; PGI<sub>2</sub>, 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.<sup>53</sup> 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.<sup>54</sup>

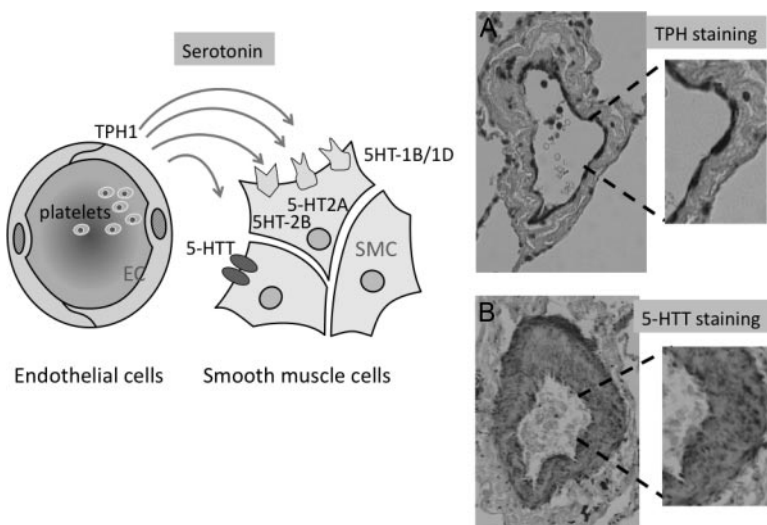
In genetically normal animals, BMPR2 expression decreases as PH develops.<sup>29</sup> One posttranscriptional mechanism that accounts for downregulation of BMPR2 protein is activation of microRNAs. microRNAs regulate gene expression by inhibiting translation. A computational algorithm on the BMPR2 gene 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.<sup>55</sup>

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<sup>56</sup>; however, nebulized BMPR2 adenovirus (with a pro-

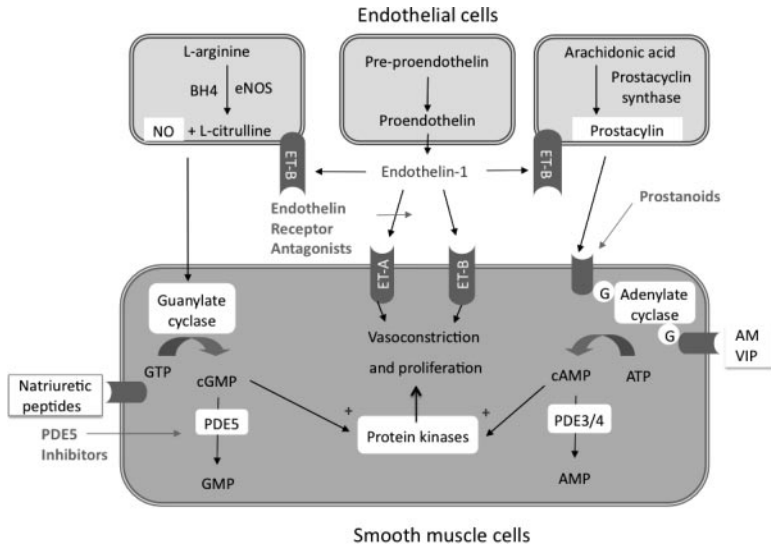
miscuous promoter) did not regress monocrotaline-induced PAH.<sup>29</sup> Further study is required and may be productive in identifying BMPR2-related targets for pharmacological manipulation in PAH. For example, inhibition of transforming growth factor- $\beta$  signaling prevents PAH in the monocrotaline model via inhibition of activin receptor-like kinase-5.<sup>57</sup>

**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,<sup>58</sup> Kv1.5,<sup>59</sup> and TRPC6 [trp cation channel, subfamily C, member 6]<sup>60</sup>) 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<sub>A</sub> receptors on SMCs, while stimulating NO and prostacyclin release via endothelial ET<sub>B</sub> receptors. Adrenomedullin (AM) and VIP are additional endothelium-derived, cAMP-dependent vasodilators that are dysregulated in PAH.

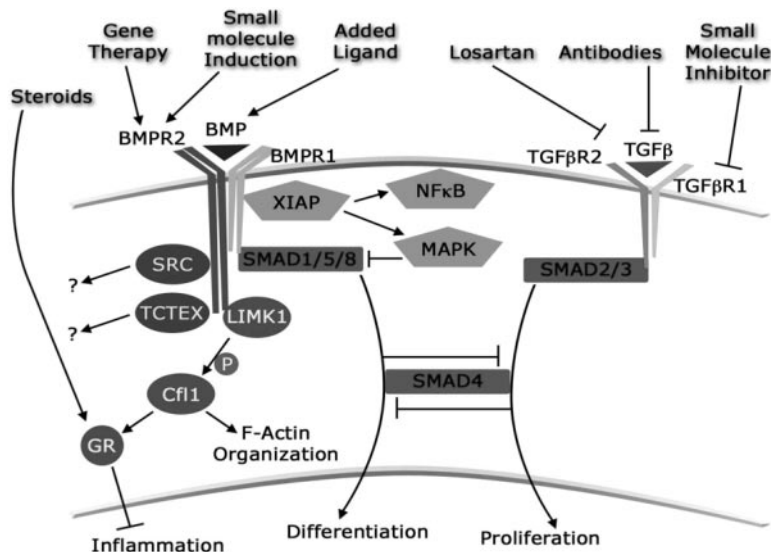
activates the inflammatory transcription factor nuclear factor- $\kappa$ B.<sup>60</sup>

**Excess Proliferation and Impaired Apoptosis Suggest Similarities to Cancer in PAH**

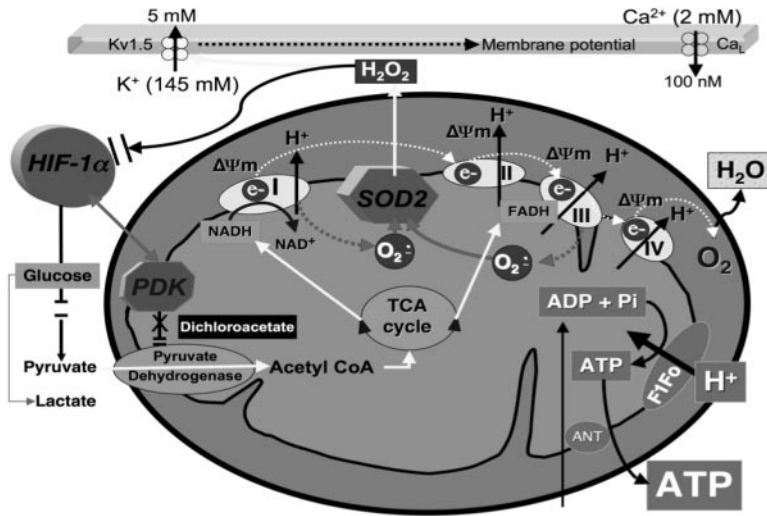
Otto Warburg, 1931 Nobel laureate, proposed that a shift in glucose metabolism from oxidative phosphorylation to glycolysis (despite adequate oxygen supply) was central to the cause and maintenance of cancers. Several observations indicate that PAH shares this “Warburg phenotype.”<sup>18,61</sup> As highlighted by Voelkel et al,<sup>62</sup> 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.<sup>18,63</sup> This enzyme phosphorylates and inhibits pyruvate dehydrogenase (PDH).<sup>64</sup> PDH catalyzes the irreversible oxidation of pyruvate, yielding acetyl-coenzyme A and CO<sub>2</sub>, 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 metab-

olism. Subversion of the mitochondrial O<sub>2</sub>-sensing mechanism, normally used to sense and respond to decreases in pO<sub>2</sub>,<sup>65</sup> appears to cause the sensor to signal hypoxia despite adequate pO<sub>2</sub>. These acquired (and reversible) mitochondrial abnormalities of fusion/fission and metabolism<sup>61</sup> are postulated to cause the observed normoxic activation of HIF-1 $\alpha$  in PAH<sup>18</sup> and cancer.<sup>63</sup> Once active, HIF-1 $\alpha$  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-1 $\alpha$  activation.<sup>18</sup> Dichloroacetate, which inactivates PDK by causing conformational changes in its nucleotide- and lipoyl-binding pockets,<sup>60</sup> regresses experimental PAH.<sup>7,33</sup>



**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)- $\beta$  signaling, and likely increase proliferation. Signaling through XIAP (X-linked inhibitor of apoptosis), which also requires BMPR1, can impact both the nuclear factor- $\kappa$ B (NF $\kappa$ B) 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- $\beta$  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<sub>2</sub>. This electron flux powers H<sup>+</sup> ion extrusion, which creates the proton-motive force responsible for creating the negative membrane potential (ΔΨ<sub>m</sub>) of mitochondria and powering F<sub>1</sub>F<sub>0</sub> ATP synthase. Side reactions between semiquinones and molecular O<sub>2</sub>, which account for ≈3% of net electron flux, create superoxide anion in proportion to pO<sub>2</sub>. Superoxide dismutase (SOD2) rapidly converts superoxide anion (produced at complexes I and III) to H<sub>2</sub>O<sub>2</sub>, 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,<sup>63</sup> and experimental PAH (chronic hypoxic PH, monocrotaline PAH, and FHR PAH).<sup>18,36,66</sup> Dichloroacetate also regresses spontaneous PH in transgenic mice that overexpress SERT in PSMCs.<sup>67</sup> 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.<sup>36</sup> 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,<sup>68</sup> 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.<sup>69</sup> 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.<sup>70,71</sup> AMP-activated protein kinase activation in ventricular hypertrophy<sup>72–74</sup> preserves ATP levels by increasing glucose transport and accelerating glycolysis and by inhibiting acetyl-coenzyme A carboxylase.<sup>71</sup> In RVH, there is a systolic flow impediment in the right coronary artery that is proportional to RV pressure and mass.<sup>75</sup> 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.<sup>76</sup> 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**

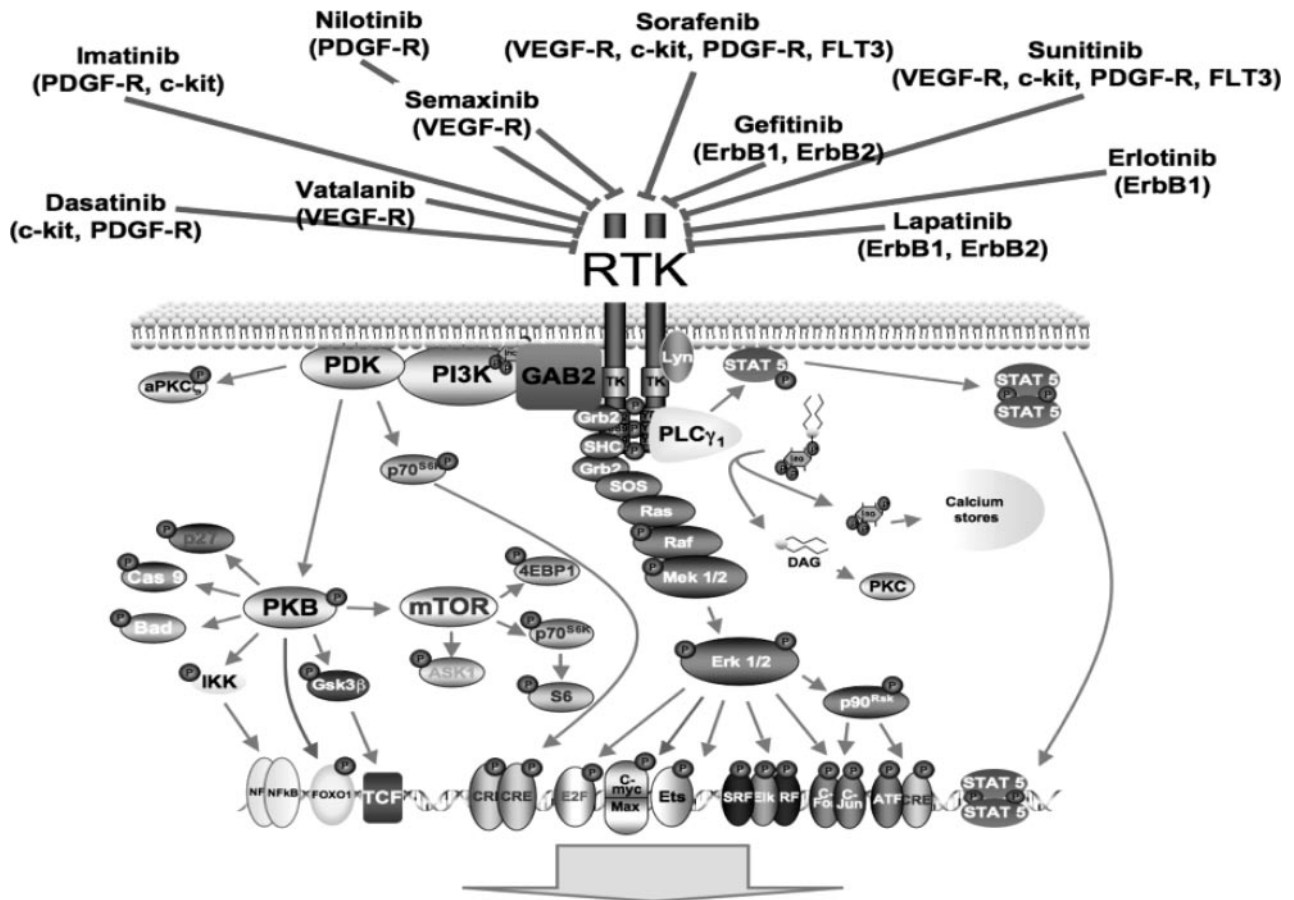
One of the most successful therapeutic strategies for PAH has been to augment endogenous prostacyclin production with exogenous prostanoids (Table; Figure 5). Fatty acid cyclooxygenase converts arachidonic acid to prostaglandin H<sub>2</sub>, a substrate for both prostaglandin I<sub>2</sub> (prostacyclin) synthase and thromboxane synthase. Prostaglandin I<sub>2</sub> synthase is expressed in pulmonary vascular endothelium and generates prostacyclin, which relaxes PSMCs and inhibits platelet aggregation through interaction with prostacyclin receptors and stimulation of cAMP. Thromboxane synthase, in platelets and endothelium, produces thromboxane A<sub>2</sub>. Thromboxane A<sub>2</sub> stimulates vasoconstriction and platelet aggregation through thromboxane/prostaglandin receptors. Endothelial dysfunction and platelet activation in PAH reduce prostacyclin levels and increase thromboxane A<sub>2</sub> production.

Continuous intravascular infusion of epoprostenol (Flolan) decreases PVR, increases cardiac output, and improves life expectancy.<sup>12</sup> 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.<sup>103,104</sup>

**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





## Proliferation, Migration, Survival, ECM synthesis

**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, I $\kappa$ B 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; NF $\kappa$ B, nuclear factor- $\kappa$ B; NHERF, sodium-hydrogen exchange regulatory factor; P, phosphorylation; 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.

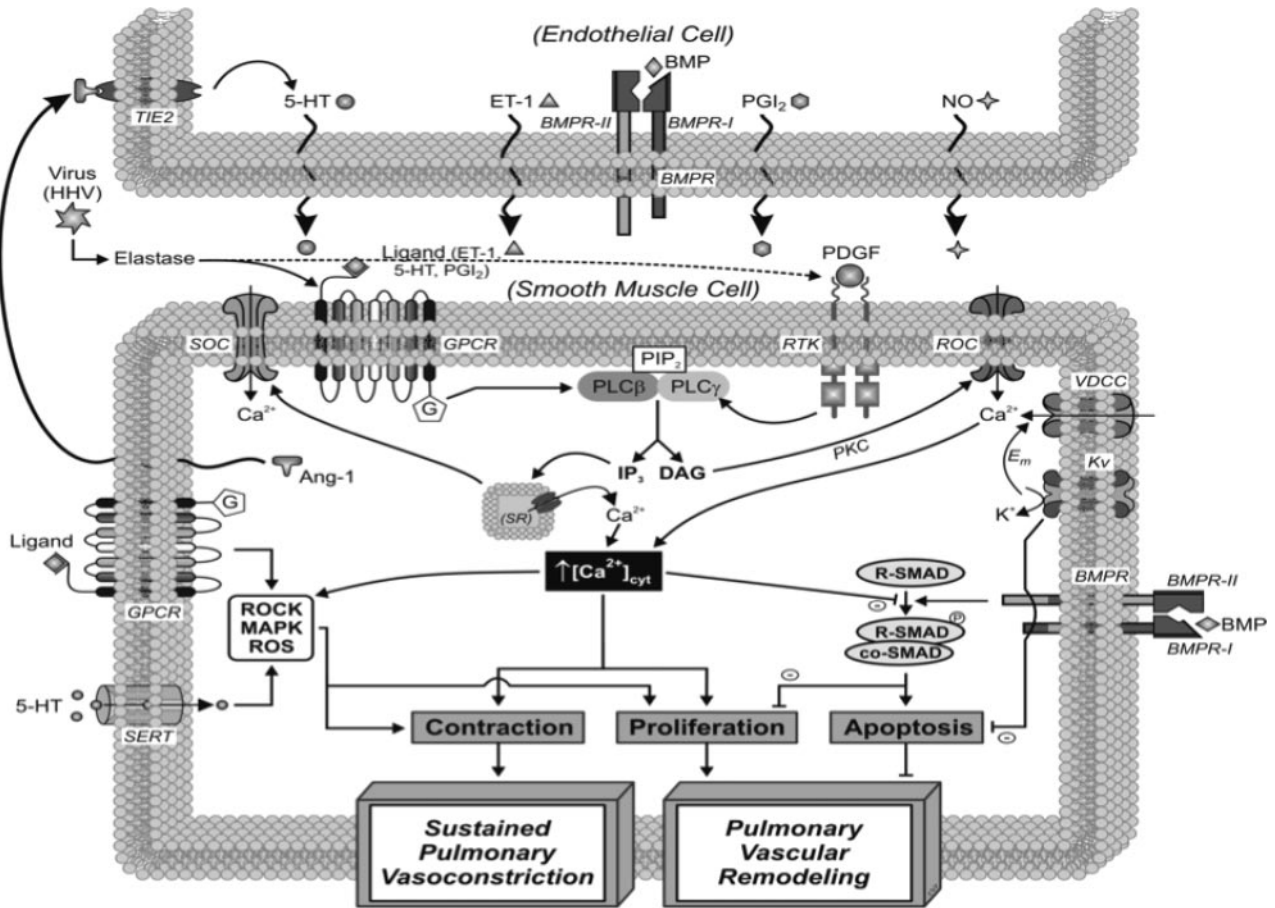
PASMCs, where it stimulates soluble guanylate cyclase (sGC) to produce cGMP (Table; Figure 5). The cardiovascular effects of cGMP are mediated by interaction with at least 3 groups of proteins: cGMP-dependent protein kinases, cGMP-regulated PDE, and cyclic nucleotide-gated ion channels. PDE5, the molecular 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<sup>105</sup> and NO bioavailability<sup>106</sup> are depressed. Moreover, PDE5 is induced both in PASMCs<sup>107</sup> and the RV,<sup>68</sup> which hastens inactivation of cGMP. Finally, production of endogenous NOS inhibitors, asymmetrical and symmetrical dimethylarginines (ADMA and SDMA), is enhanced in PH.<sup>108,109</sup>

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 cyclohydrolase-1 (GTP-CH1, the rate-limiting enzyme in synthesis of the NOS cofactor tetrahydrobiopterin [BH<sub>4</sub>]), or dimethylarginine dimethylaminohydrolase (DDAH, the enzyme responsible for eliminating endogenous NOS inhibitors).<sup>110–112</sup> Inhibition of NO production in humans by use of a competitive NOS antagonist (*N*<sup>G</sup>-monomethyl-L-arginine) increases PVR.<sup>113,114</sup> Sustained pharmacological NOS inhibition causes PH in rats, although there is disproportionate systemic hypertension.<sup>115</sup>

### *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.<sup>116</sup> Although



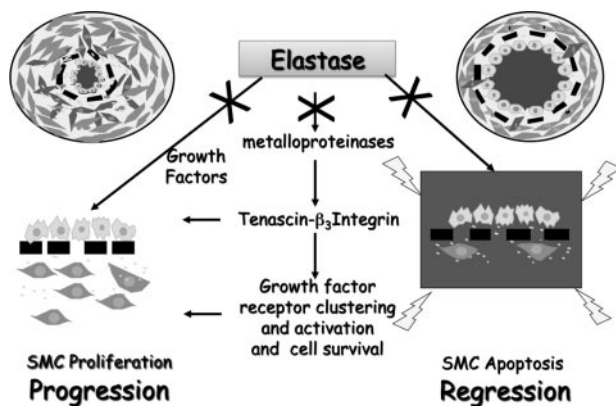
**Figure 9.** Ion channels in PAH. Schematic depiction of the cellular mechanisms linked with vasoconstriction and remodeling in pulmonary endothelial cells (PAECs) and PSMCs in PAH. Central themes of interest for the development of PAH include the following: (1) Impaired ion channel expression and function in PSMCs (Kv, VDCC, SOC, ROC); (2) increased cytosolic calcium ( $[Ca^{2+}]_{cyt}$ ) in PSMCs (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 PSMCs; (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 PSMCs 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; PGI<sub>2</sub>, prostaglandin I<sub>2</sub>; PIP<sub>2</sub>, phosphatidylinositol-4,5-bisphosphate; PKC, protein kinase C; PLC, phospholipase C; ROC, receptor-operated Ca<sup>2+</sup> channels; ROCK, Rho-associated kinase; ROS, reactive oxygen species; RTK, receptor tyrosine kinase; SERT, 5-HT transporter; SOC, store-operated Ca<sup>2+</sup> channels; SR, sarcoplasmic reticulum; and VDCC, voltage-dependent Ca<sup>2+</sup> channels.

long-term therapy with inhaled NO for PAH is feasible,<sup>84</sup> 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.<sup>117</sup> Diethylenetriamine/NO has been used effectively to improve pulmonary hemodynamics in intubated patients with adult respiratory distress syndrome.<sup>118</sup> 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.<sup>119</sup> 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.<sup>120</sup> Infusion of the PDE1 inhibitor 8-methoxymethyl-isobutyl-1-methylxanthine reduces PVR and RVH in rodent PH models.<sup>120</sup>

PDE5 expression, normally absent in cardiac myocytes, is upregulated in the RV in PAH.<sup>68</sup> PDE5 inhibition in PAH models increases RV contractility through a cGMP-mediated inhibition of PDE3.<sup>68</sup> 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.<sup>121</sup> 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 PSMCs. Heightened elastase activity also activates matrix metalloproteinases, which upregulate the glycoprotein tenascin-C. When tenascin-C binds cell-surface integrins, such as  $\alpha$ -v  $\beta$ 3 on PSMCs, 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 PSMCs 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<sup>122</sup>). 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.<sup>104,123,124</sup>

#### sGC Activators

sGC is a heterodimer that consists of  $\alpha$ - and  $\beta$ -subunits. sGC expression is upregulated as a compensatory mechanism in human PAH.<sup>125</sup> Experimental hypoxic PH is exacerbated in mice that lack sGC $\alpha$ 1.<sup>126</sup> 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).<sup>127</sup> 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.<sup>128,129</sup> 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.<sup>130</sup> BAY 63-2521 (Riociguat), an sGC stimulator, is available orally, has a favorable safety profile, and has entered phase III trials in PAH.<sup>91</sup>

#### Enhancing NOS Activity: BH<sub>4</sub> and Transcription Enhancers

BH<sub>4</sub> is an important NOS cofactor, essential for dimerization and for oxygenation of L-arginine to create NO and L-citrulline. Without BH<sub>4</sub>, NOS becomes uncoupled

and produces superoxide anion, which rapidly reacts with NO, producing peroxynitrite, further attenuating NO bioavailability.<sup>131</sup>

The rate-determining step for the de novo production of BH<sub>4</sub> is catalyzed by GTP-CH1. Mice with impaired GTP-CH1 activity exhibit reduced lung BH<sub>4</sub> and spontaneously develop PH with vascular remodeling.<sup>111</sup> Conversely, congenital overexpression of GTP-CH1 in vascular endothelium protects mice from hypoxic PH.<sup>111</sup> In a porcine model of persistent PH of the newborn, combined therapy with BH<sub>4</sub> and a superoxide dismutase mimetic (which enhances survival of endogenous NO) restores endothelial function.<sup>132</sup>

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,<sup>133</sup> and this may result in conversion of BH<sub>4</sub> to dihydrobiopterin. BH<sub>4</sub> is a cofactor for several enzymes and is well tolerated when administered in its synthetic form, sapropterin, to patients with phenylketonuria.<sup>134</sup> This observation paves the way for studies in PAH patients.

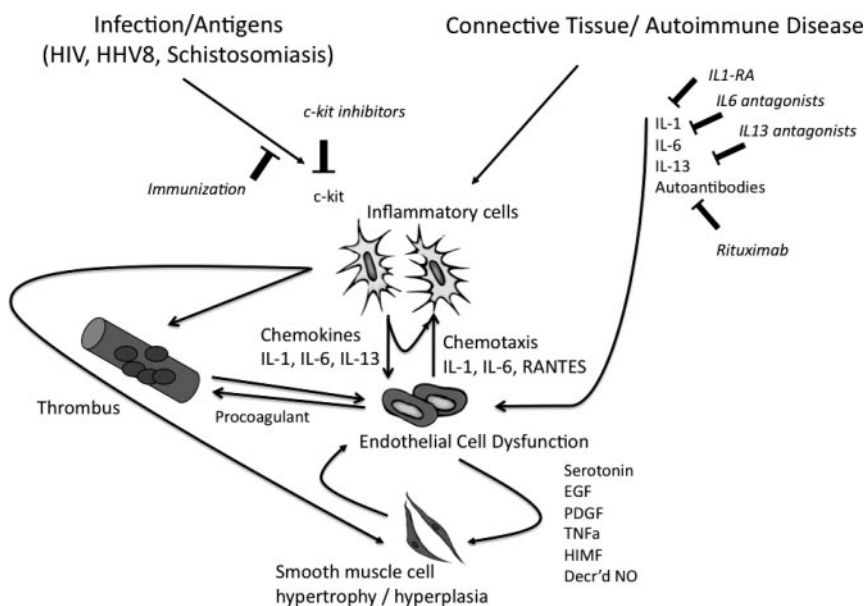
eNOS transcription enhancers, such as AVE9488 and AVE3085, similarly aim to increase NO signaling. Theoretically, an increase in eNOS without corresponding increases in cofactors such as BH<sub>4</sub> could lead to uncoupling and the formation of superoxide ions. However, AVE9488 treatment also increases BH<sub>4</sub> levels and improves eNOS coupling in apolipoprotein E-knockout mice.<sup>135</sup> 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<sub>A</sub> and ET<sub>B</sub>, to regulate vascular tone and cell proliferation (Figure 5). Both receptor subtypes are found on PSMCs and mediate vasoconstriction, whereas the ET<sub>B</sub> receptor on endothelial cells mediates NO and prostacyclin release, causing vasodilation. Lung and circulating endothelin-1 levels are increased in PAH patients.<sup>136</sup> 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<sub>A</sub> antagonism offers advantages over combined ET<sub>A</sub> and ET<sub>B</sub> antagonism (bosentan), nor is the relative efficacy compared with PDE5 inhibitors known (although a small trial suggests some benefits of sildenafil).<sup>137</sup> Liver toxicity and teratogenicity are class effects. Although comparisons with historical control data suggest that bosentan monotherapy increases survival,<sup>138</sup> 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 PASM proliferation. The triad of endothelial cell proliferation, PASM 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 TNF $\alpha$ , tumor necrosis factor- $\alpha$ .

**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).<sup>139</sup> 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.<sup>140</sup> Genetic inactivation of NPR-A is associated with PH, whereas sustained administration of atrial natriuretic peptide attenuates PAH in animal models.<sup>141</sup> 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,<sup>142</sup> but this combination is untested in patients.

**Adrenomedullin**

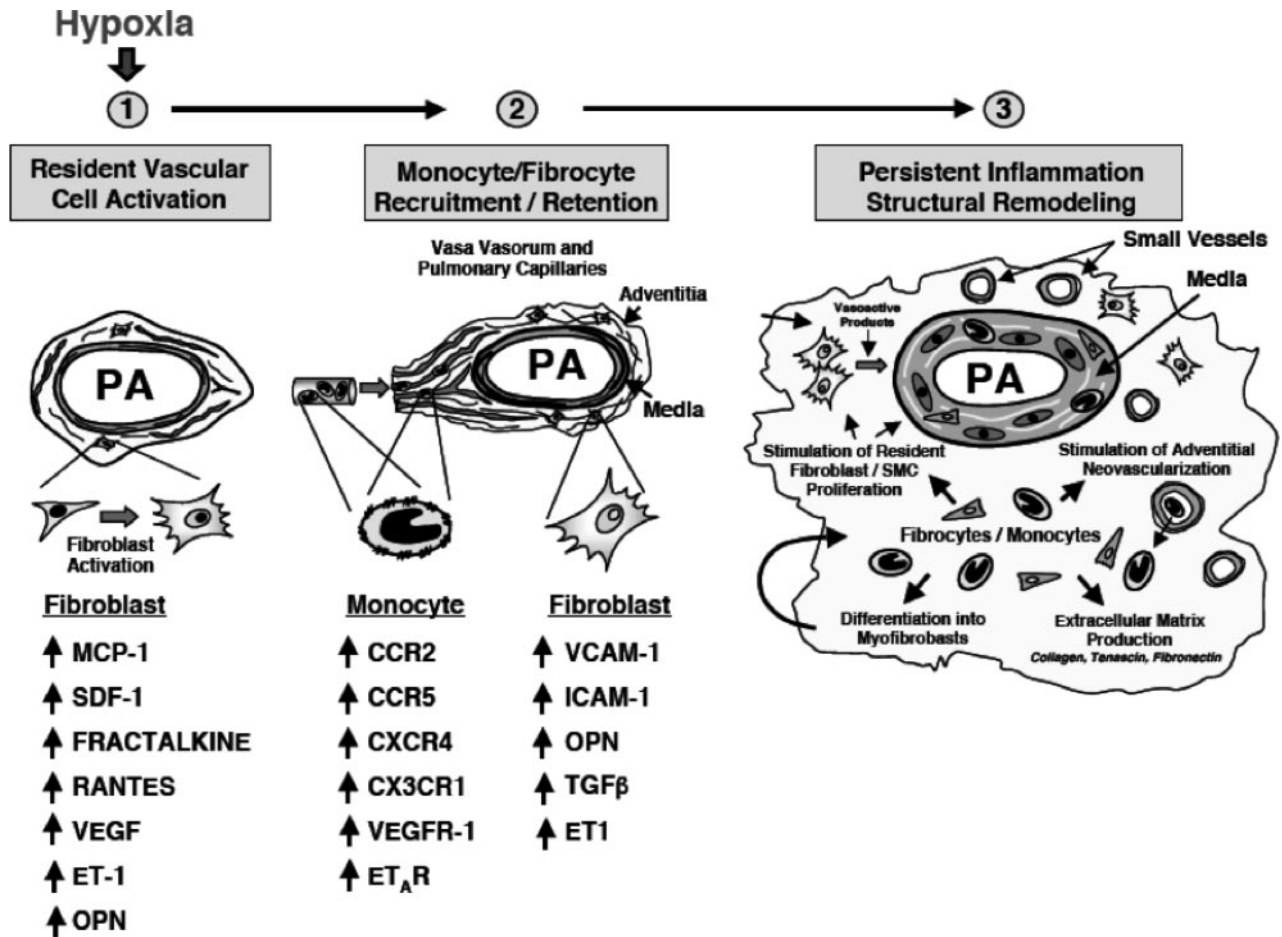
This vasodilator peptide activates several signaling pathways, such as cAMP, NO-cGMP, and PI3K (phosphatidylinositol 3-kinase)/Akt. It decreases mean PAP and RVH in hypoxic rats and exhibits antiproliferative properties.<sup>143</sup> Adrenomedullin-2, a novel peptide, acts by the same receptors as adrenomedullin, 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.<sup>144</sup> In humans with PAH, inhaled adrenomedullin causes a modest reduction in PVR

and increases peak O<sub>2</sub> consumption during exercise without exerting significant effects on the systemic vasculature.<sup>98</sup>

**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.<sup>145</sup> They overexpress proinflammatory genes and genes involved in pulmonary vascular remodeling and underexpress antiproliferative genes,<sup>146</sup> 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 PSMCs from PAH patients; conversely, serum and lung VIP levels are low in PAH. VIP inhibits the proliferation of PSMCs from PAH patients.<sup>97</sup> Nebulized VIP (200  $\mu$ g 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 PSMCs) 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 PSMCs 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).<sup>147</sup> In cystic fibrosis, in which impaired protein trafficking also occurs, sodium 4-phenylbutyrate can improve membrane trafficking of the chloride channel.<sup>148</sup> 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.<sup>147</sup>

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 proliferative pathways that are unchecked by BMPR2 dysfunction. PSMCs 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.<sup>149</sup>

**Inhibitors of Serotonin and SERT**

Plasma serotonin is increased in iPAH patients, even after lung transplantation,<sup>23</sup> 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
<b>Current therapies</b>				
L-type Ca <sup>2+</sup> channels	Decrease SMC Ca <sup>2+</sup>	L-type Ca <sup>2+</sup> 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
<b>Novel targets</b>				
Rho kinase	Decrease Ca <sup>2+</sup> sensitivity in SMCs	Rho kinase inhibitors: fasudil	Yes, acute trial	85, 86
Rho A prenylation	Decrease Ca <sup>2+</sup> 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 <sub>2</sub>	Aspirin	Yes*	27
Ornithine decarboxylase	Inhibit polyamine synthesis	α-Difluoromethylornithine	No	92
Cyclin-dependent-kinase inhibitor γ27	Inhibit SMC proliferation	Heparin	Yes*	93
PPAR-γ	Increase PPAR-γ 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 a 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.<sup>150</sup> 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<sup>150</sup>; however, in 2 separate iPAH cohorts, this relationship was not detected.<sup>151,152</sup> 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.<sup>31</sup> Fluoxetine reduces hypoxic PH in rats.<sup>153</sup> 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.<sup>96</sup> 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.<sup>154</sup> The 5-hydroxytryptamine 2A (5-HT<sub>2A</sub>) receptor mediates serotonin-induced proliferation in rat pulmonary artery fibroblasts.<sup>42</sup> Genetic deficiency of the 5-HT<sub>2B</sub> serotonin receptor reduces hypoxic PH in mice.<sup>155</sup> Terguride, a potent antagonist of 5-HT<sub>2B</sub> and 5-HT<sub>2A</sub> 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-HT<sub>2B</sub> 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 PSMCs, 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.<sup>156</sup> Given that PAH was still uncommon even among those who consumed anorexigens,<sup>157</sup> 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.<sup>54</sup> 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 and respond favorably to therapy with the PDK inhibitor dichloroacetate.<sup>67</sup>

### Rho Kinase Inhibitors

In response to calcium/calmodulin, MLC kinase phosphorylates myosin light chain (MLC), which causes PSMC 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<sub>2</sub>. 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.<sup>85</sup> In humans with PAH, fasudil, a rho kinase inhibitor, causes modest, immediate reductions in PVR.<sup>86</sup> 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 PSMC BMPR1A receptor and downstream-signaling Smads 1/5/8.<sup>158</sup> 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.<sup>159</sup>

### Restoration of Potassium Channels

Downregulation of the expression and activity of voltage-gated K<sup>+</sup> 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<sub>M</sub>) but are also involved in survival signaling, which suggests that K<sup>+</sup>

channel activation or augmentation therapy could be beneficial in PAH (Figures 7 and 9).<sup>160</sup>

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

PASMCs express a diverse array of K<sup>+</sup> 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.<sup>65</sup> Interestingly, anorexigens such as dexfenfluramine, which promote PAH, also acutely inhibit PSMC K<sup>+</sup> 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<sup>161</sup> and experimental<sup>35,160,162,163</sup> PAH. Restoration of Kv1.5 expression reduces hypoxic PH.<sup>160</sup>

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

### Inhibition of Transcription Factors

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

chronic hypoxia.<sup>40</sup> HIF-1 $\alpha$  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.<sup>39,168–171</sup> Chronic increases in calcium, in part via trp channels and in part via calcineurin-dependent pathways involving NFAT activation,<sup>40</sup> drive PASM C proliferation, which makes trp channel inhibition an interesting therapeutic strategy (Figure 9).

#### **Mitochondria-Metabolic Dysfunction in PAH**

PASMCs from FHR and PASMCs<sup>18</sup> and endothelial cells<sup>172</sup> from human PAH exhibit dysmorphic and hyperpolarized mitochondria and a glycolytic shift in metabolism. Such a shift to glycolysis, which occurs independent of pO<sub>2</sub>, 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-1 $\alpha$ , which in turn activates transcription of PDK (Figure 7).

Increased expression of HIF-1 $\alpha$  activates a panel of glycolytic genes (such as the glucose transporter, glut 1). HIF-1 $\alpha$  simultaneously suppresses the activity of the mitochondrial electron transport chain by transactivating the PDK gene, which phosphorylates and inhibits the PDH complex.<sup>64</sup> PDH catalyzes the irreversible oxidation of pyruvate, yielding acetyl-coenzyme A and CO<sub>2</sub>. Phosphorylation of any of the 3 regulatory serines of PDH by PDK completely inhibits PDH.<sup>173</sup>

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).<sup>18,63,90</sup> 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).<sup>174,175</sup> Inhibition of epidermal growth factor and PDGF receptors has beneficial effects on hemodynamics, remodeling, and survival in experimental

PAH.<sup>32,176</sup> In humans, there are case reports of a beneficial effect of adding imatinib to baseline therapy.<sup>87</sup> 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<sub>50</sub> 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.<sup>33</sup> 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.<sup>88</sup> 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.<sup>177</sup> Endogenous elastases may contribute to the development of PAH by liberating mitogens (eg, tenascin c<sup>41</sup>) 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,<sup>178</sup> but synthetic inhibitors with acceptable toxicity in humans have yet to be developed. Augmentation of the expression of endogenous elastin inhibitors, such as elafin,<sup>179</sup> 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 PPAR $\gamma$  could be a drug target in PAH.<sup>95,180</sup> PPAR $\gamma$  is a downstream target of BMP2 in human PASMCs.<sup>95</sup> PPAR $\gamma$  is important for BMP2-mediated inhibition of PDGF-induced vascular SMC proliferation.<sup>180</sup> Mice lacking SMC PPAR $\gamma$  develop PAH.<sup>180</sup> PPAR $\gamma$  activation stimulates apolipoprotein E expression. Recombinant apolipoprotein E inhibits PDGFR- $\beta$ -mediated SMC proliferation and migration.<sup>181</sup> PPAR $\gamma$  targets, independent of apolipoprotein E, may also be important in the suppression of pulmonary vascular remodeling, because male apolipoprotein E<sup>-/-</sup> mice fed a high-fat diet develop PAH that is reversed by rosiglitazone, a PPAR $\gamma$



agonist.<sup>95</sup> PPAR $\gamma$  agonists have direct antiinflammatory and proapoptotic effects. PPARs can also interact with signaling molecules to regulate gene expression, independent of DNA binding. PPAR $\gamma$  can impair the phosphorylation of extracellular signal-regulated protein kinase,<sup>182</sup> which is implicated in PASMC proliferation and migration. iPAH patients have reduced lung expression of PPAR $\gamma$  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.<sup>183</sup>

### 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  $\gamma$ -murine herpes virus-68 (the murine homolog of human herpes virus 8).<sup>184</sup> 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.<sup>185</sup>

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.<sup>186</sup> A protective role for T cells was established by the administration of splenocytes from euthymic rats. In iPAH, regulatory T cells (T<sub>reg</sub> cells) are increased, whereas CD8<sup>+</sup> cytotoxic T cells are decreased.<sup>187</sup> T<sub>reg</sub> cells maintain immunotolerance and are potent inhibitors of antitumor and possibly antiviral immune responses. The increase in T<sub>reg</sub> 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<sup>188</sup>; 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,<sup>189</sup> or a vector for cell-based therapy. Circulating EPC numbers (defined by CD34<sup>+</sup>/KDR<sup>+</sup>-positive and CD34<sup>+</sup>/CD133<sup>+</sup>/KDR<sup>+</sup>-positive cells) are significantly lower in patients with Eisenmenger syndrome than in normal

control subjects.<sup>190</sup> 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.<sup>190–193</sup> Whether these differences are beneficial, promoting revascularization in the hypertensive lung,<sup>102</sup> or contribute to the pathology, by augmenting pulmonary vascular remodeling,<sup>192</sup> 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,<sup>190</sup> and potential new therapies for PAH, such as statins and PPAR $\gamma$  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.<sup>102</sup> 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*).<sup>100–102</sup> Even fibroblasts can be made somewhat therapeutic when they are transfected with VEGF. These modified fibroblasts prevent worsening of monocrotaline-induced PAH.<sup>102</sup>

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.<sup>194,195</sup> A therapeutic trial (PHAcET [Pulmonary Hypertension: Assessment of Cell Therapy], ClinicalTrials.gov identifier NCT00469027) to assess 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 $\alpha$ ); 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.

### References

- Simonneau G, Robbins IM, Beghetti M, Channick RN, Delcroix M, Denton CP, Elliott CG, Gaine SP, Gladwin MT, Jing ZC, Krowka MJ, Langleben D, Nakanishi N, Souza R. Updated clinical classification of pulmonary hypertension. *J Am Coll Cardiol*. 2009;54:S43–S54.
- Humbert M, Sitbon O, Chaouat A, Bertocchi M, Habib G, Gressin V, Yaici A, Weitzenblum E, Cordier JF, Chabot F, Dromer C, Pison C, Reynaud-Gaubert M, Haloun A, Laurent M, Hachulla E, Simonneau G. Pulmonary arterial hypertension in France: results from a national registry. *Am J Respir Crit Care Med*. 2006;173:1023–1030.
- Peacock AJ, Murphy NF, McMurray JJ, Caballero L, Stewart S. An epidemiological study of pulmonary arterial hypertension. *Eur Respir J*. 2007;30:104–109.
- Butrous G, Ghofrani HA, Grimminger F. Pulmonary vascular disease in the developing world. *Circulation*. 2008;118:1758–1766.
- Fruchter O, Yigla M. Underlying aetiology of pulmonary hypertension in 191 patients: a single centre experience. *Respirology*. 2008;13:825–831.
- Robbins IM, Newman JH, Johnson RF, Hemnes AR, Fremont RD, Piana RN, Zhao DX, Byrne DW. Association of the metabolic syndrome with pulmonary venous hypertension. *Chest*. 2009;136:31–36.
- Thenappan T, Shah SJ, Rich S, Gombert-Maitland M. A USA-based registry for pulmonary arterial hypertension: 1982–2006. *Eur Respir J*. 2007;30:1103–1110.
- Hopkins WE, Ochoa LL, Richardson GW, Trulock EP. Comparison of the hemodynamics and survival of adults with severe primary pulmonary hypertension or Eisenmenger syndrome. *J Heart Lung Transplant*. 1996;15:100–105.
- Kuhn KP, Byrne DW, Arbogast PG, Doyle TP, Loyd JE, Robbins IM. Outcome in 91 consecutive patients with pulmonary arterial hypertension receiving epoprostenol. *Am J Respir Crit Care Med*. 2003;167:580–586.
- Condliffe R, Kiely DG, Peacock AJ, Corris PA, Gibbs JS, Vrapic F, Das C, Elliot CA, Johnson M, DeSoyza J, Torpy C, Goldsmith K, Hodgkins D, Hughes RJ, Pepke-Zaba J, Coghlan JG. Connective tissue disease-associated pulmonary arterial hypertension in the modern treatment era. *Am J Respir Crit Care Med*. 2009;179:151–157.
- Sitbon O, Humbert M, Jais X, Iosif V, Hamid AM, Provencher S, Garcia G, Parent F, Herve P, Simonneau G. Long-term response to calcium channel blockers in idiopathic pulmonary arterial hypertension. *Circulation*. 2005;111:3105–3111.
- Barst RJ, Rubin LJ, Long WA, McGoan MD, Rich S, Badesch DB, Groves BM, Tapson VF, Bourge RC, Brundage BH, Koerner SK, Langleben D, Keller CA, Murali S, Uretsky BF, Clayton LM, Jöbsis MM, Blackburn SD, Shortino D, Crow JW; Primary Pulmonary Hypertension Study Group. A comparison of continuous intravenous epoprostenol (prostacyclin) with conventional therapy for primary pulmonary hypertension. *N Engl J Med*. 1996;334:296–302.
- Tuder RM, Chacon M, Alger L, Wang J, Taraseviciene-Stewart L, Kasahara Y, Cool CD, Bishop AE, Geraci M, Semenza GL, Yacoub M, Polak JM, Voelkel NF. Expression of angiogenesis-related molecules in plexiform lesions in severe pulmonary hypertension: evidence for a process of disordered angiogenesis. *J Pathol*. 2001;195:367–374.
- Levy NT, Liapis H, Eisenberg PR, Botney MD, Trulock EP. Pathologic regression of primary pulmonary hypertension in left native lung following right single-lung transplantation. *J Heart Lung Transplant*. 2001;20:381–384.
- Nishimura T, Faul JL, Berry GJ, Vaszar LT, Qiu D, Pearl RG, Kao PN. Simvastatin attenuates smooth muscle neointimal proliferation and pulmonary hypertension in rats. *Am J Respir Crit Care Med*. 2002;166:1403–1408.
- van Albada ME, du Marchie Sarvaas GJ, Koster J, Houwertjes MC, Berger RM, Schoemaker RG. Effects of erythropoietin on advanced pulmonary vascular remodelling. *Eur Respir J*. 2008;31:126–134.
- Sato K, Webb S, Tucker A, Rabinovitch M, O'Brien RF, McMurtry IF, Stelzner TJ. Factors influencing the idiopathic development of pulmonary hypertension in the fawn hooded rat. *Am Rev Respir Dis*. 1992;145:793–797.
- Bonnet S, Michelakis ED, Porter CJ, Andrade-Navarro MA, Thébaud B, Bonnet SN, Haromy A, Harry G, Moudgil R, McMurtry MS, Weir E, Archer SL. An abnormal mitochondrial-HIF-1-Kv channel pathway disrupts oxygen-sensing and triggers pulmonary arterial hypertension (PAH) in fawn-hooded rats: similarities to human PAH. *Circulation*. 2006;113:2630–2641.
- Sakao S, Taraseviciene-Stewart L, Lee JD, Wood K, Cool CD, Voelkel NF. Initial apoptosis is followed by increased proliferation of apoptosis-resistant endothelial cells. *FASEB J*. 2005;19:1178–1180.
- Guignabert C, Izikki M, Tu LI, Li Z, Zadigue P, Barlier-Mur AM, Hanoun N, Rodman D, Hamon M, Adnot S, Eddahibi S. Transgenic mice overexpressing the 5-hydroxytryptamine transporter gene in smooth muscle develop pulmonary hypertension. *Circ Res*. 2006;98:1323–1330.

21. West J, Fagan K, Steudel W, Fouty B, Lane K, Harral J, Hoedt-Miller M, Tada Y, Ozimek J, Tuder R, Rodman DM. Pulmonary hypertension in transgenic mice expressing a dominant-negative BMPRII gene in smooth muscle. *Circ Res*. 2004;94:1109–1114.
22. Greenway S, van Suylen RJ, Du Marchie Sarvaas G, Kwan E, Ambarsumian N, Lukanidin E, Rabinovitch M. S100A4/Mts1 produces murine pulmonary artery changes resembling plexogenic arteriopathy and is increased in human plexogenic arteriopathy. *Am J Pathol*. 2004;164:253–262.
23. Herve P, Launay JM, Scrobobaci ML, Brenot F, Simonneau G, Petitpretz P, Poubreau P, Cerrina J, Duroux P, Drouet L. Increased plasma serotonin in primary pulmonary hypertension. *Am J Med*. 1995;99:249–254.
24. Christman BW, McPherson CD, Newman JH, King GA, Bernard GR, Groves BM, Loyd JE. An imbalance between the excretion of thromboxane and prostacyclin metabolites in pulmonary hypertension. *N Engl J Med*. 1992;327:70–75.
25. Steudel W, Ichinose F, Huang PL, Hurford WE, Jones RC, Bevan JA, Fishman MC, Zapol WM. Pulmonary vasoconstriction and hypertension in mice with targeted disruption of the endothelial nitric oxide synthase (NOS 3) gene. *Circ Res*. 1997;81:34–41.
26. Stewart DJ, Levy RD, Cernacek P, Langleben D. Increased plasma endothelin-1 in pulmonary hypertension: marker or mediator of disease? *Ann Intern Med*. 1991;114:464–469.
27. White RJ, Meoli DF, Swarthout RF, Kallop DY, Galaria II, Harvey JL, Miller CM, Blaxall BC, Hall CM, Pierce RA, Cool CD, Taubman MB. Plexiform-like lesions and increased tissue factor expression in a rat model of severe pulmonary arterial hypertension. *Am J Physiol Lung Cell Mol Physiol*. 2007;293:L583–L590.
28. Morrell NW, Yang X, Upton PD, Jourdan KB, Morgan N, Sheares KK, Trembath RC. Altered growth responses of pulmonary artery smooth muscle cells from patients with primary pulmonary hypertension to transforming growth factor-beta(1) and bone morphogenetic proteins. *Circulation*. 2001;104:790–795.
29. McMurtry MS, Moudgil R, Hashimoto K, Bonnet S, Michelakis ED, Archer SL. Overexpression of human bone morphogenetic protein receptor 2 does not ameliorate monocrotaline pulmonary arterial hypertension. *Am J Physiol Lung Cell Mol Physiol*. 2007;292:L872–L878.
30. McMurtry MS, Archer SL, Altieri DC, Bonnet S, Haromy A, Harry G, Bonnet S, Puttagunta L, Michelakis ED. Gene therapy targeting survivin selectively induces pulmonary vascular apoptosis and reverses pulmonary arterial hypertension. *J Clin Invest*. 2005;115:1479–1491.
31. Eddahibi S, Raffestin B, Hamon M, Adnot S. Is the serotonin transporter involved in the pathogenesis of pulmonary hypertension? *J Lab Clin Med*. 2002;139:194–201.
32. Schermuly RT, Dony E, Ghofrani HA, Pullamsetti S, Savai R, Roth M, Sydykov A, Lai YJ, Weissmann N, Seeger W, Grimminger F. Reversal of experimental pulmonary hypertension by PDGF inhibition. *J Clin Invest*. 2005;115:2811–2821.
33. Moreno-Vinasco L, Gomber-Maitland M, Maitland M, Desai A, Singleton P, Sammani S, Sam L, Liu Y, Husain A, Lang R, Ratain M, Lussier Y, Garcia J. Genomic assessment of a multikinase inhibitor, sorafenib, in a rodent model of pulmonary hypertension. *Physiol Genomics*. 2008;33:278–291.
34. Yuan XJ, Wang J, Juhaszova M, Gaine SP, Rubin LJ. Attenuated K<sup>+</sup> channel gene transcription in primary pulmonary hypertension. *Lancet*. 1998;351:726–727.
35. Reeve HL, Michelakis E, Nelson DP, Weir EK, Archer SL. Alterations in a redox oxygen sensing mechanism in chronic hypoxia. *J Appl Physiol*. 2001;90:2249–2256.
36. Michelakis ED, McMurtry MS, Wu XC, Dyck JR, Moudgil R, Hopkins TA, Lopaschuk GD, Puttagunta L, Waite R, Archer SL. Dichloroacetate, a metabolic modulator, prevents and reverses chronic hypoxic pulmonary hypertension in rats: role of increased expression and activity of voltage-gated potassium channels. *Circulation*. 2002;105:244–250.
37. Young KA, Ivester C, West J, Carr M, Rodman DM. BMP signaling controls PASMVC KV channel expression in vitro and in vivo. *Am J Physiol Lung Cell Mol Physiol*. 2006;290:L841–L848.
38. Archer SL, Wu XC, Thebaud B, Nsair A, Bonnet S, Tyrrell B, McMurtry MS, Hashimoto K, Harry G, Michelakis ED. Preferential expression and function of voltage-gated, O<sub>2</sub>-sensitive K<sup>+</sup> channels in resistance pulmonary arteries explains regional heterogeneity in hypoxic pulmonary vasoconstriction: ionic diversity in smooth muscle cells. *Circ Res*. 2004;95:308–318.
39. Landsberg JW, Yuan JX. Calcium and TRP channels in pulmonary vascular smooth muscle cell proliferation. *News Physiol Sci*. 2004;19:44–50.
40. Bonnet S, Rochefort G, Sutendra G, Archer SL, Haromy A, Webster L, Hashimoto K, Bonnet SN, Michelakis ED. The nuclear factor of activated T cells in pulmonary arterial hypertension can be therapeutically targeted. *Proc Natl Acad Sci U S A*. 2007;104:11418–11423.
41. Cowan KN, Jones PL, Rabinovitch M. Elastase and matrix metalloproteinase inhibitors induce regression, and tenascin-C antisense prevents progression, of vascular disease. *J Clin Invest*. 2000;105:21–34.
42. Welsh DJ, Harnett M, MacLean M, Peacock AJ. Proliferation and signaling in fibroblasts: role of 5-hydroxytryptamine<sub>2A</sub> receptor and transporter. *Am J Respir Crit Care Med*. 2004;170:252–259.
43. Dorfmueller P, Perros F, Balabanian K, Humbert M. Inflammation in pulmonary arterial hypertension. *Eur Respir J*. 2003;22:358–363.
44. Davie NJ, Crossno JT Jr, Frid MG, Hofmeister SE, Reeves JT, Hyde DM, Carpenter TC, Brunetti JA, McNiece IK, Stenmark KR. Hypoxia-induced pulmonary artery adventitial remodeling and neovascularization: contribution of progenitor cells. *Am J Physiol Lung Cell Mol Physiol*. 2004;286:L668–L678.
45. Deng Z, Morse JH, Slager SL, Cuervo N, Moore KJ, Venetos G, Kalachikov S, Cayanis E, Fischer SG, Barst RJ, Hodge SE, Knowles JA. Familial primary pulmonary hypertension (gene PPH1) is caused by mutations in the bone morphogenetic protein receptor-II gene. *Am J Hum Genet*. 2000;67:737–744.
46. Thomson JR, Machado RD, Pauculo MW, Morgan NV, Humbert M, Elliott GC, Ward K, Yacoub M, Mikhail G, Rogers P, Newman J, Wheeler L, Higenbottam T, Gibbs JS, Egan J, Crozier A, Peacock A, Allcock R, Corris P, Loyd JE, Trembath RC, Nichols WC. Sporadic primary pulmonary hypertension is associated with germline mutations of the gene encoding BMPRII, a receptor member of the TGF-beta family. *J Med Genet*. 2000;37:741–745.
47. Lane KB, Machado RD, Pauculo MW, Thomson JR, Phillips JA III, Loyd JE, Nichols WC, Trembath RC; International PPH Consortium. Heterozygous germline mutations in BMPR2, encoding a TGF-beta receptor, cause familial primary pulmonary hypertension. *Nat Genet*. 2000;26:81–84.
48. Yang J, Davies RJ, Southwood M, Long L, Yang X, Sobolewski A, Upton PD, Trembath RC, Morrell NW. Mutations in bone morphogenetic protein type II receptor cause dysregulation of Id gene expression in pulmonary artery smooth muscle cells: implications for familial pulmonary arterial hypertension. *Circ Res*. 2008;102:1212–1221.
49. Zhang S, Fantozzi I, Tigno DD, Yi ES, Platoshyn O, Thistlethwaite PA, Kriett JM, Yung G, Rubin LJ, Yuan JX. Bone morphogenetic proteins induce apoptosis in human pulmonary vascular smooth muscle cells. *Am J Physiol Lung Cell Mol Physiol*. 2003;285:L740–L754.
50. Attisano L, Wrana JL. Signal transduction by the TGF-beta superfamily. *Science*. 2002;296:1646–1647.
51. Hong KH, Lee YJ, Lee E, Park SO, Han C, Beppu H, Li E, Raizada MK, Bloch KD, Oh SP. Genetic ablation of the BMPR2 gene in pulmonary endothelium is sufficient to predispose to pulmonary arterial hypertension. *Circulation*. 2008;118:722–730.
52. Newman JH, Trembath RC, Morse JA, Grunig E, Loyd JE, Adnot S, Cocco F, Ventura C, Phillips JA III, Knowles JA, Janssen B, Eickelberg O, Eddahibi S, Herve P, Nichols WC, Elliott G. Genetic basis of pulmonary arterial hypertension: current understanding and future directions. *J Am Coll Cardiol*. 2004;43:33S–39S.
53. Nunes H, Humbert M, Sitbon O, Morse JH, Deng Z, Knowles JA, Le Gall C, Parent F, Garcia G, Herve P, Barst RJ, Simonneau G. Prognostic factors for survival in human immunodeficiency virus-associated pulmonary arterial hypertension. *Am J Respir Crit Care Med*. 2003;167:1433–1439.
54. Long L, MacLean MR, Jeffery TK, Morecroft I, Yang X, Rudarakanchana N, Southwood M, James V, Trembath RC, Morrell NW. Serotonin increases susceptibility to pulmonary hypertension in BMPR2-deficient mice. *Circ Res*. 2006;98:818–827.
55. Brock M, Trenkmann M, Gay RE, Michel BA, Gay S, Fischler M, Ulrich S, Speich R, Huber LC. Interleukin-6 modulates the expression of the bone morphogenetic protein receptor type II through a novel STAT3-microRNA cluster 17/92 pathway. *Circ Res*. 2009;104:1184–1191.
56. Reynolds AM, Xia W, Holmes MD, Hodge SJ, Danilov S, Curiel DT, Morrell NW, Reynolds PN. Bone morphogenetic protein type 2 receptor gene therapy attenuates hypoxic pulmonary hypertension. *Am J Physiol Lung Cell Mol Physiol*. 2007;292:L1182–L1192.

57. Long L, Crosby A, Yang X, Southwood M, Upton PD, Kim DK, Morrell NW. Altered bone morphogenetic protein and transforming growth factor-beta signaling in rat models of pulmonary hypertension: potential for activin receptor-like kinase-5 inhibition in prevention and progression of disease. *Circulation*. 2009;119:566-576.
58. Eddahibi S, Chauvat A, Morrell N, Fadel E, Fuhrman C, Bugnet A, Darteville P, Housset B, Hamon M, Weitzenblum E, Adnot S. Polymorphism of the serotonin transporter gene and pulmonary hypertension in chronic obstructive pulmonary disease. *Circulation*. 2003;108:1839-1844.
59. Remillard CV, Tigno DD, Platoshyn O, Burg ED, Brevnova EE, Conger D, Nicholson A, Rana BK, Channick RN, Rubin LJ, O'Connor DT, Yuan JX. Function of Kv1.5 channels and genetic variations of KCNA5 in patients with idiopathic pulmonary arterial hypertension. *Am J Physiol Cell Physiol*. 2007;292:C1837-C1853.
60. Yu Y, Keller SH, Remillard CV, Safrina O, Nicholson A, Zhang SL, Jiang W, Vangala N, Landsberg JW, Wang JY, Thistlethwaite PA, Channick RN, Robbins IM, Loyd JE, Ghofrani HA, Grimminger F, Schermuly RT, Cahalan MD, Rubin LJ, Yuan JX. A functional single-nucleotide polymorphism in the TRPC6 gene promoter associated with idiopathic pulmonary arterial hypertension. *Circulation*. 2009;119:2313-2322.
61. Archer SL, Gombert-Maitland M, Maitland ML, Rich S, Garcia JG, Weir EK. Mitochondrial metabolism, redox signaling, and fusion: a mitochondria-ROS-HIF-1 $\alpha$ -Kv1.5 O<sub>2</sub>-sensing pathway at the intersection of pulmonary hypertension and cancer. *Am J Physiol Heart Circ Physiol*. 2008;294:H570-H578.
62. Voelkel NF, Cool C, Lee SD, Wright L, Geraci MW, Tudor RM. Primary pulmonary hypertension between inflammation and cancer. *Chest*. 1998;114:225S-230S.
63. Bonnet S, Archer SL, Allalunis-Turner J, Haromy A, Beaulieu C, Thompson R, Lee CT, Lopaschuk GD, Puttagunta L, Bonnet S, Harry G, Hashimoto K, Porter CJ, Andrade MA, Thebaud B, Michelakis ED. A mitochondria-K<sup>+</sup> channel axis is suppressed in cancer and its normalization promotes apoptosis and inhibits cancer growth. *Cancer Cell*. 2007;11:37-51.
64. Kim JW, Tchernyshyov I, Semenza GL, Dang CV. HIF-1-mediated expression of pyruvate dehydrogenase kinase: a metabolic switch required for cellular adaptation to hypoxia. *Cell Metab*. 2006;3:177-185.
65. Weir EK, Lopez-Barneo J, Buckler KJ, Archer SL. Acute oxygen-sensing mechanisms. *N Engl J Med*. 2005;353:2042-2055.
66. McMurtry MS, Bonnet S, Wu X, Dyck JR, Haromy A, Hashimoto K, Michelakis ED. Dichloroacetate prevents and reverses pulmonary hypertension by inducing pulmonary artery smooth muscle cell apoptosis. *Circ Res*. 2004;95:830-840.
67. Guignabert C, Tu L, Izikki M, Dewachter L, Zadigue P, Humbert M, Adnot S, Fadel E, Eddahibi S. Dichloroacetate treatment partially regresses established pulmonary hypertension in mice with SM22 $\alpha$ -targeted overexpression of the serotonin transporter. *FASEB J*. 2009;23:4135-4147.
68. Nagendran J, Archer SL, Soliman D, Gurtu V, Moudgil R, Haromy A, St Aubin C, Webster L, Rebeyka IM, Ross DB, Light PE, Dyck JR, Michelakis ED. Phosphodiesterase type 5 is highly expressed in the hypertrophied human right ventricle, and acute inhibition of phosphodiesterase type 5 improves contractility. *Circulation*. 2007;116:238-248.
69. Sharma S, Taegtmeier H, Adroge J, Razeghi P, Sen S, Ngumbela K, Essop MF. Dynamic changes of gene expression in hypoxia-induced right ventricular hypertrophy. *Am J Physiol Heart Circ Physiol*. 2004;286:H1185-H1192.
70. Young LH, Li J, Baron SJ, Russell RR. AMP-activated protein kinase: a key stress signaling pathway in the heart. *Trends Cardiovasc Med*. 2005;15:110-118.
71. Evans AM. AMP-activated protein kinase and the regulation of Ca<sup>2+</sup> signalling in O<sub>2</sub>-sensing cells. *J Physiol*. 2006;574:113-123.
72. Allard MF, Parsons HL, Saedi R, Wambolt RB, Brownsey R. AMPK and metabolic adaptation by the heart to pressure overload. *Am J Physiol Heart Circ Physiol*. 2007;292:H140-H148.
73. Nascimben L, Ingwall JS, Lorell BH, Pinz I, Schultz V, Tornheim K, Tian R. Mechanisms for increased glycolysis in the hypertrophied rat heart. *Hypertension*. 2004;44:662-667.
74. Bayrak F, Komurcu-Bayrak E, Mutlu B, Kahveci G, Basaran Y, Erginel-Nalutuna N. Ventricular pre-excitation and cardiac hypertrophy mimicking hypertrophic cardiomyopathy in a Turkish family with a novel PRKAG2 mutation. *Eur J Heart Fail*. 2006;8:712-715.
75. van Wolferen SA, Marcus JT, Westerhof N, Spreeuwenberg MD, Marques KM, Bronzwaer JG, Henkens IR, Gan CT, Boonstra A, Postmus PE, Vonk-Noordegraaf A. Right coronary artery flow impairment in patients with pulmonary hypertension. *Eur Heart J*. 2008;29:120-127.
76. Piao L, Fang YH, Cadete VJ, Wietholt C, Urboniene D, Toth PT, Marsboom G, Zhang HJ, Haber I, Rehman J, Lopaschuk GD, Archer SL. The inhibition of pyruvate dehydrogenase kinase improves impaired cardiac function and electrical remodeling in two models of right ventricular hypertrophy: resuscitating the hibernating right ventricle. *J Mol Med*. 2010;88:47-60.
77. Rich S, Brundage B. High dose calcium blocking therapy for primary pulmonary hypertension: evidence for long-term reduction in pulmonary arterial pressure and regression of right ventricular hypertrophy. *Circulation*. 1987;76:135-141.
78. Fuster V, Steele PM, Edwards WD, Gersh BJ, McGoon MD, Frye RL. Primary pulmonary hypertension: natural history and the importance of thrombosis. *Circulation*. 1984;70:580-587.
79. Rich S, Kaufmann E, Levy PS. The effect of high doses of calcium-channel blockers on survival in primary pulmonary hypertension. *N Engl J Med*. 1992;327:76-81.
80. Rubin L, Mendoza J, Hood M, McGoon M, Barst R, Williams W, Diehl J, Crow J, Long W. Treatment of primary pulmonary hypertension with continuous intravenous prostacyclin (epoprostenol): results of a randomized trial. *Ann Intern Med*. 1990;112:485-491.
81. Channick R, Simonneau G, Sitbon O, Robbins I, Frost A, Tapson V, Badesch D, Roux S, Rainisio M, Bodin F, Rubin L. Effects of the dual endothelin-receptor antagonist bosentan in patients with pulmonary hypertension: a randomised placebo-controlled study. *Lancet*. 2001;358:1119-1123.
82. Barst RJ, Langleben D, Badesch D, Frost A, Lawrence EC, Shapiro S, Naeije R, Galie N. Treatment of pulmonary arterial hypertension with the selective endothelin-A receptor antagonist sitaxsentan. *J Am Coll Cardiol*. 2006;47:2049-2056.
83. Galie N, Ghofrani HA, Torbicki A, Barst RJ, Rubin LJ, Badesch D, Fleming T, Parpia T, Burgess G, Branzi A, Grimminger F, Kurzyna M, Simonneau G. Sildenafil citrate therapy for pulmonary arterial hypertension. *N Engl J Med*. 2005;353:2148-2157.
84. Perez-Penate G, Julia-Serda G, Ojeda-Betancort N, Garcia-Quintana A, Pulido-Duque J, Rodriguez-Perez A, Cabrera-Navarro P, Gomez-Sanchez M. Long-term inhaled nitric oxide plus phosphodiesterase 5 inhibitors for severe pulmonary hypertension. *J Heart Lung Transplant*. 2008;27:1326-1332.
85. Oka M, Homma N, Taraseviciene-Stewart L, Morris KG, Kraskauskas D, Burns N, Voelkel NF, McMurtry IF. Rho kinase-mediated vasoconstriction is important in severe occlusive pulmonary arterial hypertension in rats. *Circ Res*. 2007;100:923-929.
86. Ishikura K, Yamada N, Ito M, Ota S, Nakamura M, Isaka N, Nakano T. Beneficial acute effects of rho-kinase inhibitor in patients with pulmonary arterial hypertension. *Circ J*. 2006;70:174-178.
87. Ghofrani H, Seeger W, Grimminger F. Imatinib for the treatment of pulmonary arterial hypertension. *N Engl J Med*. 2005;353:1412-1413.
88. Gombert-Maitland M, Maitland ML, Barst RJ, Sugeng L, Coslet S, Perrino TJ, Bond L, Lacouture ME, Archer SL, Ratain MJ. A dosing/cross-development study of the multikinase inhibitor sorafenib in patients with pulmonary arterial hypertension. *Clin Pharmacol Ther*. 2010;87:303-310.
89. Suzuki K, Takahashi M, Morimoto H, Izawa A, Ise H, Hongo M, Hoshikawa Y, Ito T, Miyashita H, Kobayashi E, Shimada K, Ikeda U. Mycophenolate mofetil attenuates pulmonary arterial hypertension in rats. *Biochem Biophys Res Commun*. 2006;349:781-788.
90. Bonnet S, Dumas de La Roque E, Begueret H, Marthan R, Fayon M, Dos Santos P, Savineau JP, Baulieu EE. Dehydroepiandrosterone (DHEA) prevents and reverses chronic hypoxic pulmonary hypertension. *Proc Natl Acad Sci U S A*. 2003;100:9488-9493.
91. Mittendorf J, Weigand S, Alonso-Alija C, Bischoff E, Feurer A, Gerisch M, Kern A, Knorr A, Lang D, Muenster K, Radtke M, Schirok H, Schlemmer KH, Stahl E, Straub A, Wunder F, Stasch JP. Discovery of Riociguat (BAY 63-2521): a potent, oral stimulator of soluble guanylate cyclase for the treatment of pulmonary hypertension. *ChemMedChem*. 2009;4:853-865.
92. Hacker AD. Inhibition of deoxyribonucleic acid synthesis by difluoromethylornithine: role of polyamine metabolism in monocrotaline-induced pulmonary hypertension. *Biochem Pharmacol*. 1992;44:965-971.

93. Olson J, Hacker A, Altieri R, Gillelspeie M. Polyamines and the development of monocrotaline-induced pulmonary hypertension. *Am J Physiol*. 1984;247:H682–H685.
94. Thompson BT, Spence CR, Janssens SP, Joseph PM, Hales CA. Inhibition of hypoxic pulmonary hypertension by heparins of differing in vitro antiproliferative potency. *Am J Respir Crit Care Med*. 1994;149:1512–1517.
95. Hansmann G, Wagner RA, Schellong S, Perez VA, Urashima T, Wang L, Sheikh AY, Suen RS, Stewart DJ, Rabinovitch M. Pulmonary arterial hypertension is linked to insulin resistance and reversed by peroxisome proliferator-activated receptor- $\gamma$  activation. *Circulation*. 2007;115:1275–1284.
96. Kawut S, Horn E, Berekashvili K, Lederer D, Widlitz A, Rosenzweig E, Barst R. Selective serotonin reuptake inhibitor use and outcomes in pulmonary arterial hypertension. *Pulm Pharmacol Ther*. 2006;19:370–374.
97. Petkov V, Mosgoeller W, Ziesche R, Raderer M, Stiebellehner L, Vonbank K, Funk G, Hamilton G, Novotny C, Burian B, Block L. Vasoactive intestinal peptide as a new drug for treatment of primary pulmonary hypertension. *J Clin Invest*. 2003;111:1339–1346.
98. Nagaya N, Kyotani S, Uematsu M, Ueno K, Oya H, Nakanishi N, Shirai M, Mori H, Miyatake K, Kangawa K. Effects of adrenomedullin inhalation on hemodynamics and exercise capacity in patients with idiopathic pulmonary arterial hypertension. *Circulation*. 2004;109:351–356.
99. Nagaya N, Okumura H, Uematsu M, Shimzu W, Ono F, Shirai M, Mori H, Miyatake K, Kangawa K. Repeated inhalation of adrenomedullin ameliorates pulmonary hypertension and survival in monocrotaline rats. *Am J Physiol Heart Circ Physiol*. 2003;285:H2125–H2131.
100. Nagaya N, Kangawa K, Kanda M, Uematsu M, Horio T, Fukuyama N, Hino J, Harada-Shiba M, Okumura H, Tabata Y, Mochizuki N, Chiba Y, Nishioka K, Miyatake K, Asahara T, Hara H, Mori H. Hybrid cell-gene therapy for pulmonary hypertension based on phagocytosing action of endothelial progenitor cells. *Circulation*. 2003;108:889–895.
101. Zhao Y, Courtman D, Deng Y, Kugathasan L, Zhang Q, Stewart D. Rescue of monocrotaline-induced pulmonary arterial hypertension using bone marrow-derived endothelial-like progenitor cells: efficacy of combined cell and eNOS gene therapy in established disease. *Circ Res*. 2005;96:442–450.
102. Zhao YD, Courtman DW, Ng DS, Robb MJ, Deng YP, Trogadis J, Han RN, Stewart DJ. Microvascular regeneration in established pulmonary hypertension by angiogenic gene transfer. *Am J Respir Cell Mol Biol*. 2006;35:182–189.
103. Ghofrani HA, Wiedemann R, Rose F, Olschewski H, Schermuly RT, Weissmann N, Seeger W, Grimminger F. Combination therapy with oral sildenafil and inhaled iloprost for severe pulmonary hypertension. *Ann Intern Med*. 2002;136:515–522.
104. Simonneau G, Rubin LJ, Galie N, Barst RJ, Fleming TR, Frost AE, Engel PJ, Kramer MR, Burgess G, Collings L, Cossons N, Sitbon O, Badesch DB. Addition of sildenafil to long-term intravenous epoprostenol therapy in patients with pulmonary arterial hypertension: a randomized trial. *Ann Intern Med*. 2008;149:521–530.
105. Giaid A, Saleh D. Reduced expression of endothelial nitric oxide synthase in the lungs of patients with pulmonary hypertension. *N Engl J Med*. 1995;333:214–221.
106. Archer SL, Djaballah K, Humbert M, Weir KE, Fartoukh M, Dall'ava-Santucci J, Mercier JC, Simonneau G, Dinh-Xuan AT. Nitric oxide deficiency in fenfluramine- and dexfenfluramine-induced pulmonary hypertension. *Am J Respir Crit Care Med*. 1998;158:1061–1067.
107. Murray F, MacLean MR, Pyne NJ. Increased expression of the cGMP-inhibited cAMP-specific (PDE3) and cGMP binding cGMP-specific (PDE5) phosphodiesterases in models of pulmonary hypertension. *Br J Pharmacol*. 2002;137:1187–1194.
108. Bulau P, Zakrzewicz D, Kitowska K, Leiper J, Gunther A, Grimminger F, Eickelberg O. Analysis of methylarginine metabolism in the cardiovascular system identifies the lung as a major source of ADMA. *Am J Physiol Lung Cell Mol Physiol*. 2007;292:L18–L24.
109. Pullamsetti S, Kiss L, Ghofrani HA, Voswinckel R, Haredza P, Klepetko W, Aigner C, Fink L, Muey JP, Weissmann N, Grimminger F, Seeger W, Schermuly RT. Increased levels and reduced catabolism of asymmetric and symmetric dimethylarginines in pulmonary hypertension. *FASEB J*. 2005;19:1175–1177.
110. Fagan KA, McMurtry I, Rodman DM. Nitric oxide synthase in pulmonary hypertension: lessons from knockout mice. *Physiol Res*. 2000;49:539–548.
111. Khoo JP, Zhao L, Alp NJ, Bendall JK, Nicoli T, Rockett K, Wilkins MR, Channon KM. Pivotal role for endothelial tetrahydrobiopterin in pulmonary hypertension. *Circulation*. 2005;111:2126–2133.
112. Leiper J, Nandi M, Torondel B, Murray-Rust J, Malaki M, O'Hara B, Rossiter S, Anthony S, Madhani M, Selwood D, Smith C, Wojciak-Stothard B, Rudiger A, Stidwill R, McDonald NQ, Vallance P. Disruption of methylarginine metabolism impairs vascular homeostasis. *Nat Med*. 2007;13:198–203.
113. Celermajer DS, Dollery C, Burch M, Deanfield JE. Role of endothelium in the maintenance of low pulmonary vascular tone in normal children. *Circulation*. 1994;89:2041–2044.
114. Stamler JS, Loh E, Roddy MA, Currie KE, Creager MA. Nitric oxide regulates basal systemic and pulmonary vascular resistance in healthy humans. *Circulation*. 1994;89:2035–2040.
115. Hampl V, Archer SL, Nelson DP, Weir EK. Chronic EDRF inhibition and hypoxia: effects on pulmonary circulation and systemic blood pressure. *J Appl Physiol*. 1993;75:1748–1757.
116. Bloch KD, Ichinose F, Roberts JD Jr, Zapol WM. Inhaled NO as a therapeutic agent. *Cardiovasc Res*. 2007;75:339–348.
117. Hampl V, Tristani-Firouzi M, Hutsell TC, Archer SL. Nebulized nitric oxide/nucleophile adduct reduces chronic pulmonary hypertension. *Cardiovasc Res*. 1996;31:55–62.
118. Lam CF, Van Heerden PV, Blott J, Roberts B, Ilett KF. The selective pulmonary vasodilatory effect of inhaled DETA/NO, a novel nitric oxide donor, in ARDS: a pilot human trial. *J Crit Care*. 2004;19:48–53.
119. Conti M, Beavo J. Biochemistry and physiology of cyclic nucleotide phosphodiesterases: essential components in cyclic nucleotide signaling. *Annu Rev Biochem*. 2007;76:481–511.
120. Schermuly RT, Pullamsetti SS, Kwapiszewska G, Dumitrascu R, Tian X, Weissmann N, Ghofrani HA, Kaulen C, Dunkern T, Schudt C, Voswinckel R, Zhou J, Samidurai A, Klepetko W, Paddenberg R, Kummer W, Seeger W, Grimminger F. Phosphodiesterase 1 upregulation in pulmonary arterial hypertension: target for reverse-remodeling therapy. *Circulation*. 2007;115:2331–2339.
121. Michelakis E, Tymchak W, Lien D, Webster L, Hashimoto K, Archer S. Oral sildenafil is an effective and specific pulmonary vasodilator in patients with pulmonary arterial hypertension: comparison with inhaled nitric oxide. *Circulation*. 2002;105:2398–2403.
122. Archer SL, Michelakis ED. Phosphodiesterase type 5 inhibitors for pulmonary arterial hypertension. *N Engl J Med*. 2009;361:1862–1869.
123. Schermuly RT, Ghofrani HA, Enke B, Weissmann N, Grimminger F, Seeger W, Schudt C, Walrath D. Low-dose systemic phosphodiesterase inhibitors amplify the pulmonary vasodilatory response to inhaled prostacyclin in experimental pulmonary hypertension. *Am J Respir Crit Care Med*. 1999;160:1500–1506.
124. Schermuly RT, Roehl A, Weissmann N, Ghofrani HA, Schudt C, Tenor H, Grimminger F, Seeger W, Walrath D. Subthreshold doses of specific phosphodiesterase type 3 and 4 inhibitors enhance the pulmonary vasodilatory response to nebulized prostacyclin with improvement in gas exchange. *J Pharmacol Exp Ther*. 2000;292:512–520.
125. Schermuly RT, Stasch JP, Pullamsetti SS, Middendorff R, Muller D, Schluter KD, Dingendorf A, Hackemack S, Kolosonek E, Kaulen C, Dumitrascu R, Weissmann N, Mittendorf J, Klepetko W, Seeger W, Ghofrani HA, Grimminger F. Expression and function of soluble guanylate cyclase in pulmonary arterial hypertension. *Eur Respir J*. 2008;32:881–891.
126. Vermeersch P, Buys E, Pokreisz P, Marsboom G, Ichinose F, Sips P, Pellens M, Gillijns H, Swinnen M, Graveline A, Collen D, Dewerchin M, Brouckaert P, Bloch KD, Janssens S. Soluble guanylate cyclase- $\alpha$  deficiency selectively inhibits the pulmonary vasodilator response to nitric oxide and increases the pulmonary vascular remodeling response to chronic hypoxia. *Circulation*. 2007;116:936–943.
127. Evgenov OV, Pacher P, Schmidt PM, Hasko G, Schmidt HH, Stasch JP. NO-independent stimulators and activators of soluble guanylate cyclase: discovery and therapeutic potential. *Nat Rev Drug Discov*. 2006;5:755–768.
128. Deruelle P, Grover TR, Abman SH. Pulmonary vascular effects of nitric oxide-cGMP augmentation in a model of chronic pulmonary hypertension in fetal and neonatal sheep. *Am J Physiol Lung Cell Mol Physiol*. 2005;289:L798–L806.
129. Deruelle P, Grover TR, Storme L, Abman SH. Effects of BAY 41-2272, a soluble guanylate cyclase activator, on pulmonary vascular reactivity in the ovine fetus. *Am J Physiol Lung Cell Mol Physiol*. 2005;288:L727–L733.

130. Dumitrascu R, Weissmann N, Ghofrani HA, Dony E, Beuerlein K, Schmidt H, Stasch JP, Gnoth MJ, Seeger W, Grimminger F, Schermuly RT. Activation of soluble guanylate cyclase reverses experimental pulmonary hypertension and vascular remodeling. *Circulation*. 2006;113:286–295.
131. Alp NJ, Channon KM. Regulation of endothelial nitric oxide synthase by tetrahydrobiopterin in vascular disease. *Arterioscler Thromb Vasc Biol*. 2004;24:413–420.
132. Nandi M, Leiper J, Arrighi F, Hislop A, Vallance P, Haworth S. Developmental regulation of GTP-CHI in the porcine lung and its relationship to pulmonary vascular relaxation. *Pediatr Res*. 2006;59:767–772.
133. Bowers R, Cool C, Murphy RC, Tuder RM, Hopken MW, Flores SC, Voelkel NF. Oxidative stress in severe pulmonary hypertension. *Am J Respir Crit Care Med*. 2004;169:764–769.
134. Sanford M, Keating GM. Spotlight on sapropterin in primary hyperphenylalaninemia. *BioDrugs*. 2009;23:201–202.
135. Wohlfart P, Xu H, Endlich A, Habermeier A, Closs EI, Hubschle T, Mang C, Strobel H, Suzuki T, Kleinert H, Forstermann U, Ruetten H, Li H. Antiatherosclerotic effects of small-molecular-weight compounds enhancing endothelial nitric-oxide synthase (eNOS) expression and preventing eNOS uncoupling. *J Pharmacol Exp Ther*. 2008;325:370–379.
136. Gaiad A, Yanagisawa M, Langleben D, Michel RP, Levy R, Shennib H, Kimura S, Masaki T, Duguid WP, Stewart DJ. Expression of endothelin-1 in the lungs of patients with pulmonary hypertension. *N Engl J Med*. 1993;328:1732–1739.
137. Wilkins MR, Paul GA, Strange JW, Tunari N, Gin-Sing W, Banya WA, Westwood MA, Stefanidis A, Ng LL, Pennell DJ, Mohiaddin RH, Nihoyannopoulos P, Gibbs JS. Sildenafil versus Endothelin Receptor Antagonist for Pulmonary Hypertension (SERAPH) study. *Am J Respir Crit Care Med*. 2005;171:1292–1297.
138. McLaughlin VV, Sitbon O, Badesch DB, Barst RJ, Black C, Galie N, Rainisio M, Simonneau G, Rubin LJ. Survival with first-line bosentan in patients with primary pulmonary hypertension. *Eur Respir J*. 2005;25:244–249.
139. Nagaya N, Nishikimi T, Okano Y, Uematsu M, Satoh T, Kyotani S, Kuribayashi S, Hamada S, Kakishita M, Nakanishi N, Takamiya M, Kunieda T, Matsuo H, Kangawa K. Plasma brain natriuretic peptide levels increase in proportion to the extent of right ventricular dysfunction in pulmonary hypertension. *J Am Coll Cardiol*. 1998;31:202–208.
140. Potter LR, Abbey-Hosch S, Dickey DM. Natriuretic peptides, their receptors, and cyclic guanosine monophosphate-dependent signaling functions. *Endocr Rev*. 2006;27:47–72.
141. Zhao L, Winter RJ, Krausz T, Hughes JM. Effects of continuous infusion of atrial natriuretic peptide on the pulmonary hypertension induced by chronic hypoxia in rats. *Clin Sci (Lond)*. 1991;81:379–385.
142. Baliga RS, Zhao L, Madhani M, Lopez-Torondel B, Visintin C, Selwood D, Wilkins MR, MacAllister RJ, Hobbs AJ. Synergy between natriuretic peptides and phosphodiesterase 5 inhibitors ameliorates pulmonary arterial hypertension. *Am J Respir Crit Care Med*. 2008;178:861–869.
143. Qi JG, Ding YG, Tang CS, Du JB. Chronic administration of adrenomedullin attenuates hypoxic pulmonary vascular structural remodeling and inhibits proadrenomedullin N-terminal 20-peptide production in rats. *Peptides*. 2007;28:910–919.
144. Shirai M, Pearson JT, Shimouchi A, Nagaya N, Tsuchimochi H, Ninomiya I, Mori H. Changes in functional and histological distributions of nitric oxide synthase caused by chronic hypoxia in rat small pulmonary arteries. *Br J Pharmacol*. 2003;139:899–910.
145. Said SI, Hamidi SA, Dickman KG, Szema AM, Lyubsky S, Lin RZ, Jiang YP, Chen JJ, Waschek JA, Kort S. Moderate pulmonary arterial hypertension in male mice lacking the vasoactive intestinal peptide gene. *Circulation*. 2007;115:1260–1268.
146. Hamidi SA, Prabhakar S, Said SI. Enhancement of pulmonary vascular remodeling and inflammatory genes with VIP gene deletion. *Eur Respir J*. 2008;31:135–139.
147. Sobolewski A, Rudarakanchana N, Upton PD, Yang J, Crilly TK, Trembath RC, Morrell NW. Failure of bone morphogenetic protein receptor trafficking in pulmonary arterial hypertension: potential for rescue. *Hum Mol Genet*. 2008;17:3180–3190.
148. Zeitlin PL, Diener-West M, Rubenstein RC, Boyle MP, Lee CK, Brass-Ernst L. Evidence of CFTR function in cystic fibrosis after systemic administration of 4-phenylbutyrate. *Mol Ther*. 2002;6:119–126.
149. Thomas M, Docx C, Holmes AM, Beach S, Duggan N, England K, Leblanc C, Lebre C, Schindler F, Raza F, Walker C, Crosby A, Davies RJ, Morrell NW, Budd DC. Activin-like kinase 5 (ALK5) mediates abnormal proliferation of vascular smooth muscle cells from patients with familial pulmonary arterial hypertension and is involved in the progression of experimental pulmonary arterial hypertension induced by monocrotaline. *Am J Pathol*. 2009;174:380–389.
150. Eddahibi S, Humbert M, Fadel E, Raffestin B, Darmon M, Capron F, Simonneau G, Darteville P, Hamon M, Adnot S. Serotonin transporter overexpression is responsible for pulmonary artery smooth muscle hyperplasia in primary pulmonary hypertension. *J Clin Invest*. 2001;108:1141–1150.
151. Machado RD, Koehler R, Glissmeyer E, Veal C, Suntharalingam J, Kim M, Carlquist J, Town M, Elliott CG, Hoepfer M, Fijalkowska A, Kurzyna M, Thomson JR, Gibbs SR, Wilkins MR, Seeger W, Morrell NW, Gruenig E, Trembath RC, Janssen B. Genetic association of the serotonin transporter in pulmonary arterial hypertension. *Am J Respir Crit Care Med*. 2006;173:793–797.
152. Willers ED, Newman JH, Loyd JE, Robbins IM, Wheeler LA, Prince MA, Stanton KC, Cogan JA, Runo JR, Byrne D, Humbert M, Simonneau G, Sztrymf B, Morse JA, Knowles JA, Roberts KE, McElroy JJ, Barst RJ, Phillips JA III. Serotonin transporter polymorphisms in familial and idiopathic pulmonary arterial hypertension. *Am J Respir Crit Care Med*. 2006;173:798–802.
153. Mitani Y, Mutlu A, Russell J, Brindley D, DeAlmeida J, Rabinovitch M. Dexfenfluramine protects against pulmonary hypertension in rats. *J Appl Physiol*. 2002;93:1770–1778.
154. Morecroft I, Dempsey Y, Bader M, Walther DJ, Kotnik K, Loughlin L, Nilsen M, MacLean MR. Effect of tryptophan hydroxylase 1 deficiency on the development of hypoxia-induced pulmonary hypertension. *Hypertension*. 2007;49:232–236.
155. Launay JM, Herve P, Peoc'h K, Tournois C, Callebort J, Nebigil CG, Etienne N, Drouet L, Humbert M, Simonneau G, Maroteaux L. Function of the serotonin 5-hydroxytryptamine 2B receptor in pulmonary hypertension. *Nat Med*. 2002;8:1129–1135.
156. Dempsey Y, Morecroft I, Welsh D, MacRitchie N, Herold N, Loughlin L, Nilsen M, Peacock A, Harmar A, Bader M, MacLean M. Converging evidence in support of the serotonin hypothesis of dexfenfluramine-induced pulmonary hypertension with novel transgenic mice. *Circulation*. 2008;117:2928–2937.
157. Abenheim L, Moride Y, Brenot F, Rich S, Benichou J, Kurz X, Higenbottam T, Oakley C, Wouters E, Aubier M, Simonneau G, Begaud B; International Primary Pulmonary Hypertension Study Group. Appetite-suppressant drugs and the risk of primary pulmonary hypertension. *N Engl J Med*. 1996;335:609–616.
158. Liu Y, Ren W, Warburton R, Toksoz D, Fanburg B. Serotonin induces Rho/ROCK-dependent activation of Smads 1/5/8 in pulmonary artery smooth muscle cells. *FASEB J*. 2009;23:2299–2306.
159. Mair K, MacLean M, Morecroft I, Dempsey Y, Palmer T. Novel interactions between the 5-HT transporter, 5-HT1B receptors and Rho kinase in vivo and in pulmonary fibroblasts. *Br J Pharmacol*. 2008;155:606–616.
160. Pozeg ZI, Michelakis ED, McMurtry MS, Thebaud B, Wu XC, Dyck JR, Hashimoto K, Wang S, Moudgil R, Harry G, Sultanian R, Koshal A, Archer SL. In vivo gene transfer of the O<sub>2</sub>-sensitive potassium channel Kv1.5 reduces pulmonary hypertension and restores hypoxic pulmonary vasoconstriction in chronically hypoxic rats. *Circulation*. 2003;107:2037–2044.
161. Yuan JX, Aldinger AM, Juhaszova M, Wang J, Conte JV Jr, Gaine SP, Orens JB, Rubin LJ. Dysfunctional voltage-gated K<sup>+</sup> channels in pulmonary artery smooth muscle cells of patients with primary pulmonary hypertension. *Circulation*. 1998;98:1400–1406.
162. Smirnov SV, Robertson TP, Ward JPT, Aaronson PI. Chronic hypoxia is associated with reduced delayed rectifier K<sup>+</sup> current in rat pulmonary artery muscle cells. *Am J Physiol*. 1994;266:H365–H370.
163. Platoshyn O, Yu Y, Golovina VA, McDaniel SS, Krick S, Li L, Wang JY, Rubin LJ, Yuan JX. Chronic hypoxia decreases K(V) channel expression and function in pulmonary artery myocytes. *Am J Physiol Lung Cell Mol Physiol*. 2001;280:L801–L812.
164. Mandegar M, Fung YC, Huang W, Remillard CV, Rubin LJ, Yuan JX. Cellular and molecular mechanisms of pulmonary vascular remodeling: role in the development of pulmonary hypertension. *Microvasc Res*. 2004;68:75–103.

165. Remillard CV, Yuan JX. Activation of K<sup>+</sup> channels: an essential pathway in programmed cell death. *Am J Physiol Lung Cell Mol Physiol*. 2004;286:L49–L67.
166. Yu Y, Platoshyn O, Zhang J, Krick S, Zhao Y, Rubin LJ, Rothman A, Yuan JX. c-Jun decreases voltage-gated K<sup>+</sup> channel activity in pulmonary artery smooth muscle cells. *Circulation*. 2001;104:1557–1563.
167. Geraci M, Moore M, Gesell T, Yeager M, Alger L, Golpon H, Gao B, Loyd J, Tuder R, Voelkel N. Gene expression patterns in the lungs of patients with primary pulmonary hypertension: a gene microarray analysis. *Circ Res*. 2001;88:555–562.
168. Fantozzi I, Zhang S, Platoshyn O, Remillard CV, Cowling RT, Yuan JX. Hypoxia increases AP-1 binding activity by enhancing capacitative Ca<sup>2+</sup> entry in human pulmonary artery endothelial cells. *Am J Physiol Lung Cell Mol Physiol*. 2003;285:L1233–L1245.
169. Golovina VA, Platoshyn O, Bailey CL, Wang J, Limsuwan A, Sweeney M, Rubin LJ, Yuan JX. Upregulated TRP and enhanced capacitative Ca<sup>2+</sup> entry in human pulmonary artery myocytes during proliferation. *Am J Physiol Heart Circ Physiol*. 2001;280:H746–H755.
170. Zhang S, Patel HH, Murray F, Remillard CV, Schach C, Thistlethwaite PA, Insel PA, Yuan JX. Pulmonary artery smooth muscle cells from normal subjects and IPAH patients show divergent cAMP-mediated effects on TRPC expression and capacitative Ca<sup>2+</sup> entry. *Am J Physiol Lung Cell Mol Physiol*. 2007;292:L1202–L1210.
171. Yu Y, Fantozzi I, Remillard CV, Landsberg JW, Kunichika N, Platoshyn O, Tigno DD, Thistlethwaite PA, Rubin LJ, Yuan JX. Enhanced expression of transient receptor potential channels in idiopathic pulmonary arterial hypertension. *Proc Natl Acad Sci U S A*. 2004;101:13861–13866.
172. Xu W, Koeck T, Lara AR, Neumann D, DiFilippo FP, Koo M, Janocha AJ, Masri FA, Arroliga AC, Jennings C, Dweik RA, Tuder RM, Stuehr DJ, Erzurum SC. Alterations of cellular bioenergetics in pulmonary artery endothelial cells. *Proc Natl Acad Sci U S A*. 2007;104:1342–1347.
173. Roche TE, Baker JC, Yan X, Hiromasa Y, Gong X, Peng T, Dong J, Turkan A, Kasten SA. Distinct regulatory properties of pyruvate dehydrogenase kinase and phosphatase isoforms. *Prog Nucleic Acid Res Mol Biol*. 2001;70:33–75.
174. Roberts KE, McElroy JJ, Wong WP, Yen E, Widlitz A, Barst RJ, Knowles JA, Morse JH. BMPR2 mutations in pulmonary arterial hypertension with congenital heart disease. *Eur Respir J*. 2004;24:371–374.
175. Garrington TP, Johnson GL. Organization and regulation of mitogen-activated protein kinase signaling pathways. *Curr Opin Cell Biol*. 1999;11:211–218.
176. Merklinger SL, Jones PL, Martinez EC, Rabinovitch M. Epidermal growth factor receptor blockade mediates smooth muscle cell apoptosis and improves survival in rats with pulmonary hypertension. *Circulation*. 2005;112:423–431.
177. Rabinovitch M. Elastase and the pathobiology of unexplained pulmonary hypertension. *Chest*. 1998;114:213S–224S.
178. Ye CL, Rabinovitch M. Inhibition of elastolysis by SC-37698 reduces development and progression of monocrotaline pulmonary hypertension. *Am J Physiol*. 1991;261:H1255–H1267.
179. Zaidi SH, You XM, Ciura S, Husain M, Rabinovitch M. Overexpression of the serine elastase inhibitor elafin protects transgenic mice from hypoxic pulmonary hypertension. *Circulation*. 2002;105:516–521.
180. Hansmann G, de Jesus Perez VA, Alastalo TP, Alvira CM, Guignabert C, Bekker JM, Schellong S, Urashima T, Wang L, Morrell NW, Rabinovitch M. An antiproliferative BMP-2/PPARγ/apoE axis in human and murine SMCs and its role in pulmonary hypertension. *J Clin Invest*. 2008;118:1846–1857.
181. Ishigami M, Swertfeger DK, Granholm NA, Hui DY. Apolipoprotein E inhibits platelet-derived growth factor-induced vascular smooth muscle cell migration and proliferation by suppressing signal transduction and preventing cell entry to G1 phase. *J Biol Chem*. 1998;273:20156–20161.
182. Wakino S, Kintscher U, Liu Z, Kim S, Yin F, Ohba M, Kuroki T, Schonthal AH, Hsueh WA, Law RE. Peroxisome proliferator-activated receptor gamma ligands inhibit mitogenic induction of p21(Cip1) by modulating the protein kinase Cdelta pathway in vascular smooth muscle cells. *J Biol Chem*. 2001;276:47650–47657.
183. Crossno JT Jr, Garat CV, Reusch JE, Morris KG, Dempsey EC, McMurtry IF, Stenmark KR, Klemm DJ. Rosiglitazone attenuates hypoxia-induced pulmonary arterial remodeling. *Am J Physiol Lung Cell Mol Physiol*. 2007;292:L885–L897.
184. Spiekerkoetter E, Alvira CM, Kim YM, Bruneau A, Pricola KL, Wang L, Ambartsumian N, Rabinovitch M. Reactivation of gammaHV68 induces neointimal lesions in pulmonary arteries of S100A4/Mts1-overexpressing mice in association with degradation of elastin. *Am J Physiol Lung Cell Mol Physiol*. 2008;294:L276–L289.
185. Marecki JC, Cool CD, Parr JE, Beckey VE, Luciw PA, Tarantal AF, Carville A, Shannon RP, Cota-Gomez A, Tuder RM, Voelkel NF, Flores SC. HIV-1 Nef is associated with complex pulmonary vascular lesions in SHIV-nef-infected macaques. *Am J Respir Crit Care Med*. 2006;174:437–445.
186. Taraseviciene-Stewart L, Nicolls MR, Kraskauskas D, Scerbavicius R, Burns N, Cool C, Wood K, Parr JE, Boackle SA, Voelkel NF. Absence of T cells confers increased pulmonary arterial hypertension and vascular remodeling. *Am J Respir Crit Care Med*. 2007;175:1280–1289.
187. Ulrich S, Nicolls MR, Taraseviciene L, Speich R, Voelkel N. Increased regulatory and decreased CD8<sup>+</sup> cytotoxic T cells in the blood of patients with idiopathic pulmonary arterial hypertension. *Respiration*. 2008;75:272–280.
188. Voelkel NF, Quaife RA, Leinwand LA, Barst RJ, McGoan MD, Meldrum DR, Dupuis J, Long CS, Rubin LJ, Smart FW, Suzuki YJ, Gladwin M, Denholm EM, Gail DB. Right ventricular function and failure: report of a National Heart, Lung, and Blood Institute working group on cellular and molecular mechanisms of right heart failure. *Circulation*. 2006;114:1883–1891.
189. Smadja DM, Gaussem P, Mauge L, Israel-Biet D, Dignat-George F, Peyrard S, Agnoletti G, Vouhe PR, Bonnet D, Levy M. Circulating endothelial cells: a new candidate biomarker of irreversible pulmonary hypertension secondary to congenital heart disease. *Circulation*. 2009;119:374–381.
190. Diller GP, van Eijl S, Okonko DO, Howard LS, Ali O, Thum T, Wort SJ, Bedard E, Gibbs JS, Bauersachs J, Hobbs AJ, Wilkins MR, Gatzoulis MA, Wharton J. Circulating endothelial progenitor cells in patients with Eisenmenger syndrome and idiopathic pulmonary arterial hypertension. *Circulation*. 2008;117:3020–3030.
191. Teichert-Kuliszewska K, Kutryk MJ, Kuliszewski MA, Karoubi G, Courtman DW, Zucco L, Granton J, Stewart DJ. Bone morphogenetic protein receptor-2 signaling promotes pulmonary arterial endothelial cell survival: implications for loss-of-function mutations in the pathogenesis of pulmonary hypertension. *Circ Res*. 2006;98:209–217.
192. Asosingh K, Aldred MA, Vasanji A, Drazba J, Sharp J, Farver C, Comhair SA, Xu W, Licina L, Huang L, nand-Apte B, Yoder MC, Tuder RM, Erzurum SC. Circulating angiogenic precursors in idiopathic pulmonary arterial hypertension. *Am J Pathol*. 2008;172:615–627.
193. Junhui Z, Xingxiang W, Guosheng F, Yunpeng S, Furong Z, Junzhu C. Reduced number and activity of circulating endothelial progenitor cells in patients with idiopathic pulmonary arterial hypertension. *Respir Med*. 2008;102:1073–1079.
194. Wang XX, Zhang FR, Shang YP, Zhu JH, Xie XD, Tao QM, Chen JZ. Transplantation of autologous endothelial progenitor cells may be beneficial in patients with idiopathic pulmonary arterial hypertension: a pilot randomized controlled trial. *J Am Coll Cardiol*. 2007;49:1566–1571.
195. Zhu JH, Wang XX, Zhang FR, Shang YP, Tao QM, Chen JZ. Safety and efficacy of autologous endothelial progenitor cells transplantation in children with idiopathic pulmonary arterial hypertension: open-label pilot study. *Pediatr Transplant*. 2008;12:650–655.

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

## On-line supplement

**Statins:** The **pleiotropic effects of** HMG CoA reductase inhibitors **include** anti-proliferative, anti-thrombotic, anti-inflammatory and anti-oxidant effects. This broad range of activity arises from the inhibition of isoprenoids, which are essential for post-translational isoprenylation of Rho and Ras family GTPases. These proteins are responsible for many cellular functions, including coupling membrane growth factor receptors to intracellular signaling pathways that affect cell proliferation.

In vivo, simvastatin reduces PAH in a monocrotaline-pneumonectomy model and has a dramatic survival advantage<sup>1</sup>. However, in another rodent study neither simvastatin nor atorvastatin plus rapamycin improved established monocrotaline-induced PAH<sup>2</sup>. Several trials of statins in human PAH are underway, including one comparing aspirin and simvastatin (NCT00384865).

**Dihydroepiandrosterone (DHEA):** DHEA increases expression of sGC and activates calcium-sensitive potassium channels (BK<sub>Ca</sub>) in PSMCs<sup>3</sup>. In general, agents that activate BK<sub>Ca</sub> channels in PSMC are vasodilatory. DHEA inhibits and reverses chronic hypoxic pulmonary hypertension in rats<sup>3</sup>. DHEA, normally secreted by the adrenal gland, is available “over the counter” in health food stores. Further testing in animal models with more refractory PAH (e.g. chronic hypoxia plus a VEGF inhibitor) is indicated before moving to human trials, but at least there is a substantial body of evidence suggesting DHEA is well tolerated in humans.



**Cell Cycle Inhibitors:** Excessive cell proliferation is a hallmark of PAH, making the use of a cell cycle inhibitor, such as rapamycin, attractive. In mice exposed to chronic hypoxia, rapamycin, given by intraperitoneal injection, reduced both RVH and the increase in PA medial thickness<sup>4</sup>. However, rapamycin, given by gavage, failed to reverse PAH induced in rats by monocrotaline<sup>5</sup>. Further long-term regression studies in animals are indicated prior to testing rapamycin in human PAH, in light of the mixed results of rodent studies and the potential for toxicity.

**Survivin inhibitors:** Survivin is a member of inhibitor apoptosis protein family, that occurs in human and experimental PAH<sup>6</sup>. Inhalation of an adenovirus containing a dominant-negative survivin mutant decreases monocrotaline-PAH and prolongs survival<sup>6</sup>. This is associated with increased apoptosis in the media of the pulmonary arteries and increased Kv current. Pharmacological inhibitors of survivin, such as YM-155 (Astellas) [NCT00328588](https://clinicaltrials.gov/ct2/show/study/NCT00328588) are currently being tested in cancer and may merit testing in PAH.

**Polyamine Inhibitors:** In rat PA endothelial cells, hypoxia decreases ornithine decarboxylase activity thereby increasing polyamine import. In rat PSMCs polyamine uptake (specifically putrescine) is necessary for hypoxic activation of p38MAP kinase, an important driver of cell proliferation. Blockade of polyamine synthesis reduces monocrotaline-induced PAH in rats<sup>7</sup>.  $\alpha$ -difluoromethylornithine (DFMO), an irreversible inhibitor of polyamine synthesis, is used in patients with trypanosomiasis and in breast

cancer. Assuming additional testing in animal models of PAH **were to be** promising, this experience in humans could inform a trial in PAH.

**STAT3 Inhibition:** Endothelial cells from the PAs of iPAH patients show less apoptosis and more proliferation and migration in culture than those of controls<sup>8</sup>. STAT3, a regulator of cell survival, is persistently activated in iPAH endothelial cells. A Janus-kinase (JAK) inhibitor tyrphostin AG490, which reduces STAT3 activity and blocks proliferation, has been proposed for the treatment of leukemia<sup>9</sup>. This is a strategy worthy of assessment in experimental PAH.

**Heparins:** Heparin reduces chronic hypoxic pulmonary hypertension in guinea pigs by an antiproliferative effect, unrelated to its anticoagulant activity. The antiproliferative effect of heparin is dependent on the presence of the cyclin-dependent kinase inhibitor p27<sup>10</sup>. Unfortunately, low molecular weight heparins are less potent stimulators of p27 and are less effective in preventing experimental pulmonary vascular remodeling than unfractionated heparin, making simple translation to a chronic PAH therapy difficult. However, dalteparin but not enoxaparin, does reduce pulmonary hypertension and vascular remodeling in hypoxic guinea pigs<sup>11</sup>.

**Angiotensin 1 blockers:** The role of angiotensin 1 in PAH is controversial with one group finding it is upregulated, driving PAH and another finding it is depressed, and that upregulation is therapeutic. The expression of angiotensin 1, a protein involved in the recruitment of smooth-muscle cells around blood vessels, and the phosphorylation of its

endothelial receptor, TIE2, are increased in the lungs of patients with several forms of human pulmonary hypertension<sup>12</sup>. In human PA endothelial cells, angiotensin 1 inhibits the expression of BMPR1A, which is necessary for the signaling of BMPR2<sup>12</sup>. These results suggest that angiotensin 1 could promote pulmonary hypertension. Supporting this hypothesis, angiotensin 1 stimulates proliferation of PASMCs and increases serotonin production by human pulmonary artery endothelial cells<sup>13</sup>. Blockade of TIE2, achieved by adenoviral gene transfer into the pulmonary artery in rats, largely prevents PAH, caused by either monocrotaline or angiotensin over-expression; however, this strategy does not reduce chronic hypoxic PAH<sup>14</sup>. These observations suggest that angiotensin 1 plays a role in the pathophysiology of some forms of pulmonary hypertension.

However, in other studies, cell-based angiotensin gene transfection reduces, rather than exacerbates, hypoxic pulmonary hypertension in rats<sup>15</sup>. This group found robust angiotensin 1 expression in healthy human lungs and no significant increase in expression in human PAH samples<sup>15</sup>. More clarity is required before this pathway is exploited therapeutically in humans.

### References:

1. Nishimura T, Faul JL, Berry GJ, Vaszar LT, Qiu D, Pearl RG, Kao PN. Simvastatin attenuates smooth muscle neointimal proliferation and pulmonary hypertension in rats. *Am J Respir Crit Care Med.* 2002;166(10):1403-1408.
2. McMurtry M, Bonnet S, Michelakis E, Bonnet S, Harmony A, Archer S. Statin therapy, alone or with rapamycin, does not reverse monocrotaline pulmonary arterial hypertension: the rapamycin-atorvastatin-simvastatin (RATS) study. *Am J Physiol Lung Cell Mol Physiol.* 2007;2007:Epub ahead of print.
3. Oka M, Karoor V, Homma N, Nagaoka T, Sakao E, Golembeski S, Limbird J, Imamura M, Gebb S, Fagan K, McMurtry I. Dehydroepiandrosterone upregulates soluble

- guanylate cyclase and inhibits hypoxic pulmonary hypertension. *Cardiovasc Res.* 2007;74:377-387.
4. Nishimura T, Faul JL, Berry GJ, Veve I, Pearl RG, Kao PN. 40-O-(2-hydroxyethyl)-rapamycin attenuates pulmonary arterial hypertension and neointimal formation in rats. *Am J Respir Crit Care Med.* 2001;163(2):498-502.
  5. McMurtry MS, Bonnet S, Michelakis ED, Haromy A, Archer SL. Statin therapy, alone or with rapamycin, does not reverse monocrotaline pulmonary arterial hypertension: the rapamycin-atorvastatin-simvastatin study. *Am J Physiol Lung Cell Mol Physiol.* 2007;293(4):L933-940.
  6. McMurtry MS, Archer SL, Altieri DC, Bonnet S, Haromy A, Harry G, Bonnet S, Puttagunta L, Michelakis ED. Gene therapy targeting survivin selectively induces pulmonary vascular apoptosis and reverses pulmonary arterial hypertension. *J Clin Invest.* 2005;115(6):1479-1491.
  7. Olson J, Hacker A, Altieri R, Gillelspeie M. Polyamines and the development of monocrotaline-induced pulmonary hypertension. *Am J Physiol.* 1984;247:H682-685.
  8. Xu W, Koeck T, Lara AR, Neumann D, DiFilippo FP, Koo M, Janocha AJ, Masri FA, Arroliga AC, Jennings C, Dweik RA, Tudor RM, Stuehr DJ, Erzurum SC. Alterations of cellular bioenergetics in pulmonary artery endothelial cells. *Proc Natl Acad Sci U S A.* 2007;104(4):1342-1347.
  9. Caceres-Cortes JR. A potent anti-carcinoma and anti-acute myeloblastic leukemia agent, AG490. *Anticancer Agents Med Chem.* 2008;8(7):717-722.
  10. Yu L, Quinn D, Garg H, Hales C. Cyclin-dependent kinase inhibitor p27Kip1, but not p21WAF1/Cip1, is required for inhibition of hypoxia-induced pulmonary hypertension and remodeling by heparin in mice. *Circ Res.* 2005;97:937-945.
  11. Al-Ansari E, Du HK, Yu L, Ochoa CD, Garg HG, Quinn DA, Hales CA. Low-molecular-weight heparin inhibits hypoxic pulmonary hypertension and vascular remodeling in guinea pigs. *Chest.* 2007;132(6):1898-1905.
  12. Du L, Sullivan CC, Chu D, Cho AJ, Kido M, Wolf PL, Yuan JX, Deutsch R, Jamieson SW, Thistlethwaite PA. Signaling molecules in nonfamilial pulmonary hypertension. *N Engl J Med.* 2003;348(6):500-509.
  13. Sullivan C, Du L, Chu D, Cho A, Kido M, Wolf P, Jamieson S, Thistlethwaite P. Induction of pulmonary hypertension by an angiotensin II/TIE2/serotonin pathway. *Proc Nat Acad Sci U S A.* 2003;100:12331-12336.
  14. Kido M, Du L, Sullivan CC, Deutsch R, Jamieson SW, Thistlethwaite PA. Gene transfer of a TIE2 receptor antagonist prevents pulmonary hypertension in rodents. *J Thorac Cardiovasc Surg.* 2005;129(2):268-276.
  15. Kugathasan L, Dutly AE, Zhao YD, Deng Y, Robb MJ, Keshavjee S, Stewart DJ. Role of angiotensin-1 in experimental and human pulmonary arterial hypertension. *Chest.* 2005;128(6 Suppl):633S-642S.