Future Perspectives for the Treatment of Pulmonary Arterial Hypertension

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Over the past 2 decades, pulmonary arterial hypertension has evolved from a uniformly fatal condition to a chronic, manageable disease in many cases, the result of unparalleled development of new therapies and advances in early diagnosis. However, none of the currently available therapies is curative, so the search for new treatment strategies continues. With a deeper understanding of the genetics and the molecular mechanisms of pulmonary vascular disorders, we are now at the threshold of entering a new therapeutic era. Our working group addressed what can be expected in the near future. The topics span the understanding of genetic variations, novel antiproliferative treatments, the role of stem cells, the right ventricle as a therapeutic target, and strategies and challenges for the translation of novel experimental findings into clinical practice.   (J Am Coll Cardiol 2009;54:S108–17) © 2009 by the American College of Cardiology Foundation

Genetic Variations in Pulmonary Arterial Hypertension

Pulmonary arterial hypertension (PAH) is characterized by extensive narrowing of the pulmonary vascular bed, leading to a progressive increase in pulmonary vascular resistance, right ventricular (RV) afterload, and cardiac failure. Vasoconstriction, structural changes in the vessel wall (remodeling), and thrombosis contribute to the increased pulmonary vascular resistance. In advanced disease, this process involves proliferation and hyperplasia of endothelial and smooth muscle cells (SMCs), with an increase in the extracellular matrix. A variety of growth factors and their receptors, neurohormones, and cytokines can produce these morphologic changes. The levels of these mediators are determined, in part, by their respective gene expression. Variations in the genes coding for (or regulating expression/activity of) bone morphogenetic protein receptor type 2 (BMPR2), serotonin (5-HT), serotonin transporters (SERT), prostacyclin (PGI_2) receptors, PGI_2 synthase, voltage-dependent potassium channel (Kv) 1.5, nitric oxide (NO), endothelin (ET)-1, ET-1 receptors A and B (ET_A and ET_B), and reactive oxygen species (ROS) may be relevant in PAH. Accordingly, understanding the genetic regulation of these proteins, including the roles of genetic polymorphisms and mutations, may provide useful insight into pathogenesis, prognosis, and treatment of PAH.

Genetic polymorphisms with potential relevance to PAH. BMPR2. BMPR2 is a member of the transforming growth factor (TGF)-β family. Studies suggest that BMPR2 suppresses growth in vascular tissue (i.e., SMCs) [1,2]. Isolated vascular SMCs from patients with idiopathic pulmonary arterial hypertension (IPAH) show enhanced cell proliferation [3]. Several mutations in the coding sequences (13 exons) have been identified in the BMPR2 gene, including deletion/insertion, nonsense, and missense [4,5]. Strong evidence has established an association between BMPR2 polymorphisms and familial PAH and IPAH [6–9]. Inactivating heterozygous mutations are distributed throughout the BMPR2 gene in at least 70% of patients with a family history of PAH (i.e., familial heritable

PAH) and have also been detected in 3.5% to 40% of sporadic cases of heritable PAH (10–13).

SMAD PROTEINS. Activated BMP receptors phosphorylate a set of BMP-restricted Smad protein, (Smad1, 5, and 8) (14,15), which then complex with the common partner Smad4 and translocate into the nucleus to regulate transcription of target genes (16). Many of the Smad-responsive genes encode for proteins that inhibit cell growth and induce apoptosis (17). Thus it has been proposed that BMPR2 signaling subserves a growth regulatory function in pulmonary vascular cells, inhibiting the proliferation and possibly enhancing apoptosis in SMCs. Mutations that interfere with BMPR2 signaling would enhance vascular remodeling. Genetic variations in the Smad4 gene have been identified in different forms of cancer (18–21). Two missense mutations in the Smad4 amino-terminal domain, L43S and R100T, result in proteins that are not efficiently translocated to the nucleus and, consequently, produce severely defective transcriptional responses to specific TGF ligands (22).

ET-1, ET$_A$, AND ET$_B$. ET-1 has been implicated in the pathogenesis of multiple vascular abnormalities, including PAH (23). ET-1 is believed to act in a paracrine manner on two G-protein–coupled receptors (GPCRs), ET$_A$ and ET$_B$, but with opposite effects (24,25). ET$_A$, which is present on vascular SMCs, mediates vasoconstriction and proliferation (26). ET$_B$ is found predominantly on endothelial cells, where it promotes vasodilation by releasing NO, PGI2, or other endothelium-dependent vasodilators (27,28).

Six polymorphisms in the ET$_A$ receptor gene and 3 in the ET$_B$ receptor gene have been identified (29), which may explain some of the differential response to drugs. Alleles at the different polymorphic sites were similarly distributed in patients with myocardial infarction (MI) and controls. A C/T substitution located in the nontranslated part of exon 8 of the ET$_A$ receptor gene was associated with pulse pressure. A G/T polymorphism (ET1 K198N) in the ET-1 gene strongly interacted with body mass index in the determination of blood pressure levels. The T allele was associated with an increase of blood pressure in overweight subjects. An insertion/deletion polymorphism in the untranslated region of exon 1 of the ET-1 gene was correlated with parameters of essential hypertension (30). Polymorphisms of the ET system have also been correlated with dilated cardiomyopathy (31). The H323H (C/T) polymorphism in exon 6 of the ET$_A$ receptor gene was significantly associated with a shorter survival time after diagnosis. Influences of polymorphisms in the ET$_A$ and ET$_B$ receptor genes on aortic stiffness and left ventricular geometric and radial artery parameters were analyzed in 528 never-treated hypertensive subjects. ET$_A$ receptor polymorphism G231A and the ET$_B$ receptor polymorphism 30G/A receptor gene variants influenced pulse wave velocity levels in women. In men, the ET$_B$ L277L receptor gene polymorphism variant was also related to radial artery parameters (32).

NO. NO dilates pulmonary and systemic vessels and inhibits vascular cell growth. There are 3 isoforms of the enzyme: endothelial NO synthase (eNOS), inducible NO synthase, and neuronal NO synthase, and all are expressed in the lung. Altered eNOS expression has been associated with systemic and pulmonary hypertension (33–35) and altered vascular remodeling (36,37). Decreased expression of eNOS in the pulmonary vascular endothelium of patients with most forms of PAH suggests that sustained attenuation of pulmonary vascular NO production is associated with clinically significant alterations in pulmonary vascular tone (38). The eNOS Glu298Asp polymorphism is reported to be a strong risk factor for coronary artery disease and hypertension (39). Moreover, this Glu298Asp polymorphism is associated with reduced basal NO production (40). A new polymorphism in the promoter of the eNOS gene (-786 T/C) significantly reduces its promoter activity (41). This mutation affects coronary arterial vasoreactivity by reducing endothelial NO synthesis.

GPCRs. G proteins are essential partners of multiple transmembrane receptors for the activation or inhibition of intracellular signaling cascades. More than one-half of all drugs target GPCRs and either activate or inactivate them. The GPCRs consist of α, β, and γ subunits, which are intracellular signals for stimuli such as hormones and chemokines. These stimuli activate GPCR by inducing or stabilizing a new conformation in the receptor (42).

Mutations in genes encoding GPCR can cause loss of function by impairing any of several steps in the normal GPCR/guanosine triphosphatase (GTPase) cycle (43). Polymorphisms in the GPCR signaling pathway have

been identified in the G protein α subunit (Gαs) (44) and in the G protein β3 subunit (45). The Gαs polymorphism leads to constitutively active α-subunit, and overexpression of Gαs induces hypertrophy and heart failure. Several studies suggest an association of the α-subunit of G proteins with hypertension (46). A study has demonstrated the association between a common silent polymorphism T393C in GNAS1 and hypertension. T/C substitution at position 393 in exon 5 changes mRNA folding structures (47). The T393C GNAS gene polymorphism was found to be more common in 268 white hypertensive patients than in 231 matched control subjects (41). Recently, a polymorphism in the G protein β3 subunit gene (GNB3) exchanging cytosine to thymidine (C825T) has been discovered in selected patients with essential hypertension and considered as a candidate mutation for both arterial hypertension and atherosclerosis (48). The T allele of the GNB3 polymorphism has been associated with increases in signal transduction.

NICOTINAMIDE ADENINE DINUCLEOTIDE PHOSPHATE (NADPH) OXIDASE SYSTEM. The ROS play important roles as signaling molecules in vascular cells, and NADPH oxidases contribute to ROS production within the vasculature (49). Enhanced production of ROS, especially $^{\bullet}O_2^-$, also decreases NO bioavailability (50).

The NADPH oxidase consists of 4 subunits (p22phox, gp91phox, p47phox, and p67phox), and a substantial proportion of the ROS generated in endothelial cells appear to be intracellular (51). Enhanced vascular NADPH oxidase activity is associated with upregulation of p22phox mRNA in several models of hypertension, including the spontaneously hypertensive rat (52). Several polymorphisms for the p22phox subunit have been described and are associated with coronary artery disease (53,54). A polymorphism in the promoter of the p22phox gene has been identified (−930 A/G) and has been associated with hypertension (55,56).

5-HT. 5-HT is a neurotransmitter that is a potent pulmonary vasoconstrictor and smooth muscle cell mitogen (57). Pulmonary vascular lesions in PAH display markedly elevated levels of SERT, and explanted pulmonary vascular SMCs exhibit increased 5-HT uptake, implicating SERT in vascular remodeling. Recent studies have shown that cultured pulmonary artery SMCs from patients with IPAH demonstrate a greater proliferative response to 5-HT in comparison with cells from subjects without PAH (58). The pulmonary vasoconstrictor effects of 5-HT are produced via binding to receptors, and the mitogenic actions of 5-HT are transduced via the SERT pathway (59,60). An insertion/deletion polymorphism in the promoter region of the SERT gene with long (L) and short (S) forms affects SERT expression and function, with the L allele driving a 2- to 3-fold higher rate of gene transcription than the S allele (61). This polymorphism has been associated with PAH (62), as the LL variant is more frequent in patients with PAH. The L-allelic variant of the SERT

gene promoter was present in homozygous form in 65% of patients but in only 27% of controls. Moreover, SMCs from the pulmonary artery of PAH patients with the LL polymorphism are highly proliferative in response to 5-HT, compared with cells from IPAH patients without the LL genotype.

PGI₂. PGI₂ is produced by the action of PGI₂ synthase on arachidonic acid in endothelial cells. PGI₂ synthase activity and PGI₂ levels are reduced in patients with PAH, which leads to a relative deficiency of its potent vasodilatory and antiproliferative effects (63). Patients with severe PAH have an imbalance in the local production of PGI₂ and reduced expression of PGI₂ synthase (63,64). In vivo studies in mice have demonstrated that overexpression of PGI₂ synthase protects against hypoxia-induced pulmonary hypertension (PH) (65). Several polymorphisms for the PGI₂ synthase gene have been described. One polymorphism resulting in an altered PGI₂ synthase protein sequence (a nonsense mutation in exon 2) has been observed in a family with essential hypertension and cerebral infarction (66) and 3 missense mutations in the coding sequence (P38L, S118R, and R379S) and 1 in the promoter region of the PGI₂ synthase (R6) (67). The human PGI₂ receptor is a GPCR that plays an important role in vascular homeostasis. Two PGI₂ receptor polymorphisms have been identified in the coding sequence, the V25M and the R212H. Recent genetic analyses have revealed 2 polymorphisms within the coding sequence, V25M and R212H of the PGI₂ receptor. In in vitro experiments, the R212H variant has been associated with a significant decrease in binding affinity for PGI₂ and G-protein activation versus the wild-type receptor (68).

Kv. Membrane potential is an important regulator of intracellular free calcium concentration ($[Ca2+]i$) and pulmonary vascular tone. The pore-forming α-subunit, Kv1.5, in human pulmonary artery SMCs (PASMCs) plays an important role in regulating membrane potential, vascular tone, and PASMC proliferation (69,70). Inhibition of Kv1.5 expression and function has been implicated in PASMCs from patients with IPAH (71,72). Recently, several genetic variations in the Kv1.5 channel gene (KCNA5) have been identified (73). Remillard et al. (73) showed an association between allele frequency of the single-nucleotide polymorphisms no. 4 (T-937a) and 17 (G2870a) in the KCNA5 gene and NO response in patients with IPAH, suggesting that variations in KCNA5 transcriptional regulation may affect pulmonary vascular reactivity to vasodilators in patients with IPAH.

NATRIURETIC PEPTIDES. The natriuretic peptide family comprises 3 major members, atrial or A-type (ANP), brain or B-type (BNP), and C-type, which interact with 3 receptor subtypes, NPR-A, NPR-B, and NPR-C (74). Both ANP and BNP reduce elevated pulmonary vascular tone and attenuate hypoxia-induced PH in mice (74–76). Thus, overexpression of ANP may protect against some

forms of experimental PH (75). Several genetic variations have been described for the ANP and the BNP genes (77,78). A significant association has been demonstrated between a GT repeat in intron 2 of the NPR-B gene with essential hypertension (79). A recent study showed an association between ANP/NPRA gene polymorphisms and left ventricular structure in human essential hypertension (77). This study showed that the ANP–C664G and the NPRA polymorphisms, both in the promoter region, have a significant effect on left ventricular MI in patients carrying the mutant alleles.

Pharmacogenomics in PAH. Clinicians and the lay public accept the notion that not all patients respond to drug therapy in the same fashion. Genetic polymorphisms in drug-metabolizing enzymes, transporters, receptors, and other drug targets have been linked to interindividual differences in the efficacy and toxicity of many medications. Pharmacogenomics and pharmacogenetics can lead to DNA-based tests to improve drug selection, identify optimal dosing, maximize drug efficacy, and minimize toxicity. For some drugs, there are clear implications of genetic information for drug therapy to avoid toxicity and to optimize response (80,81). In addition, understanding genetic contributors to variability in drug response provides a new tool in drug development that carries the hope of decreasing the risk for unexpected toxicities, identifying patients most likely to respond, and streamlining drug development (82). This is a relatively new area of study in PAH, and a large study investigating pharmacogenomics in PAH is now underway.

Antiangiogenesis Strategies for PAH

Angiogenesis in PAH. The role of angiogenesis in PAH remains controversial (83). In support of dysregulated angiogenesis, circulating and platelet levels of vascular endothelial growth factor (VEGF) are increased in PAH and are further increased with prostanoid treatment (84,85). In support of this hypothesis, Tuder et al. (86,87) cite evidence of increased VEGF, VEGF receptor 2, endothelial cell monoclonality, loss of tumor suppressor genes in endothelial cells, and diminished endothelial cell apoptosis.

The converse hypothesis is that angiogenesis is protective in PH. This hypothesis is supported by the demonstration that inhibition of angiogenesis factors (VEGF receptor 2) promotes hypoxia-induced PH, whereas overexpression of proangiogenesis factors (VEGF, angiopoeitin-1) reduces and/or reverses monocrotaline (MCT) and hypoxic PH (88,89).

Other angiogenic pathways that may play a role in PAH include the epidermal growth factor receptor (EGFR). MCT-induced PH in rats was attenuated by an EGFR inhibitor (90). Thalidomide inhibits angiogenesis through as yet undetermined pathways and has been used in some patients with polyneuropathy, organomegaly, endocrinopathy, monoclonal gammopathy, and skin changes (POEMS

syndrome) and multiple myeloma with mixed results (91,92). In rats with severe PAH, thalidomide failed to improve PH (93).

Statins decrease angiogenesis in systemic atherosclerotic vascular disease (94). In MCT, hypoxia, and VEGF receptor blockade + hypoxia models, statins inconsistently attenuate PAH (95–98). One clinical study of statins in PAH suggested improvement (99).

Antiangiogenesis strategies. Antiangiogenesis strategies can approach the pathway from several different angles. The VEGF is the most well-studied angiogenesis factor, and several antiangiogenesis strategies to date target either VEGF itself or its receptors. Bevacizumab (anti-VEGF antibody) is approved for the treatment of colorectal and nonsmall-cell lung cancers as an adjuvant to conventional chemotherapy. Unfortunately, bevacizumab has been associated with increased risk of vascular events, including acute hypertension and cerebrovascular and coronary events, especially in patients with established disease or risk factors for vascular disease. The mechanism of these complications is not known (100,101).

The oral multireceptor tyrosine kinase inhibitors sunitinib and sorafenib are used in the treatment of renal and gastrointestinal tumors. These agents act to inhibit the VEGF receptor and have also been associated with acute systemic hypertension and cardiac ischemia (102). Sorafenib has been evaluated in a rodent model of PAH (103). Cetuximab (monoclonal antibody that binds to the EGFR) is approved for use in head and neck and colorectal cancers. Panitumumab is another anti-EGFR antibody used in colorectal cancer. Cetuximab has been associated with fatal cardiac arrest in one patient (101).

Angiogenesis may also be a target of inhibitors of mammalian target of rapamycin, which signals through PI3K/AKT. Inhibition of mammalian target of rapamycin with rapamycin decreased hypoxia-induced angiogenesis and neointimal formation in systemic arteries (104,105). In models of PH, rapamycin has been reported to attenuate hypoxic PH and either has had no effect (when combined with a statin) or has attenuated MCT-induced PH associated with decreased pulmonary vascular resistances and inhibition of neointimal formation (98,106–108).

Unresolved questions
1. In PAH, is angiogenesis protective, harmful, or both?
2. What angiogenic targets should be considered?
3. Is the risk of treatment-induced heart disease a reason to abandon antiangiogenesis strategies in PAH?

Growth factor inhibitors: role of platelet-derived growth factor (PDGF) signaling in PAH. In the MCT rat model of PH, thrombotic lesions and platelet dysfunction appear to play significant roles (109). Abnormalities in procoagulant activity and fibrinolytic function due to shear stress may generate a thrombogenic surface, with the subsequent development of thrombotic lesions. Increased plasma levels of fibrinopeptide A- and D-dimers support this hypothesis,

with more recent studies suggesting that the interactions between platelets and vessels contribute to the vascular changes in PAH (109). These perturbations may also accelerate vasoconstriction by releasing thromboxane A2, platelet-activating factor, 5-HT, PDGF, TGF-β, and VEGF.

The PDGF receptor antagonist STI571 (imatinib mesylate) reversed pulmonary vascular remodeling in 2 different animal models of PH (110). Up-regulation of the PDGF receptor β was found in both tissue from experimental models of pulmonary hypertension (108) and in human lungs from patients with PAH (110,111). In several case reports, addition of imatinib to approved PAH drugs was shown to improve pulmonary hemodynamics and functional capacity of patients with severe PAH (112–114). A recently completed phase II clinical trial evaluating the safety and efficacy of imatinib mesylate in PAH failed to meet the primary efficacy end point of improvement in exercise capacity; however, many secondary end points, including pulmonary hemodynamics, were significantly improved. Phase III randomized controlled trials with tyrosine kinase inhibitors in PAH are expected to begin soon.

Questions for clinical research

1. In addition to PDGF, how significant are various other growth factors, such as basic fibroblast growth factor, insulin-like growth factor 1, and epidermal growth factor (90), in PAH?
2. Angiogenesis, apoptosis, and proteolysis may all be important in the pathobiology of PAH. Is targeting increased elastase activity using elastase inhibitors (115,116) another possible strategy that warrants exploration?
3. How, if at all, do growth factor inhibitors interact with the disease-specific targeted PAH treatments currently in use?
4. Can early intervention with growth factor inhibitors arrest vascular injury, allowing restoration of endothelial function?

Endothelial Progenitor Cells/ Stem Cells in Lung Repair

Regeneration of lung microvasculature may be a novel and effective therapeutic strategy for restoring pulmonary hemodynamics in patients with advanced PAH. Somatic cell-based gene therapy with eNOS (117) or various angiogenic factors, including VEGF and angiopoietin-1 (88,118), can reduce MCT-induced PAH in prevention models, possibly by protecting against endothelial cell apoptosis or inducing microvascular angiogenesis. Delivery of fibroblasts transduced with eNOS significantly improved RV systolic pressure in rats with established PAH, associated with evidence of regeneration of the lung microcirculation and consistent with the now well-accepted role of eNOS and NO in angiogenesis (119–121).

Recently it has been shown that circulating bone marrow–derived endothelial progenitor cells (EPCs) play an important role in repair of endothelial injury and participate directly in postnatal vasculogenesis and angiogenesis in systemic vascular beds (122,123). The administration of EPCs after MCT-induced PAH in rats almost completely prevented the increase in RV systolic pressure seen with MCT alone (122). Delayed administration of progenitor cells after MCT-induced PAH prevented the further progression of PAH, whereas only animals receiving EPCs transduced with human eNOS exhibited significant reversal of established disease.

In contrast with these promising results, other experimental findings indicate that bone marrow–derived stem cells may contribute not only to the maintenance of pulmonary vascular homeostasis, but to the pathogenesis of PAH as well. Acute, severe PAH is a frequent complication of allogenic bone marrow stem-cell transplantation for malignant infantile osteopetrosis (124), and late-onset PAH also occurs in association with graft-versus-host disease after allogeneic stem-cell transplantation (125). These conflicting observations suggest that further studies are needed to determine whether stem cells have a beneficial role in PAH, which cell types contribute to the unregulated vessel remodeling, and whether a feasible and affordable strategy for vascular lung repair can be developed.

Molecular imaging. Monitoring stem cells in vivo remains problematic due to limitations of conventional histologic assays and imaging modalities. These limitations may be circumvented by novel methods of molecular imaging in vivo, encompassing micro positron emission tomography (PET) analysis and the use of suitable tracers, PET reporter genes, and probes to monitor both changes in tissue perfusion and stem-cell homing and engraftment. Noninvasive imaging reporter genes are useful for many medical and biologic research applications (126,127). The PET reporter genes and probes offer potential for long-term imaging of therapeutic transgenes and cells in patients (128). Integration of molecular cell imaging into studies of PAH-directed cell therapy holds promise to facilitate further growth of the field toward a broadly clinically useful application.

Clinical impact. A successful cell therapy for lung repair will require the development of multiple interconnected strategies that will improve stem-cell culturing conditions and enhance the inherent technological content in Good Manufacturing Practice cell factories. This will result in the development of populations of human stem cells that will make feasible both vasculogenesis and paracrine release of trophic mediators for the treatment of patients with PAH.

Mechanisms of RV Remodeling: Developing Therapeutic Antiremodeling Strategies

Irrespective of the etiology of the PAH, most patients die from intractable right heart failure. Despite its profound clinical consequences, little is known about RV adaptation and failure within the context of PH. Relatively few mechanistic studies have addressed the role of the right ventricle in this disease and,

specifically, the role of the interaction of the right ventricle with the pulmonary vasculature. Moreover, there is a paucity of information about the interaction between the pulmonary vasculature and the right ventricle (RV–PA coupling). Recent data suggest that exercise limitation in PH may primarily be related to poor RV–PA coupling.

A critical aspect to the future understanding of the nature of RV function/failure is to better delineate the differences and similarities between RV and left ventricular hypertrophy and failure. An understanding of RV hypertrophy and failure signaling will allow for future therapies that will promote the growth of the adult heart (hypertrophy) to produce a stable molecular and cellular response to adverse hemodynamic and/or neurohormonal stress. Accordingly, disrupted intracellular signaling along this signaling axis leads to decompensation, maladaptive remodeling, and RV failure.

PAH and the heart. Although the distinctive pathologic abnormality in PAH is the degree and distribution of the pulmonary arteriopathy, the level of pulmonary artery pressure has only modest prognostic significance (129). Rather, it is the ability of the right ventricle to function under this increased load that determines both the severity of symptoms and survival (130). With this in mind, novel and practical ways to assess the presence and extent of subclinical RV failure are desperately needed before the stage of overt RV failure. Moreover, the role of pulmonary vascular stiffening and wave reflectance in increasing RV hydraulic load appears to be under-recognized and may be particularly important in other hypoxemic lung diseases.

Pulmonary artery wave reflection as a component of RV load. Several studies have shown that the pulsatile load is increased in chronic PH, as suggested by the increased characteristic impedance and enhanced wave reflection (131,132). This has generally been attributed to decreased pulmonary artery compliance and complex changes in reflection sites. This abnormal pulsatile load may have detrimental effects on ventricular-vascular coupling by increasing the pulsatile part of ventricular power and thus unfavorably loading the still-ejecting right ventricle. The role of pulmonary arterial input impedance has been under-recognized in the past, and there are compelling reasons why this measure should now be evaluated.

Cardiac hypertrophy and failure. Cardiomyocyte hypertrophy occurs in response to an increased load, such as that associated with hypertension and other forms of pressure overload, or to compensate for loss of myocardial tissue after MI. This response has been considered to be adaptive to increased load, because hypertrophy normalizes the increase in wall stress induced by mechanical overload. However, in humans increased cardiac mass is a strong independent risk factor for morbidity and mortality, and prolongation of this hypertrophic response in animals inevitably leads, on the one hand, to contractile dysfunction and heart failure through poorly understood mechanisms. On the other

hand, normal postnatal growth of the heart or exercise-induced cardiac growth also occurs through hypertrophy of individual cardiac muscle cells (133). These forms of so-called "physiologic" cardiac hypertrophy are not associated with contractile dysfunction and are morphologically and molecularly distinct from stress-induced hypertrophy.

The distinctions between physiologic hypertrophy and that associated with decompensation in response to excessive hemodynamic stressors and increased neurohormonal stimulation, commonly known as "pathologic" hypertrophy, are many. On the one hand, "pathologic" hypertrophy is characterized by large increases in myocyte size and ventricular thickness that is accompanied by increases in interstitial fibrosis and the induction of the fetal cardiac gene program. "Physiologic" hypertrophy, on the other hand, is characterized by smaller increases in myocyte size and ventricular thickness, no increase in interstitial fibrosis, and no induction of the fetal cardiac gene program. In addition, "physiologic" hypertrophy is reversible, whereas "pathologic" hypertrophy in animals might not be reversible, perhaps as the result of irreversible damage to the heart, such as loss of cardiomyocytes by necrosis and apoptosis.

Almost all the pathways studied involving cardiac hypertrophy and failure have been studies in the left ventricle, with a relative paucity of information validated or confirmed in the right ventricle. This leaves few answers regarding the relative importance of many of these pathways in RV failure. A critical aspect of future study will require comparisons in human RV samples.

Heart failure and oxidative stress. Increased ROS generation is a major feature of the transition from hypertrophy to heart failure. In a pro-oxidative environment, the formation of peroxynitrite from superoxide and NO can occur. Peroxynitrite in turn promotes NOS3 uncoupling, such that its synthase activity is redirected from NO production to the generation of superoxide (O_2^-). This uncoupling of NOS3 converts the enzyme from an important prosurvival, antihypertrophic, and proangiogenic (via NO) molecule to one that promotes cardiac dysfunction and destruction, including maladaptive hypertrophy, extracellular matrix remodeling, and probably myocyte cell death, although such a direct connection has not been reported. The target for peroxynitrite modification may be the Zn-thiolate cluster of NOS3 itself or the essential cofactor tetrahydrobiopterin (BH4). It has recently been shown that NOS3 uncoupling occurs in chronic pressure overload of the left ventricle, and that oral BH4 supplementation restored NO bioavailability, suppressed NO synthase-derived ROS, and prevented both cardiac dysfunction and maladaptive matrix remodeling (134,135). This may provide a rationale for exploring a similar strategy in right heart failure due to PAH.

Influence of current and emerging PH therapies on RV function. With enhanced ability to investigate RV function, there is interest in evaluating the effects of current PAH therapies on RV function. Expression of RV phosphodiesterase-5 (PDE5) is increased in patients with

PAH, and inhibition of this enzyme improves inotropy in animal models. Moreover, magnetic resonance imaging studies have shown that sildenafil acutely promotes RV relaxation. Several other studies have shown improved RV systolic and diastolic function in response to acute and chronic treatment with PGI$_2$ analogs, PDE5 inhibitors, and ET receptor antagonists (136). Further studies are needed to translate these observations to clinical PAH.

Author Disclosures

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