HHS Public Access

Author manuscript

J Am Coll Cardiol. Author manuscript; available in PMC 2016 May 27.

Published in final edited form as:

J Am Coll Cardiol. 2009 June 30; 54(1 Suppl): S108–S117. doi:10.1016/j.jacc.2009.04.014.

Future Perspectives for the Treatment of Pulmonary Arterial Hypertension

Hossein A. Ghofrani, MD[*], **Robyn J. Barst, MD**[†], **Raymond L. Benza, MD, FACC**[‡], **Hunter C. Champion, MD, PhD**[§], **Karen A. Fagan, MD**[‖], **Friedrich Grimminger, MD, PhD**[*], **Marc Humbert, MD, PhD**[¶], **Gérald Simonneau, MD**[¶], **Duncan J. Stewart, MD**[**], **Carlo Ventura, MD, PhD**[††], and **Lewis J. Rubin, MD**[‡‡]

[*]University of Giessen Lung Center, University Hospital Giessen and Marburg GmbH, Giessen, Germany; [†]Columbia University, New York, New York [‡]Drexel University College of Medicine, Section of Heart Failure, Transplantation, and Pulmonary Hypertension Program, Allegheny General Hospital, Pittsburgh, Pennsylvania [§]Division of Cardiology, Johns Hopkins University, Baltimore, Maryland [‖]Division of Pulmonary and Critical Care Medicine, University of South Alabama, Mobile, Alabama [*]Medical Clinic IV and V, University Hospital Giessen and Marburg GmbH, Giessen, Germany [¶]Université Paris-Sud, Service de Pneumologie, Hôpital Antoine-Béclère, Clamart, France [**]Ottawa Health Research Institute, Ottawa, Canada [††]University of Bologna, Laboratory of Molecular Biology and Stem Cell Engineering, National Institute of Biostructures and Biosystems, Institute of Cardiology, S.Orsola - Malpighi Hospital, Bologna, Italy [‡‡]University of California, San Diego, Medical Center, La Jolla, California

Reprint requests and correspondence: Hossein-Ardeschir Ghofrani, MD, Head, Pulmonary Hypertension Division, Medical Clinic II/V, University of Giessen Lung Center (UGLC), University Hospital Giessen and Marburg GmbH, Klinikstrasse 36, 35392 Giessen, Germany, Phone: +49 641 99 42 422, Fax: +49 49 641 99 42 419, ; Email: ardeschir.ghofrani@uglc.de, ; Email: ardeschir.ghofrani@innere.med.uni-giessen.de

Conflicts of Interest:

Dr. Barst has received honoraria for serving as a consultant, advisory board member, and/or speaker from Actelion Pharmaceuticals, Eli Lilly, GlaxoSmithKline, Gilead Sciences, Novartis, and Pfizer.

Dr. Benza has received grant support from Actelion Pharmaceuticals, Gilead Sciences, LungRx, and United Therapeutics; and speaking honoraria from Actelion, Gilead, and United Therapeutics.

Dr. Champion has indicated no conflict of interest to disclose.

Dr. Fagan TK

Dr. Ghofrani has received honoraria and research funds from Actelion Pharmaceuticals, Bayer Schering, Encysive Pharmaceuticals, ErgoNex Pharma, GlaxoSmithKline, Novartis, and Pfizer.

Dr. Grimminger has received honoraria and research funds from Actelion Pharmaceuticals, Bayer Schering, Novartis, and Pfizer.

Dr. Humbert has received honoraria and research grants from Actelion Pharmaceuticals, Bayer Schering, GlaxoSmithKline, Novartis, Pfizer, and United Therapeutics.

Dr. Rubin has received research grants from Actelion Pharmaceuticals, Gilead Sciences, the National Heart, Lung and Blood Institute, Pfizer, and United Therapeutics; and has served on advisory committees for Actelion, Gilead, and Pfizer; and as a consultant for Actelion, Aires Pharmaceuticals, Bayer Schering Pharma, Cerulean Biosciences, Gilead, mondoBIOTECH, the National Heart, Lung and Blood Institute, Onyx Pharmaceuticals, Pfizer, Solvay Pharmaceuticals, and United Therapeutics. He owns stock in United Therapeutics.

Prof. Simonneau has received honoraria and research grants from Actelion Pharmaceuticals, Bayer Schering, GlaxoSmithKline, Pfizer, and United Therapeutics.

Dr. Stewart has received honoraria from Lung Rx and is a shareholder in Northern Therapeutics.

Dr. Ventura has received funds from “Regione Emilia Romagna, Italy.”

Abstract

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.

Keywords

Treatment; Pulmonary Arterial Hypertension; PAH

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 II (BMPR-II), serotonin (5-HT), serotonin transporters (SERT), prostacyclin receptors, prostacyclin synthase, voltage-dependent potassium channel Kv1.5, nitric oxide (NO), endothelin 1 (ET-1), ET-1 receptor 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-β (TGF-β) family. Studies suggest that *BMPR2* suppresses growth in vascular tissue (ie, 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 pulmonary arterial hypertension (FPAH) 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 BMPR receptors phosphorylate a set of BMP restricted Smad proteins, (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 BMPR-II 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, prostacyclin, 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 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, eNOS, inducible (iNOS) and neuronal nitric oxide synthase (nNOS), 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 Glu 298 Asp 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.

G-protein coupled receptors (GPCRs)—G proteins are essential partners of multiple transmembrane receptors for the activation or inhibition of intracellular signaling cascades. More than half of all drugs target GPCRs and either activate or inactivate them. GPCR 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/GTPase cycle (43). Polymorphisms in the GPCR signaling pathway have been identified in the Gα subunit (Gαs) (44) and in the Gβ-3 subunit (Gβ-3) (45). The Gαs polymorphism leads to constitutively active α-subunit, and overexpression of Gsα induces hypertrophy and heart failure. Several studies suggest an association of the α-subunit of Gs 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.

NADPH oxidase system—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).

NADPH oxidase consists of four 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 twofold to threefold 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.

Prostacyclin (PGI2)—PGI2 is produced by the action of prostacyclin 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 PH (65). Several polymorphisms for the PGI2 synthase gene have been described. One polymorphism resulting in an altered prostacyclin synthase protein sequence (a nonsense mutation in exon 2) has been observed in a family with essential hypertension and cerebral infarction (66) and three missense mutations in the coding sequence (P38L, S118R, and R379S) and one in the promoter region of the PGI2 synthase (R6) (67). The human PGI2 receptor is a G-protein-coupled receptor 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 two polymorphisms within the coding sequence, V25M and R212H of the prostacyclin receptor. In in vitro experiments, the R212H variant has been associated with a significant decrease in binding affinity for prostacyclin and G-protein activation versus the wild-type receptor (68).

Voltage-dependent potassium channels (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 idiopathic pulmonary arterial hypertension (IPAH) (71,72). Recently several genetic variations in the Kv1.5 channel gene (*KCNA5*) have been identified (73). Remillard et al showed an association between allele frequency of the SNPs *no. 4* (*T*-937*a*) and *17* (*G*2870*a*) in the *KCNA5* gene and NO response in IPAH patients, suggesting that variations in *KCNA5* transcriptional regulation may affect pulmonary vascular reactivity to vasodilators in IPAH patients.

Natriuretic peptides—The natriuretic peptide family comprises 3 major members, atrial or A-type (ANP), brain or B-type (BNP) and C-type (CNP), 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 pulmonary hypertension (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 under way.

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 cite evidence of increased VEGF, VEGFR-2, endothelial cell monoclonality, loss of tumor suppressor genes in endothelial cells, and diminished endothelial cell apoptosis (86,87).

The converse hypothesis is that angiogenesis is protective in PH. This hypothesis is supported by the demonstration that inhibition of angiogenesis factors (VEGFR-2) promotes hypoxia-induced PH, while 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 VEGFR 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. 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 non-small 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 GI 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 Ab that binds to the EGFR) is approved for use in head and neck and colorectal cancers. Panitumumab is another anti-EGFR Ab 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 (mTOR), which signals through PI3K/AKT. Inhibition of mTOR 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 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, platelet-derived growth factor (PDGF), TGF-β, and VEGF.

The PDGF receptor antagonist STI571 (imatinib mesylate) reversed pulmonary vascular remodeling in 2 different animal models of PH (110). Upregulation of the PDGFR-ß was found in both tissue from experimental models of pulmonary hypertension (108) and in human lungs from patients with pulmonary arterial hypertension (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 endpoints including pulmonary hemodynamics were significantly improved. Phase III randomised controlled trials with tyrosine kinase inhibitors in PAH are supposed to start soon.

Questions for clinical research

1. In addition to PDGF, how significant are various other growth factors, such as basic FGF, IGF-1, and EGF (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 owing 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 (MicroPET) 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). 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 towards 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 underrecognized 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 pulmonary hypertension, 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 underrecognized 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 following 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 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. “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, while “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 as to 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 (O2−). 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 NOS-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. RV phosphodiesterase-5 expression 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 prostacyclin analogs, PDE5 inhibitors, and ET receptor antagonists (136). Further studies are needed to translate these observations to clinical PAH.

Abbreviations and Acronyms

ANP	atrial natriuretic peptide
BMPR	bone morphogenetic protein receptor

BNP	brain natriuretic peptide
EGFR	epidermal growth factor receptor
EPC	endothelial progenitor cell
eNOS	endothelial nitric oxide synthase
ET	endothelin
GPCR	G-protein coupled receptor
5-HT	serotonin
iNOS	inducible nitric oxide synthase
IPAH	idiopathic pulmonary hypertension
Kv	voltage-dependent potassium channel
MCT	monocrotaline
MI	myocardial infarction
mTOR	mammalian target of rapamycin
nNOS	neuronal nitric acid synthase
PAH	pulmonary arterial hypertension
PASMC	pulmonary artery smooth muscle cell
PDGF	platelet-derived growth factor
PET	positron emission tomography
PG12	prostacyclin
PH	pulmonary hypertension
ROS	reactive oxygen species
RV	right ventricular
SERT	serotonin transporter
SMC	smooth muscle cell
TGF	transforming growth factor
VEGF	vascular endothelial growth factor

References

1. Willette RN, Gu JL, Lysko PG, Anderson KM, Minehart H, Yue T. BMP-2 gene expression and effects on human vascular smooth muscle cells. J Vasc Res. 1999; 36:120–5. [PubMed: 10213907]
2. Nakaoka T, Gonda K, Ogita T, et al. Inhibition of rat vascular smooth muscle proliferation in vitro and in vivo by bone morphogenetic protein-2. J Clin Invest. 1997; 100:2824–32. [PubMed: 9389748]
3. Morrell NW, Yang X, Upton PD, et al. Altered growth responses of pulmonary artery smooth muscle cells from patients with primary pulmonary hypertension to transforming growth factor-β 1 and bone morphogenetic proteins. Circulation. 2001; 104:790–5. [PubMed: 11502704]

4. Cogan JD, Pauciulo MW, Batchman AP, et al. High frequency of BMPR2 exonic deletions/duplications in familial pulmonary arterial hypertension. Am J Respir Crit Care Med. 2006; 174:590–8. [PubMed: 16728714]
5. Trembath RC, Harrison R. Insights into the genetic and molecular basis of primary pulmonary hypertension. Pediatr Res. 2003; 53:883–8. [PubMed: 12621102]
6. Deng Z, Morse JH, Slager SL, et al. Familial primary pulmonary hypertension (gene PPH1) is caused by mutations in the bone morphogenetic protein receptor-II gene. Am J Hum Genet. 2000; 67:737–44. [PubMed: 10903931]
7. Thomson JR, Machado RD, Pauciulo MW, et al. Sporadic primary pulmonary hypertension is associated with germline mutations of the gene encoding BMPR-II, a receptor member of the TGF-β family. J Med Genet. 2000; 37:741–5. [PubMed: 11015450]
8. Cogan JD, Vnencak-Jones CL, Phillips JA 3rd, et al. Gross BMPR2 gene rearrangements constitute a new cause for primary pulmonary hypertension. Genet Med. 2005; 7:169–74. [PubMed: 15775752]
9. Machado RD, Pauciulo MW, Thomson JR, et al. BMPR2 haploinsufficiency as the inherited molecular mechanism for primary pulmonary hypertension. Am J Hum Genet. 2001; 68:92–102. [PubMed: 11115378]
10. Runo JR, Loyd JE. Primary pulmonary hypertension. Lancet. 2003; 361:1533–44. [PubMed: 12737878]
11. Sztrymf B, Coulet F, Girerd B, et al. Clinical outcomes of pulmonary arterial hypertension in carriers of *BMPR2* mutation. Am J Respir Crit Care Med. 2008; 177:1377–83. [PubMed: 18356561]
12. Rosenzweig EB, Morse JH, Knowles JA, et al. Clinical implications of determining BMPR2 mutation status in a large cohort of children and adults with pulmonary arterial hypertension. J Heart Lung Transplant. 2008; 27:668–74. [PubMed: 18503968]
13. Thompson J, Machado R, Pauciulo N, et al. Familial and sporadic primary pulmonary hypertension is caused by *BMPR2* gene mutations resulting in haploinsufficiency of the bone morphogenetic protein type II receptor. J Heart Lung Transplant. 2001; 20:149. Abstract.
14. Kawabata M, Imamura T, Miyazono K. Signal transduction by bone morphogenetic proteins. Cytokine Growth Factor Rev. 1998; 9:49–61. [PubMed: 9720756]
15. Massagué J, Seoane J, Wotton D. Smad transcription factors. Genes Dev. 2005; 19:2783–810. [PubMed: 16322555]
16. Shi Y, Massagué J. Mechanisms of TGF-β signaling from cell membrane to the nucleus. Cell. 2003; 113:685–700. [PubMed: 12809600]
17. Derynck R, Zhang YE. Smad-dependent and Smad-independent pathways in TGF-beta family signalling. Nature. 2003; 425:577–84. [PubMed: 14534577]
18. Cullingworth J, Hooper ML, Harrison DJ, et al. Carcinogen-induced pancreatic lesions in the mouse: effect of Smad4 and Apc genotypes. Oncogene. 2002; 21:4696–701. [PubMed: 12096346]
19. de Bosscher K, Hill CS, Nicolas FJ. Molecular and functional consequences of Smad4 C-terminal missense mutations in colorectal tumour cells. Biochem J. 2004; 379:209–16. [PubMed: 14715079]
20. Maliekal TT, Antony ML, Nair A, Paulmurugan R, Karunagaran D. Loss of expression, and mutations of Smad 2 and Smad 4 in human cervical cancer. Oncogene. 2003; 22:4889–97. [PubMed: 12894231]
21. de Winter JP, Roelen BA, ten Dijke P, van der Burg B, van den Eijnden-van Raaij AJ. DPC4 (SMAD4) mediates transforming growth factor-β1 (TGF-β1) induced growth inhibition and transcriptional response in breast tumour cells. Oncogene. 1997; 14:1891–9. [PubMed: 9150356]
22. Moren A, Itoh S, Moustakas A, ten Dijke P, Heldin CH. Functional consequences of tumorigenic missense mutations in the amino-terminal domain of Smad4. Oncogene. 2000; 19:4396–404. [PubMed: 10980615]
23. Haynes WG, Webb DJ. Endothelin as a regulator of cardiovascular function in health and disease. J Hypertens. 1998; 16:1081–98. [PubMed: 9794709]
24. Sakurai T, Yanagisawa M, Takuwa Y, et al. Cloning of a cDNA encoding a non-isopeptide-selective subtype of the endothelin receptor. Nature. 1990; 348:732–5. [PubMed: 2175397]

25. Arai H, Hori S, Aramori I, Ohkubo H, Nakanishi S. Cloning and expression of a cDNA encoding an endothelin receptor. Nature. 1990; 348:730–2. [PubMed: 2175396]
26. Zamora MA, Dempsey EC, Walchak SJ, Stelzner TJ. BQ123, an ETA receptor antagonist, inhibits endothelin-1-mediated proliferation of human pulmonary artery smooth muscle cells. Am J Respir Cell Mol Biol. 1993; 9:429–33. [PubMed: 8398181]
27. Sato K, Oka M, Hasunuma K, Ohnishi M, Sato K, Kira S. Effects of separate and combined ETA and ETB blockade on ET-1-induced constriction in perfused rat lungs. Am J Physiol. 1995; 269:L668–72. [PubMed: 7491987]
28. Sato K, Rodman DM, McMurtry IF. Hypoxia inhibits increased ET_B receptor-mediated NO synthesis in hypertensive rat lungs. Am J Physiol Lung Cell Mol Physiol. 1999; 276:L571–81.
29. Nicaud V, Poirier O, Behague I, et al. Polymorphisms of the endothelin-A and -B receptor genes in relation to blood pressure and myocardial infarction: the Etude Cas-Témoins sur l'Infarctus du Myocarde (ECTIM) Study. Am J Hypertens. 1999; 12:304–10. [PubMed: 10192234]
30. Stevens PA, Brown MJ. Genetic variability of the ET-1 and the ETA receptor genes in essential hypertension. J Cardiovasc Pharmacol. 1995; 26:S9–12. [PubMed: 8587478]
31. Herrmann SM, Schmidt-Petersen K, Pfeifer J, et al. A polymorphism in the endothelin-A receptor gene predicts survival in patients with idiopathic dilated cardiomyopathy. Eur Heart J. 2001; 22:1948–53. [PubMed: 11601839]
32. Lajemi M, Gautier S, Poirier O, et al. Endothelin gene variants and aortic and cardiac structure in never-treated hypertensives. Am J Hypertens. 2001; 14:755–60. [PubMed: 11497190]
33. Shesely EG, Maeda N, Kim HS, et al. Elevated blood pressures in mice lacking endothelial nitric oxide synthase. Proc Natl Acad Sci U S A. 1996; 93:13176–81. [PubMed: 8917564]
34. Huang PL, Huang Z, Mashimo H, et al. Hypertension in mice lacking the gene for endothelial nitric oxide synthase. Nature. 1995; 377:239–42. [PubMed: 7545787]
35. Fagan KA, Tyler RC, Sato K, et al. Relative contributions of endothelial, inducible, and neuronal NOS to tone in the murine pulmonary circulation. Am J Physiol Lung Cell Mol Physiol. 1999; 277:L472–8.
36. Rudic RD, Shesely EG, Maeda N, Smithies O, Segal SS, Sessa WC. Direct evidence for the importance of endothelium-derived nitric oxide in vascular remodeling. J Clin Invest. 1998; 101:731–6. [PubMed: 9466966]
37. Quinlan TR, Li D, Laubach VE, Shesely EG, Zhou N, Johns RA. eNOS-deficient mice show reduced pulmonary vascular proliferation and remodeling to chronic hypoxia. Am J Physiol Lung Cell Mol Physiol. 2000; 279:L641–50. [PubMed: 11000123]
38. Giaid A, Saleh D. Reduced expression of endothelial nitric oxide synthase in the lungs of patients with pulmonary hypertension. N Engl J Med. 1995; 333:214–21. [PubMed: 7540722]
39. Rossi GP, Taddei S, Virdis A, et al. The T-786C and Glu298Asp polymorphisms of the endothelial nitric oxide gene affect the forearm blood flow responses of Caucasian hypertensive patients. J Am Coll Cardiol. 2003; 41:938–45. [PubMed: 12651037]
40. Veldman BA, Spiering W, Doevendans PA, et al. The Glu298Asp polymorphism of the NOS 3 gene as a determinant of the baseline production of nitric oxide. J Hypertens. 2002; 20:2023–7. [PubMed: 12359981]
41. Yoshimura M, Nakayama M, Shimasaki Y, et al. A T-786–>C mutation in the 5′-flanking region of the endothelial nitric oxide synthase gene and coronary arterial vasomotility. Am J Cardiol. 2000; 85:710–4. [PubMed: 12000044]
42. Gether U. Uncovering molecular mechanisms involved in activation of G protein-coupled receptors. Endocr Rev. 2000; 21:90–113. [PubMed: 10696571]
43. Spiegel AM, Weinstein LS. Inherited diseases involving g proteins and g protein-coupled receptors. Annu Rev Med. 2004; 55:27–39. [PubMed: 14746508]
44. Jia H, Hingorani AD, Sharma P, et al. Association of the $G_s\alpha$ gene with essential hypertension and response to -blockade. Hypertension. 1999; 34:8–14. [PubMed: 10406816]
45. Hengstenberg C, Schunkert H, Mayer B, et al. Association between a polymorphism in the G protein β3 subunit gene (GNB3) with arterial hypertension but not with myocardial infarction. Cardiovasc Res. 2001; 49:820–7. [PubMed: 11230982]

46. Feldman RD, Tan CM, Chorazyczewski J. G protein alterations in hypertension and aging. Hypertension. 1995; 26:725–32. [PubMed: 7591010]
47. Frey UH, Alakus H, Wohlschlaeger J, et al. GNAS1 T393C polymorphism and survival in patients with sporadic colorectal cancer. Clin Cancer Res. 2005; 11:5071–7. [PubMed: 16033819]
48. Siffert W. G protein polymorphisms in hypertension, atherosclerosis, and diabetes. Annu Rev Med. 2005; 56:17–28. [PubMed: 15660499]
49. Rueckschloss U, Duerrschmidt N, Morawietz H. NADPH oxidase in endothelial cells: impact on atherosclerosis. Antioxid Redox Signal. 2003; 5:171–80. [PubMed: 12716477]
50. Cai H, Harrison DG. Endothelial dysfunction in cardiovascular diseases: the role of oxidant stress. Circ Res. 2000; 87:840–4. [PubMed: 11073878]
51. Li JM, Shah AM. Intracellular localization and preassembly of the NADPH oxidase complex in cultured endothelial cells. J Biol Chem. 2002; 277:19952–60. [PubMed: 11893732]
52. Xu JW, Ikeda K, Yamori Y. Genistein inhibits expressions of NADPH oxidase p22phox and angiotensin II type 1 receptor in aortic endothelial cells from stroke-prone spontaneously hypertensive rats. Hypertens Res. 2004; 27:675–83. [PubMed: 15750262]
53. Zafari AM, Davidoff MN, Austin H, et al. The A640G and C242T p22(phox) polymorphisms in patients with coronary artery disease. Antioxid Redox Signal. 2002; 4:675–80. [PubMed: 12230880]
54. Inoue N, Kawashima S, Kanazawa K, Yamada S, Akita H, Yokoyama M. Polymorphism of the NADH/NADPH oxidase p22 phox gene in patients with coronary artery disease. Circulation. 1998; 97:135–7. [PubMed: 9445163]
55. San José G, Moreno MU, Oliván S, et al. Functional effect of the p22phox–930A/G polymorphism on p22phox expression and NADPH oxidase activity in hypertension. Hypertension. 2004; 44:163–9. [PubMed: 15210651]
56. Zalba G, San José G, Moreno MU, Fortuño A, Díez J. NADPH oxidase-mediated oxidative stress: genetic studies of the p22(phox) gene in hypertension. Antioxid Redox Signal. 2005; 7:1327–36. [PubMed: 16115038]
57. Marcos E, Fadel E, Sanchez O, et al. Serotonin-induced smooth muscle hyperplasia in various forms of human pulmonary hypertension. Circ Res. 2004; 94:1263–70. [PubMed: 15059929]
58. Eddahibi S, Hanoun N, Lanfumey L, et al. Attenuated hypoxic pulmonary hypertension in mice lacking the 5-hydroxytryptamine transporter gene. J Clin Invest. 2000; 105:1555–62. [PubMed: 10841514]
59. MacLean MR, Sweeney G, Baird M, McCulloch KM, Houslay M, Morecroft I. 5-Hydroxytryptamine receptors mediating vasoconstriction in pulmonary arteries from control and pulmonary hypertensive rats. Br J Pharmacol. 1996; 119:917–30. [PubMed: 8922741]
60. 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. [PubMed: 12024106]
61. Lesch KP, Bengel D, Heils A, et al. Association of anxiety-related traits with a polymorphism in the serotonin transporter gene regulatory region. Science. 1996; 274:1527–31. [PubMed: 8929413]
62. Eddahibi S, Humbert M, Fadel E, et al. Serotonin transporter overexpression is responsible for pulmonary artery smooth muscle hyperplasia in primary pulmonary hypertension. J Clin Invest. 2001; 108:1141–50. [PubMed: 11602621]
63. Christman BW, McPherson CD, Newman JH, et al. An imbalance between the excretion of thromboxane and prostacyclin metabolites in pulmonary hypertension. N Engl J Med. 1992; 327:70–5. [PubMed: 1603138]
64. Tuder RM, Cool CD, Geraci MW, et al. Prostacyclin synthase expression is decreased in lungs from patients with severe pulmonary hypertension. Am J Respir Crit Care Med. 1999; 159:1925–32. [PubMed: 10351941]
65. Geraci MW, Gao B, Shepherd DC, et al. Pulmonary prostacyclin synthase overexpression in transgenic mice protects against development of hypoxic pulmonary hypertension. J Clin Invest. 1999; 103:1509–15. [PubMed: 10359560]

66. Nakayama T, Soma M, Takahashi Y, Uwabo J, Izumi Y, Kanmatsuse K. Novel polymorphic CA/TG repeat identified in the human prostacyclin synthase gene. Hum Hered. 1997; 47:176–7. [PubMed: 9156330]

67. Chevalier D, Cauffiez C, Bernard C, et al. Characterization of new mutations in the coding sequence and 5′-untranslated region of the human prostacyclin synthase gene (CYP8A1). Hum Genet. 2001; 108:148–55. [PubMed: 11281454]

68. Stitham J, Stojanovic A, Hwa J. Impaired receptor binding and activation associated with a human prostacyclin receptor polymorphism. J Biol Chem. 2002; 277:15439–44. [PubMed: 11854299]

69. Archer SL, Souil E, Dinh-Xuan AT, et al. Molecular identification of the role of voltage-gated K+ channels, Kv1.5 and Kv2.1, in hypoxic pulmonary vasoconstriction and control of resting membrane potential in rat pulmonary artery myocytes. J Clin Invest. 1998; 101:2319–30. [PubMed: 9616203]

70. Archer SL, Wu XC, Thébaud B, et al. Preferential expression and function of voltage-gated, O2-sensitive K+ channels in resistance pulmonary arteries explains regional heterogeneity in hypoxic pulmonary vasoconstriction: ionic diversity in smooth muscle cells. Circ Res. 2004; 95:308–18. [PubMed: 15217912]

71. Yuan JXJ, Aldinger AM, Juhaszova M, et al. Dysfunctional voltage-gated K+ channels in pulmonary artery smooth muscle cells of patients with primary pulmonary hypertension. Circulation. 1998; 98:1400–6. [PubMed: 9760294]

72. Yuan XJ, Wang J, Juhaszova M, Gaine SP, Rubin LJ. Attenuated K+ channel gene transcription in primary pulmonary hypertension. Lancet. 1998; 351:726–7. [PubMed: 9504523]

73. Remillard CV, Tigno DD, Platoshyn O, et al. Function of Kv1.5 channels and genetic variations of KCNA5 in patients with idiopathic pulmonary arterial hypertension. Am J Physiol Cell Physiol. 2007; 292:C1837–53. [PubMed: 17267549]

74. Wilkins MR, Nunez DJ, Wharton J. The natriuretic peptide family: turning hormones into drugs. J Endocrinol. 1993; 137:347–59. [PubMed: 8371073]

75. Klinger JR, Petit RD, Curtin LA, et al. Cardiopulmonary responses to chronic hypoxia in transgenic mice that overexpress ANP. J Appl Physiol. 1993; 75:198–205. [PubMed: 7690745]

76. Jin H, Yang RH, Chen YF, Jackson RM, Oparil S. Atrial natriuretic peptide attenuates the development of pulmonary hypertension in rats adapted to chronic hypoxia. J Clin Invest. 1990; 85:115–20. [PubMed: 2136863]

77. Rubattu S, Bigatti G, Evangelista A, et al. Association of atrial natriuretic peptide and type A natriuretic peptide receptor gene polymorphisms with left ventricular mass in human essential hypertension. J Am Coll Cardiol. 2006; 48:499–505. [PubMed: 16875975]

78. Rubattu S, Stanzione R, Di Angelantonio E, et al. Atrial natriuretic peptide gene polymorphisms and risk of ischemic stroke in humans. Stroke. 2004; 35:814–8. [PubMed: 15017020]

79. Rehemudula D, Nakayama T, Soma M, et al. Structure of the type B human natriuretic peptide receptor gene and association of a novel microsatellite polymorphism with essential hypertension. Circ Res. 1999; 84:605–10. [PubMed: 10082481]

80. Weinshilboum R. Inheritance and drug response. N Engl J Med. 2003; 348:529–37. [PubMed: 12571261]

81. Evans WE, McLeod HL. Pharmacogenomics—drug disposition, drug targets, and side effects. N Engl J Med. 2003; 348:538–49. [PubMed: 12571262]

82. Roses AD. Pharmacogenetics and drug development: the path to safer and more effective drugs. Nat Rev Genet. 2004; 5:645–56. [PubMed: 15372086]

83. Papaioannou AI, Kostikas K, Kollia P, Gourgoulianis KI. Clinical implications for vascular endothelial growth factor in the lung: friend or foe? Respir Res. 2006; 7:128. [PubMed: 17044926]

84. Eddahibi S, Humbert M, Sediame S, et al. Imbalance between platelet vascular endothelial growth factor and platelet-derived growth factor in pulmonary hypertension: effect of prostacyclin therapy. Am J Respir Crit Care Med. 2000; 162:1493–9. [PubMed: 11029367]

85. Voelkel NF, Douglas IS, Nicolls M. Angiogenesis in chronic lung disease. Chest. 2007; 131:874–9. [PubMed: 17356107]

86. Lee SD, Shroyer KR, Markham NE, Cool CD, Voelkel NF, Tuder RM. Monoclonal endothelial cell proliferation is present in primary but not secondary pulmonary hypertension. J Clin Invest. 1998; 101:927–34. [PubMed: 9486960]

87. Tuder RM, Chacon M, Alger L, et al. Expression of angiogenesis-related molecules in plexiform lesions in severe pulmonary hypertension: evidence for a process of disordered angiogenesis. J Path. 2001; 195:367–74. [PubMed: 11673836]

88. Campbell AI, Zhao Y, Sandhu R, Stewart DJ. Cell-based gene transfer of vascular endothelial growth factor attenuates monocrotaline-induced pulmonary hypertension. Circulation. 2001; 104:2242–8. [PubMed: 11684638]

89. Zhao YD, Courtman DW, Ng DS, et al. Microvascular regeneration in established pulmonary hypertension by angiogenic gene transfer. Am J Respir Cell Mol Biol. 2006; 35:182–9. [PubMed: 16543611]

90. 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; 112:423–31. [PubMed: 16027270]

91. Hattori Y, Shimoda M, Okamoto S, Satoh T, Kakimoto T, Ikeda Y. Pulmonary hypertension and thalidomide therapy in multiple myeloma. Br J Haematol. 2005; 128:885–7. [PubMed: 15755296]

92. Antonioli E, Nozzoli C, Gianfaldoni G, et al. Pulmonary hypertension related to thalidomide therapy in refractory multiple myeloma. Ann Oncol. 2005; 16:1849–50. [PubMed: 16012178]

93. Vescovo G, Ravara B, Angelini A, et al. Effect of thalidomide on the skeletal muscle in experimental heart failure. Eur J Heart Fail. 2002; 4:455–60. [PubMed: 12167383]

94. Koutouzis M, Nomikos A, Nikolidakis S, et al. Statin treated patients have reduced intraplaque angiogenesis in carotid endarterectomy specimens. Atherosclerosis. 2007; 192:457–63. [PubMed: 17335827]

95. Girgis RE, Li D, Zhan X, et al. Attenuation of chronic hypoxic pulmonary hypertension by simvastatin. Am J Physiol Heart Circ Physiol. 2003; 285:H938–45. [PubMed: 12750068]

96. Girgis RE, Ma SF, Ye S, et al. Differential gene expression in chronic hypoxic pulmonary hypertension: effect of simvastatin treatment. Chest. 2005; 128:579S. [PubMed: 16373842]

97. Girgis RE, Mozammel S, Champion HC, et al. Regression of chronic hypoxic pulmonary hypertension by simvastatin. Am J Physiol Lung Cell Mol Physiol. 2007; 292:L1105–10. [PubMed: 17277047]

98. McMurtry MS, Bonnet S, Michelakis ED, Bonnet S, 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:L933–40. [PubMed: 17675370]

99. Kao PN. Simvastatin treatment of pulmonary hypertension: an observational case series. Chest. 2005; 127:1446–52. [PubMed: 15821229]

100. Cilley JC, Barfi K, Benson AB 3rd, Mulcahy MF. Bevacizumab in the treatment of colorectal cancer. Expert Opin Biol Ther. 2007; 7:739–49. [PubMed: 17477810]

101. Willett CG, Duda DG, Czito BG, Bendell JC, Clark JW, Jain RK. Targeted therapy in rectal cancer. Oncology (Williston Park). 2007; 21:1055–65. [PubMed: 17910311]

102. Zhong H, Bowen JP. Molecular design and clinical development of VEGFR kinase inhibitors. Curr Top Med Chem. 2007; 7:1379–93. [PubMed: 17692027]

103. Moreno-Vinasco L, Gomberg-Maitland M, Maitland ML, et al. Genomic assessment of a multikinase inhibitor, sorafenib, in a rodent model of pulmonary hypertension. Physiol Genomics. 2008; 33:278–91. [PubMed: 18303084]

104. Weis M, Heeschen C, Glassford AJ, Cooke JP. Statins have biphasic effects on angiogenesis. Circulation. 2002; 105:739–45. [PubMed: 11839631]

105. Burke SE, Lubbers NL, Chen YW, et al. Neointimal formation after balloon-induced vascular injury in Yucatan minipigs is reduced by oral rapamycin. J Cardiovasc Pharmacol. 1999; 33:829–35. [PubMed: 10367584]

106. Humar R, Kiefer FN, Berns H, Resink TJ, Battegay EJ. Hypoxia enhances vascular cell proliferation and angiogenesis in vitro via rapamycin (mTOR)-dependent signaling. FASEB J. 2002; 16:771–80. [PubMed: 12039858]

107. 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; 163:498–502. [PubMed: 11179130]
108. Paddenberg R, Stieger P, von Lilien AL, et al. Rapamycin attenuates hypoxia-induced pulmonary vascular remodeling and right ventricular hypertrophy in mice. Respir Res. 2007; 8:15. [PubMed: 17319968]
109. Hervé P, Humbert M, Sitbon O, et al. Pathobiology of pulmonary hypertension: the role of platelets and thrombosis. Clin Chest Med. 2001; 22:451–8. [PubMed: 11590840]
110. Schermuly RT, Dony E, Ghofrani HA, et al. Reversal of experimental pulmonary hypertension by PDGF inhibition. J Clin Invest. 2005; 115:2811–21. [PubMed: 16200212]
111. Perros F, Montani D, Dorfmüller P, et al. Platelet-derived growth factor expression and function in idiopathic pulmonary arterial hypertension. Am J Respir Crit Care Med. 2008; 178:81–8. [PubMed: 18420966]
112. Ghofrani HA, Seeger W, Grimminger F. Imatinib for the treatment of pulmonary arterial hypertension. N Engl J Med. 2005; 353:1412–13. [PubMed: 16192491]
113. Patterson KC, Weissmann A, Ahmadi T, Farber HW. Imatinib mesylate in the treatment of refractory idiopathic pulmonary arterial hypertension. Ann Intern Med. 2006; 145:152–153. [PubMed: 16847299]
114. Souza R, Sitbon O, Parent F, Simonneau G, Humbert M. Long term imatinib treatment in pulmonary arterial hypertension. Thorax. 2006; 61:736. [PubMed: 16877696]
115. Cowan KN, Heilbut A, Humpl T, Lam C, Ito S, Rabinovitch M. Complete reversal of fatal pulmonary hypertension in rats by a serine elastase inhibitor. Nat Med. 2000; 6:698–702. [PubMed: 10835689]
116. 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. [PubMed: 10619858]
117. Campbell AI, Kuliszewski MA, Stewart DJ. Cell-based gene transfer to the pulmonary vasculature: endothelial nitric oxide synthase overexpression inhibits monocrotaline-induced pulmonary hypertension [see comments]. Am J Respir Cell Mol Biol. 1999; 21:567–75. [PubMed: 10536116]
118. Zhao YD, Campbell AI, Robb M, Ng D, Stewart DJ. Protective role of angiopoietin-1 in experimental pulmonary hypertension. Circ Res. 2003; 92:984–91. [PubMed: 12690034]
119. Babaei S, Stewart DJ. Overexpression of endothelial NO synthase induces angiogenesis in a co-culture model. Cardiovasc Res. 2002; 55:190–200. [PubMed: 12062722]
120. Cooke JP. NO and angiogenesis. Atheroscler Suppl. 2003; 4:53–60. [PubMed: 14664903]
121. Ziche M, Morbidelli L, Choudhuri R, et al. Nitric oxide synthase lies downstream from vascular endothelial growth factor-induced but not basic fibroblast growth factor-induced angiogenesis. J Clin Invest. 1997; 99:2625–34. [PubMed: 9169492]
122. Zhao YD, Courtman DW, Deng Y, Kugathasan L, Zhang Q, Stewart DJ. 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; 96:442–50. [PubMed: 15692087]
123. Asahara T, Kawamoto A. Endothelial progenitor cells for postnatal vasculogenesis. Am J Physiol Cell Physiol. 2004; 287:C572–9. [PubMed: 15308462]
124. Steward CG, Pellier I, Mahajan A, et al. Severe pulmonary hypertension: a frequent complication of stem cell transplantation for malignant infantile osteopetrosis. Br J Haematol. 2004; 124:63–71. [PubMed: 14675409]
125. Grigg A, Buchanan M, Whitford H. Late-onset pulmonary arterial hypertension in association with graft-versus-host disease after allogeneic stem-cell transplantation. Am J Hematol. 2005; 80:38–42. [PubMed: 16138351]
126. Massoud TF, Gambhir SS. Molecular imaging in living subjects: seeing fundamental biological processes in a new light. Genes Dev. 2003; 17:545–80. [PubMed: 12629038]
127. Serganova I, Blasberg R. Reporter gene imaging: potential impact on therapy. Nucl Med Biol. 2005; 32:763–80. [PubMed: 16243653]

128. Peñuelas I, Haberkorn U, Yaghoubi S, Gambhir SS. Gene therapy imaging in patients for oncological applications. Eur J Nucl Med Mol Imaging. 2005; 32:S384–403. [PubMed: 16180032]

129. Gaine SP, Rubin LJ. Primary pulmonary hypertension. Lancet. 1998; 352:719–25. [PubMed: 9729004]

130. Chin KM, Kim NH, Rubin LJ. The right ventricle in pulmonary hypertension. Coron Artery Dis. 2005; 16:13–8. [PubMed: 15654194]

131. Parmley WW, Tyberg JV, Glantz SA. Cardiac dynamics. Annu Rev Physiol. 1977; 39:277–99. [PubMed: 322599]

132. Piene H. Pulmonary arterial impedance and right ventricular function. Physiol Rev. 1986; 66:606–52. [PubMed: 3526365]

133. Kelly RP, Ting CT, Yang TM, et al. Effective arterial elastance as index of arterial vascular load in humans. Circulation. 1992; 86:513–21. [PubMed: 1638719]

134. Janssens S, Pokreisz P, Schoonjans L, et al. Cardiomyocyte-specific overexpression of nitric oxide synthase 3 improves left ventricular performance and reduces compensatory hypertrophy after myocardial infarction. Circ Res. 2004; 94:1256–62. [PubMed: 15044322]

135. Moens AL, Takimoto E, Tocchetti CG, et al. Reversal of cardiac hypertrophy and fibrosis from pressure overload by tetrahydrobiopterin: efficacy of recoupling nitric oxide synthase as a therapeutic strategy. Circulation. 2008; 117:2626–36. [PubMed: 18474817]

136. Chin KM, Kim NH, Rubin LJ. The right ventricle in pulmonary hypertension. Coron Artery Dis. 2005; 16:13–18. [PubMed: 15654194]