

Mechanisms of disease: pulmonary arterial hypertension

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Abstract | Our understanding of, and approach to, pulmonary arterial hypertension has undergone a paradigm shift in the past decade. Once a condition thought to be dominated by increased vasoconstrictor tone and thrombosis, pulmonary arterial hypertension is now seen as a vasculopathy in which structural changes driven by excessive vascular cell growth and inflammation, with recruitment and infiltration of circulating cells, play a major role. Perturbations of a number of molecular mechanisms have been described, including pathways involving growth factors, cytokines, metabolic signaling, elastases, and proteases, that may underlie the pathogenesis of the disease. Elucidating their contribution to the pathophysiology of pulmonary arterial hypertension could offer new drug targets. The role of progenitor cells in vascular repair is also under active investigation. The right ventricular response to increased pressure load is recognized as critical to survival and the molecular mechanisms involved are attracting increasing interest. The challenge now is to integrate this new knowledge and explore how it can be used to categorize patients by molecular phenotype and tailor treatment more effectively.

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Introduction

Pulmonary hypertension is a progressive disease of various origins that is associated with a poor prognosis and results in right heart dysfunction. In all its variant presentations, this disease is estimated to affect up to 100 million people worldwide.¹ According to the current classification of pulmonary hypertension, which was agreed upon at the 4th World Symposium on Pulmonary Hypertension in 2008, five categories of chronic pulmonary hypertension exist (Box 1).²

In this Review, we focus on molecular mechanisms involved in the pathogenesis of group 1, pulmonary arterial hypertension (PAH), which includes a variety of diseases that share several pathophysiological, histological, and prognostic features. Although rare, idiopathic PAH (IPAH; group 1.1) defines the group and is a disease in which there is neither a family history of, nor an identified risk factor for, PAH. In heritable PAH (group 1.2; formerly known as familial PAH), loss-of-function mutations in the transforming growth factor β /bone morphogenetic protein (TGF- β /BMP)

receptor superfamily and, more rarely, in activin receptor-like kinase type 1 (ALK-1; also called ACVRL1) have been identified as underlying mechanisms. Drug-induced and toxin-induced forms of PAH are included in group 1.3, and group 1.4 comprises PAH associated with identified diseases (for example, HIV and schistosomiasis infections). Persistent pulmonary hypertension of the newborn is included in group 1.5, and patients with pulmonary veno-occlusive disease and/or pulmonary capillary hemangiomatosis are classified as being in group 1'.^{1,3–5}

In this article, we highlight findings from patients with PAH. However, we also discuss preclinical data from a number of animal models of pulmonary hypertension. Notably, although the existing animal models inform on certain aspects of PAH, no animal model of pulmonary hypertension recapitulates the human condition. For example, mice exposed to hypoxia develop pulmonary hypertension and are widely used as a disease model, but although they display certain features in common with PAH, they are more closely related to group 3 pulmonary hypertension. Rats treated with the toxin monocrotaline, another frequently used model of pulmonary hypertension, may exhibit direct damage to the liver, kidneys, and heart, in addition to the pulmonary vasculature. For an in-depth discussion of the merits and limitations of the different animal models of pulmonary hypertension and their relevance to human PAH, the reader is referred to an article by Stenmark *et al.*⁶

Pathways of disease

The subcategories of PAH differ in their underlying causes. However, all are characterized by excessive pulmonary vasoconstriction and abnormal vascular remodeling

Competing interests

R. T. Schermuly declares associations with Actelion, Bayer Healthcare, Ergonex, Excellence Cluster Cardiopulmonary System, German Research Foundation, Gilead, GlaxoSmithKline, Lilly, Novartis, Pfizer, Solvay Pharmaceuticals, and the University of Giessen and Marburg Lung Center. H. A. Ghofrani declares associations with Bayer Healthcare, Ergonex, Excellence Cluster Cardiopulmonary System, German Research Foundation, GlaxoSmithKline, Novartis, Pfizer, and the University of Giessen and Marburg Lung Center. M. R. Wilkins declares associations with Bayer Healthcare, Biomarin, GlaxoSmithKline, and Pfizer. F. Grimminger declares associations with Actelion, Bayer Healthcare, Encysive, Excellence Cluster Cardiopulmonary System, German Research Foundation, GlaxoSmithKline, Novartis, Parexel International GmbH, Pfizer, and the University of Giessen and Marburg Lung Center.

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Key points

- Pulmonary hypertension is a progressive disease of various origins, which has a poor prognosis and affects, in its different forms, more than 100 million people worldwide
- Pulmonary arterial hypertension (PAH) is now considered to be a vasculopathy in which structural changes driven by excessive vascular cell growth and inflammation have a major role
- A number of proproliferative signaling pathways involving growth factors, cytokines, metabolic signaling, and elastases and proteases have been identified in the pathophysiology of PAH
- Clinical studies with tyrosine kinase inhibitors, serotonin antagonists, and soluble guanylate cyclase stimulators are underway in patients with PAH
- The benefits of progenitor cells for vascular repair in PAH are under active investigation
- The right ventricular response to increased pressure load is recognized as critical to survival in patients with PAH, and strategies for preserving myocardial function are increasingly attracting interest

Box 1 | Current classification of pulmonary hypertension

As agreed at the 4th World Symposium on Pulmonary Hypertension in Dana Point, CA, USA, in 2008, pulmonary hypertension is now classified into five groups:⁵

Group 1—Pulmonary arterial hypertension

- Group 1.1—Idiopathic pulmonary arterial hypertension
- Group 1.2—Heritable pulmonary arterial hypertension (formerly known as familial pulmonary arterial hypertension)
- Group 1.3—Drug-induced and toxin-induced forms of pulmonary arterial hypertension
- Group 1.4—Pulmonary arterial hypertension associated with identified diseases (for example, HIV and schistosomiasis infections)
- Group 1.5—Persistent pulmonary hypertension of the newborn

Group 1'—Pulmonary veno-occlusive disease and/or pulmonary capillary hemangiomatosis

Group 2—Pulmonary hypertension resulting from left heart disease; this group represents the large group of patients with pulmonary venous hypertension

Group 3—All forms of pulmonary hypertension associated with alveolar hypoxia and/or disorders of the respiratory system

Group 4—All (thrombo)embolic diseases leading to pulmonary hypertension

Group 5—Pulmonary hypertension with unclear multifactorial mechanisms (a group of diseases directly affecting the pulmonary vessels)

processes that usually affect all vessel layers (intima, media, and adventitia) and result in severe loss of cross-sectional area and, therefore, increased right ventricular afterload (Figure 1).⁷ Large pulmonary artery compliance is also decreased, contributing to the strain on the right ventricle.⁸ Intimal changes include endothelial injury, endothelial cell proliferation, invasion of the intima by (myo)fibroblast-like cells, enhanced matrix deposition with intimal fibrosis and, sometimes, obstruction of the vascular lumen by

unique plexiform lesions.⁹ Vascular smooth muscle cell (SMC) proliferation is a prominent feature of PAH. These structural changes suggest a switch from a quiescent state to a proliferative, apoptosis-resistant cellular phenotype. Lung vascular remodeling can be associated with chronic inflammatory events and recruitment of progenitor cell types, which might fuel proliferative changes (for example, in circulating fibrocytes and mesenchymal progenitor cells)¹⁰ or which might have an antiproliferative effect and thus offer a prospective therapeutic intervention (for example, endothelial progenitor cells).¹¹

Imbalance in vasoactive mediators

In patients with PAH, the generation of vasodilatory mediators, in particular prostaglandin I₂, is reduced.^{12,13} Reduced levels of the vasodilatory and antiproliferative nitric oxide (NO; generated via NO synthases), and cyclic guanosine monophosphate (the second messenger downstream of NO) are also well described in pulmonary vascular disease, including PAH.^{14,15} Increased levels of asymmetric dimethyl-arginine (an endogenous inhibitor of NO synthase) might also have a functional role in PAH (Figure 2).^{16,17}

In addition to reduced generation of vasodilatory mediators in patients with PAH, levels of the potent vasoconstrictors thromboxane and endothelin 1 are increased.^{12,13} Moreover, 5-hydroxytryptamine (5-HT) causes vasoconstriction, and abnormalities in endothelial cell-SMC cross-talk have been observed in humans with IPAH and a mouse model of hypoxia-induced pulmonary hypertension, with enhanced endothelial 5-HT production, transport, and paracrine activity in adjacent pulmonary vascular SMCs.^{18,19} Certain forms of drug-induced or toxin-induced PAH (group 1.3) also involve the 5-HT pathway. Fenfluramine and aminorex, anorexigenic drugs associated with an increased risk of PAH, are both 5-HT transporter substrates and increase extracellular concentrations of 5-HT.²⁰

Abnormalities in both K⁺ channels and Ca²⁺ channels have been linked with pathological pulmonary vascular tone, dysregulation of cellular homeostasis, and induction of fibroproliferative sequelae, particularly in SMCs. A selective downregulation of pulmonary artery smooth muscle voltage-gated K⁺ channels has been described in tissue from humans with IPAH and from animal models of pulmonary hypertension, with subsequent membrane depolarization, opening of voltage-gated Ca²⁺ channels, and induction of muscle contraction via Ca²⁺-calmodulin and myosin light chain kinase.^{21–23} Moreover, Ca²⁺ signaling via transient receptor potential (TRP) ion channels has an important role in IPAH. *TRPC3* and *TRPC6* expression were found to be upregulated in pulmonary arterial SMCs from patients with IPAH, and inhibition of *TRPC6* expression markedly reduced SMC proliferation.²⁴ The acute hypoxic vasoconstrictor response was also reported to be exclusively dependent on *TRPC6* in mice with hypoxia-induced pulmonary hypertension.²⁵

Cell proliferation and vascular remodeling

Some vasoconstrictors are also able to exert proproliferative actions. Endothelin stimulates SMC proliferation

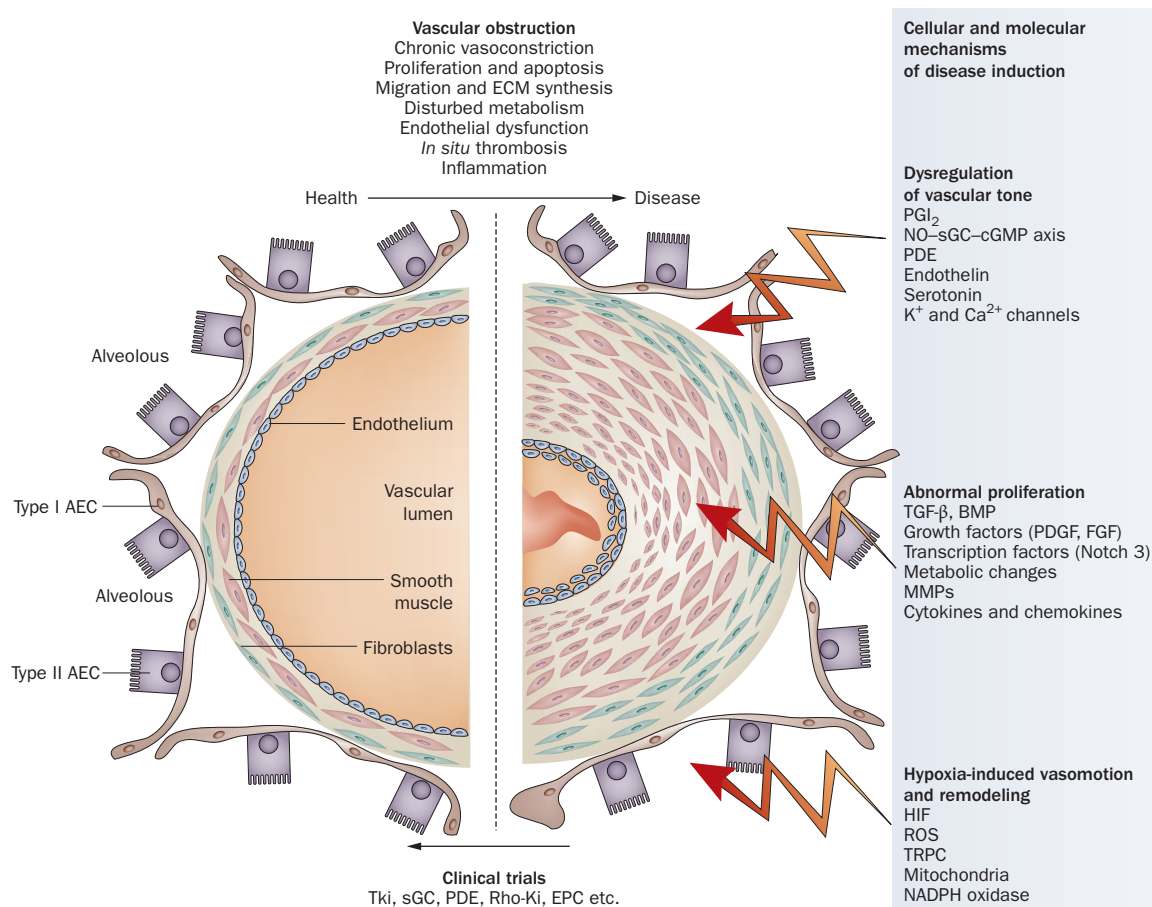


Figure 1 | Vascular remodeling in pulmonary arterial hypertension. Putative therapeutic targets are indicated. Abbreviations: 5-HT, 5-hydroxytryptamin; K- and Ca-channels, potassium and calcium channels; AEC, alveolar epithelial cells; BMP, bone morphogenetic protein; cGMP, cyclic guanosine monophosphate; ECM, extracellular matrix; EGF, epidermal growth factor; EPC, endothelial progenitor cells; HIF, hypoxia inducible factor; MMPs, matrix metalloproteinases; NADPH, nicotinamide adenine dinucleotide phosphate; NO, nitric oxide; PDE, phosphodiesterase; PDGF, platelet-derived growth factor; PGI₂, prostaglandin I₂; Rho-Ki, Rho kinases; ROS, reactive oxygen species; sGC, soluble guanylate cyclase; TGF, transforming growth factor- β ; TK, tyrosine kinase; TKi, tyrosine kinase inhibitor; TRPC, transient receptor potential cation channels; VEGF, vascular endothelial growth factor.

via downstream signaling cascades involving endothelin type A and type B receptors.²⁶ In addition to the vasoconstriction described above, which mainly occurs via the SMC 5-HT receptors, 5-HT also causes SMC proliferation, mostly via 5-HT-transporter-mediated 5-HT uptake.^{18–20} Sustained Ca²⁺ flux, whether from the extracellular space or from intracellular stores, may also exert mitogenic effects in SMCs, with Ca²⁺-calmodulin-dependent stimulation of the cell cycle, activation of mitogen-activated protein kinase pathways, and stimulation of the activating protein 1 family of transcription factors (upstream of several growth factors).²⁷

Remodeling is seen in the large pulmonary arteries as well in the smaller distal pulmonary arteries. In mice with hypoxic pulmonary hypertension, compliance of the extralobar pulmonary arteries was found to be reduced as a result of narrowing and wall thickening, rather than vasoconstriction.²⁸ Excessive vascular collagen might contribute to this phenotype; large pulmonary arteries from rats with hypoxic pulmonary hypertension showed increased collagen alongside decreased compliance,²⁹ and

expression of a degradation-resistant form of collagen in hypoxic mice prevented the mechanical properties of the extralobar pulmonary arteries returning to normal after removal from hypoxic conditions.³⁰

Growth factors

Several growth factors are implicated in PAH and vascular remodeling (Figure 2). By binding and activating cell surface tyrosine kinase receptors, growth factors act as potent mitogens and chemoattractants for vascular cells, such as SMCs, fibroblasts, and endothelial cells. Activation of the tyrosine kinase receptor initiates major intracellular signaling cascades, resulting in cellular proliferation, migration, and resistance to apoptosis. Only the growth factors most clearly implicated in PAH will be discussed in this Review.

Vascular endothelial growth factor (VEGF) and VEGF receptor 2 (VEGFR-2) are expressed in the plexiform lesions of patients with PAH,^{31,32} indicating a role for VEGF in the pathogenesis of PAH. However, preclinical data have been inconsistent. VEGF is upregulated in rats

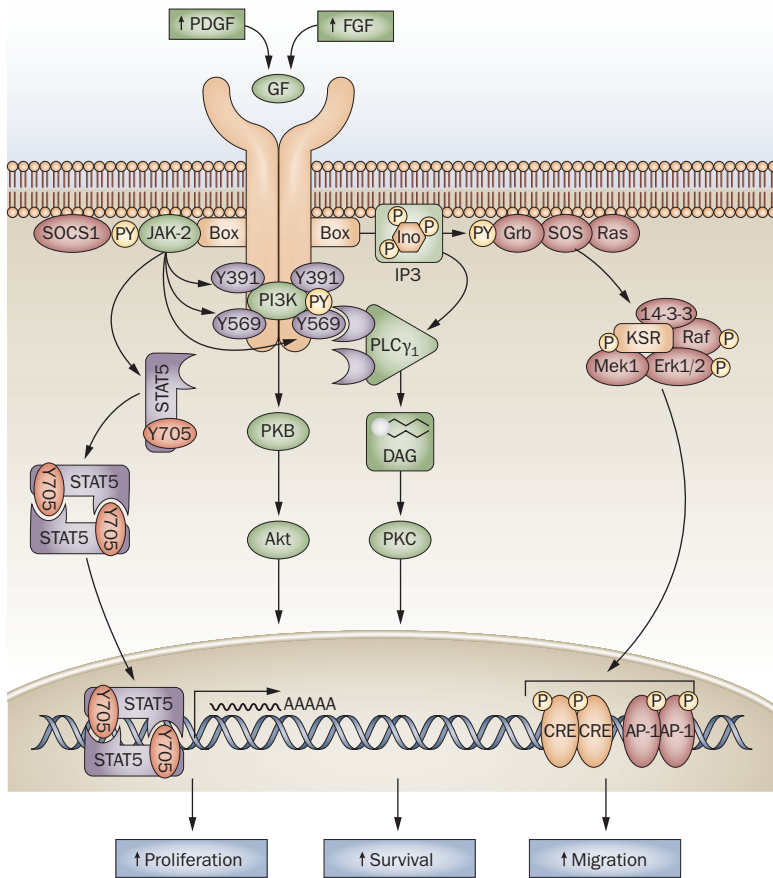


Figure 2 | Growth factor signaling in pulmonary arterial hypertension. Expression of certain growth factors is increased in pulmonary arterial hypertension, promoting cell proliferation, survival, and migration via a number of signaling pathways, and thus contributing to pulmonary vascular remodeling. Abbreviations: Akt, v-akt murine thymoma viral oncogene homolog; AP-1, activator protein-1; CaM, calmodulin; CamKII, calcium/calmodulin-dependent kinase II; CRE, cyclic adenosine monophosphate response element; DAG, diacylglycerol; Erk 1/2, extracellular signal-regulated kinase 1/2; FGF, fibroblast growth factor; GF, growth factor; Grb2, growth factor receptor-bound protein 2; IP3, inositol triphosphate; JAK-2, Janus-activated kinase 2; KSR, kinase suppressor of Ras; Mek 1/2, mitogen-activated protein kinase 1/2; MLCK, myosin light chain kinase; PDGF, platelet-derived growth factor; PI3K, phosphoinositide-3-kinase; PKB, protein kinase B; PKC, protein kinase C; PLC, phospholipase C; Raf, rapidly growing fibrosarcoma; Ras, Ras protein; SOS, son of sevenless; STAT, signal transducer and activator of transcription.

with chronic hypoxic pulmonary hypertension, but down-regulated in rats with monocrotaline-induced pulmonary hypertension.^{33,34} The inhibition of VEGFR in rats *in vivo* results in septal cell apoptosis and emphysema formation.^{35,36} However, when VEGFR inhibition is combined with chronic hypoxia in rats, severe pulmonary hypertension with angioproliferative lesions develops,^{37,38} and when rats with established pulmonary hypertension are treated with VEGF, the disease is aggravated.³⁸ The controversies regarding the role of VEGF in the pathogenesis of pulmonary hypertension continue, as is evident from findings that gene-transfer-mediated VEGF overexpression in rats attenuates the development of experimental pulmonary hypertension (in monocrotaline-induced, pulmonary fibrosis, and hypoxic models),^{39–41} whereas inhibition of

VEGF signaling by the small molecule multikinase inhibitor sorafenib in rats also attenuates experimental pulmonary hypertension (generated by monocrotaline or VEGFR inhibition combined with hypoxia).^{42,43} Overall, results indicate a unique and complex role for VEGF in pulmonary pathophysiology,⁴⁴ but therapeutic applications are unlikely until the precise role of VEGF is elucidated.

Basic fibroblast growth factor (bFGF) is a ubiquitously expressed member of the FGF family. Substantial alterations in plasma and urine bFGF levels have been observed in patients with PAH.⁴⁵ Upregulation of bFGF in response to hypoxia and shear stress is observed in rat and in ovine pulmonary arterial SMCs,^{46,47} and upregulation of FGF-2 occurs in lambs with pulmonary hypertension,⁴⁸ but not hypoxic mice.⁴⁹ Repeated intravenous FGF-2 small interfering RNA administration prevents (and nearly reverses) monocrotaline-induced pulmonary hypertension in rats, and pharmacological inhibition of FGF receptor 1 reverses established pulmonary hypertension.⁵⁰ Thus, FGF-2 is a possible therapeutic target.

Pulmonary overexpression of TGF- α , a member of the epidermal growth factor (EGF) family, results in the development of pulmonary hypertension and vascular remodeling in mice,⁵¹ and might be associated with EGF-induced pulmonary vascular SMC proliferation.^{52,53} Inhibition of the EGF receptor attenuates monocrotaline-induced pulmonary hypertension in rats,^{54,55} but it does not improve chronic hypoxia-induced pulmonary hypertension in mice,⁵⁴ undermining EGF as a therapeutic target in PAH.

Platelet-derived growth factor (PDGF) acts as a potent mitogen and chemoattractant for SMCs.^{56,57} Pulmonary mitogen expression of the PDGF ligand and receptor is increased in patients with severe PAH,^{58–60} and PDGF signaling is up-regulated in the pulmonary arteries of patients with IPAH.⁶⁰ Moreover, the activation of Wnt signaling (which regulates lung SMC development) in mice increases expression of PDGF receptors (PDGFRs) through the transcriptional activity of tenascin C.⁶¹ Upregulation of PDGFR has also been shown in lambs with pulmonary hypertension induced by partial ligation of the ductus arteriosus,⁶² and inhibition of PDGFR improves survival in rats with monocrotaline-induced pulmonary hypertension.^{42,59}

Hepatocyte growth factor (HGF) signals through c-Met (a tyrosine kinase receptor). HGF expression decreases under hypoxic conditions in cell and organ culture systems,⁶³ and HGF production is downregulated in monocrotaline-injected rats.⁶⁴ Furthermore, HGF gene transfer attenuates the development of monocrotaline-induced pulmonary hypertension in rats.^{64,65} Thus, HGF might have a protective role in pulmonary vascular remodeling, but preclinical and clinical studies in this area are needed.

The TGF- β superfamily comprises a large series of cytokine growth factors that control many cellular functions, including proliferation, migration, differentiation, and extracellular matrix secretion and deposition. BMP receptor type 2 (BMPR2), ALK-1, and endoglin have been implicated as causal factors in hereditary and associated forms of PAH.⁶⁶ Stem cell factor acts through its receptor c-kit, which is expressed on stem and progenitor cells as well as mast cells. Cells expressing c-kit are found in pulmonary

artery walls, both in clinical PAH and in animals with hypoxic pulmonary hypertension.^{67–70} Further studies are needed to define the role of stem cell factor and c-kit.

Proteases and elastases

Matrix metalloproteinases (MMPs) have key roles in modulating structural extracellular matrix (ECM) proteins. They also enable release of growth factors from ECM-associated stores by revealing previously cryptic ECM binding sites, and by targeting non-ECM substrates. Upregulation of MMPs and endogenous vascular elastase activity occurs in remodeled lung vasculature in experimental and clinical settings of pulmonary hypertension,^{71–73} and inhibitors of MMPs and elastase exert beneficial effects in rats with monocrotaline-induced pulmonary hypertension.^{74,75} The broad spectrum of MMP activities and their roles in developmental biology and tissue homeostasis mandates a more detailed understanding of their physiological and pathophysiological functions in lung parenchyma and vasculature.

Bone morphogenetic protein receptor type 2

Mutations in the *BMPR2* gene have been found in the majority of patients with heritable PAH and some forms of IPAH, providing a strong stimulus for further research in this field.^{76–78} To date, more than 140 distinct mutations have been identified in *BMPR2* in patients with IPAH. Moreover, mutations in the gene for type I TGF- β superfamily receptor ALK-1 have been linked with severe PAH development in families with hereditary hemorrhagic telangiectasia.⁷⁸ The mechanisms by which *BMPR2* and *ACVRL1* mutations disrupt physiological intracellular signaling are not well defined, and might vary between cell types. Some potential mechanisms suggested by *in vitro* studies include upregulation of proliferative pathways involving p38 mitogen-activated protein kinase,⁷⁹ reduced activation of the transcription factor Smad 1,⁸⁰ and disruption of *BMPR2* binding to Tctex-1 (a light chain of the motor complex dynein)⁸¹ and LIM kinase 1, which regulates actin dynamics.⁸² Further investigation is needed into the molecular mechanisms linking these mutations with the pulmonary vasculopathy, the growth of pulmonary arterial SMCs, and abnormal endothelial cell proliferation in PAH.⁸³

Notch

Notch signaling is a conserved signaling mechanism, essential for cell-fate determination during embryonic development. Four mammalian Notch receptors (Notch 1–4) and five ligands (Jagged 1 and 2, and delta-like protein 1, 3, and 4) have been described. These receptors and ligands are membrane bound; therefore, signaling occurs by cell-to-cell contact, leading to proteolytic cleavages in the Notch receptor, including a final cleavage by γ secretase to release the receptor's intracellular domain. The intracellular domain of the Notch receptor then translocates to the nucleus and forms an active transcription activator complex with the DNA binding protein CBF-1 and activates transcription of the *HES* and *HEY* genes, its downstream targets (Figure 3).⁸⁴

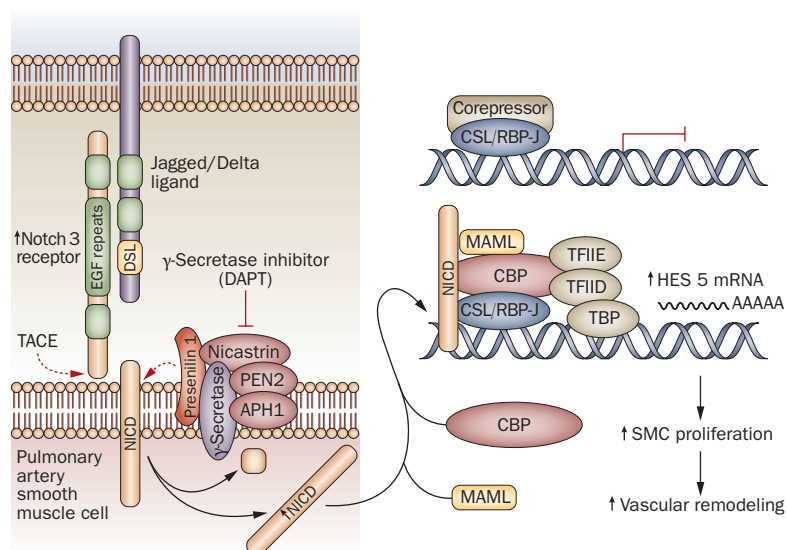


Figure 3 | Notch signaling pathway and its proposed role in pulmonary hypertension. In the absence of activated Notch signaling, the DNA binding protein CBF-1/RBP-J κ forms a complex with co-repressors to prevent transcription of target genes. Interaction of ligand (via its DSL motif) with the EGF-like repeats on the receptor triggers two successive proteolytic cleavages by TACE, followed by gamma secretase. These cleavages result in release of NICD, which translocates to the nucleus and interacts with CBF-1/RBP-J κ , removing the co-repressors. Coactivators, including MAML, CBP, and other transcription factors, are subsequently recruited, leading to transcription of Notch target genes (the HES family of transcription repressors). Notch 3 is the predominantly expressed Notch receptor in vascular SMCs, including pulmonary artery SMCs. Arrows indicate changes observed in pulmonary arterial hypertension. Abbreviations: APH 1, anterior pharynx-defective 1; CBF-1, Cp-binding factor 1; CBP, CREB binding protein; CREB, cyclic adenosine monophosphate response element-binding; DSL, Delta Serrate Ligand; EGF, epidermal growth factor; MAML, mastermind-like protein; NICD, Notch Intracellular Domain; PEN 2, presenilin 2; RBP-J κ , recombination signal sequence-binding protein-J κ ; SMC, smooth muscle cell; TACE, tumor necrosis factor α -converting enzyme; TBP, TATA box-binding protein; TF, transcription factor.

Notch signaling is involved in multiple aspects of vascular development, including vasculogenesis, angiogenesis, and differentiation of vascular SMCs.⁸⁵ Notch 3 is expressed exclusively in adult human and rodent SMCs,⁸⁶ and might regulate arterial SMC identity and maturation during development.⁸⁷ Notch 3 acts to repress expression of contractile protein genes⁸⁸ and to promote proliferation of vascular SMCs.⁸⁹ During hypoxia, hypoxia-inducible factor 1 α activates Notch-responsive genes, maintaining cells in an undifferentiated state.⁹⁰ Notch 3 also regulates expression of PDGF receptor β in vascular SMCs.⁹¹

Notch 3 and its target gene, *HES5*, are expressed in lung biopsies from patients with nonfamilial PAH, and in the lungs of two rodent models of pulmonary hypertension (hypoxic mice and monocrotaline-treated rats).⁹² The extent of overexpression at the protein level correlates with disease severity in these rodent models, and lung immunofluorescent stains show confinement of Notch 3 and transcription factor HES-5 expression to the media of small pulmonary arteries and arterioles. Cultures of pulmonary arteriole SMCs from patients with PAH display higher levels of mRNA and protein for Notch 3 and transcription factor HES-5 than SMCs from healthy lungs. Overexpression of the Notch 3 intracellular domain leads to an increased

growth rate of these SMCs, whereas knock down of *HES5* obliterates this proliferative effect.⁹² Moreover, treatment with a γ -secretase inhibitor that blocks cleavage of the Notch receptor attenuates hypoxia-induced pulmonary hypertension in mice.⁹² In summary, these data indicate that upregulation of the Notch 3–transcription factor HES-5 signaling pathway is associated with development of PAH in humans and is needed for development of the disease in the rodent models of pulmonary hypertension.

Peroxisome proliferator-activated receptor γ

Peroxisome proliferator-activated receptors (PPARs) are ligand-activated transcription factors involved in physiological processes ranging from lipogenesis to inflammation.⁹³ PPAR γ is expressed in many tissues, including vascular endothelial cells, vascular SMCs, and macrophages,^{94,95} and has crucial roles in cell growth, inflammation, and angiogenesis.^{96–98} PPAR γ is activated by several natural ligands,⁹⁹ and is also stimulated by synthetic ligands termed thiazolidinediones (for example, troglitazone and rosiglitazone) that have been used as therapeutic agents in type 2 diabetes mellitus.¹⁰⁰

PPAR γ has a potential role in the pathogenesis of PAH. This transcription factor is expressed abundantly in pulmonary vascular endothelial cells in normal human lung tissue, but not in the angiogenic plexiform lesions of lungs from patients with PAH or in the vascular lesions of rats with severe pulmonary hypertension induced by inhibition of VEGFR combined with chronic hypoxia.¹⁰¹ Global deletion of PPAR γ results in embryonic lethality,¹⁰² but the critical role of PPAR γ in maintaining pulmonary vascular homeostasis was demonstrated in mice that spontaneously developed pulmonary hypertension as a result of conditional knockout of PPAR γ in SMCs or in endothelial cells.^{103,104}

If, as these reports suggest, reduced PPAR γ in cells of the pulmonary vascular wall causes PAH, then the stimulation of PPAR γ might alleviate PAH. Apolipoprotein E deficiency, in association with a high-fat diet, results in insulin resistance and PAH in mice, and PPAR γ activation reversed PAH in this animal model.¹⁰⁵ Endogenous expression of PPAR γ in SMCs protects against the spontaneous development of PAH; studies using a PPAR γ antagonist and PPAR γ -deficient pulmonary artery SMCs showed that PPAR γ is required for BMP-2-mediated inhibition of SMC proliferation.¹⁰³ PPAR γ activation also reduces levels of two factors critical in PAH pathogenesis: endothelin 1¹⁰⁶ and asymmetric dimethylarginine (an endogenous NO synthase inhibitor).^{107,108} Activation of PPAR γ by rosiglitazone induces significant expression of heme oxygenase 1 in primary cultured pulmonary arterial SMCs, which upregulates p21 (*WAF1*) expression, leading to inhibition of 5-HT-stimulated SMC proliferation.¹⁰⁹ Moreover, PPAR γ agonists partially reverse pulmonary hypertension induced by monocrotaline and chronic hypoxia in rats,^{110,111} attenuate the development of hypoxic pulmonary hypertension in mice, and reduce hypoxic Nox4 induction and generation of reactive oxygen species in the lung of mice.¹¹² Concerns about the risks of cardiovascular events from the use of PPAR γ agonists in older patients with diabetes temper enthusiasm for this therapeutic drug class in patients.¹¹³

Identifying downstream targets regulated by PPARs in pulmonary vasculature might enable development of PPAR-related therapeutic strategies for PAH and perhaps circumnavigate concerns over the adverse effects of PPAR γ agonists reported in patients with diabetes.

Endothelial repair and angiogenesis

Bone-marrow-derived endothelial progenitor cells (EPCs) circulate in adult peripheral blood,¹¹⁴ and have a role in the maintenance of endothelial integrity and function and repair of endothelial injury. These cells also participate directly in postnatal vasculogenesis and angiogenesis in systemic vascular beds.¹¹⁵ Over a decade ago, Tuder *et al.* reported that plexiform lesions might result from deregulated growth of endothelial cells, and that the presence of perivascular inflammatory cells might influence lesion development.¹¹⁶ After VEGF and VEGFR-2 expression were demonstrated in plexiform lesions from patients with severe PAH, the lesions were hypothesized to perhaps be attributable to a process of disordered angiogenesis.³² Thus, proangiogenic factors (particularly progenitor cells) became a focus of research.

Animal studies involving transplantation of bone-marrow-derived cells suggest that bone-marrow progenitor cells are recruited during pulmonary vascular remodeling.^{117–119} Selective depletion of monocytes and fibrocytes attenuated hypoxia-induced pulmonary vascular remodeling in rats, indicating a role for fibrocytes in the remodeling process.¹²⁰ By contrast, defective mobilization and recruitment of EPCs resulted in potentiation of hypoxic pulmonary hypertension in mice that lacked the erythropoietin receptor in nonerythroid lineages.¹²¹ Furthermore, bone-marrow-derived cells did not contribute substantially to pathological pulmonary arterial remodeling induced by monocrotaline with unilateral subpneumectomy in rats.¹²² However, EPC dysfunction was sustained after chronic hypoxia-induced pulmonary hypertension in mice.¹²³ It seems that compensatory EPC mobilization and recruitment might occur in PAH, but might not be sufficient to prevent disease development as the EPCs are dysfunctional.

Patients with Eisenmenger syndrome and IPAH have reduced numbers of EPCs.^{124,125} However, some investigators have found increased EPC numbers in patients with PAH,^{126,127} which may reflect an unsuccessful effort to repair damage to the pulmonary endothelium, as EPC function can be impaired in patients with PAH.¹²⁶ Specifically, lung tissue from patients with IPAH showed increased expression of progenitor cell markers compared with controls, particularly within plexiform lesions.¹²⁶ A higher level of circulating proangiogenic progenitor cells was also observed in patients with IPAH, compared with healthy individuals.^{126,127} Dysfunctional BMP2-mediated Wnt signaling¹²⁸ might partly explain the altered endothelial cell behavior and angiogenesis; however, the precise mechanism of the (dys)regulation of angiogenesis in the pathogenesis of PAH remains unresolved. At the experimental level, the beneficial effects of proangiogenic therapies in animal models of pulmonary hypertension (associated with pulmonary fibrosis or induced by hypoxia

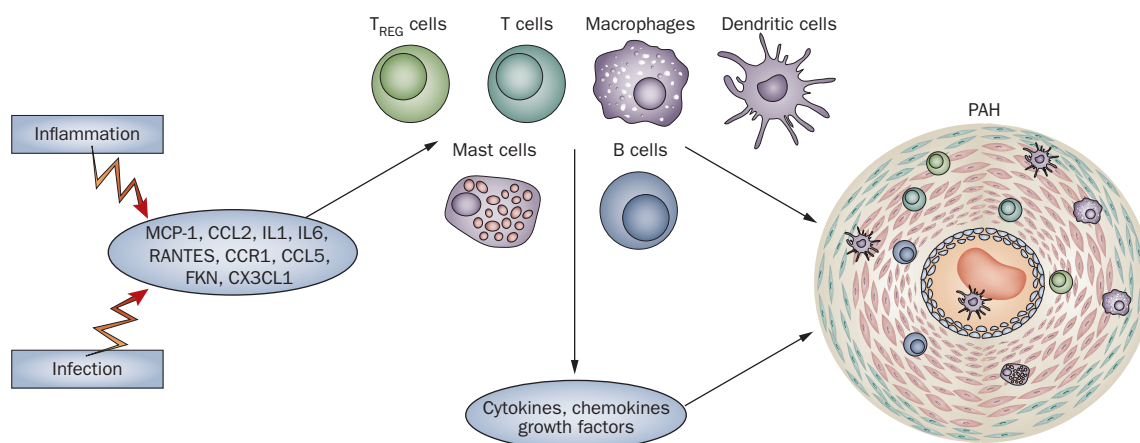


Figure 4 | Inflammation-mediated vascular remodeling in PAH. In response to infection and inflammatory events, lung vascular cells produce inflammatory mediators (chemokines and cytokines), thereby recruiting inflammatory cells (macrophages, dendritic cells, mast cells, B cells, T cells, and T regulatory cells). With the coordination of inflammatory mediators, inflammatory cells might perpetuate the release of cytokines, chemokines, and growth factors. Finally, these processes lead to vascular remodeling in PAH by matrix remodeling, collagen deposition, vascular cell proliferation, migration, and *in situ* thrombosis. Abbreviations: CCL2, chemokine (C-C motif) ligand 2; CCL5, chemokine (C-C motif) ligand 5, also known as Regulated upon Activation, Normally T cell Expressed and Secreted (RANTES); CCR1, chemokine (C-C motif) receptor 1; FKN, fractalkine (also known as C-X3-C motif chemokine 1); IL, interleukin; MCP-1, monocyte chemoattractant protein-1; PAH, pulmonary arterial hypertension.

or monocrotaline treatment) suggest that dysregulation of angiogenesis might be associated with pathogenesis.^{40,129,130} Impaired angiogenesis might be attributable to increased oxidative stress,¹³¹ among many other factors, but precise elucidation of the link between angiogenesis and PAH pathogenesis needs further study, and the role of stem and progenitor cells in the pathogenesis of PAH remains far from clear.

Inflammation as a trigger

Infectious organisms can affect the lung circulation directly by obliterating lung vessels or indirectly via inflammation. Inflammation has a key role in human PAH as well as in experimental models of pulmonary hypertension (Figure 4).^{132,133} Monocytes, macrophages, T lymphocytes, and dendritic cells are found in plexiform lesions, and other vascular lesions of PAH-affected human lungs.¹³⁴ Furthermore, inflammation contributes to the growth of pulmonary plexiform lesions.¹³⁵

PAH is associated with latent viral infections (for example, HIV, human herpes virus [HHV], Epstein–Barr virus, and cytomegalovirus).¹³⁶ The causative role of viruses in PAH induction is not well characterized, but HHV-8—a vasculotropic herpes virus associated with angioproliferative disorders—has provided some insight. HHV-8 has a particular tropism for endothelial cells and B lymphocytes, and might regulate angiogenesis. In one study, HHV-8 latency-associated nuclear antigen 1 (LANA-1) was identified in the lung tissue of 10 of 16 (62.5%) patients with IPAH;¹³⁷ however, other researchers have not found HHV-8 LANA-1.¹³⁸ *In vitro* studies found that a HHV-8-encoded viral G-protein-coupled receptor induces MMP2 activation and promotes angiogenesis in human pulmonary artery endothelial cells, implicating this receptor as a causative agent in IPAH.¹³⁹ Moreover, HHV-8 infection results in an apoptosis-resistant phenotype, which may be important in the development of plexiform lesions.¹⁴⁰

PAH is also associated with schistosomiasis, a parasitic infection that causes substantial inflammation and pulmonary vascular disease. Over 200 million people are currently infected with schistosomiasis and up to 20% of those with long-term infection develop PAH.^{1,3,5,141} Lung tissue parasitic egg burden induces inflammatory and immune processes, and may underlie PAH pathogenesis in infected patients. *Schistosoma* eggs modulate regulatory T cell activity and express a novel TGF- β superfamily member: *Schistosoma mansoni* inhibin/activin (SmInAct).¹⁴² Infiltration of T and B cells, plus increased serum levels of interleukin (IL)-1, IL-6, connexin 36, fractalkine (FKN) and chemokine (C-C motif) ligand 5 (also known as Regulated upon Activation, Normally T cell Expressed and Secreted; RANTES), have been reported in the pulmonary vascular lesions of patients with *Schistosoma*-related PAH.¹ Interestingly, the chronically infected schistosomiasis mouse model showed extensive pulmonary vascular remodeling in the absence of pulmonary hypertension and a correlation between cytokine levels (particularly IL-13) and the extent of remodeling.¹⁴³

Cytokines

Substantial evidence indicates a role for inflammatory cytokines in IPAH. Increased circulating levels of monocyte chemoattractant protein 1, tumor necrosis factor, IL-1 β , and IL-6 were found in patients with IPAH.^{144,145} T and B lymphocytes and macrophages are described in plexiform lesions characteristic of PAH.¹⁴⁶

Animal models also support the role of inflammatory cytokines in the initiation and progression of PAH. Monocrotaline-treated rats showed increased IL-6 expression in the lungs, and rats injected with IL-6 developed pulmonary hypertension.^{147,148} Furthermore, transgenic overexpression of IL-6 led to severe pulmonary hypertension in mice,¹⁴⁹ and IL-6-deficient mice were protected from hypoxia-driven experimental pulmonary hypertension.¹⁵⁰ Both *in-vitro* and *in-vivo* models also

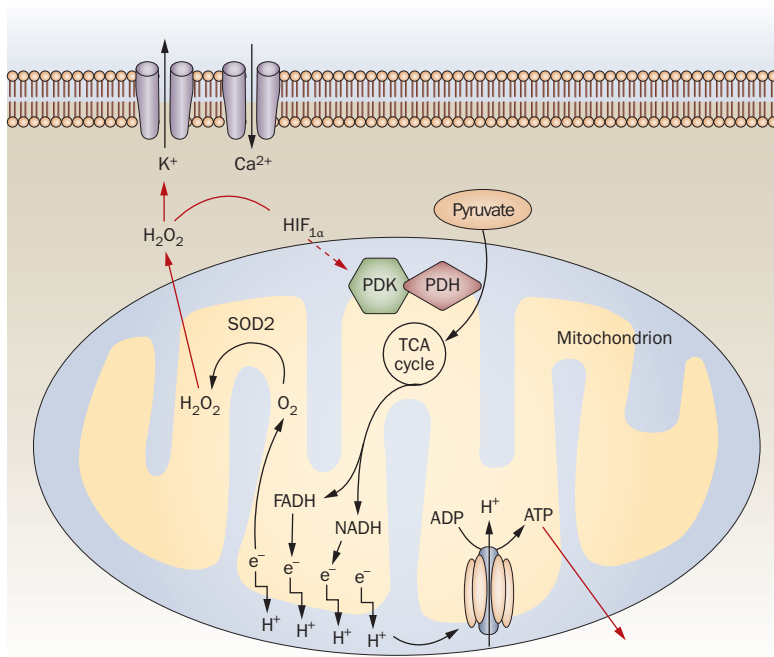


Figure 5 | Metabolic pathways in the mitochondrion. The PDH complex converts pyruvate, derived from glycolysis, to acetyl-coenzyme A in the mitochondrion, thus allowing it to enter the TCA Cycle and generate up to 36 moles of ATP per molecule of glucose in the presence of oxygen. Electron donors (mitochondrial NADH and FADH) produced by the TCA cycle pass electrons down a redox-potential gradient in the electron transport chain to molecular O₂. This electron flux powers H⁺ ion extrusion, powering ATP synthase. SOD2 converts superoxide anion (produced at complexes I and III) to H₂O₂, which serves as a redox messenger signaling 'normoxia'. In hypoxia, there is activation of HIF-1 and PDK which inhibits PDH. If pyruvate remains in the cytoplasm, it may complete glycolysis, producing lactic acid and generating 2 moles of ATP per glucose molecule. Dichloroacetate inhibits PDK and increases the ratio of glucose oxidation to glycolysis. Abbreviations: ATP, adenosine triphosphate; FADH, flavine adenine dinucleotide; HIF-1, hypoxia-inducible factor 1; NADH, nicotinamide adenine dinucleotide; PDH, pyruvate dehydrogenase; PDK, pyruvate dehydrogenase kinase; SOD2, superoxide dismutase 2; TCA, tricarboxylic acid.

suggest an interaction between BMPR2 and IL-6: transgenic mice overexpressing a dominant-negative BMPR2 had increased lung IL-6 expression and enhanced susceptibility to pulmonary hypertension,¹⁵¹ and increased IL-6 levels were found to suppress the expression of BMPR2 via induction of a microRNA cluster pathway.¹⁵²

Chemokines

Chemokines have a major role in the various steps of leukocyte recruitment, including initial reversible adherence to the endothelium (rolling), activation, firm adherence, and extravasation into the inflamed tissue.¹⁵³ Chemokine-dependent mechanisms leading to inflammatory cell recruitment in the lungs of patients with PAH have been analyzed.^{154,155}

FKN (also known as C-X3-C motif chemokine 1) is a soluble chemotactic protein that is also found as a membrane-anchored cell-adhesion molecule on endothelial cells.^{156,157} The actions of FKN are mediated by CX3C chemokine receptor 1 (CX3CR1), a transmembrane receptor expressed by monocytes, microglial cells, neurons, natural killer cells, mast cells, and subpopulations of T lymphocytes. Unlike other chemokines, FKN can

mediate the rapid-capture, integrin-independent adhesion, and activation of circulating CX3CR1⁺ leukocytes under high blood flow.¹⁵⁸ FKN expression is increased in circulating CD4⁺ and CD8⁺ T lymphocytes from patients with PAH, in inflammatory cells surrounding plexiform lesions, and in pulmonary arterial SMCs of remodeled arteries.^{155,159} A polymorphism in CX3CR1 is associated with a reduced risk of acute coronary artery disease in humans, indicating that FKN has a critical role in monocyte and T cell recruitment to the vessel wall.¹⁶⁰ Other cytokines overproduced in patients with PAH are RANTES and chemokine ligand 2 (CCL-2).^{154,161} CCL-2 overproduction is a feature of the abnormal pulmonary endothelial cell phenotype in IPAH and, compared with healthy controls, pulmonary artery SMCs from patients with IPAH exhibited stronger migratory and proliferative responses to CCL-2.¹⁶¹

Thrombosis

Thrombosis is a common feature of PAH, and research using calibrated automated thrombography demonstrated that patients with IPAH have a hypercoagulable phenotype.¹⁶² Tissue factor (which initiates coagulation) is upregulated in the pulmonary vasculature of rats with pulmonary hypertension induced by monocrotaline and pneumonectomy, and is also strongly expressed in the pulmonary vasculature of patients with IPAH.¹⁶³ Increased plasma levels of fibrinopeptide A, a marker of fibrin generation, have been reported in patients with IPAH.¹⁶⁴ Fibrinolysis might also be impaired, since patients with IPAH were shown to have increased levels of plasminogen activator inhibitor 1, compared with controls.¹⁶⁴ Increased activity of von Willebrand factor, which is essential for the interaction between platelets and endothelial cells, has been noted in IPAH.¹⁶⁴ Platelet aggregation might also be increased as a result of imbalance in vasoactive mediators; thromboxane A₂ (levels of which are increased in patients with PAH) is proaggregatory, whereas NO and prostacyclin (levels of which are decreased in patients with PAH) inhibit aggregation.^{12,165}

Warburg hypothesis: role of mitochondria

In 1924, Otto Warburg proposed that a shift in glucose metabolism from oxidative phosphorylation to glycolysis (despite adequate oxygen) was central to the growth of cancers, and this phenomenon became known as the 'Warburg effect' (Figure 5).¹⁶⁶ Glycolysis is less efficient at the generation of ATP than at oxidative phosphorylation, which results in increased glucose uptake, a phenomenon that can be tracked using ¹⁸F-fluorodeoxyglucose (¹⁸F-FDG). Cancers typically show increased ¹⁸F-FDG uptake and data suggest that proliferating pulmonary vascular endothelial cells from patients with PAH have a similar phenotype.¹⁶⁷ Reduced oxidative phosphorylation inhibits the electron transport chain and reduces the generation of superoxide, which might confer resistance to apoptosis and so perpetuate the phenotype. If so, then restoration of oxidative phosphorylation would be expected to render cells more susceptible to apoptosis and offer a novel strategy for treating cancer and vascular remodeling in PAH.

Dichloroacetate, a pyruvate dehydrogenase kinase inhibitor, has been used to test this hypothesis: dichloroacetate

decreases tumor growth *in vitro* and *in vivo*, without affecting noncancer mitochondria and tissues.¹⁶⁸ Studies of this drug in several experimental models of pulmonary hypertension have also been encouraging. Chronic dosing of dichloroacetate markedly attenuated pulmonary hypertension in rats exposed to hypoxia or monocrotaline, the fawn-hooded rat, which has deficient uptake of serotonin into platelets and develops pulmonary hypertension spontaneously, and mice overexpressing the serotonin transporter.^{169–171}

Clinical studies in glioblastoma multiforme have begun,¹⁷² and a clinical trial of dichloroacetate in PAH is also underway.¹⁷³ The right ventricle in patients with PAH also exhibits a metabolic shift to glycolysis, which might impair function.¹⁷⁴ Inhibition of pyruvate dehydrogenase kinase might, therefore, have beneficial effects on myocardial function in addition to effects on pulmonary vascular resistance.^{175,176}

Right ventricular hypertrophy

Right ventricular hypertrophy (RVH) develops when the right ventricle works against increased resistance in the pulmonary circulation. Cardiac hypertrophy—defined as an increase in myocyte size without cell replication—develops in PAH, at first as a compensatory mechanism. The right ventricle accumulates muscle mass (via elevated protein synthesis and an increase in myocyte size), and the right ventricle wall thickens.¹⁷⁷ The right ventricle also assumes a more-rounded shape, compressing the left ventricle.¹⁷⁸ Cardiac output is initially maintained, but with persistent elevated resistance, progressive contractile dysfunction occurs, leading to decompensation, dilatation, and right heart failure.^{177,179}

Elevated pressure within the right ventricle increases right ventricular wall stress and myocardial oxygen demand.¹⁷⁷ The increase in wall stress also impedes myocardial perfusion; impaired right coronary artery systolic flow and right ventricular ischemia have both been demonstrated in patients with PAH.^{180,181} Mechanical stretch is translated into intracellular signaling pathways via integrins,¹⁸² and integrin signaling to focal adhesion kinase has been implicated in right ventricular myocardial hypertrophy in rats with monocrotaline-induced pulmonary hypertension.¹⁸³ Metabolic changes have been noted, with upregulation of glucose transporter protein 4 (GLUT4) in the right ventricle of rats with monocrotaline-induced pulmonary hypertension,¹⁸⁴ and increased right ventricular uptake of ¹⁸F-FDG in patients with PAH.¹⁷⁴

Little is currently known about the processes governing the transition from compensated RVH to decompensated right heart failure in PAH. The development of right heart failure might be accelerated by maladaptive neurohormonal signaling, formation of reactive oxygen and nitrogen species, and exaggerated inflammatory responses.¹⁷⁷ Although elevated right ventricular afterload leads to RVH, experimental data suggest it might not be sufficient to cause progression to right heart failure. Rats with afterload increased by pulmonary arterial banding developed RVH but not right heart failure, whereas rats with pulmonary hypertension induced by VEGFR inhibition combined

with hypoxia did develop right heart failure, indicating that the remodeled vasculature in PAH might release mediators that interfere with adaptive RVH.¹⁸⁵ The monocrotaline rat model might also help to shed light on the transition from RVH to right heart failure. Rats treated with 30 mg/kg monocrotaline develop compensated RVH, whereas rats treated with a higher dose of 80 mg/kg monocrotaline develop right heart failure. This model has been used to demonstrate that right heart failure is associated with myocardial expression of tenascin C and downregulation of integrin alpha 6, and the investigators suggested that the deadhesive properties of tenascin C might cause myocardial slippage and thus lead to ventricular dilatation.¹⁸⁶

Harnessing molecular technologies

The power of genetics to aid our understanding of disease mechanisms is illustrated by the revelation of a link between mutations in *BMPR2* and PAH. Evolving technology and expertise in genomics, transcriptomics, and proteomics offer promise of further insights into the pathogenesis of this disease. These tools are being employed with enthusiasm for discovery research on serum and plasma samples, circulating cells, and human tissue;^{187,188} animal models provide an alternative source of tissue for understanding molecular mechanisms.¹⁸⁷ For example, a screen to detect altered protein expression in mice with hypoxic pulmonary hypertension led to the identification of a novel protein with a role in IPAH. Four and a half LIM domains protein 1 (FHL-1), a protein involved in muscle development, was upregulated in the lungs after 24 h of hypoxia (that is, at the onset of pulmonary hypertension).¹⁸⁷ Strong upregulation of FHL-1 was also observed in pulmonary arteries from patients with IPAH, and *in vitro* studies in human pulmonary arterial SMCs showed that FHL-1 promotes cellular migration and proliferation.¹⁸⁷ Proteomic screening of plasma samples and lung tissue from patients with PAH and controls has also identified a range of differentially expressed proteins with roles in growth, proliferation, and metabolism, providing new avenues for future research.^{188,189} One protein found to be upregulated in lungs from patients with IPAH, chloride intracellular channel protein 4, is important for vascular endothelial cell integrity and merits further study.¹⁸⁹

Advances in imaging also permit studies of disease processes *in vivo*.¹⁹⁰ Molecular imaging using PET is an emerging illustration of a novel approach to investigating the pathology of PAH *in vivo*. PET imaging is widely used in oncology, and a variety of PET tracers in development permit measurement of cell metabolism, proliferation, apoptosis, tissue hypoxia, and angiogenesis *in vivo*. ¹⁸F-FDG is an example of a PET tracer that is used frequently. This glucose analog is transported into metabolically active cells by the same transporter as glucose and converted to ¹⁸F-FDG-6-phosphate. In contrast to glucose, however, ¹⁸F-FDG cannot be further metabolized and ¹⁸F-FDG-6-phosphate becomes trapped in cells at a rate proportional to glucose use. FDG-PET in patients with cancer exploits the observation that many cancers use glycolysis in the cytoplasm instead of mitochondrial glucose oxidation as a major energy source. Studies in patients with PAH show

increased uptake in the lung,¹⁶⁷ but at this time it is unclear whether this uptake represents vascular cell proliferation or inflammation. In an ongoing study in patients with IPAH,¹⁷³ dichloroacetate is being used, and ¹⁸F-FDG uptake in lung and heart measured, to probe the relationship between aberrant glucose metabolism and cardiopulmonary hemodynamics. Studies using new tracers (for example, ¹⁸F-labeled fluorothymidine and ¹⁸F-labeled fluciclatide) might inform us further on the relationship between active proliferation and angiogenesis and the natural history of PAH. Interestingly, FDG uptake is increased in the right ventricle of patients with PAH, and uptake is reduced by targeted PAH therapy (such as prostacyclin).¹⁷⁴ FDG uptake, alone or combined with cardiac MRI, might prove a useful biomarker of cardiac function in PAH.

Conclusions

PAH is a treatable disease. The rapid pace of advance in our understanding of the condition in the past few years reflects, at least in part, the modern strategy of pursuing molecular mechanisms across 'disease boundaries'. As this pursuit continues, new therapies and management strategies will emerge for robust examination in the clinical setting.

A key challenge going forward will be to integrate this new knowledge in a systems biology approach, to prioritize key molecular pathways and link to personalized therapeutic interventions. The current assessment of patients with PAH is based on clinical phenotyping and fails to take into consideration the fact that PAH is a heterogeneous disorder. In neoplastic disorders, molecular signature analysis has proven useful in assigning prognosis and likely response to treatment. This assignment should be the goal in PAH management; patients with PAH are widely recognized

to vary considerably in their response to treatment. For example, a small, but important, minority of patients with IPAH exhibit a pronounced acute hemodynamic response to NO inhalation, which predicts an approximately 50% probability of a favorable long-term response to calcium-channel blockers.¹⁹¹ Ongoing work to understand the molecular basis of this response, by comparing genomic sequences from responders and nonresponders, might provide a noninvasive test to guide the prescription of calcium antagonists for patients with IPAH. Circulating protein biomarkers, such as B-type natriuretic peptide (BNP) or N-terminal-proBNP, and growth differentiation factor 15, can be used to stratify patients according to prognosis but have limited clinical utility for diagnosis or predicting optimal drug therapy. Genomic markers carry more information and offer greater promise, whether based on haplotypes, circulating cell transcriptomes, or microRNA profiles. The individualization of therapy in PAH is an exciting prospect, but we are still at the very beginning of this story.

Review criteria

Articles were selected from literature published in English and listed by PubMed Central. Search terms included, but were not limited to, "pulmonary arterial hypertension", "hypoxia", "monocrotaline", "rat", "mouse", "human", "proliferation", "apoptosis", "genetics", "genomics", "mRNA", "transcriptomics", "proteomics", and "imaging", in various combinations. No date limitations were used. All cited articles reflect established or emerging opinions discussed in various fora (for example, international meetings) by recognized experts in the field of pulmonary vascular disease.

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Author contributions

All authors contributed to researching data for the article, made a substantial contribution to the discussion of content, wrote, and reviewed and edited the manuscript before submission.