

Insights Into the Pathobiology of Pulmonary Hypertension

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ABSTRACT

Pulmonary hypertension is a complex disorder defined by an abnormal increase of pulmonary arterial pressure that may result in right ventricular failure and death. Pulmonary hypertension has a multifactorial aetiology and is currently classified into five groups based on histopathological appearance and treatment modalities. Our understanding of the pathobiology of pulmonary hypertension has evolved enormously in recent years. A condition that in the past was considered mainly determined by increased vascular tone is now seen as a vasculopathy in which structural changes are driven by excessive cell growth. In the present review we analyse mechanisms that may contribute to the pathobiology of pulmonary hypertension, including imbalance between vasoactive mediators, altered cell proliferation and apoptosis, dysfunctional endothelial repair and angiogenesis, and contributing factors such as inflammation. (BRN Rev. 2015;1:63-77)

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INTRODUCTION

Pulmonary hypertension (PH) is defined by an abnormal increase of pulmonary arterial pressure that may progress in right ventricular impairment, right ventricular failure, and

death. Pulmonary hypertension is a progressive disease of multifactorial aetiology, currently classified into five groups based on histopathological appearance and treatment modalities: (i) pulmonary arterial hypertension (PAH), (ii) PH due to left heart disease,

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TABLE 1. Classification of pulmonary hypertension

1. Pulmonary arterial hypertension
– Idiopathic PAH
– Heritable PAH
– Drug and toxin induced
– Associated with:
• Connective tissue disease
• HIV infection
• Portal hypertension
• Congenital heart diseases
• Schistosomiasis
1'. Pulmonary veno-occlusive disease and/or pulmonary capillary hemangiomatosis
1''. Persistent pulmonary hypertension of the newborn
2. Pulmonary hypertension due to left heart disease
– Left ventricular systolic dysfunction
– Left ventricular diastolic dysfunction
– Valvular disease
– Congenital/acquired left heart inflow/outflow tract obstruction and congenital cardiomyopathies
3. Pulmonary hypertension due to lung diseases and/or hypoxia
– Chronic obstructive pulmonary disease
– Interstitial lung disease
– Other pulmonary diseases with mixed restrictive and obstructive pattern
– Sleep-disordered breathing
– Alveolar hypoventilation disorders
– Chronic exposure to high altitude
– Developmental lung diseases
4. Chronic thromboembolic pulmonary hypertension
5. Pulmonary hypertension with unclear multifactorial mechanisms
– Hematologic disorders: chronic haemolytic anaemia, myeloproliferative disorders, splenectomy
– Systemic disorders: sarcoidosis, pulmonary histiocytosis, lymphangioleiomyomatosis
– Metabolic disorders: glycogen storage disease, Gaucher disease, thyroid disorders
– Others: tumoral obstruction, fibrosing mediastinitis, chronic renal failure, segmental pulmonary hypertension

Adopted in the 5th World Symposium on Pulmonary Hypertension, Nice 2013¹.
HIV: human immunodeficiency virus; PAH: pulmonary arterial hypertension.

(iii) PH due to lung diseases and/or hypoxia, (iv) chronic thromboembolic PH (CTEPH), and (v) PH with unclear or multifactorial mechanisms¹ (Table 1).

Pulmonary hypertension develops as a consequence of the increase of pulmonary vascular resistance produced by the reduction of effective surface in pulmonary vessels. Two major mechanisms account for such a reduction: occlusion by chronic thrombotic lesions or narrowing by the remodelling of the vessel wall. By far, remodelling of resistance vessels is the major determinant of increased pulmonary vascular resistance in the different forms of PH.

In the present review we will address the different cellular and molecular mechanisms underlying the remodelling process of pulmonary vessels and account for the development of PH.

VASOACTIVE MEDIATORS

The endothelium plays a key role in regulating the reduced vascular tone of pulmonary circulation and controlling cell proliferation in the vessel wall. Vascular tone is governed by the balanced release of endothelium-derived vasoactive agents with either vasoconstrictive or vasodilator properties (Fig. 1). Different signalling pathways have been identified as potential mechanisms that may produce a vasoconstrictive/vasodilator imbalance and contribute to the development of PH.

Nitric oxide pathway

Nitric oxide (NO) is synthesized in the endothelial cell from L-arginine by the action of

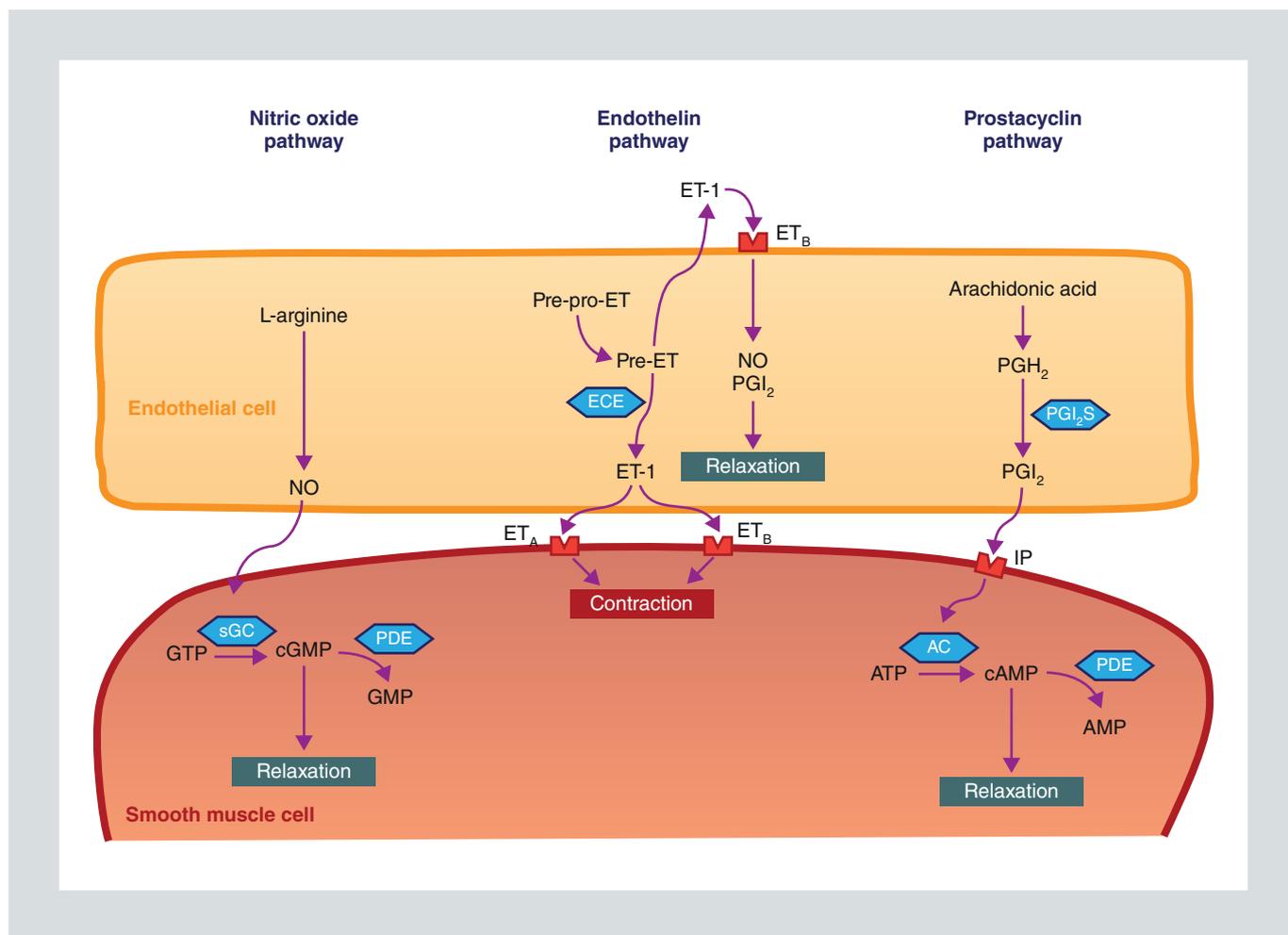


FIGURE 1. Major pathways involved in the regulation of pulmonary vascular tone that conform current targets for pulmonary hypertension treatment. Substances with vasorelaxing action also possess antiproliferative effects on smooth muscle cells, whereas vasoconstrictive agents promote their proliferation.

AC: adenylate cyclase; AMP: adenosine monophosphate; ATP: adenosine triphosphate; cAMP: cyclic AMP; cGMP: cyclic GMP; ECE: endothelin converting enzyme; eNOS: endothelial nitric oxide synthase; ET: endothelin; ET_A: endothelin receptor A; ET_B: endothelin receptor B; GMP: guanosine monophosphate; GTP: guanosine triphosphate; IP: prostaglandin I₂ receptor; NO: nitric oxide; PDE: phosphodiesterase; PGH₂: prostaglandin H₂; PGI₂: prostacyclin; PGI₂S: prostacyclin synthase; sGC: soluble guanylate cyclase.

endothelial nitric oxide synthase (eNOS) (Fig. 1). In smooth muscle cells (SMCs) of the vessel wall, NO activates soluble guanylate cyclase (sGC) that converts guanosine triphosphate (GTP) to cyclic guanosine monophosphate (cGMP). Cyclic GMP exerts a number of actions through protein kinase G (PKG)-dependent mechanisms, or through other pathways related to regulated ion channels or phosphodiesterases. Cyclic GMP is degraded to 5'GMP

by the action of phosphodiesterases (PDE), in particular PDE-5, which is abundantly expressed in the lung². Major effects of cGMP are vasodilation, inhibition of platelet aggregation, and anti-remodelling, anti-apoptotic and anti-inflammatory effects.

Patients with PH show reduced expression of eNOS³ and increased levels of asymmetric dimethyl-arginine (ADMA), an endogenous

competitive inhibitor of eNOS⁴. Currently, the NO signalling pathway is a major target for the treatment of PH by means of PDE-5 inhibitors, such as sildenafil and tadalafil⁵, or sGC stimulators (riociguat)⁶, both resulting in a net increase of the bioavailability of cGMP.

Endothelin pathway

Endothelin-1 (ET-1) is also synthesized in the endothelial cell and regulates vascular tone by activating ET_A and ET_B receptors located on pulmonary artery SMCs, acting as potent vasoconstrictor and inducing cell proliferation (Fig. 1). The expression of ET-1 is increased in lungs of patients with PH⁷, and circulating levels of ET1 are also increased in patients with PH⁸. In addition to its vasoconstrictor effect, ET-1 can also induce fibrotic changes by interacting with metalloproteinase⁹. The ET_B receptor is also present on endothelial cells and is involved in the release of NO and prostacyclin, thereby producing a vasodilator effect.

The endothelin pathway is also a major target for the current treatment of PH with the use of dual ET_A and ET_B receptor antagonists (bosentan, macitentan), or selective ET_A receptor antagonists (ambrisentan)^{5,10}.

Prostacyclin pathway

In the endothelial cell, prostaglandin H₂ (PGH₂) is generated from arachidonic acid by fatty acid cyclooxygenase. Prostaglandin H₂ is a substrate for prostacyclin synthase to generate prostacyclin (PGI₂). In SMCs, PGI₂ binds to the prostaglandin I₂ receptor (IP) that activates

adenylate cyclase (AC) to produce cyclic adenosine monophosphate (cAMP) that in turn activates protein kinase A (PKA), which produces a vasodilator effect, inhibits platelet aggregation, and exerts antiproliferative effects (Fig. 1). Prostacyclin synthase expression is reduced in lungs of patients with severe PH¹¹.

Prostaglandin H₂ may also be the substrate of thromboxane A₂ (TXA₂) through the action of thromboxane synthase. Thromboxane A₂ stimulates vasoconstriction and platelet aggregation via thromboxane/PG receptors.

The prostacyclin pathway is also a current target for PH treatment. Synthetic prostacyclin (epoprostenol) and prostacyclin analogues (iloprost, treprostinil) are given to patients with PH to enhance cAMP-PKA activity and exert vasodilator and antiproliferative effects. Recently, non-prostanoid agonists of the IP receptor have been developed (selexipag) and tested in randomized controlled trials¹².

Serotonin pathway

Serotonin or 5-hydroxytryptamine (5-HT) is a neurotransmitter that may act as vasoconstrictor and prothrombotic agent. Serotonin production is increased in PH¹³. Most of the cardiovascular effects of serotonin are mediated through their binding to the 5-HTB₂ receptor. Stimulation of the 5-HTB₂ receptor causes vasoconstriction and stimulation of fibroblasts, which may produce remodelling of pulmonary vessels and fibrotic changes in cardiac valves. Indeed, stimulators of the 5-HTB₂ receptor, such as appetite suppressants (fenfluramine, aminorex), may produce PH and cardiac valve disease.

Whereas in experimental models 5-HTB₂ receptor antagonists have shown to reduce PH¹⁴, in humans a phase II study with the 5-HTB₂ receptor antagonist terguride was not associated with clinical or hemodynamic improvements in patients with PAH¹⁵.

Potassium and calcium channels

The concentration of free calcium ions (Ca²⁺) in the cytoplasm is an important determinant of contraction, migration, and proliferation in SMC. Abnormalities in both potassium (K⁺) and Ca²⁺ channels have been linked to increased pulmonary vasoconstriction, dysregulation of vascular cell homeostasis, and proliferation of SMCs in PH^{16,17}. Pulmonary artery SMCs from patients with PH may show a selective downregulation of voltage-gated K⁺ (Kv) channels, which is associated with opening of voltage-gated Ca²⁺ channels, increased Ca²⁺ cytoplasmic concentration, and induction of SMC contraction¹⁸.

Transient receptor potential channels (TRPC) may contribute to the intracellular influx of Ca²⁺ and are associated with proliferation of SMCs. The TRCP3 and TRCP6 are upregulated in pulmonary artery SMCs from patients with PH¹⁹.

Currently, Ca²⁺ channel blockers are used to treat a reduced group of patients with idiopathic PAH who show positive response in the acute vasoreactivity testing⁵. Exploration of other ways to manipulate Ca²⁺ signalling pathways by means of Kv gene therapy, dichloroacetate, or anti-survivin are currently under way²⁰.

CELL PROLIFERATION

Arterial obliteration by vessel remodelling is a hallmark of PH pathogenesis²¹. Vascular remodelling involves all layers of the vessel wall. Each compartment of the pulmonary arterial wall entitles a wide range of cellular heterogeneity, which further enhances the remodelling process²¹. A common feature to all forms of PH remodelling is the formation of a layer of smooth muscle-like cells (or myofibroblasts) embedded in extracellular matrix, between the endothelium and the internal elastic lamina, termed the *neointima*. In addition, clusters of hyperproliferative endothelial cells resembling small tumours further expand the wall of the compromised pulmonary artery^{22,23}.

The specific biological processes in each PH type that enable vessel remodelling are unclear, but there is evidence to involve changes in SMC/endothelial cell differentiation, migration, proliferation, and apoptosis. Of all, cell proliferation has been more extensively investigated in PH. Pulmonary artery SMC and endothelial cells are characterized by a pro-proliferative and anti-apoptotic phenotype²⁴.

Growth factors

Growth factors are key players in the regulation of cell proliferation in PH. Several growth factors, including platelet-derived growth factor (PDGF)^{25,26}, epidermal growth factor (EGF)²⁷, and vascular endothelial growth factor (VEGF)²⁸, have been implicated in the abnormal proliferation and migration of pulmonary artery vascular cells (Fig. 2). These factors act as potent mitogens and chemoattractants for

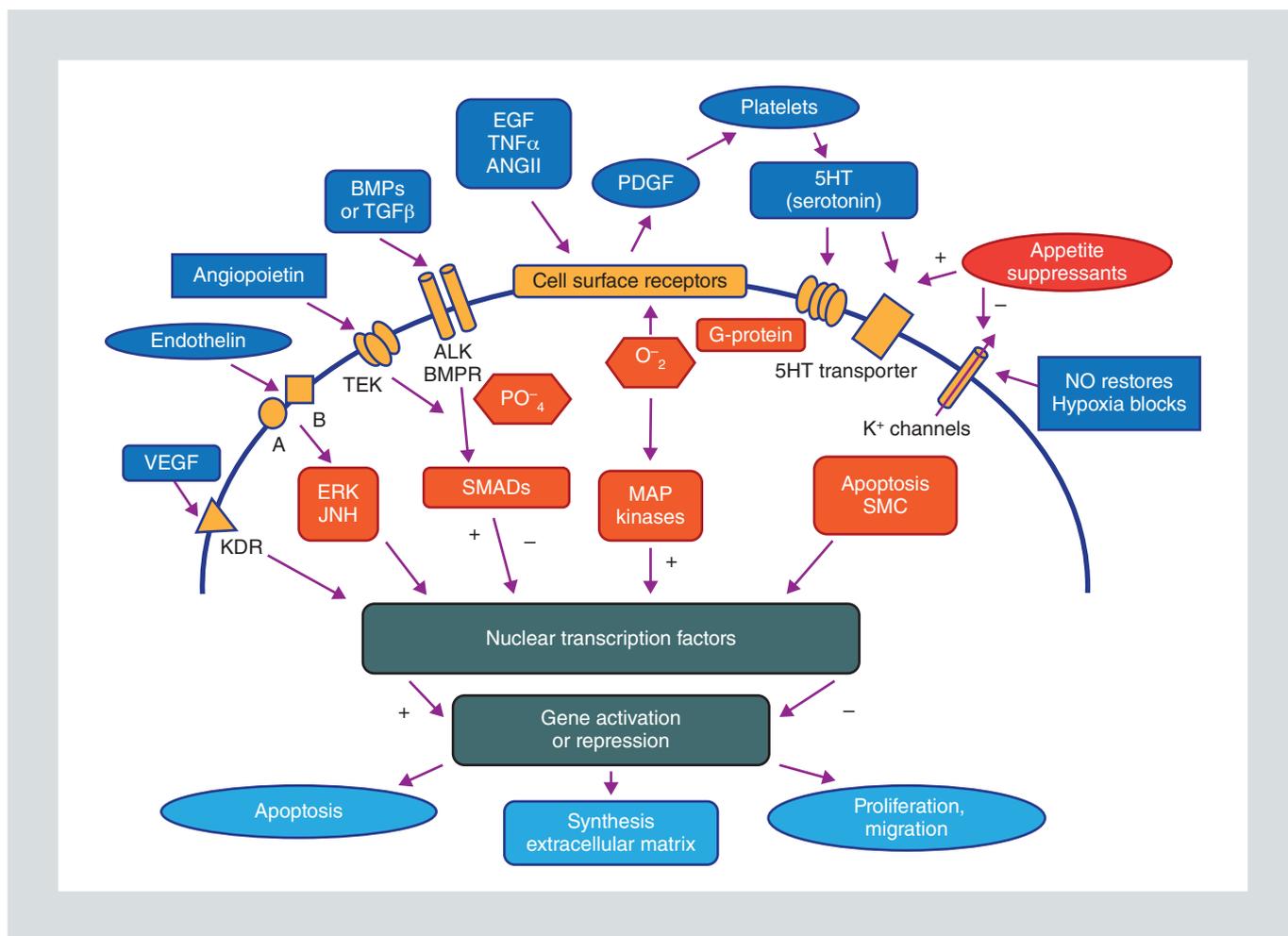


FIGURE 2. Major mediators in pulmonary hypertension pathophysiology.

ALK: activin-like receptor kinase; BMP: bone morphogenetic protein; BMPR: bone morphogenetic protein receptor; EGF: epidermal growth factor; 5HT: 5-hydroxytryptamine; KDR: kinase insert domain receptor; MAP: mitogen-activated protein; SMAD: mothers against decapentaplegic; SMC: smooth muscle cell; TGF: transforming growth factor; TNF: tumour necrosis factor; VEGF: vascular endothelial growth factor.

fibroblasts, SMCs, and endothelial cells and cause resistance to apoptosis.

Although there is consensus of a role of VEGF in PH, the pathogenic role of VEGF could be dependent on its isoform. The VEGF expression is upregulated within the pulmonary vasculature, as well as in plexiform lesions²⁹. However, a recent study identified a pathogenic role for VEGF-B. Unlike VEGF-A, VEGF-B appears to exacerbate remodelling in VEGF-B

knockout mice (VEGF-B^{-/-}) exposed to chronic hypoxia. They exhibit significantly less pulmonary vascular remodelling compared with wild-type mice (VEGF-B^{+/+})³⁰.

Other growth factors including PDGF, basic fibroblast growth factor (FGF), insulin-like growth factor-1, and EGF have also been implicated in the development of remodelling and all have been reported to be increased in PH. The mechanism that leads to induction

of these growth factors in the pulmonary vasculature is unclear. Recent studies have shown that reactive oxygen species play an important role in PH. Hypoxia, mechanical stretch, shear stress, and hydrogen peroxide have the capacity to induce PDGF expression in human pulmonary endothelial cells.

Receptor tyrosine kinases for growth factors form potential targets for PH treatment. The tyrosine kinase inhibitor imatinib reverses vessel remodelling in experimental PH²⁶. The effects of imatinib have been evaluated in a multicenter, randomized controlled trial in patients with PAH. Results show that imatinib improves pulmonary haemodynamics, symptoms, and exercise tolerance in PAH³¹. However, imatinib has not been approved for PAH treatment due to serious adverse effects. Along with this, nilotinib, a second generation tyrosine kinase inhibitor, EGF receptor blockers and FGF receptor inhibitors have been shown to attenuate PH in experimental models^{32,33}.

Angiopoietin-1 is an angiogenic factor essential for lung vascular development³⁴. Highly expressed by SMCs, angiopoietin-1 stabilizes the development of blood vessels by recruiting muscle cells, inducing cellular migration and division, enhancing the formation of endothelial tubes, and creating mature arterial structures. Interestingly, evidence suggests that all forms of non-familial PH are characterized by the upregulation of angiopoietin-1, correlating directly with the severity of the disease³⁴. Furthermore, angiopoietin-1 can stimulate endothelial cells to produce and secrete serotonin (5-HT)³⁵. These findings suggest that PH occurs through an angiopoietin-1/TIE2/5-HT paracrine pathway.

Apoptosis

Several abnormalities that have been described in PH contribute to a proliferation-apoptosis imbalance and to resistance to apoptosis within the vascular wall and may explain the remodelling in pulmonary arteries. Survivin, a member of the inhibitor of apoptosis gene family, is involved in cell proliferation and promotes cell survival in cancer by blocking programmed cell death. Survivin exhibits differential expression in nearly all human cancers, but not in normal tissues³⁶. In cancer, inhibition of survivin reduces cell proliferation, increases apoptosis, and sensitizes cells to cytotoxic agents and radiotherapy. The administration of the pro-apoptotic sphingolipid ceramide (encapsulated nanoliposomes) reduces the expression of survivin and cures a type of leukaemia of natural killer cells (NK-LGL leukaemia) in an experimental rat model³⁷. Likewise, treatment with sepantromium bromide (YM155), a new survivin inhibitor, or other anti-survivins have therapeutic efficacy, leading to apoptosis and inhibition of the proliferation in SK-NEP-1 Wilms tumour³⁸. Phase I and II studies have shown that the administration of YM155 is safe, reaches plasma concentrations, and has antitumor activity in patients with different types of solid tumours^{39,40}.

Survivin is expressed in pulmonary arteries of patients with PAH, but not in control subjects⁴¹. Accordingly, it has been hypothesized that the survivin signalling pathway may play a pivotal role in cell proliferation and cell division, thereby contributing to the development of PAH^{41,42}. *In vitro*, survivin inhibition induces pulmonary artery SMC apoptosis and reduces its proliferation⁴¹. In rats with

monocrotaline-induced PH, survivin is over-expressed in pulmonary artery SMCs and its inhibition with the nebulization of an adenovirus carrying a survivin suppressor mutant prolongs their survival⁴¹, thereby suggesting that the survivin pathway could be a potential therapeutic target in PH.

GENETIC MECHANISMS

Pulmonary arterial hypertension is a complex disease with both genetic and environmental components. In idiopathic PAH, about 75% of the cases are sporadic and about 25% are familial. Inheritance of heritable PAH appears to be autosomal dominant with a penetrance of approximately 20%, suggesting that the mutation alone is insufficient to result in expression of the disease phenotype.

Bone morphogenetic protein receptor type 2 (BMPR2) is a member of the transforming growth factor beta (TGF- β) superfamily, a group of serine kinase transmembrane signals that have also been implicated in other pathologies. The BMPR2 receptor was originally described to be involved in the regulation of growth and differentiation of bone and cartilage⁴³, but more recently, it was also shown to play a critical role in the regulation of growth, differentiation, and apoptosis of other cell types including endothelial cells and pulmonary artery SMCs⁴⁴ (Fig. 3). The BMPR2 mutation is present in more than 50% of heritable PAH. The gene is located on the long arm of chromosome 2 (2q31,32) has 13 exons, and 298 mutations have been described at different points⁴⁵. A phenomenon of genetic anticipation occurs, meaning that later generations have the disease at younger ages.

Patients with the mutated gene have also some differences from those without: worse hemodynamic profile and less response to acute vasodilator test; however, there are no differences in survival and clinical characteristics at diagnosis⁴⁶.

In recent years there have been great advances in this field and other rare mutations in PAH have been described, mainly genes belonging to the TGF- β superfamily: activin-like receptor kinase-1 (ALK1), endoglin (ENG), and mothers against decapentaplegic 9 (SMAD9)⁴⁷⁻⁵⁰. Also, mutations have been described in caveolin-1 (CAV1)⁵¹, which encodes a membrane protein of caveolae, abundant in the endothelial cells of the lung, and KCNK3, a gene encoding potassium channel super family K member-3⁵².

ENDOTHELIAL REGENERATION AND ANGIOGENESIS

The endothelium is considered a genetically stable, quiescent cell line that plays a critical role in the regulation and maintenance of vascular homeostasis. It acts as a non-adhesive surface for platelets and leukocytes and produces important factors in the regulation of inflammation, thrombosis, and blood flow⁵³. Despite the uncertainty of the exact mechanisms causing PH, the current belief suggests that an injury occurring in a genetically predisposed individual could initiate microvascular degeneration or result in proliferative apoptosis-resistant endothelial cells⁵⁴. Endothelial dysfunction favours an increase in vascular tone and mediates structural changes in the pulmonary vasculature and drives an abnormal endothelial cellular

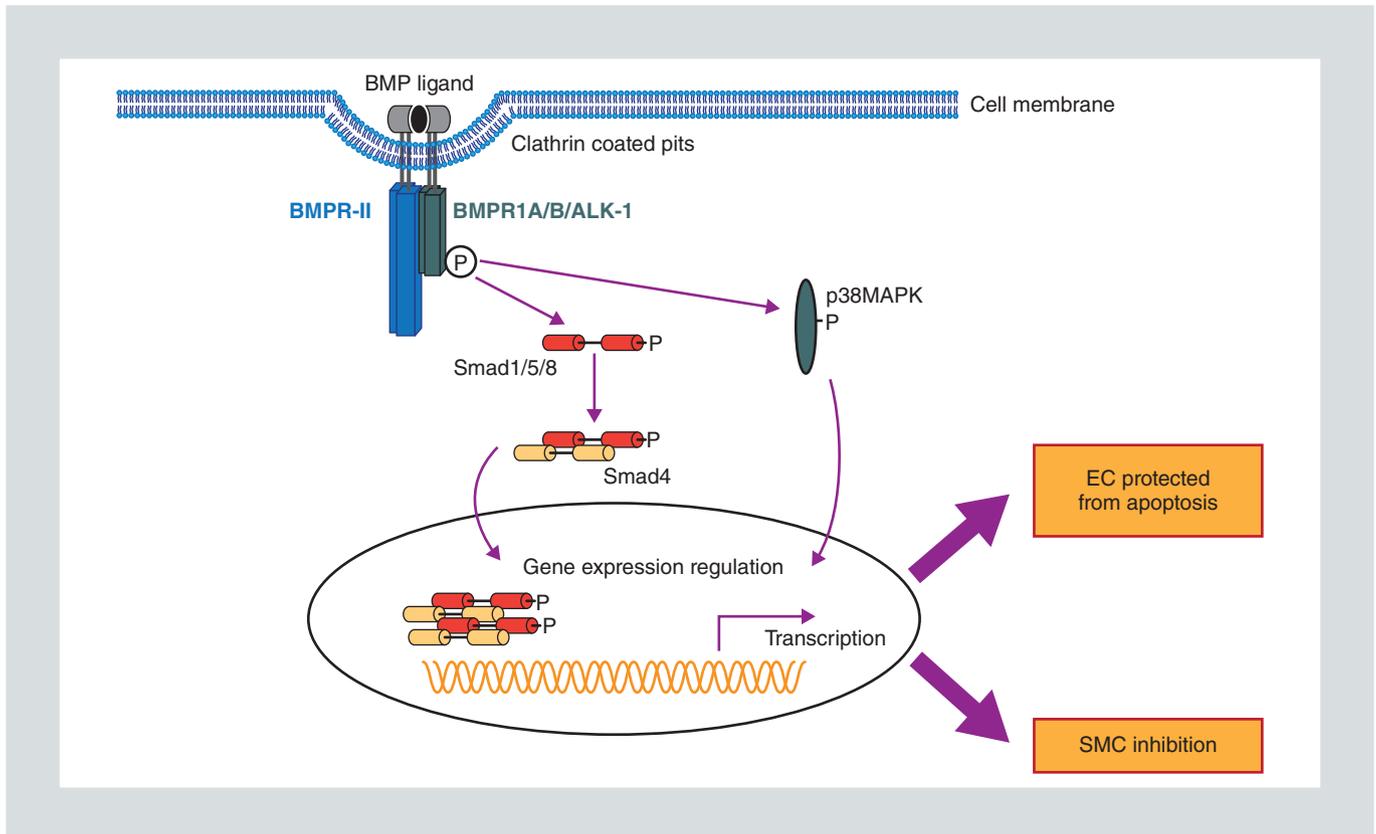


FIGURE 3. Bone morphogenetic protein-2 signalling pathway. Signalling in the bone morphogenetic protein pathway begins with the binding of a bone morphogenetic protein to the type II receptor. This causes the recruitment of a bone morphogenetic protein type I receptor, which it phosphorylates. The type I receptor phosphorylates a receptor-mothers against decapentaplegic a transcriptional regulator. ALK: activin-like receptor kinase; BMP: bone morphogenetic protein; BMPR: bone morphogenetic protein receptor; EC: endothelial cell; MAPK: mitogen-activated protein kinase; SMAD: mothers against decapentaplegic; SMC: smooth muscle cell.

proliferation, usually resulting in vascular remodelling and the development of proliferative lesions such as the plexiform structures⁵⁵. Disrupted endothelium leave the underlying vascular tissue defenceless against various blood-borne factors that may further promote pathological changes.

Integrity of the vascular endothelium depends partly on the extent of injury, but also on the capacity for endothelial regeneration and repair following vascular damage. The traditional paradigm of vascular repair is based on the proliferation and migration of pre-existing mature endothelial cells from

the adjacent vasculature⁵⁶ (Fig. 4). However, mature endothelial cells are terminally differentiated cells and are thought to have low proliferative potential, with limited capacity to contribute to endothelial reconstruction. In a seminal study, Asahara et al.⁵⁷ described a population of circulating cells that could differentiate into mature endothelial cells *ex vivo*. These circulating endothelial progenitor cells (EPC) are positive for the stem cell marker CD34 and the VEGF receptor 2 (VEGFR-2). They are thought to originate from the bone marrow, circulate in peripheral blood, and home to sites of endothelial damage where they can differentiate into cells with endothelial-like

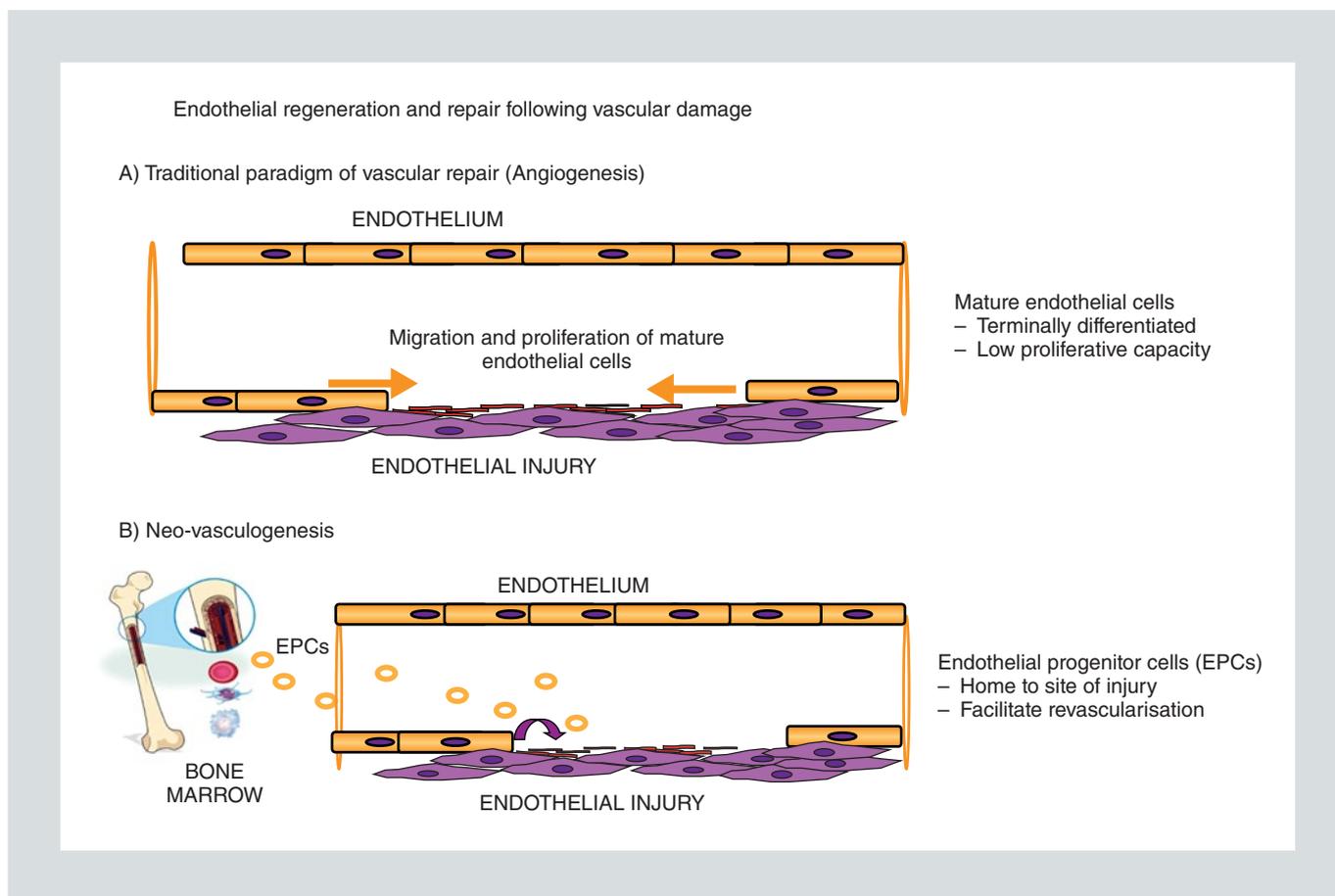


FIGURE 4. Endothelial regeneration capacity following endothelial injury. **A)** Traditional paradigm of vascular repair by the locally outgrowth of pre-existing vessels; angiogenesis. **B)** Neo-vasculogenesis, *de novo* formation of endothelial cells. Mobilized bone marrow derived endothelial progenitor cells have the capacity to migrate and incorporate into the injured tissue vascular bed.

phenotype to facilitate re-endothelialization (Fig. 4). The exact origin and phenotype of these progenitors remains uncertain.

It is considered that numbers of circulating EPCs reflect the ability of the organism to repair the endothelium⁵⁸. Reduced number of circulating EPCs has been established as an independent prognostic risk factor associated with endothelial dysfunction and high cardiovascular risk⁵⁹. Therefore, circulating progenitor cells have been suggested as biological markers of vascular dysfunction. Their presence in peripheral circulation offers the opportunity to monitor their number as a

marker of disease state and, importantly, as a marker of response to therapy.

Studies of circulating EPCs in PH have provided conflicting results (Table 2). While some studies indicate greater levels of circulating EPCs in patients with PH⁶⁰⁻⁶², others found that EPCs were reduced compared to controls⁶³⁻⁶⁶. This discrepancy could be due to methodological differences and failure to reliably match for cardiovascular risk factors, sex, and age. In recent years, the idea that the vasculature is capable of regeneration has raised the possibility that EPC-based therapies may provide an alternative to conventional

TABLE 2. Putative circulating endothelial progenitor cells in patients with pulmonary hypertension

Author Year	Age	Sex	Disease type	Sample size	Antibody used to define an EPC	EPC	CFU-EPC
Asosingh et al. ⁶⁰ 2008	Matched between groups	Matched between groups	IPAH	IPAH (n = 16) HV (n = 16)	CD34/CD133	IPAH > HV	IPAH > HV
Smadja et al. ⁶¹ 2008	Matched between groups	Matched between groups	PAH/CTEPH	PAH (n = 9) HV (n = 9)	CD146, CD34/ CD133	(CD146)-PAH > HV, CTEPH ~HV (CD34/CD133)-PAH/CTEPH ~HV	PAH/CTEPH ~HV
Toshner et al. ⁶² 2009	Matched between groups	Matched between groups	IPAH	IPAH (n = 7) HV (n = 7)	CD34/CD133/ KDR	IPAH > HV	Not available
Fadini et al. ⁶³ 2007	Not described	Not available	IPF/PH	IPF/PAH (n = 5) HV (n = 25)	CD34/CD133/ KDR	IPF/PAH < HV	Not available
Diller et al. ⁶⁴ 2008	Matched between groups	Matched between groups	IPAH	IPAH (n = 55) HV (n = 47)	CD34/KDR	IPAH < HV	IPAH > HV
Junhui et al. ⁶⁵ 2008	Matched between groups	Matched between groups	IPAH	IPAH (n = 20) HV (n = 20)	CD133/KDR	IPAH < HV	Not analysed
Hansmann et al. ⁶⁶ 2011	Matched between groups	Matched between groups	PAH	IPAH (n = 43) HV (n = 6)	CD34/KDR	IPAH < HV	Not analysed

CFU-EPC: endothelial outgrowth colony-forming units; CTEPH: chronic thromboembolic pulmonary hypertension; EPC: endothelial progenitor cell; HV: healthy volunteers; IPAH: idiopathic pulmonary arterial hypertension; IPF: idiopathic pulmonary fibrosis; KDR: kinase insert domain receptor; PAH: pulmonary artery hypertension.

treatments. Despite this ambiguity, *ex vivo* expanded endothelial-like progenitor cells have shown therapeutic benefit in short-term studies in animal models of PH^{67,68} and in a randomized controlled trial in humans with idiopathic PAH⁶⁹. Whether decreased vascular damage is caused by a direct endothelial repair by EPCs or indirectly by the release of paracrine signals is unknown. At present, it is too premature to draw any definitive conclusion as to the utility of EPCs in PH treatment. Additional evidence is required to better define and understand the role of EPC as a biomarker or diagnostic tool and to understand its physiological and functional characteristics in PH.

An accurate assessment of the severity of endothelial dysfunction may have diagnostic and prognostic value for PH. Thus, it is

important to identify novel circulating biological targets in blood that specifically indicate the pathologic mechanism, the severity of the disease, and the treatment response. Novel potential biomarkers, such as endothelium-derived microparticles (EMP), have been recently suggested as alternative indicators of endothelial dysfunction in PH. The EMPs are small membrane vesicles released from endothelial cells in response to cell activation, damage, or apoptosis⁷⁰. Patients with PH show higher numbers of circulating EMPs, thereby reflecting the patient's endothelial impairment⁷¹. They also have been correlated to some extent with established parameters of endothelial dysfunction, vascular damage, and disease severity⁷¹.

As endothelial dysfunction is a major determinant in the design of new therapeutic drugs,

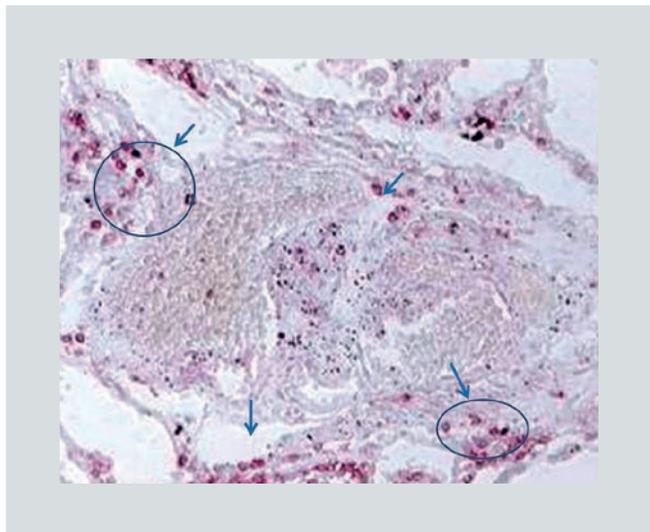


FIGURE 5. Perivascular CD45+ inflammatory cells (arrows) in a plexiform lesion from a patient with pulmonary arterial hypertension.

understanding mechanisms of endothelial repair is critical towards developing a cure based on PH-targeted therapies. The combined assessment of potential surrogate markers for vascular dysfunction and early detection (EMPs and EPCs) could therefore provide valuable information about the patient's endothelial impairment, endogenous repair capacity, and disease severity.

INFLAMMATION

Inflammatory mechanisms appear to play a significant role in some types of PH, including monocrotaline-induced PH in rats and PAH of various origins in humans, including connective tissue diseases and human immunodeficiency virus (HIV) infection⁷². In fact, a wide range of cytokines has been shown to be elevated and to correlate with survival in PAH⁷³. As is well documented in tissue-based studies, both early and persistent inflammation contributes to pulmonary vascular disease. The idea of an inflammatory

process as a mechanism of vascular remodelling derived from studies demonstrating an increased number of inflammatory cells infiltrating the adventitia of pulmonary arteries in many forms of PH (Fig. 5). In these studies, the amount of perivascular inflammatory infiltrate, largely of lymphocytes, correlated with parameters of pulmonary vascular remodelling and haemodynamics in PH⁷⁴. In perivascular tissues from subjects with PH, the most common cellular components of inflammation are described⁷²: macrophages (CD68, CD14), dendritic cells (CD209), T-lymphocytes (CD3, CD4 and CD8) are usually increased as compared with control subjects⁷⁵. The action of secreted cytokines, including TGF- β , in the adventitia mediates specific homing for leukocytes in this vascular compartment, leading to their inappropriate/pathologic retention and survival⁷⁶. The adventitia is, therefore, suited to harbour canonical innate immune cells, specifically macrophages and dendritic cells, which with adventitial fibroblasts, are all equipped with the necessary machinery (e.g., toll-like receptors and inflammasome components [like the nod-like receptors]) to potently respond to a variety of exogenous and endogenous danger signals.

The innate inflammatory system appears to also participate in PH. Natural killer cells, which target stressed, virally infected, or oncogenically transformed cells, are dysfunctional, with reduced number and cytolytic capacity, in patients with idiopathic PAH and in mouse and rat models of PH⁷⁷. The complement system, which bridges innate and adaptive immunity, is also activated in PAH, and deficiency of complement C3 protects mice from hypoxia-induced PH⁷⁸.

Finally, inflammation is closely associated with pulmonary vascular disease in the setting of autoimmune diseases, such as scleroderma, and paradigmatic of the interplay of inflammation and PH is its link with schistosomiasis (the most frequent cause of PAH worldwide)⁷⁹ and HIV infection⁸⁰.

A further pathway by which remote signaling might target the pulmonary vasculature is by the release of cell-derived exosomes from distant sources, exerting anti- or proinflammatory depending on their cargo. Exosomes derived from mesenchymal stem cells were found to inhibit the hypoxic activation of the signal transducers and activators of transcription 3 (STAT3) pathway in hypoxic mice and prevent the development of PH⁸¹.

CONCLUSIONS

In summary, multiple factors are involved in the pathobiology of PH. The progression of the disease is driven by a combination of changes in the balance of vasoactive mediators, altered cell proliferation and apoptosis, dysfunctional endothelial repair and angiogenesis, and contributing factors such as inflammation. The past decade has witnessed major therapeutic advances in the treatment of PH and currently available pharmacological agents have provided significant improvement in survival and well-being of patients. Yet, further progression in the understanding of the molecular mechanisms underlying vascular changes in PH will furnish the basis for future innovative approaches to the treatment of this devastating disease.

CONFLICT OF INTEREST

Dra. I. Blanco reports personal fees from Bayer, personal fees from Actelion, outside the submitted work. Dr. Barberà reports personal fees from Actelion, personal fees from Bayer, personal fees from GlaxoSmithKline, personal fees from Pfizer, grants from Actelion, grants from Bayer, grants from GlaxoSmithKline, grants from Pfizer, outside the submitted work. All other authors declare no relevant conflict of interest.

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