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TGF β and BMPRII signalling pathways in the pathogenesis of pulmonary arterial hypertension

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Highlights:

- Dysregulation of the BMP–TGF β axis is a major concern in pulmonary arterial hypertension
- Reduced BMPRII expression causes an imbalance of BMP–TGF β signalling
- Impaired BMP–TGF β signalling results in endothelial dysfunction and inflammation
- Innovative therapeutic approaches could incorporate broader aspects of the PAH pathogenesis

Keywords: Pulmonary arterial hypertension; BMPRII; transforming growth factor; therapies; signalling; endothelial dysfunction.

Teaser: This review highlights the latest findings regarding the role of TGF β and BMPRII signalling in the pathogenesis of pulmonary arterial hypertension and emphasises new therapeutic strategies targeting TGF β /BMPRII pathways.

Pulmonary arterial hypertension (PAH) is a severe condition characterised by remodelling of precapillary pulmonary arteries sometimes associated with mutations in the bone morphogenetic protein receptor type 2 (*BMPR2*) gene. Even in the absence of *BMPR2* mutations, increased transforming growth factor (TGF) β receptor signalling and decreased *BMPRII* signalling have been shown to contribute to PAH pathogenesis. In this Keynote, we review the potential mechanisms by which the imbalance of BMP/TGF β signalling contributes to endothelial dysfunction, vascular remodelling, inflammation and disordered angiogenesis in PAH. Additionally, we highlight how currently used drugs can influence BMP/TGF β signalling. Finally, we browse the newly developed therapeutic approaches targeting *BMPRII* and TGF β signalling pathways by focusing on preclinical studies and clinical trials.

Introduction

Pulmonary hypertension (PH) is characterised by increased blood pressure in the pulmonary arteries, veins or capillaries. Different pathological features and therapeutic approaches segregate five categories of PH: (i) pulmonary arterial hypertension (PAH); (ii) PH as a result of left heart disease; (iii) PH caused by lung disease and/or hypoxia; (iv) chronic thromboembolic PH (CTEPH); and (v) PH with unclear multifactorial mechanisms [1]. PAH is defined by a mean pulmonary arterial pressure (mPAP) ≥ 25 mmHg at rest, a normal capillary wedge pressure < 15 mmHg and a pulmonary vascular resistance (PVR) above 3 Wood units [1]. It is a progressive, severe and incurable disorder affecting the pulmonary vasculature, as well as the right ventricle (RV). The disease is characterised by an obstruction of the small precapillary pulmonary arteries resulting in increased PVR, RV hypertrophy, right heart failure and death if left untreated [2]. Idiopathic PAH (IPAH) corresponds to sporadic cases without any familial history or identified risk factors [1]. When PAH occurs in a hereditary context (HPAH), germline mutations are identified. In addition, PAH can be associated with drug or toxin intake such as anorexigens (see Glossary), dasatinib or interferon, or connective tissue disease (CTD), congenital heart disease (CHD), portal hypertension, schistosomiasis and HIV [1]. According to the updated classification, PAH also includes pulmonary veno-occlusive disease/pulmonary capillary hemangiomatosis (PVOD/PCH) and persistent PH of the newborn [1].

PAH is a rare disease with a prevalence estimated between 15 and 50 subjects per million inhabitants in western countries, occurring more frequently in females (60–76% of IPAH patients are female) [3]. Although there is a significant increase in the awareness of the disease, there remains a diagnostic delay. Patients with PAH generally display unspecific symptoms including fatigue, syncope or dyspnoea and can be diagnosed at any age. In the absence of specific treatment, the average survival time is ~ 2.8 years [4]. Even with effective vasodilator therapies, mortality rates remain high with a 5-year survival rate of 49–67% [5]. Currently, there is no curative treatment available for PAH with lung transplantation being the only option in patients with severe PAH refractory to current medical therapies [6].

When PAH occurs in a hereditary context, germline mutations in the bone morphogenetic protein receptor type 2 (*BMPR2*) gene, a member of the transforming growth factor (TGF) β superfamily, are detected in 70% of cases. However, *BMPR2* mutations have also been detected in 11–40% of sporadic cases without any familial history [7]. The pathophysiological process of PAH that leads to increased PVR and RV hypertrophy involves vasoconstriction, vascular remodelling, inflammation and thrombosis, contributing to the obstruction of the precapillary arteries [8]. Pulmonary vascular remodelling is a complex process triggered by different stimuli-like hypoxia, shear stress, inflammation, drugs or toxins and genetic susceptibility [9]. Vascular remodelling involves thickening

of intima, media and adventitia, attributable to hypertrophy and hyperplasia of pulmonary arterial smooth muscle cells (PASMCs), PA endothelial cells (PAECs) and fibroblasts, consequently contributing to vascular obstruction. Additionally, muscularisation of peripheral arteries, loss of precapillary arteries, neointima formation and formation of plexiform lesions are observed in severe PAH [10]. The presence of inflammatory cells such as T and B lymphocytes, macrophages and dendritic cells is frequently observed in remodelled pulmonary vessels, suggesting that the inflammatory process can contribute to the pathogenesis of PAH [11]. Altered angiogenesis is another important aspect of the pathophysiology of PAH; whether vascular endothelial growth factor (VEGF), one of the most important angiogenic factors, plays a mechanistic part in the development of PAH remains unclear. However, VEGF and VEGF receptor (VEGFR) expression levels are elevated within plexiform lesions from patients with PAH [12], and a combination of hypoxia with administration of the antiangiogenic VEGFR blocker Sugen (SU5416) causes obliterative PAH in rodents [13]. Endothelial dysfunction is considered a hallmark of the pathophysiology of PAH and is defined by an imbalance between vasodilators and vasoconstrictors. This is an important aspect in PAH characterised by sustained reduction in vasodilators such as prostacyclin (PGI_2) or nitric oxide (NO) and upregulation of vasoconstrictors, such as endothelin (ET)-1, serotonin or thromboxane A_2 . These pathways also exert, to a variable degree, effects on vascular remodelling and coagulation homeostasis [14]. The identification of molecules involved in the regulation of pulmonary vascular tone, including PGI_2 , NO and ET-1, has led to the development of several approved PAH therapies targeting three major pathways (Figure 1).

PGI_2 pathway

PGI_2 analogues include epoprostenol, iloprost and treprostinil and the selective agonist of the prostacyclin receptor (IP) selexipag. PGI_2 analogues activate the IP receptor and downstream adenylate cyclase (AC), resulting in the production of cyclic adenosine monophosphate (cAMP), which induces vasodilation and reduces vascular SMC proliferation [15].

NO pathway

(i) Phosphodiesterase type 5 inhibitors (PDE₅I) including sildenafil and tadalafil target the NO pathway by enhancing the production of cyclic guanosine monophosphate (cGMP), resulting in PASMC relaxation and vasodilation [16]; (ii) activator of the soluble guanylate cyclase (sGC) riociguat directly activates sGC in the absence of NO, resulting in the production of cGMP, PASMC relaxation and vasodilation [17].

ET-1 pathway

ET-1 receptor antagonists (ERAs) inhibit the vasoconstrictive effect of ET-1 by antagonising ET-1 receptor type A (ET_A) and/or B (ET_B). ET_A and ET_B receptors expressed by SMCs stimulate

vasoconstriction, whereas ET_B receptors stimulate release of vasodilator agents by ECs. ERAs can be selective of ET_A (ambrisentan) or nonselective blockers of ET_A and ET_B (bosentan and macitentan) [18]. In pathological conditions like PAH, ET_B receptors are upregulated on PSMCs and downregulated on PAECs [19].

One important issue is that PAH patients can respond differently to treatment; currently, only acute vasoreactivity testing with NO or PGI₂ analogues is performed to orientate treatment towards low-priced calcium channel blockers. In addition, currently approved drugs exhibit vasodilator effects but do not reverse pulmonary vascular remodelling with further insight into the pathogenesis of PAH required to advance drug development and improve patient management. This review focuses on TGF β signalling, its dysregulation in PAH and potential interventions.

TGF β superfamily

TGF β family classification

BMPR2 encodes for BMPRII, which is part of the TGF β superfamily. This is a large family of structurally similar polypeptide growth factors and related receptors with >30 different ligands including TGF β s, activins, inhibins, Nodal, Lefty (left-right determination factors), bone morphogenetic proteins (BMPs), growth and differentiation factors (GDFs) and anti-Müllerian hormone (AMH) [20]. These ligands are known to signal via a heterocomplex of two groups of transmembrane serine/threonine kinase receptors (i.e., type I and type II receptors). This dimeric complex comprises two type I (ALK1-7) and two type II receptors (TGF β receptor type II, BMPRII, ActRIIA, ActRIIB or anti-Müllerian hormone receptor II). Co-receptors to this complex include endoglin or betaglycan, involved in specific ligand–receptor binding and in regulating receptor signalling pathways. TGF β signalling is further complicated by expression of endogenous antagonists (i.e., noggin, gremlin-1, chordin or follistatin) that can modulate ligand–receptor interactions and signalling. Noggin and chordin bind and antagonise BMP2, 4 and 7; noggin, by competitively interacting with BMP4, inhibits its activity and prevents BMP4 from binding to cell-surface receptors [21] and chordin antagonises normal signalling by blocking receptor binding. Additionally, follistatin can inhibit the effect of BMP2, 4 and 7 by forming a trimeric complex with the receptors [22]. Gremlin-1 is part of the deadenylating nuclease (DAN)/Cerberus protein family and can block BMP2, 4 and 7 *in vivo* and *in vitro* [23]. Altogether, this highlights the numerous combinations and complexity of TGF β signalling (Figure 2).

TGF β signalling

Upon ligand binding, the type II receptor subunit phosphorylates type I receptors, which initiates two intracellular signalling cascades: the canonical (Smad-dependent) and noncanonical (Smad-independent) pathways (Figure 3). Small mothers against decapentaplegic (Smad) protein is a transcription factor that transduces the signal into the nucleus [24]. Once phosphorylated, Smad1/5/8

or Smad2/3 proteins form a complex with Smad4 and translocate into the nucleus. It is commonly accepted that BMPs and GDFs signal through activation of Smad1, Smad5 and Smad8 by phosphorylation; and that TGF β s, activins and Nodal signal through Smad2 and Smad3. Activation of downstream noncanonical pathways include phosphatidylinositol 3-kinase/protein kinase B (PI3K/Akt), extracellular signal-regulated kinase (ERK), p38 mitogen-activated protein kinase (MAPK), c-Jun N-terminal kinases (JNK), TGF-activated kinase (TAK)1 and protein kinase C (PKC) pathways. Regulation of these signalling cascades involves multiple partners like inhibitory Smad proteins (Smad6 and Smad7), protein ligases (SMURF1 and SMURF2), protein phosphatases (PPA1 α) and miRNAs. Cross-communication with Wnt, Notch, MAPK, Rho/Rho-kinase, TGF β , hypoxia-inducible factor (HIF) and nuclear factor- κ B (NF- κ B) signalling pathways could also contribute to the regulation of canonical and noncanonical TGF β signalling [25].

The wide range of TGF β signalling pathways implies that its effects on tissues are complex and opposing effects can often occur. Receptor dimerisation plays a major part in determining the activation of canonical or noncanonical signalling. Hence, preformed BMP receptor complexes, encompassing homodimers of type I and II receptors, would preferentially induce canonical Smad-dependent signalling. By contrast, ligand-induced oligomerisation of type I and type II receptors would predominantly activate noncanonical non-Smad signalling [26]. Therefore, the ligand will first bind a type I receptor complex and then recruit a type II receptor for phosphorylation and downstream signalling (Figure 3). This might explain the increase in noncanonical signalling in *BMPR2* mutation carriers because decreased BMPRII expression favours *de novo* heterodimer formation.

TGF β superfamily ligands modulate a wide range of developmental programmes, cellular processes and disease states. TGF β ₁ is one of the three subtypes and is known to have key physiological roles in embryonic development, angiogenesis, wound healing, inflammation and immune cell function by T-cell regulation and differentiation. However, excessive TGF β ₁ production is associated with lung fibrotic diseases [27]. All TGF β s are synthesised as precursor molecules that will form a latent TGF β complex. Once bound to latent TGF β -binding protein (LTBP), the complex will be secreted as a large latent complex (LLC), which can be activated by proteases, integrins, reactive oxygen species (ROS) or change in pH. Impairment of these activating factors can result in dysregulated TGF β signalling, potentially inducing inflammation, autoimmune disorders, fibrosis, cancer or PAH [28].

TGF β signalling in PAH

Mutations in the BMPR2 gene

BMPRII is known to be involved in osteogenesis and cell differentiation. Within the pulmonary circulation, the BMPRII pathway inhibits SMC cell proliferation, primarily within the small pulmonary

arterioles. When mutated, BMPRII is associated with an increased susceptibility to develop PAH [29]; *BMPR2* mutation carriers develop PAH 10 years earlier than noncarriers and display a more severe haemodynamic profile [30]. In patients with a familial history of PAH, germline mutations in the *BMPR2* gene are detected in 70% of HPAH patients. *BMPR2* is located on chromosome 2q33 and consists of 13 exons encoding four functional domains: an N-terminal ligand-binding domain, a single transmembrane region, a serine/threonine kinase and a cytoplasmic domain. Lastly, a compilation of 384 distinct variants, including mutations and polymorphisms, has been identified across the *BMPR2* locus in different cohorts of patients worldwide (Figure 4). Mutations are spread across the gene, with an exception in exon 13, and include missense mutations, nonsense-mutations, splice defects, deletions and duplications [31]. Whereas a large proportion of variants and polymorphisms are pathogenic, whether all mutations are disease-causing has yet to be elucidated [31].

The majority of *BMPR2* mutations are frameshift and nonsense mutations, triggering nonsense-mediated mRNA decay (NMD), resulting in degradation of the mutated mRNA and leading to haploinsufficiency [32]. Approximately 40% of *BMPR2* mutations are missense mutations affecting highly conserved amino acids that produce stable mRNA transcripts and mutant proteins. HPAH patients carrying these mutations harbour more-severe forms of PAH with lower age at diagnosis and reduced time to lung transplantation [33], suggesting dominant-negative effects on BMPRII function [32]. NMD-negative mutants can affect the ligand-binding domain, the kinase domain or the cytoplasmic tail [34]. Missense mutations located in the cytoplasmic tail result in disrupted interaction with signalling partners of Smad-independent pathways [32] but do not impair the capacity of the protein to be localised to the cell surface or its ability to activate BMP/Smad signalling [34].

Cysteine substitutions in the ligand-binding domain, missense mutations in the kinase domain or in-frame deletion of exon 2 are associated with dysfunctional intracellular membrane trafficking. This results in retention of dysfunctional mutant receptors within the endoplasmic reticulum (ER) or Golgi compartments [35], without reduction in the global expression of BMPRII. The dominant-negative effects of intracellular retention could be attributed to chaperone proteins [36], or changes in protein-folding kinetics. Accordingly, chemical chaperones can restore the trafficking of mutated BMPRII protein to the cell surface and increase Smad1/5 signalling [36]. Therefore, enhancing protein trafficking offers a narrow window for therapeutic intervention in HPAH associated with mutations located within the ligand-binding domain; it also implies tailored individual analysis of *BMPR2* mutations to identify patients who could benefit from chaperone therapy.

Mutations in TGF β family and partners of the BMP signalling pathway

Mutations in the *ACVRL1* gene encoding the activin-receptor-like kinase (ALK)1 protein and endoglin (ENG) have been identified in patients with PAH [37]. Interestingly, *ACVRL1* and *ENG* mutations have

been identified in PAH patients with a history of hereditary haemorrhagic telangiectasia [37], a disease characterised by abnormal blood vessel formation, suggesting that TGF β signalling might have an important role in angiogenesis and vessel formation. *ALK-1* mutations are rare in PAH, mostly affect the functional kinase domain and result in a more severe phenotype compared with *BMPR2* mutations. ALK-1, which is a type I receptor, promotes proliferation and migration via the type II receptor Smad1/5/8 pathway in ECs. Mutations in *ACVRL1* result in decreased ALK1 expression, reduced Smad1/5/8 activation and increased Smad2/3 signalling through increased ALK5 activity [38]. Endoglin, predominantly expressed on ECs, acts as a co-receptor to stabilise the receptor complexes and ensure correct receptor signalling. Splice-site mutations cause haploinsufficiency, resulting in impaired receptor signalling, dysregulated angiogenesis, vascular malformations and aberrant EC proliferation [25].

Mutations in proteins that do not belong to the TGF β family but are associated with TGF β /BMP signalling have also been associated with PAH. Mutations in Smad8 (*smad9* gene), an intracellular transcription factor, are associated with a heritable form of PAH; however, the consequences of *smad9* mutations are currently poorly understood because Smad1 and Smad5 can compensate for the loss of Smad8 function [39]. In 2012, a novel genetic mutation in the *CAV1* gene was found in families in which no known mutations in the TGF β signalling pathway were identified. *CAV1* encodes for caveolin-1, a membrane protein of caveolae, regulating endothelial function and PAEC permeability [40]. Caveolin-1 expression is reduced in remodelled pulmonary arteries from PAH patients, suggesting that loss of caveolin-1 reflects the proliferating and apoptosis-resistant phenotype of pulmonary vascular cells in severe PH. Its downregulation in mouse aortic SMCs results in impaired BMPRII membrane localisation and reduced BMP-dependent Smad signalling, suggesting a direct interaction of caveolin-1 with BMPRII [41]. Accordingly, elafin, an elastase-specific protease inhibitor, amplifies BMP signalling in a caveolin-dependent way in SU5416-hypoxic (SuHx) rats [42].

Biallelic mutations in the eukaryotic translation initiation factor 2-alpha kinase 4 (*EIF2AK4*) gene have been detected in patients with PVOD/PCH, and PAH patients with reduced diffusion capacities [43] who were probably misdiagnosed PVOD patients. *EIF2AK4* encodes a kinase termed general control nonderepressable (GCN)2, which phosphorylates the eukaryotic translation initiation factor 2 α leading to a global protein synthesis downregulation in response to amino acid starvation, hypoxia or viral infection. EIF2AK4 can regulate the BMPRII/TGF β pathway by activating Tribbles homolog 3 (TRB3), which interacts with the tail domain of BMPRII in human primary PSMCs [44]. However, the understanding of EIF2AK4 involvement in the pathogenesis of PAH remains incomplete.

Impaired BMPRII signalling in PAH

Despite the impact of *BMPR2* as a genetic factor in PAH, currently we do not completely understand the molecular mechanisms by which loss of BMP receptor function impairs pulmonary vascular cell function in PAH. Unravelling these uncertainties could explain why only 20% of *BMPR2* mutation carriers develop advanced PAH [44] and might predict which mutation carriers will further develop PAH. So far, *BMPR2* gene mutations result in impaired Smad signalling and activation of alternative pathways such as the p38 MAPK pathway [34].

BMP and TGF β receptor pathways are involved in PAH pathogenesis [46]; consequently, a loss of homeostasis by decreased BMPRII and/or increased TGF β signalling would contribute to PAH pathogenesis. TGF β is involved in inflammation, angiogenesis, wound healing and immune cell function, and exerts potent effects on PASMC proliferation, extracellular matrix synthesis and differentiation *in vitro* [47]. Growth, migration and excess matrix deposition by pulmonary vascular cells contribute to vascular wall remodelling in PH [10]. Interestingly, PASMCs isolated from patients harbouring disease-causing mutations exhibit an enhanced mitogenic response to TGF β [48]; accordingly, a loss of BMPRII in explant-derived PASMCs from PAH patients harbouring *BMPR2* mutations opposed the normal growth-inhibitory effect of TGF β [49]. *BMPR2* transient silencing in human lung microvascular ECs (HLMVECs) did not affect total TGF β concentration levels. However, addition of TGF β causes an increase in downstream TGF β signalling, even without *BMPR2* knockdown [50], suggesting the importance of the balance between BMPRII and TGF β (Figure 5). In addition, TGF β signalling has been assessed *in vivo* by performing immunohistochemical detection of phosphorylated active downstream effectors of TGF β receptors (Smad 1/5/8 and Smad2) in normal lungs and lungs with remodelled pulmonary arteries in chronic cigarette smokers or in patients with IPAH. This showed that ECs from remodelled pulmonary arteries expressed all TGF β signalling molecules, whereas ECs from occlusive lesions displayed altered expression of downstream effectors of TGF β receptors [51].

Animal models of PAH, such as monocrotaline (MCT) and the SuHx rat models, have highly contributed to unravelling the role of BMPRII in the pathogenesis of PAH. MCT, an alkaloid derived from *Crotalaria spectabilis*, induces an obliterative vasculitis, resulting in endothelial dysfunction, medial thickening, loss of peripheral arteries, increased PVR and RV hypertrophy [52]. Combination of chronic hypoxia with a single subcutaneous injection of VEGFR2 inhibitor SU5416 (SuHx) in rats results in severe PAH, progressive obstructive pulmonary vasculopathy characterised by intimal hyperplasia, fibrosis and plexiform lesion formation comparable with human lesions [52]. Homozygous *BMPR2*^(-/-) knockout mice die *in utero* whereas heterozygous *BMPR2*^(+/-) mice are viable but do not develop PH spontaneously [52]. By contrast, conditional smooth-muscle-specific transgenic mice, expressing a dominant-negative BMPRII, display a reproducible PAH phenotype characterised by increased RV systolic pressure (RVSP), higher Fulton index and pulmonary arterial muscularisation [53]. Similarly,

conditional endothelial-specific expression of a truncated BMPRII resulted in increased RVSP and pulmonary arterial muscularisation in mice [54]. Most recently, rats with a monoallelic deletion of 71 bp in exon 1 of *BMPR2* were shown to display reduced BMPRII expression and downstream signalling; these rats also develop age-dependant PH with a low penetrance, are more susceptible to hypoxia-induced PH and display progressive pulmonary vascular remodelling. In addition, they exhibit intrinsic RV dysfunction. This study highlighted how *BMPR2* mutations can alter the pulmonary vasculature as well as the RV function, and the deleterious effect in patients bearing *BMPR2* mutations with compromised response to therapy [55].

Dysfunctional BMPRII/TGF β signal transduction results in altered expression of transcription factors, including Smads and Id (inhibitor of DNA binding) and decreased expression of BMPRII in hypoxic and MCT rat models of PAH. By contrast, reduced BMP signalling and enhanced TGF β ₁ signalling is only observed in MCT rats. Additionally, ALK5 inhibition prevents disease progression in MCT rats, suggesting a shift from BMPRII/Smad1/5/8 signalling to TGF β /Smad2/3 signalling as a potential mechanism for PAH development [47]. The attenuation of hypoxic PH in rats by adenoviral-mediated overexpression of BMPRII highlights the crucial role of BMPRII loss in the pathogenesis of PAH. Targeted *BMPR2* gene delivery to the endothelium also reduced PH in MCT and hypoxic rats, resulting in increased Smad1/5/8 phosphorylation, reduced Smad3 phosphorylation and changes in non-Smad pathways such as PI3K and p38 MAPK [56]. The current hypothesis is that BMPRII and TGF β work in a strict equilibrium in which BMPRII buffers the effects of TGF β , with both cascades competing for Smad4 protein availability. When an imbalance occurs, either by decreased levels of BMPRII or increased TGF β levels, the predominant effect shifts to TGF β signalling owing to its increased binding with Smad4 [47] (Figure 5).

TGF β signalling and endothelial dysfunction

Considering endothelial dysfunction as a hallmark of PAH and the crucial role of TGF β signalling in the pathogenesis of the disease, it is of major interest to investigate whether both pathways interact, taking into account that endothelial dysfunction could have effects on vascular remodelling and homeostasis [14]. Currently, there is clinical evidence that BMPRII dysfunction impairs pulmonary vasoreactivity – a large majority of PAH patients with a *BMPR2* mutation do not respond to acute vasodilators [54].

Firstly, decreased NO production observed in PAH could be caused by uncoupling of endothelial nitric oxide synthase (eNOS) or reduced eNOS activation. Interestingly, NO production by HLMVECs expressing different *BMPR2* mutants is significantly decreased and mutations in *BMPR2* resulted in impaired eNOS activation in human PAECs [58]. Consequently, eNOS uncoupling might explain the increased ROS production by transgenic mice overexpressing *BMPR2* mutants [59]. It is suggested that

BMP2/4-induced eNOS activation is protein kinase A (PKA)-dependent and can be regulated by direct interaction with BMPRII [58]. Because BMPRII and eNOS closely interact with caveolin-1, which plays an important part in TGF β signalling and pulmonary endothelial barrier function [60], increased TGF β signalling inhibiting caveolin-1 expression would indirectly affect eNOS activation [61].

Secondly, transient *BMPR2* silencing in HLMVECs results in increased ET-1 secretion [50] and HLMVECs from PAH patients with *BMPR2* mutations produce significantly higher levels of ET-1 [62]. TGF β is known to stimulate ET-1 production in ECs and, similarly, *BMPR2* silencing induced ET-1 production in HLMVECs but not TGF β production. Reduced BMPRII expression or TGF β stimulation in HLMVECs resulted in elevated ET-1 levels [50], suggesting that TGF β activity could be counterbalanced by BMPRII. Accordingly, adenoviral-mediated BMPRII overexpression restores the imbalance between ET-1 and eNOS by increasing eNOS phosphorylation and limiting TGF β_1 -triggered ET-1 stimulation [63], such that disruption of the homeostasis between NO/eNOS and ET-1 could play an important part in PAH pathogenesis (Figure 6). Besides the effect of ET-1 on NO release via the ET $_B$ receptor, NO can also inhibit the production of ET-1 by endothelin-converting enzyme inactivation depending on the sGC/cGMP pathway [64], fulfilling the potential feedback loop accounting for BMPRII-loss-induced endothelial dysfunction.

TGF β signalling and inflammation

Endothelial dysfunction implies a loss of the endothelial barrier function. Interestingly, a loss of BMPRII results in impaired endothelial barrier function *in vitro* and *in vivo* [60,65]. Loss of endothelial barrier function increased permeability to circulating cytokines and growth factors, which could result in infiltration of inflammatory cells within the pulmonary vascular wall. Furthermore, chronic inflammation contributes to the pathogenesis of PAH and to the structural pulmonary vascular remodelling characterised by perivascular inflammatory infiltrates comprising T and B lymphocytes, macrophages, dendritic cells, mast cells and monocytes [66]. Circulating levels of several cytokines and chemokines are also elevated in PAH and could be associated with a worse clinical prognosis [66–69]. Whether impaired BMPRII function alters immune function or increases susceptibility to inflammatory triggers remains debateable. Circulating levels of inflammatory mediators, including interleukins or C-reactive protein (CRP), are similar in PAH patients with or without a *BMPR2* mutation [62,68]. Similarly, *BMPR2*^{+/-} knockout mice, which do not develop PAH spontaneously, do not elicit increased levels of interleukin (IL)-6 or keratinocyte chemoattractant (KC) – the mouse equivalent to IL-8 – compared with control mice [70]. However, endothelial-specific loss of BMPRII results in increased CXC chemokine receptor type 2 (CCR2) expression, enhanced leukocyte infiltration and circulating KC in mice [65]. In addition, endothelial-specific expression of a truncated BMPRII protein in mice induced infiltration of inflammatory cells including macrophages and CD3⁺ T cells in remodelled pulmonary vessels [54].

Considering the low penetrance of *BMPR2* mutations in PAH, a role for inflammation as a trigger has been suggested. Accordingly, *BMPR2*^{+/-} knockout mice overexpressing 5-lipoxygenase are more susceptible to developing PH, wherein enhanced endothelial injury and inflammatory response could work in concert with *BMPR2* heterozygosity to promote persistent PH [71]. Similarly, acute and chronic exposure of *BMPR2*^{+/-} knockout mice to lipopolysaccharide resulted in increased IL-6 and KC/IL-8 production and increased RVSP [70]. Another consequence of *BMPR2* mutations could be an enhanced vulnerability to an inflammatory second hit, as suggested by upregulated IL-6 in transgenic mice expressing an inducible dominant-negative *BMPR2* in smooth muscle [72]. Accordingly, a CCR1/2 antagonist has been shown to reduce pulmonary vascular leakage and leukocyte infiltration in mice with a specific EC loss of BMPRII [73]. Additionally, during aging, rats bearing a monoallelic deletion in *BMPR2* exon 1 display pulmonary perivascular leukocyte accumulation [55].

In vitro, PASMCs from *BMPR2*^{+/-} knockout mice or from PAH patients with *BMPR2* mutations exposed to lipopolysaccharide, or from transgenic mice expressing a mutated *BMPR2* in smooth muscle, display an increased production of inflammatory mediators including IL-6, KC/IL-8, MCP-1 and TGFβ [70,72]. Moreover, in response to tumour necrosis factor (TNF)α, transient *BMPR2* silencing in human PAECs enhanced transmigration of leukocytes by disrupting barrier function and releasing proinflammatory cytokines including IL-6 and IL-8 [65] or production of the potent chemokine granulocyte macrophage colony-stimulating factor (GM-CSF) [74]. In response to inflammatory mediators such as TNFα or CRP, HLMVECs from PAH patients with a *BMPR2* mutation displayed enhanced adhesiveness to monocytes and IL-6 release, supporting the hypothesis that reduced BMPRII expression in pulmonary endothelium elicits an exacerbated inflammatory response [62]. In human PASMCs, transient *BMPR2* silencing enhanced TNFα-induced cytokine mRNA levels, whereas BMP4, a ligand of BMPRII, reduced these levels through myocardin-related transcription factor A (MRTF-A) by inhibiting NF-κB expression [75]. CCL5 deficiency inhibited PASMC proliferation and migration, increased PAEC apoptosis *in vitro* and attenuated SuHx-induced PAH in rats *in vivo* by restoring BMPRII signalling [76]. This is in accordance with the previous demonstration of the CCL5/CCR5 pathway as a potential therapeutic target in PAH [69].

Proinflammatory effects elicited by TGFβ are illustrated by NF-κB-induced release of IL-6 and IL-8 in response to enhanced TGFβ signalling in PASMCs deficient in BMPRII [49]. Prolonged TGFβ expression reduced the activity of AC and increased cyclooxygenase-2, inactivating AC by increased expression of Smad6 [77], an inhibitor of Smad1/5 activation. Interestingly, a loss of BMPRII in ECs promoted inflammation and atherosclerosis *in vitro* and *in vivo* by increasing expression of ICAM and VCAM and consequently monocyte adhesion or infiltration through NF-κB signalling [78]. This is in favour of the potential anti-inflammatory role of BMPRII, compared with TGFβ. In accordance with the equilibrium between BMPRII and TGFβ, loss of BMPRII favours TGFβ and NF-κB signalling, resulting in reduced

BMPRII signalling and eNOS uncoupling that lead to enhanced ROS production, adhesion molecule expression and monocyte adhesion and infiltration. Although various arguments favour inflammation or the increased expression of cytokines as a consequence, there are currently various clues demonstrating that a coincidence of *BMPR2* heterozygosity with an inflammatory insult is required to develop PAH. Nevertheless, targeting inflammatory pathways appears to be a promising therapeutic strategy, as recently highlighted [79].

TGF β signalling and angiogenesis

PAH has been initially described as a proliferative disease triggered by endothelial cell injury and apoptosis and compared to a cancer-like disorder. Lately, this paradigm has shifted towards alternative hypotheses. It has been recently stipulated that microvascular rarefaction could be attributed to a degenerative process and that angiogenesis could thereby be beneficial [80]. Although angioproliferative pulmonary vascular remodelling and microvascular rarefaction contribute to increased pulmonary vascular resistance, a paradox about the deleterious vs the beneficial role of angiogenesis in the pathogenesis of PAH remains a matter of debate. It is generally accepted that genetic factors and environmental stress contribute to the progression of PAH pathogenesis by inducing pulmonary EC apoptosis [80]. Although PAH patients display increased circulating VEGF levels [12], the combination of hypoxia with the administration of SU5416, a VEGF receptor inhibitor, resulted in severe PAH in rats [13]. In addition, anticancer drugs such as imatinib or dasatinib inhibit angiogenesis but only dasatinib was responsible for drug-induced PAH [81]. Despite the encouraging effects of imatinib as an add-on therapy in PAH, ~31% had to discontinue the long-term safety and efficacy study, owing to serious and unexpected adverse events further discouraging the use of imatinib to treat PAH [82].

VEGF is considered the major angiogenic factor, with five isoforms, termed A, B, C, D and placental growth factor, and three VEGF receptors: VEGFR1, 2 and 3. VEGF-A mainly binds to VEGFR2 and displays several splice variants with some exhibiting angiogenic (VEGF_{xxx} isoforms) or antiangiogenic (VEGF_{xxx}b isoforms) properties; VEGF₁₆₅b being the major antiangiogenic isoform. VEGFB preferably binds to VEGFR1 and is weakly angiogenic in most tissues, except the heart. VEGFC, which binds VEGFR3, and VEGFD, which can bind to VEGFR2 and VEGFR3, are largely considered angiogenic [83].

Angioproliferative effects

Apoptosis and injury of pulmonary vascular ECs can have indirect consequences including the emergence of hyperproliferative apoptosis-resistant cells that further form occlusive lesions. Accordingly, increased expression of VEGF and its receptors is observed in occlusive lesions, accompanied by disordered angiogenesis [12]. In addition, upregulation of remodelling-associated genes, such as TGF β ₁, HIF1 α , VEGF α , VEGFR1/2, thrombospondin, angiopoietin-1 and their receptor

Tie-2, as well as sprouting-associated markers, including NOTCH and matrix metalloproteinase, was found in human occlusive lesions [84]. Because BMPRII is mainly expressed within the endothelium of pulmonary arteries, it is involved in the progression of occlusive lesions. Accordingly, loss of functional BMPRII is associated with increased PAEC apoptosis and emergence of apoptosis-resistant vascular cells resulting in obliterative arterial remodelling [85].

Microvascular rarefaction

PAEC apoptosis can result in EC dropout resulting in precapillary regression and then microvascular rarefaction. There is clinical evidence that the amount of occlusive lesions poorly correlates with compromised haemodynamics in PAH patients [80]. Interestingly, impaired neovascularisation has been associated with adverse outcomes in CTEPH patients [86]. Considering that BMPRII deficiency results in PAEC apoptosis, one can hypothesise that impaired BMPRII signalling would contribute to disordered angiogenesis in PAH. Accordingly, circulating levels of VEGF_{165b}, known to inhibit migration and proliferation of ECs as well as revascularisation, are significantly increased in patients with PAH [87]. More recently, decreased VEGFR3 expression was observed in lung tissue and PAECs from PAH patients, although *BMPR2* mutations did not appear to determine VEGFR3 expression [88]. VEGFR3 is physically associated with BMPRII, thus facilitating ligand-induced endocytosis of BMPRII, Smad protein phosphorylation and transcription of Id genes. Deletion of endothelial VEGFR3 worsened hypoxia-induced PH in mice and impaired BMP signalling, whereas reconstitution of VEGF3 in PAECs from PAH patients with a *BMPR2* mutation partially restored BMP signalling [88]. Angiogenesis is significantly reduced in progenitor stem cells differentiated *in vitro* into ECs issued from *BMPR2* mutation carriers and BMPRII enhances angiogenesis [89]. FK506 or tacrolimus, known to activate BMPRII signalling, induced angiogenesis *in vitro* similarly to the angiogenic factor VEGF [90]. Reduced pulmonary microvascular density was observed in rats with a monoallelic deletion in *BMPR2* exon 1 [55].

Regarding TGF β signalling, TGF β can induce Smad2/3 phosphorylation through ALK5, which results in reduced angiogenesis by inhibiting EC proliferation, migration and tube formation [38]. An ALK5 kinase inhibitor can also counteract the antiangiogenic effect of TGF β by enhancing growth and monolayer integrity of stem-cell-derived ECs from mouse embryos [91]. Consequently, proliferative and degenerative concepts contribute to the pathogenesis of PAH. Although a loss of BMPRII results in hyperproliferation of PSMCs, it promotes PAEC apoptosis. Similarly, although BMPRII signalling promotes angiogenesis, TGF β displays antiangiogenic properties, strengthening the hypothesis that a fair balance between TGF β and BMPRII signalling is required to maintain intact endothelium barrier function. A lack of equilibrium in the expression of pro- and anti-angiogenic isoforms of VEGF can contribute either to angioproliferative arterial remodelling or to regression of precapillary arterioles

throughout the pathophysiological process of PAH. Thereby, angiogenesis could be considered detrimental in early pathogenesis of PAH by contributing to occlusive arteriopathy and beneficial at more-advanced stages of the disease to reduce microvascular rarefaction and promote revascularisation of existing obstructive lesions, thus restraining an increase in PVR.

Effects of current therapies on TGF β pathway signalling

Throughout the current review, we aim to highlight the importance of the role of the TGF β pathway in the pathophysiology of PAH. Consequently, unravelling the mechanisms by which current vasodilatory PAH-specific therapies could interact with the BMPRII/TGF β pathway is highly relevant.

Endothelin pathway

Bosentan, a dual ET-1 receptor antagonist, can restore BMPRII expression, Smad1/5/8 protein activation and Id1 transcription in response to hypoxia and BMP2 in PSMCs [92]. Macitentan, a more efficient dual ET-1 receptor antagonist, can inhibit the TGF β profibrotic action, by blocking the ETR–TGF β RI-complex-mediated signalling in systemic sclerosis dermal fibroblasts [93]. Accordingly, bosentan and macitentan can prevent endothelial–mesenchymal transition in microvascular ECs by interfering with ET-1/TGF β signalling and restoring tube formation ability [94].

Nitric oxide pathway

Sildenafil potentiates the antiproliferative effects of BMP4 by enhancing canonical BMP signalling and restores the antiproliferative response to BMP4 in human PSMCs harbouring a *BMPR2* mutation [95]. In addition, sildenafil can restore connexin-40 levels in PSMCs from MCT rats [96]. Interestingly, addition of a BMPRII antagonist (LDN193189) prevents sildenafil from increasing connexin-40 levels, indicating that BMPRII signalling can regulate the expression of connexin-40, connexins being transmembrane proteins of communication channels between adjacent cells. Moreover, sildenafil can stimulate angiogenic properties in human umbilical vein ECs (HUVECs) through a protein kinase G/MAPK-dependent way [97]. Riociguat can inhibit TGF β expression, Smad-independent TGF β signalling and reduce fibroblast activation and collagen release [98].

Prostacyclin pathway

Prostanoids are known for their vasodilatory, antiproliferative and anticoagulant properties, mainly occurring through the AC/PKA pathway [15]. In addition, prostanoids can inhibit the TGF β pathway [99]. Beraprost, a PGI₂ analogue, selectively inhibits proliferation of murine primary PSMCs harbouring a pathogenic *BMPR2* nonsense mutation in the presence or absence of TGF β ₁ stimulation. More specifically, beraprost inhibits TGF β ₁-induced Smad-dependent and Smad-independent signalling via a PKA-dependent pathway by reducing Smad2/3 and p38 MAPK protein phosphorylation. Treprostinil inhibits the TGF β pathway by reducing Smad3 phosphorylation in MCT rats [100]. By

inhibiting TGF β signalling, prostanoids favour the beneficial BMPRII pathway; beraprost and iloprost inhibit Smad3 activity and induce *Id1/2* gene in a cAMP/PKA-dependent manner [100].

Drugs targeting the TGF β pathway in PAH

Restoring the equilibrium between TGF β and BMPRII signalling in pulmonary vascular cells could represent future potential therapeutic targets to reverse PAH. This could include suppression of TGF β signalling and/or downstream pathways, upregulation of BMPRII signalling or a combination of both.

Therapeutics that target BMPRII signalling

Upregulation of BMPRII by adenoviral gene delivery showed beneficial effects in experimental chronic hypoxia and MCT rat models [56]. Interestingly, BMP9 binds ALK1 and BMPRII with high affinity and can reverse experimental PAH in mice bearing a heterozygous knock-in allele of a human *BMPR2* mutation, R899X, as well as in MCT and SuHx rats, by enhancing BMPRII signalling. Additionally, BMP9 prevents apoptosis and enhances monolayer integrity of PAECs from PAH patients bearing *BMPR2* mutations [101]. Because BMP9 can increase *BMPR2* gene expression, in addition to protecting PAECs from apoptosis and promoting vascular stability, it provides a proof of concept to treat PAH by directly enhancing BMPRII signalling using recombinant BMP9 protein, as an example. Recently, rescuing BMPRII in progenitor stem cells differentiated *in vitro* into ECs, issued from *BMPR2* mutation carriers, has been shown to improve endothelial function, enhance angiogenesis and ameliorate endothelial barrier function [89]. This suggests that rescuing BMPRII-driven endothelial function combining gene and cell therapy could be a novel treatment strategy for PAH in the future [102].

Activation of BMPRII signalling by tacrolimus, which reverses dysfunctional BMPRII signalling, enhances downstream Smad signalling and *Id1* gene expression and reverses experimental PAH in conditional *BMPR2* knockout mice and in MCT and SuHx rats [90]. Recent clinical trials using tacrolimus in PAH have shown an improved condition in a limited number of patients with end-stage PAH and improvement in exercise capacity and RV function in a subset of PAH patients [103]. More recently, the expression of a novel *BMPR2* gene modifier: fragile histidine triad (FHIT), was shown to be reduced in PAH patients; reduced FHIT expression was associated with impaired BMPRII signalling and endothelial and smooth muscle cell dysfunction. Interestingly, enzastaurin, a cancer drug that can prevent lymphoma relapse, can enhance FHIT and *BMPR2* expression, as well as prevent and reverse experimental PH in mice exposed to hypoxia and SuHx rats [104]. This suggests that direct or indirect pharmaceutical elevation of BMPRII expression could be considered a potential novel treatment strategy for PAH therapy and even prevention.

Baicalin, an organic compound known to inhibit the inflammatory response by blocking NF- κ B expression, attenuated lung damage in MCT rats by inhibiting proliferation and inducing apoptosis of

PASMCs. Baicalin decreases the expression of gremlin-1, a BMP antagonist, and increases the expression of the inhibitor of NF- κ B, I- κ B α , BMPRII, BMP-4, BMP-9 and Smad1/5/8. Additionally, Bcl2 antiapoptotic gene is downregulated by baicalin, suggesting its proangiogenic properties. Furthermore, baicalin inhibits SMC proliferation *in vivo* and *in vitro* and attenuates endothelial–mesenchymal transition in MCT rats [105].

An innovative experimental approach is improving the trafficking and restoring the signalling of mutated BMPRII. Accordingly, functional rescue of mis-trafficked BMPRII mutants resulted in increased membrane expression and the restoration of Smad signalling [36]. Similarly, improving BMPRII trafficking by administration of chaperone proteins seemed to be beneficial in PAH [106]. Ataluren, which induces ribosomal read-through of nonsense mutations, can restore BMP signalling [107]. Chloroquine, known to inhibit autophagy, prevented lysosomal degradation of expressed BMPRII, resulting in sustained expression of BMPRII at the cell surface and restored the proapoptotic, antiproliferative phenotype of PASMCs [108]. Recently, upregulation of chloride intracellular channel (CLIC)4 expression and ADP ribosylation factor (Arf)6 activity, regulators of endosomal trafficking, has been observed in endothelial-colony-forming cells from IPAH patients. As a result of Arf6-mediated reduction, CLIC4 reduced BMPRII expression and signalling, and increased lysosomal targeting of BMPRII. Lung-endothelium-targeted knockdown of CLIC4 attenuated development of PH and restored lung expression of BMPRII in SuHx mice. HecinH3, an inhibitor of Arf6, also prevented development of PH in SuHx mice and attenuated PH in MCT rats [109]. Thereby, pharmacological therapies that can restore the trafficking of BMPRII protein appear as promising strategies to improve the treatment of PAH.

The protease inhibitor, elafin, a caveolin-1-dependent inhibitor of elastase, can amplify BMPRII signalling and reverse experimental PH [42]. Statins, commonly used to reduce low density lipoprotein (LDL) circulating levels in humans, were shown to significantly increase expression levels of BMPRII mRNA and protein in HUVEC cells [78]. However, randomised placebo-controlled clinical trials failed to show any beneficial effect of statins on exercise capacity or haemodynamics in PAH patients [110]. Sepiapterin, a substrate of sepiapterin reductase with a role in tetrahydrobiopterin (BH₄) synthesis, can restore BMPRII-dependent eNOS uncoupling and induce angiogenesis *in utero* [111]. Dexamethasone, used as an anti-inflammatory drug, can improve haemodynamics and pulmonary vascular remodelling. Experiments in MCT rats show that early PH prevention was through increasing BMPRII mRNA levels, suggesting a BMPRII-induced negative feedback loop reducing IL-6 expression [112]. Loss of BMPRII results in eNOS uncoupling and decreased NO availability. Accordingly, eNOS overexpression attenuates PH in MCT rats [14,113]. Thereby, the Pulmonary Hypertension and Cell-Therapy (PHACeT) trial aimed to investigate the tolerability and safety of the administration of culture-derived endothelial progenitor cells (EPCs) transiently transfected with eNOS on haemodynamics,

exercise capacity and immune surveillance in seven PAH patients and showed an improvement in exercise capacity [114]. Most recently, susceptibility to PAH can be conferred onto a wild-type animal by transplantation of *BMPR2*-deficient mouse bone marrow; in addition, transplantation of bone marrow from wild-type mice into *BMPR2*-deficient mice prevents the development of PAH. This therefore suggests that haematopoietic stem cell transplantation might be considered as a potential treatment strategy in genetic forms of PAH [115].

Therapeutics that target TGF β signalling

Instead of upregulating *BMPRII* expression, inhibition of TGF β activity is another potential therapeutic approach to treat PAH. The TGF β -neutralising antibody 1D11 and SB431542, an inhibitor of ALK5, decrease RVSP and media thickness in a mouse model of *Schistosoma*-induced pulmonary vascular disease, although ALK5 blockade was less effective [116]. Accordingly, a selective TGF β ligand trap was able to attenuate vascular remodelling, haemodynamics and improve survival using prophylactic and rescuing approaches in MCT and SuHx rats [117], suggesting that antagonism of TGF β ligand could be an effective strategy to mitigate PAH. Sotatercept, another TGF β ligand trap that can restore BMP signalling and was successful in a Phase I clinical trial, is currently being tested in a Phase II trial in PAH patients (PULSAR study; NCT03496207). As recently highlighted, whether a TGF β ligand trap therapeutic strategy can translate to the clinic remains to be investigated; the recent experimental findings and the launch of the PULSAR study provide promising rationale to consider successful treatment of hereditary and idiopathic PAH [118].

Schisandrin B, a molecule isolated from *Schisandra chinensis*, commonly used in traditional Chinese medicine, inhibited hypoxia-induced arterial SMC proliferation and migration by attenuating TGF β_1 levels and reversing activation of ERK and p38 MAPK proteins [119]. Potential therapeutic approaches targeting *BMPRII* and TGF β signalling pathways are summarised in Figure 7.

Concluding remarks and future perspectives

This review highlights the importance of the TGF β family signalling in the pathogenesis of PAH. Various preclinical data support the concept of restoring normal TGF β signalling and enhancing the *BMPRII* axis as new therapeutic approaches for PAH. However, most strategies are not tissue specific and comprehensive investigation is required to better target the pulmonary vasculature and, importantly, the RV. Despite its complexity and involvement at several stages of the pathogenesis of PAH, the TGF β signalling pathway remains an appealing therapeutic target.

PAH is a heterogeneous disorder with morphological similarities but also distinct genomic variations. The heterogeneous and/or opposing signalling effects of the downstream effectors of the TGF β receptors can contribute to the heterogeneity observed in response to treatment in PAH patients.

Accordingly, pharmacogenomic approaches have been implemented to better understand variable response patterns to these therapies and investigate how genetic variations could influence the response to drugs [120]. The real utility of pharmacogenomic approaches in the treatment of PAH remains to be defined in basing treatment selection and clinical trial inclusion on individual patient gene expression patterns and associated targeted therapies.

Rather than targeting pulmonary vasculature vasoconstriction, innovative therapeutic approaches could encompass broader aspects of the pathophysiology of PAH. The recent demonstration that reprogramming skin fibroblasts, from PAH patients with a *BMPR2* mutation, into ECs in combination with *in vitro* correction of the mutation [89], the recent identification of the potentially ‘druggable’ *BMPR2* gene modifier [104], the current PULSAR clinical trial based on the use of the TGF β ligand trap, sotatercept or the more recent targeting of regulators of endosomal trafficking [109] open new perspectives for the future treatment of PAH. It could become a reality to implement innovative and tailored therapeutic approaches by combining gene editing, cell therapy and oral medication to definitely cure PAH targeting vasoconstriction, TGF β /BMPRII imbalance, pulmonary vascular remodelling and RV failure [102].

Conflicts of interest

The authors have no conflicts of interest to declare.

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Figure legends

Figure 1. Overview of current therapeutic targets. Abbreviations: AC, adenylate cyclase; ATP, adenosine triphosphate; cAMP, cyclic adenosine monophosphate; cGMP, cyclic guanosine monophosphate; COX I, cyclooxygenase I; ECE, endothelin-converting enzyme; eNOS, endothelial nitric oxide synthase; ET, endothelin; ET1, endothelin-1; ETA, endothelin receptor A; ETb, endothelin receptor B; GMP, guanosine monophosphate; GTP, guanosine triphosphate; IP, prostaglandin I₂ receptor; NO, nitric oxide; PDE5, phosphodiesterase 5; PGI₂, prostacyclin; PKA, protein kinase A; PKG, protein kinase G; sGC, soluble guanosine cyclase.

Figure 2. Schematic overview of the TGF β superfamily. Abbreviations: ActRII, activin type 2 receptor; ALK, activin-like kinase; AMH, anti-Müllerian hormone; AMHRII, anti-Müllerian hormone receptor type II; BMP, bone morphogenetic protein; BMPRII, bone morphogenetic protein receptor type II; GDF, growth differentiation factor; IS, inhibiting substance; MIS, Müllerian-inhibiting substance; Smad, Small mothers against decapentoplegic; TGF β , transforming growth factor beta; TGF β RII, transforming growth factor beta receptor type II.

Figure 3. Schematic overview of different TGF β and BMPRII signalling pathways. Abbreviations: BMPRII, bone morphogenetic protein receptor type II; ERK, extracellular-signal-regulated kinase; Id1, inhibitor of DNA binding; JNK, c-Jun N-terminal kinases; MAPK, mitogen-activated protein kinase; NF- κ B, nuclear factor kappa-light-chain-enhancer of activated B cells; p38MAPK, p38-mitogen activated protein kinase; Smad, small mothers against decapentoplegic; TGF β RII, transforming growth factor beta receptor type II.

Figure 4. BMPRII mutations associated with PAH. Red bars represent all different mutation categories and the relative occurrence in the exon. Abbreviation: TM region, transmembrane region.

Figure 5. Equilibrium (left) and disequilibrium (right) between TGF β and BMPRII signalling pathways. Disequilibrium is due to either increased TGF β or decreased BMPRII signalling resulting in proliferative phenotype in favour of progression of PAH. Abbreviations: BMPRII, bone morphogenetic protein receptor type II; PAH, pulmonary arterial hypertension; TGF β RII, transforming growth factor beta receptor type II.

Figure 6. Overview of the cooperation of the BMPRII/TGF β pathway with the NO and ET pathway. Whereas TGF β and BMPRII are in homeostasis in normal conditions, loss of BMPRII breaks the equilibrium and shifts the predominant effect towards the TGF β pathway, resulting in increased ET-1 production and in a defect in the homeostasis between ET-1 and eNOS. Abbreviations: BMPRII, bone

morphogenetic protein receptor type II; eNOS, endothelial nitric oxide synthase; ET-1 endothelin-1; TGF β RII, transforming growth factor beta receptor type II.

Figure 7. Potential therapeutic strategies targeting the TGF β pathway. Inhibiting ALK5 (V) or using TGF β capturing antibodies (II) can decrease TGF β signalling. Increasing BMPRII signalling can be performed by adding BMPRII ligand, BMP9 (I) or chloroquine (III) to inhibit endocytosis and lysosomal degradation of BMPRII receptors, or tacrolimus (IV) to trap FKBP12 and increase downstream signalling pathway, or ataluren (VI) with ribosomal read-through of nonsense mutations, or chemical chaperones (VII) to assist correct folding of the BMPRII protein. Abbreviations: ALK5, activin-receptor-like kinase type 5; BMP9, bone morphogenetic protein 9; BMPRII, bone morphogenetic protein receptor type II; FKBP, FK506 binding protein 12; Smad, small mothers against decapentoplegic; TGF β , transforming growth factor beta; TGF β RII, transforming growth factor beta receptor type II.

Figures

Boxes

Glossary

Anorexigens: drugs used to suppress appetite.

Hemangiomatosis: a condition where a benign tumor made up of blood vessels is present in several parts of the body.

Haploinsufficiency: mechanism of action to explain a phenotype when a diploid organism has lost one copy of a gene and is left with a single functional copy of that gene.

Hereditary haemorrhagic telangiectasia: also known as Osler–Weber–Rendu syndrome, is a rare autosomal-dominant genetic disorder that leads to abnormal blood vessel formation.

Vasculopathy: any disorder of the blood vessels.

Arteriopathy: any disease of the arteries.

Prostanoids: subclass of eicosanoids consisting of the prostaglandins, the thromboxanes and the prostacyclins.

Tacrolimus: an immunosuppressive drug used mainly after allogeneic organ transplant to lower the risk of organ rejection.

Ataluren: pharmaceutical drug for the treatment of Duchenne muscular dystrophy.

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