

Review Article

# Bone morphogenetic protein signalling in pulmonary arterial hypertension: revisiting the BMPRII connection

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Pulmonary arterial hypertension (PAH) is a rare and life-threatening vascular disorder, characterised by abnormal remodelling of the pulmonary vessels and elevated pulmonary artery pressure, leading to right ventricular hypertrophy and right-sided heart failure. The importance of bone morphogenetic protein (BMP) signalling in the pathogenesis of PAH is demonstrated by human genetic studies. Many PAH risk genes are involved in the BMP signalling pathway and are highly expressed or preferentially act on vascular endothelial cells. Endothelial dysfunction is recognised as an initial trigger for PAH, and endothelial BMP signalling plays a crucial role in the maintenance of endothelial integrity. *BMPR2* is the most prevalent PAH gene, found in over 80% of heritable cases. As BMPRII protein is the major type II receptor for a large family of BMP ligands and expressed ubiquitously in many tissues, dysregulated BMP signalling in other cells may also contribute to PAH pathobiology. Sotatercept, which contains the extracellular domain of another transforming growth factor- $\beta$  family type II receptor ActRIIA fused to immunoglobulin Fc domain, was recently approved by the FDA as a treatment for PAH. Neither its target cells nor its mechanism of action is fully understood. This review will revisit BMPRII function and its extracellular regulation, summarise how dysregulated BMP signalling in endothelial cells and smooth muscle cells may contribute to PAH pathogenesis, and discuss how novel therapeutics targeting the extracellular regulation of BMP signalling, such as BMP9 and Sotatercept, can be related to restoring BMPRII function.

## Pulmonary arterial hypertension and bone morphogenetic protein signalling

Pulmonary arterial hypertension (PAH) is a rare but debilitating condition with a high mortality rate, affecting 15–26 people per million of the population in western countries [1,2]. The pathology is characterised by the abnormal muscularisation of pre-capillary pulmonary arteries, formation of concentric and plexiform lesions and narrowing of the pulmonary vascular lumen, resulting in an increase in pulmonary vascular resistance, elevated pulmonary artery pressure, right ventricle hypertrophy, and progressive right heart failure [3]. The FDA approved PAH therapies prior to 2024 target three pathways that predominantly affect vascular tone: endothelin 1, nitric oxide and prostacyclin. Although these therapies have improved exercise capacity and delayed clinical worsening time, they do not provide a cure for most patients and survival at three years post-diagnosis remains unacceptably low [4]. Therapies directly targeting the underlying disease pathophysiology are urgently needed.

Genetic studies suggest that PAH can be caused by pathogenic germline mutations. The most prevalent disease gene is *BMPR2* (bone morphogenetic protein (BMP) receptor 2) [5,6], encoding the type II receptor for the large family of BMP ligands. *BMPR2* mutations are found in over 80% of familial cases and ~17% of idiopathic PAH (IPAH) patients [7–9]. Among the 12 validated PAH

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genes that have been recognised by the International Consortium for Genetic Studies in PAH [10], many are related to BMP signalling. Apart from *BMPR2*, *ACVRL1* encodes BMP type I receptor activin receptor-like kinase 1 (ALK1), *ENG* encodes co-receptor endoglin, *GDF2* encodes ligand BMP9, and *SMAD9* is a component of BMP signalling machinery [6,11]. Of note, mutations in PAH genes are predisposing factors with incomplete penetrance. *BMPR2* variants penetrance is estimated to be 42% for heterozygous women and 14% for heterozygous men [6,12].

## Introduction to BMP signalling

BMPs are members of the transforming growth factor- $\beta$  (TGF- $\beta$ ) superfamily. These ligands are mostly homodimers, initiating cellular signalling by forming a complex with cell surface receptors comprising two copies of a type I receptor and two copies of a type II receptor. Both types of receptors are serine/threonine kinases. After formation of the signalling complex, the constitutively active type II receptor will phosphorylate and activate the type I receptor, which will in turn phosphorylate the receptor-regulated SMADs (R-SMADs, including SMAD1, SMAD5, SMAD9, SMAD2 and SMAD3). The phosphorylated R-SMADs then form a complex with the common mediator SMAD, SMAD4, and translocate to the nucleus to regulate gene expression. Typically, BMP signals are mediated by SMAD1, SMAD5 and SMAD9, whereas signals from TGF- $\beta$ s, Nodal, activins and some growth and differentiation factors (GDFs) are mediated by SMAD2 and SMAD3. Signalling from the TGF- $\beta$  family ligands can also involve non-SMAD pathways such as p38, ERK1/2 and PI3K which impact on cell proliferation, apoptosis and migration [13]. A more comprehensive review on TGF- $\beta$  family signalling has been published recently [14].

TGF- $\beta$  family ligands are encoded by a total of 33 genes in humans, yet there are only 7 type I and 5 type II receptors mediating their signals, hence there is a high degree of promiscuity in ligand-receptor interactions. One BMP ligand can signal via different type I and type II receptor pairs, and the same type I and type II receptor pair can mediate signals from different ligands. In addition to ligand-receptor interaction, each ligand is synthesised and secreted as a prodomain bound complex; the prodomain may modify ligand bioactivity or localisation [15,16]. The extracellular regulation of BMP signalling also involves ligand traps (inhibitors) which limit ligand availability to the receptors, and cell surface co-receptors (also called type III receptors) which can modify ligand-receptor interactions [11]. Therefore, the overall signalling outcome is highly context dependent and determined by local concentrations of different ligands, ligand traps, and cell surface receptors and co-receptors [17] (Figure 1).

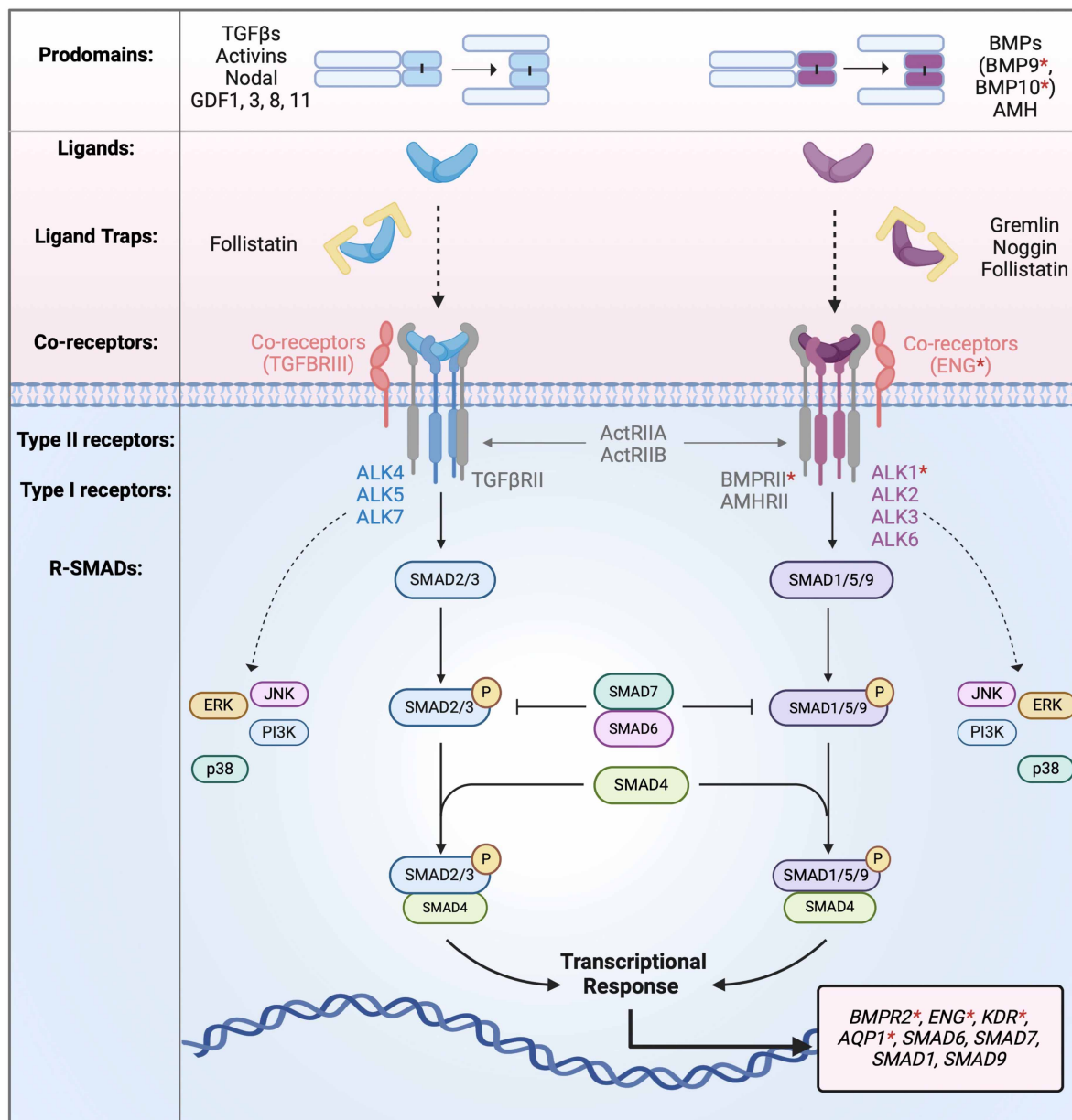
TGF- $\beta$  and BMP signalling are also regulated at intracellular levels. Inhibitory SMADs, such as SMAD6 and SMAD7, are target genes of many ligands and can directly inhibit BMP and TGF- $\beta$  signalling. Some BMPs also regulate the expression of their own receptors and co-receptors, for example, BMP9 and BMP10 induce the expression of *BMPR2*, *ENG*, *SMAD9*, and suppress *SMAD1* expression in endothelial cells (Figure 1). Such intracellular regulation provides feedback loops, ensuring a highly dynamic yet tightly controlled BMP signalling outcome.

With such complex regulation mechanisms, it is essential to establish how mutations in different genes lead to the dysregulated BMP signalling and contribute to the pathobiology of PAH. In addition, it is essential to understand how *BMPR2* mutations might affect signals from different TGF- $\beta$  family ligands and in different cell types, which could contribute to the initiation or exacerbation of PAH.

## Dysregulated endothelial BMP signalling in the pathogenesis of PAH

Genetic findings strongly support the crucial role of dysregulated endothelial BMP signalling in the initiation of PAH. Several genes that are mutated in PAH encode proteins that are part of BMP signalling complex and highly expressed in vascular endothelial cells, such as *BMPR2*, *ACVRL1* and *ENG*. Importantly, *ACVRL1* is almost exclusively expressed in endothelial cells, mediating signals from two specific ligands, BMP9 and BMP10. Mutations in both *GDF2* (encoding BMP9) and *BMP10* have been identified in PAH patients. The clinical phenotypes of PAH patients with *GDF2* and *BMP10* mutations have been characterised in a recent report [18].

Endothelial dysfunction, which includes endothelial cell apoptosis, compromised barrier function, and altered vasoactive mediator release, etc, plays a central role in the initiation of PAH [19]. Reduced or loss of endothelial *BMPR2* expression leads to endothelial dysfunction. *In vitro*, a reduction or loss of *BMPR2* in human pulmonary



**Figure 1. Introduction to the highly dynamic TGF-β and BMP signalling.**

TGF-β family signalling is regulated at multiple levels including: (1) TGF-β family ligands are synthesised and secreted as the prodomain bound forms; (2) there are a large number of ligands signalling through a limited number of type I and type II receptor pairs with a high degree of promiscuity in ligand-receptor interaction; (3) extracellular ligand traps can bind ligands and prevent them from binding to the receptors; (4) co-receptors can modify ligand-receptor interactions through direct protein-protein interactions; (5) TGF-β and BMP signalling can regulate the gene expression of components on the signalling pathways, such as *SMAD9*, *BMPR2* and *ENG*, or inhibitors of the signalling pathways, such as *SMAD6* and *SMAD7*. Genes (and encoded proteins) that are mutated in PAH are highlighted by \*.

vascular endothelial cells induces mitochondrial dysfunction and promotes a pro-inflammatory and pro-apoptotic state [20], causes endothelial-to-mesenchymal transition [21], and induces apoptosis [22,23] and excess permeability [22,24]. *BMPRII* deficiency impairs apoptosis via the *BMPRII*-*ALK1*-*BclX* (B-cell lymphoma X)-mediated pathway and the *Bcl-xL* isoform could be a potential biomarker for PAH [25]. *In vivo*, loss of

*Bmpr2* causes increased lung vascular permeability [22]. Conditional deletion of *Bmpr2* in the pulmonary endothelium [26] or knocking-in human mutation R899X into *Bmpr2* gene [22] predisposes mice to PAH. Circulating BMP ligands, mostly BMP9 and BMP10, act constitutively and potently on vascular endothelial cells [27,28], inducing *BMPR2* expression [29] and have a plethora of endothelial protective functions, including anti-apoptosis, anti-migration, anti-proliferation, and anti-angiogenesis [22]. Administration of BMP9 neutralising antibody in adult mice leads to excess permeability in lung vasculature [30].

## **Crystal structures of the BMPRII signalling complex reveal the highly dynamic interaction between BMP10 and BMPRII**

Although *BMPR2* mutations in PAH were first published in 2000, structural insights into how BMPRII interacts with a BMP ligand were only reported in 2022 [31]. Crystal structures of BMPRII extracellular domain (ECD) in complex with BMP10, and in complex with both BMP10 and ALK1 ECD, revealed an unprecedented degree of plasticity in the BMPRII:BMP10 interaction [31]. This suggests that stabilising the interaction between BMPRII and BMP10 requires high concentrations of BMPRII, and that under normal physiological conditions, BMPRII-dependent signalling is most active in tissues with the highest *BMPR2* expression. As lung vascular endothelial cells have the highest expression of *BMPR2*, along with the high expression of BMP10 in the right atrium, together they partly explain why *BMPR2* mutations which cause haploinsufficiency will have the most impact on lung vasculature [31].

## ***BMPR2* mutations also cause dysregulated BMP signalling in pulmonary smooth muscle cells**

BMPRII is the type II receptor for all BMPs, and ubiquitously expressed in many cell types. Germline mutations in *BMPR2* also affect its expression in non-endothelial cells. In pulmonary artery smooth muscle cells (PASMCs) isolated from PAH patients harbouring *BMPR2* mutations, BMP4-induced SMAD1 phosphorylation and *ID1* gene expression were reduced [32,33]. The growth suppressive response to BMP4 was lost in proximal PASMCs harbouring *BMPR2* mutations [32].

BMP6 and BMP7 also signal in PASMCs. In one study, it was shown that the induction of *ID1* and *ID3* gene expression by BMP6 treatment was reduced in *BMPR2* mutant PASMCs [33]. In another report, the result was more complicated [34]. In this latter study using mouse *Bmpr2*<sup>-/-</sup> and *Bmpr2*<sup>+/-</sup> PASMCs, it was shown that while BMP7 signalling was reduced in *Bmpr2*<sup>+/-</sup> PASMCs, there was a gain of BMP6 and BMP7 signalling in *Bmpr2*<sup>-/-</sup> PASMCs, even when BMP2 and BMP4 signalling remained reduced upon complete knockout of *Bmpr2*. This suggests that after a threshold change of cell surface BMPRII to somewhere below 50%, BMP6 and BMP7 gain of signal appears. More interestingly, in these *Bmpr2*<sup>-/-</sup> PASMCs, ActRIIA took over to mediate BMP4 and BMP6 signalling, and the type I receptor preference changed. ActRIIA can pair up with both ALK2 and ALK3 to mediate BMP4 signalling in *Bmpr2*<sup>-/-</sup> cells, whereas BMP6 (or BMP7) employs ALK2 only when BMPRII is absent [34]. Such gain of BMP6 signalling was also observed in PASMCs when BMPRII was shed from cell surface and *BMPR2* mRNA levels were reduced by more than 50% upon tumour necrosis factor- $\alpha$  (TNF- $\alpha$ ) treatment [35].

## **Altered inflammatory response exacerbates loss of BMPRII function and contributes to PAH pathogenesis**

The penetrance of gene mutations causing PAH is low, and a second hit is often present in the pathogenesis of PAH. Inflammation is widely accepted as a major second hit for PAH, and multiple studies have shown inflammation further contributes to dysregulated BMP signalling in pulmonary vascular cells. BMPRII deficiency promotes an exaggerated inflammatory response in human and mouse SMCs, producing higher levels of IL-6 and IL-8 after LPS-stimulation compared with controls [36]. TNF- $\alpha$  causes reduced mRNA expression of *BMPR2* in both human pulmonary artery endothelial cells (hPAECs) and PASMCs [35], thus exacerbating the loss of BMPRII protein function in these vascular cells. *BMPR2* deficiency in PASMCs conferred insensitivity to TGF- $\beta$  induced growth inhibition, and this process is associated with enhanced IL-6 and IL-8 induction by TGF- $\beta$  [37]. IL1- $\beta$  drives an exaggerated inflammatory response when *BMPR2* is deficient in PASMCs [38]. On the endothelial cell front, loss of *BMPR2* leads to increased permeability in PAEC monolayers [24], and mice with an endothelial-specific knockout of *Bmpr2* showed increased leukocyte recruitment and reduced barrier

function [39]. In humans, aberrant immune regulation is a key feature in a significant proportion of patients with IPAH and associated with clinical outcomes, with a small subset of patients showing immunoglobulin reactivity to BMPRII [40].

## Therapeutic strategies targeting BMP signalling in PAH

There are many ongoing efforts targeting BMP signalling for PAH treatment which have been reviewed recently [41–44]. Some focus on directly enhancing the cell surface BMPRII expression and such efforts include: (1) ataluren/PTC124 which promotes the read-through of stop-gain *BMPR2* mutations [45,46]; (2) small chemical chaperons such as 4-phenylbutyrate (4BA) which promote the secretion of misfolded BMPRII mutant proteins trapped in the endoplasmic reticulum [47]; (3) chloroquine and hydroxychloroquine which prevent lysosomal degradation of wild type BMPRII proteins [48]. However, these approaches are not specific to BMPRII, and the efficacy and potential side effects for treating PAH are yet to be seen in humans. Another approach employs low-dose FK506 which enhanced BMP signalling and reversed PAH in rodent models [49]. FK506 binds to FKBP12 and releases it from BMP type I receptors thereby enhancing BMP signalling. In a Phase IIa randomised placebo-controlled trial, FK506 was shown to be safe, increased *BMPR2* expression and improved 6-min walk distance in a subset of patients, but the overall efficacy is yet to be evaluated in a larger, multicentre trial [50]. Two approaches will be discussed further here: (1) targeting BMP9 signalling, and (2) Sotatercept, which has been approved by the FDA in March 2024 for treating PAH. Both approaches target the extracellular regulation of the TGF- $\beta$  family signalling complex.

## Targeting BMP9 signalling in PAH and controversies over BMP9 signalling

Genetic and clinical evidence strongly supports that loss of BMP9 signalling contributes to the pathogenesis of PAH. Rare heterozygous detrimental mutations in *GDF2* (encoding BMP9) have been found in several large cohort genomic studies [51–53]. Patients with pathogenic BMP9 mutations have lower plasma levels of BMP9 and BMP10 [52,54]. Several homozygous null mutations in *GDF2* have also been identified in paediatric PAH patients and circulating BMP9 is unmeasurable in these patients [54–57].

BMP9 is secreted from the liver and circulates at active concentrations, acting constitutively on vascular endothelium as a vascular quiescence factor [27]. BMP9 and BMP10 are the only two known high affinity ligands for ALK1. They form a signalling complex with ALK1 and BMPRII in endothelial cells and signal potently with an EC<sub>50</sub> below 0.1 ng/ml [30,58]. While BMPRII protein levels in endothelial cells reduce rapidly after protein synthesis inhibition [59], BMP9 induces *BMPR2* mRNA expression in endothelial cells [29], thus forming a dynamic balance. Based on the hypothesis that the loss of endothelial BMPRII and circulating BMP9 could be rescued by supplementation of BMP9, it was reported in 2015 that administration of recombinant BMP9 reversed PAH in three different rodent models: a genetic mouse knock-in model containing a human *BMPR2* mutation, the monocrotaline (MCT) — induced rat model, and a rat model induced by Sugen alongside chronic hypoxia (Sugen-Hypoxia) [22]. Here, BMP9 was also shown to confer protection against endothelial dysfunction. For example, *in vitro*, treatment of hPAECs with BMP9 offered protection against apoptosis induced by TNF- $\alpha$  and cycloheximide co-treatment, and BMP9 prevented excessive permeability in PAEC monolayer induced by TNF- $\alpha$  or LPS [22]. Of note, a potential beneficial role of BMP9 has also been reported in sepsis. Human patients with sepsis have lower BMP9 concentrations at admission, and lower BMP9 concentrations are associated with higher risk of death. BMP9 treatment improved the outcome in mice with experimental sepsis [60].

However, Tu et al. [61] reported in 2019 that BMP9 knockout mice, or mice administered with a neutralising anti-BMP9 antibody, were significantly protected against chronic hypoxia-induced pulmonary hypertension. Furthermore, they showed that ALK1-Fc treatment rescued rat PAH models induced either by MCT or Sugen-Hypoxia. Such results are intriguing as they are different from hypotheses derived from human genetics. Further studies using BMP9 and BMP10 double knockout mice revealed even more complex picture where the double knockout mice developed high-output heart failure [62]. Here they also showed that BMP9 contributed to the hypoxia-induced pulmonary vascular remodelling, whereas BMP10 played a role in hypoxia-induced cardiac remodelling. In a separate study, BMP9 and BMP10 were shown to directly act on vascular smooth muscle cells and affect the contractility state of the SMCs [63]. Table 1 summarises *in vivo* studies supporting BMP9 agonist or antagonist approaches in the context of PAH.

**Table 1. Summary of *in vivo* studies related to BMP9 agonist and antagonist approaches in PAH preclinical models**

PMID	Journal, year	Title of paper, reference number	Key findings related to BMP9 agonist or antagonist approaches in PAH models
26076038	<i>Nat. Med.</i> , 2015	Selective enhancement of endothelial BMPR-II with BMP9 reverses pulmonary arterial hypertension [22]	Administration of recombinant BMP9 reversed established PAH in <i>Bmpr2</i> R899X knock-in mice, as well as in rat PAH models induced by monocrotaline or Sugen-Hypoxia.
30636542	<i>Circ. Res.</i> , 2019	Selective BMP-9 inhibition partially protects against experimental pulmonary hypertension [61]	<ul style="list-style-type: none"> <li>• Bmp9<sup>-/-</sup> mice and its inhibition in C57BL/6 mice using neutralising anti-BMP9 antibodies substantially prevent against chronic hypoxia-induced pulmonary hypertension.</li> <li>• The BMP9/BMP10 ligand trap ALK1 ECD administered in monocrotaline or Sugen/Hypoxia (SuHx) rats substantially attenuates proliferation of pulmonary vascular cells, inflammatory cell infiltration, and regresses established pulmonary hypertension in rats.</li> </ul>
30312106	<i>Am. J. Respir. Crit. Care Med.</i> , 2019	Bone morphogenetic protein 9 is a mechanistic biomarker of portopulmonary hypertension [64]	<ul style="list-style-type: none"> <li>• Administration of BMP9 ligand trap ALK1-Fc exacerbated pulmonary hypertension and pulmonary vascular remodelling in mice treated with hypoxia.</li> </ul>
33334130	<i>Circulation</i> , 2021	BMP9 and BMP10 act directly on vascular smooth muscle cells for generation and maintenance of the contractile state [63]	<ul style="list-style-type: none"> <li>• BMP9 KO/BMP10 iKO in right atrium: dramatic changes in vascular tone and diminution of the VSMC layer with attenuated contractility and decreased systemic as well as right ventricular systolic pressure.</li> <li>• Deletion of <i>Acrv11</i> (encoding Alk1) in VSMCs recapitulated the Bmp9/10 phenotype in pulmonary but not in aortic and coronary arteries.</li> </ul>
34086873	<i>Cardiovasc. Res.</i> , 2022	Different cardiovascular and pulmonary phenotypes for single- and double-knock-out mice deficient in BMP9 and BMP10 [62]	<ul style="list-style-type: none"> <li>• BMP9 contributes to chronic hypoxia-induced pulmonary vascular remodelling, whereas BMP10 plays a role in hypoxia-induced cardiac remodelling in mice.</li> <li>• Combined deficiency in Bmp9 and Bmp10 led to vascular defects resulting in a decrease in peripheral vascular resistance and blood pressure and the progressive development of high-output heart failure and pulmonary hemosiderosis.</li> </ul>

The controversial observations on BMP9 extend to *in vitro* cell biological studies. A recent report suggests that loss of endothelial *BMPR2* expression reverses the endothelial response to BMP9, causing enhanced proliferation [65]. It is difficult to compare this study with previous published data as it uses very different treatment conditions, i.e. 1 ng/ml of BMP9 treatment which is well above the concentrations measured in human plasma [54], and the experiments were performed in full growth media which already contain high concentrations of BMP9. Of note, most of the reports use serum-restricted conditions when evaluating BMP9 signalling. Altered BMP9 response in endothelial cells derived from PAH patients was also observed in another study. Here BMP9

induced unfavourable endothelial-to-mesenchymal transition only in endothelial cells isolated from PAH patients but not healthy controls [66].

With the current understanding of BMP9 and BMP10 signalling [67,68], it is difficult to reconcile these controversial observations. The functions of BMP9 and BMP10 may have more complexity than hitherto recognised, i.e. more cell types might be involved when investigating BMP9 and BMP10 effects *in vivo*. One might interpret such controversial findings as BMP9 exerts different roles at different stages of PAH; a beneficial role during the initial stage of PAH (where genetic studies are very powerful in identifying the underlying cause of the disease) and a more complicated role during the late stage of the disease which can often be demonstrated in cells isolated from patients who are at advanced stages of PAH. Such a scenario was seen for TGF- $\beta$ , where it could act as either a tumour suppressor or a tumour promotor depending on different stages of the tumour development [69].

## Sotatercept and ActRIIA-mediated signalling

Sotatercept, a fusion protein comprising the extracellular ligand binding domain of ActRIIA fused to the Immunoglobulin Fc domain, is approved by the FDA for treating Group 1 PAH and is the first PAH therapy targeting the TGF- $\beta$  superfamily. In a Phase III clinical trial for PAH [70], Sotatercept improved the 6-min walk distance primary endpoint as well as in eight out of nine secondary efficacy endpoints compared with placebo controls, including time to death and clinical worsening [70,71]. Adverse events include epistaxis, telangiectasia, increased haemoglobin levels, thrombocytopenia and increased blood pressure, some of which may be related to the known affinity of ActRII-A with BMP and GDF ligands.

The mechanism of action of Sotatercept is still not fully understood. An early study suggests that it restores the balance between SMAD1/5/9 and SMAD2/3 signalling in PAH [72]. Here the authors showed that treatment with ActRIIA-Fc reversed elevated phospho-SMAD2/3 in a rat MCT model, but no restoration of reduced phospho-SMAD1/5/9 was observed in either rat MCT or rat Sugen-Hypoxia models [72]. Of note, Sotatercept is a ligand trap for activins and potentially also BMP9 and BMP10, so increased phospho-SMAD1/5/9 is not a direct outcome expected from Sotatercept treatment. Another study showed that treatment of ActRIIA-Fc in a Sugen-Hypoxia rat model normalised inflammatory response in the lungs, and importantly, the treatment suppressed the elevation of *Inhba* (encoding activin A, or ActA) and *Inhbb* (encoding ActB) expression in the right ventricle of Sugen-Hypoxia rats [73]. However, Sotatercept is an extracellular ligand trap and SMAD proteins are the intracellular mediators of signalling; these data still do not reveal the mode of action of Sotatercept at direct protein-protein interaction levels. We still do not know which target ligand or ligands are trapped by Sotatercept for its efficacy in PAH, nor do we know the major cell type that is responsible for the efficacy of Sotatercept.

Ligands with high affinities for ActRIIA are more likely to be bound and inhibited by Sotatercept. ActRIIA and ActRIIB are the major type II receptors for Activins, and ActRIIA has been shown to mediate signals from multiple BMP ligands using siRNA approaches [29,34,74]. In Biacore direct binding assays, ActRIIA-Fc has been shown to bind multiple TGF- $\beta$  family ligands with high affinities (Table 2). For activin ligands, ActA and ActB bind to ActRIIA-Fc with the highest affinity [77,79], whereas ActC only binds to ActRIIA transiently and no reported data on ActE. ActRIIA-Fc binds tightly to several GDF and BMP ligands, with  $K_D$  in the sub-nanomolar range for GDF11 and BMP10, and in the nanomolar range for GDF8, BMP7, BMP4, BMP9 and BMP6 (Table 2, Figure 2). Interestingly, many of these ligands also bind BMPRII with high affinity (Table 3, Figure 2); for example, ActB binds to BMPRII-Fc with comparable affinity to BMP10 and stronger than many other BMP ligands [77,79]. ActA also binds BMPRII-Fc with very high affinity, but weaker than ActB or BMP10. Serum levels of both ActA and ActB are significantly elevated in PAH patients [84]. *INHBA* (encoding ActA) is highly expressed in lung microvascular endothelial cells [85]. PAECs isolated from the lungs of patients with IPAH synthesised more *INHBA* mRNA and released more ActA protein into the culture medium [85]. ActA has been shown to be capable of inhibiting BMP9 but not BMP2 and BMP4 signalling in two multiple myeloma cell lines [86], but such inhibition was not observed in endothelial cells [87]. Interestingly, it was suggested that BMPRII inhibits activin signalling via ALK2 because knocking down *BMPRII* by siRNA lead to enhanced ActA-phospho-SMAD1/5 signalling via ALK2 in multiple myeloma cells [88]. This observation agrees with another report that ActA forms a non-signalling complex with ALK2 and type II Activin/BMP receptors [89]. Taken together, these reports could potentially point to a hypothesis that elevated ActA and ActB contribute to the disease progression of PAH, partly by competitive binding to BMPRII thereby further reducing the availability of BMPRII for BMP signalling and exacerbating BMPRII loss. Another potential

**Table 2. Affinities of different ligands for ActRIIA measured by surface plasmon resonance (Biacore)**

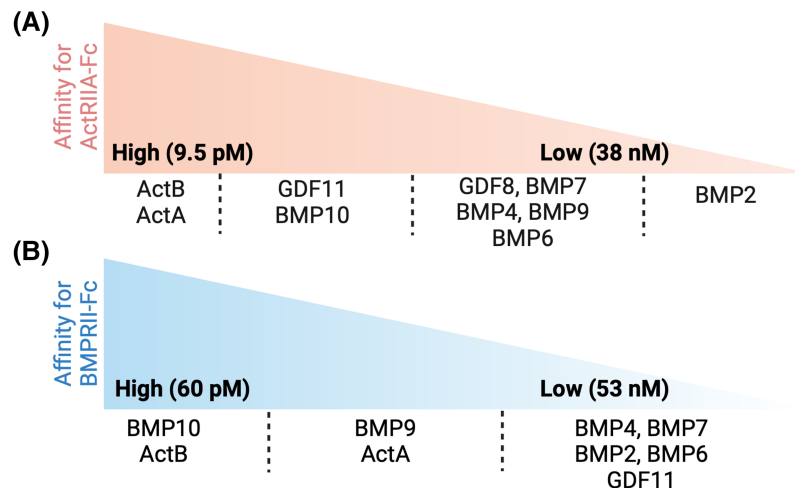
Ligands	Dissociation constant ( $K_D$ ) and reference paper
ActA	22 pM [75], 23 pM [76], 43 pM [77] <sup>a</sup> , 59 pM [78] <sup>b</sup> , 90 pM [79] <sup>a</sup>
ActB	9.5 pM [75], 61 pM [77] <sup>a</sup> , 53 pM [79] <sup>a</sup>
ActC	Transient binding [75]
ActE	No report
GDF8	3.77 nM [77] <sup>a</sup>
GDF11	573 pM [77] <sup>a</sup> , 52 pM [79] <sup>a</sup>
BMP2	38 nM [78] <sup>b</sup> , 20.6 nM [80] <sup>c</sup> , ~68 nM [81] <sup>b</sup>
BMP4	~3 nM [82], 3.51 nM [77] <sup>a</sup> , 11.9 nM [80] <sup>c</sup>
BMP6	10.5 nM [77] <sup>a</sup>
BMP7	1.59 nM [77] <sup>a</sup> , 1.2 nM [78] <sup>b</sup> , No binding [79] <sup>a</sup>
BMP9	6.43 nM [83] <sup>c</sup> (25°C)
BMP10	88.6 pM [83] (25°C), 381 pM [77], 1 nM [79] <sup>a</sup>

Data were obtained using human ActRIIA-Fc immobilised on the Biacore chip and titrating a range of ligand concentrations apart from those specified in the footnotes below. Data using immobilised ligands are not included here. Although it is difficult to directly compare the exact  $K_D$  values from different studies, the reported values are in general agreement, and the values obtained from the same study under the same conditions are directly comparable.<sup>a</sup>From single injection on Biacore.

<sup>b</sup>Used mouse ActRIIA, and not Fc fusion.

<sup>c</sup>By Steady-state analysis.

mechanism suggested by a recent report is that binding of ActA to BMPRII leads to endocytosis of BMPRII protein, hence further reducing the cell surface BMPRII [85]. However, increased BMPRII levels or BMPRII-mediated signalling after ActRIIA-Fc or Sotatercept treatment has not been reported in either preclinical or clinical data. Nevertheless, both hypotheses predict that Sotatercept should have a beneficial effect in PAH by directly sequestering the elevated ActA and ActB. Of interest, treatment of PAECs with either BMP9 or BMP10, both in the physiologically relevant prodomain-bound forms and at physiologically relevant



**Figure 2. Summary of different ligand binding affinities for ActRIIA and BMPRII.**

Ligands with high to low affinities for ActRIIA-Fc (A) and BMPRII-Fc (B), based on the binding data in Table 2 and Table 3.

$K_D$  values for the high and low affinities are taken from Table 2 and Table 3.



**Table 3. Affinities of different ligands for BMPRII measured by surface plasmon resonance (Biacore)**

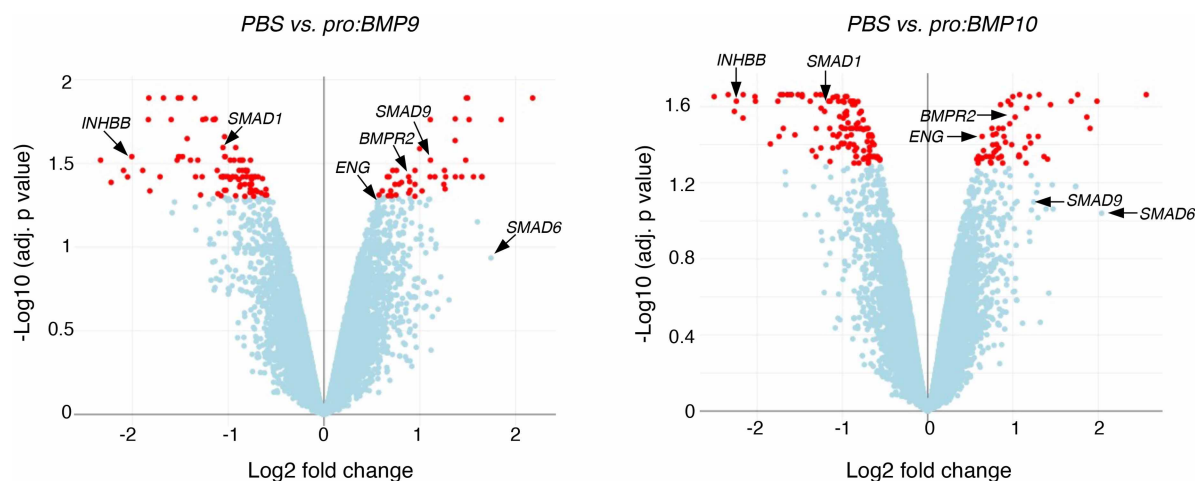
Ligands	Dissociation constant ( $K_D$ ) and reference paper
ActA	9.0 nM [77] <sup>a</sup> , 9.6 nM [79] <sup>a</sup>
ActB	0.7 nM [79], 0.6 nM [77], 2.3 nM [79] <sup>a</sup> , 0.7 nM [79]
GDF11	53.3 nM [77] <sup>a</sup>
BMP2	41.4 [80] <sup>b</sup>
BMP4	7.4 nM [82], 26.3 nM [80] <sup>b</sup>
BMP6	44.5 nM [77] <sup>a</sup>
BMP7	39 nM [77] <sup>a</sup> , 13 nM [79] <sup>a</sup>
BMP9	7.4 nM [77] <sup>a</sup> , 0.6 nM [83]
BMP10	2.4 nM [79], 2.1 nM [79] <sup>a</sup> , 0.2 nM [77] <sup>a</sup> , 0.06 nM [83]

Data were obtained using human BMPRII-Fc immobilised on the Biacore chip and titrating a range of ligand concentrations apart from those specified in the footnotes below. Data using immobilised ligands are not included here. Although it is difficult to directly compare the exact  $K_D$  values from different studies, the reported values are in general agreement, and the values obtained from the same study under the same conditions are directly comparable.<sup>a</sup>From single injection on Biacore.  
<sup>b</sup>By Steady-state analysis.

concentrations, can suppress the expression of the *INHBB* gene which encodes ActB (Figure 3) [90], in agreement with a beneficial effect of a BMP9 agonist approach for treating PAH.

## Summary and future directions

Human genetics and pre-clinical studies both support a fundamental role of BMP signalling in the pathogenesis of PAH. BMP and activin signalling complexes are intertwined at multiple levels, involving the competitive binding of all three BMP type II receptors, BMPRII, ActRIIA and ActRIIB. The positive outcome from the



**Figure 3. BMP9 and BMP10 suppress ActB expression in human pulmonary artery endothelial cells (hPAECs).**

Serum-starved hPAECs were treated with PBS, prodomain-bound BMP9 (pro:BMP9), or pro:BMP10 at concentrations equivalent to 0.4 ng/ml growth factor domain alone. After 5 hours, cells were harvested, and RNA was extracted for microarray analysis. Volcano plots showing the differential gene expression. Hits with adjusted P values less than 0.05 are shown in red. *INHBB*, which encoding ActB, is highlighted. Also highlighted are the components of BMP signalling (Figure 1) that are regulated by BMP9 and BMP10. Data for PBS vs pro:BMP9 has been published previously [30].

Sotatercept Phase III trial strongly suggests that dysregulated BMP signalling in PAH also involves activin signalling. A more in-depth investigation of the effect of ActA and ActB in PAH models and patient samples is warranted. Another area of BMP signalling in PAH that is not covered in this review is the shared mutations of endothelial BMP signalling components in PAH and hereditary haemorrhagic telangiectasia, which will be another important and intriguing topic to review.

## Perspectives

- *The importance of the field:* pathogenic mutations in multiple components of BMP signalling pathways have been identified in human genetic studies on PAH, supporting a crucial role of dysregulated BMP signalling in the pathogenesis of PAH. Sotatercept is the first FDA-approved therapeutic modality that directly targets extracellular regulation of TGF- $\beta$ /BMP signalling.
- *Summary of the current thinking:* compromised endothelial BMP signalling involving ALK1 and BMPRII is likely an initial trigger for PAH. Aberrant BMP signalling in other cell types and other TGF- $\beta$  family ligands and receptors may also contribute to the pathogenesis of PAH.
- *Future directions:* a deeper mechanistic insight into the extracellular regulation of signalling from BMPs, activins and GDFs may provide novel therapeutic opportunities. This could be achieved by *in vitro* cell signalling assays in a physiologically relevant context, such as using human primary cells, patient cells and co-culture models, and coupled with biochemical and structural studies to address the direct protein–protein interactions amongst ligands and receptors.

## Competing Interests

The authors declare that there are no competing interests associated with the manuscript.

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## Author Contribution

W.L. wrote the manuscript. K.Q. generated [Figures 1](#) and [2](#) and reviewed the manuscript.

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## Abbreviations

ALK1, activin receptor-like kinase 1; BMP, bone morphogenetic protein; ECD, extracellular domain; GDF, growth and differentiation factor; hPAEC, human pulmonary artery endothelial cell; IPAH, idiopathic PAH; MCT, monocrotaline; PAH, pulmonary arterial hypertension; PASMC, pulmonary artery smooth muscle cells; TGF- $\beta$ , transforming growth factor- $\beta$ ; TNF- $\alpha$ , tumour necrosis factor- $\alpha$ .

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