Targeting transforming growth factor-β receptors in pulmonary hypertension

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A review of the role of the TGF-β–activin–nodal branch in pulmonary arterial hypertension and how this knowledge has not only provided insight into understanding its pathogenesis, but has also paved the way for possible novel therapeutic approaches https://bit.ly/2Pujk8O

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ABSTRACT The transforming growth factor-β (TGF-β) superfamily includes several groups of multifunctional proteins that form two major branches, namely the TGF-β–activin–nodal branch and the bone morphogenetic protein (BMP)–growth differentiation factor (GDF) branch. The response to the activation of these two branches, acting through canonical (small mothers against decapentaplegic (Smad) 2/3 and Smad 1/5/8, respectively) and noncanonical signalling pathways, are diverse and vary for different environmental conditions and cell types. An extensive body of data gathered in recent years has demonstrated a central role for the cross-talk between these two branches in a number of cellular processes, which include the regulation of cell proliferation and differentiation, as well as the transduction of signalling cascades for the development and maintenance of different tissues and organs. Importantly, alterations in these pathways, which include heterozygous germline mutations and/or alterations in the expression of several constitutive members, have been identified in patients with familial/heritable pulmonary arterial hypertension (PAH) or idiopathic PAH (IPAH). Consequently, loss or dysfunction in the delicate, finely-tuned balance between the TGF-β–activin–nodal branch and the BMP–GDF branch are currently viewed as the major molecular defect playing a critical role in PAH predisposition and disease progression. Here we review the role of the TGF-β–activin–nodal branch in PAH and illustrate how this knowledge has not only provided insight into understanding its pathogenesis, but has also paved the way for possible novel therapeutic approaches.

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Introduction

Pulmonary arterial hypertension (PAH) is a severe cardiopulmonary condition with a median survival time of 7 years despite best standards of care [1–3]. In the updated pulmonary hypertension (PH) classification, PAH corresponds to Group 1 PH, defined by right-heart catheterisation as precapillary PH in the absence of other causes such as chronic lung disease or chronic thromboembolic disease [2]. PAH may be idiopathic (IPAH), familial/heritable, induced by drugs and toxins, or associated with different conditions (such as portal hypertension, connective tissue disease, congenital heart disease, HIV infection and schistosomiasis) [2].

Several pulmonary vasodilators have been developed for the treatment of PAH over the past decades, but there is currently no cure [4]. Treatments commonly used in PAH include calcium channel blockers (for a small subset of patients with an acute vasodilator response) and different drugs that target three well-identified pathways of endothelial cell dysfunction [5, 6]. The latter are: 1) endothelin receptor antagonists (ERAs) targeting the endothelin pathway; 2) phosphodiesterase Type-5 inhibitors (PDE5i) and guanylate cyclase stimulators targeting the nitric oxide pathway; and 3) prostacyclin analogues and prostacyclin receptor agonists targeting the prostacyclin pathway. Even if the development of approved PAH therapies has improved quality of life and clinical outcomes in PAH, they do not reverse the progressive obliteration of the lung vasculature which causes the increases in pulmonary vascular resistance (PVR) that ultimately lead to right-heart overload, hypertrophy, fibrosis and finally failure.

There is an unmet need to identify mechanisms underlying structural and functional pulmonary vascular remodelling in PAH. In recent years, the respective roles of endothelial cell and smooth muscle cell (SMC) dysfunction, unresolved inflammation, and loss or dysfunction in the bone morphogenetic protein (BMP) and transforming growth factor-β (TGF-β) signalling pathways have been better understood [7]. Indeed, there is now clear evidence that heterozygous *BMPR2* germline mutation represents the main susceptibility gene for PAH. *BMPR2* encodes BMP receptor Type II (BMPRII), a Type-II receptor of the TGF-β superfamily. More recently, mutations of other members of the TGF-β superfamily have been identified in heritable PAH (table 1), but a full mechanistic understanding of how loss and/or dysfunction in the TGF-β–activin–nodal and BMP–growth differentiation factor (GDF) signalling contributes to disease pathogenesis is lacking.

As alterations in the signalling of the TGF-β superfamily are well recognised in PAH, several agents targeting the BMPRII axis and its balance with the activin–inhibin–nodal branch are currently being tested [7–9]. This review examines the current state of the art regarding the dysfunctions that affect BMP and TGF-β signalling systems, critical knowledge that might help in designing novel approaches for PAH treatment.

Signalling by the TGF-β–activin–nodal and BMP–GDF branches

The TGF-β superfamily forms a unique group of at least 37 structurally related proteins that are classified into four different subfamilies based on their sequence similarities and affinity for specific receptors. These are: BMP–GDF (Group 1), activin–inhibin–nodal (Group 2), TGF-β (Group 3) and other (Group 4) (figure 1a). In mammals, the TGF-β superfamily is highly conserved and widely distributed throughout the body [10, 11].

These family member proteins are made as inactive pro-peptide homodimers or heterodimers covalently linked *via* a disulfide bridge. During the secretory pathway, these dimers are proteolytically cleaved from the C-terminus by proteases of the pro-protein convertase family. Often, however, the pro-peptide remains associated with the mature dimer by non-covalent interactions (figure 1b). This interaction between the

TABLE 1 Genes causing heritable pulmonary arterial hypertension (PAH). Reproduced from [8] with permission

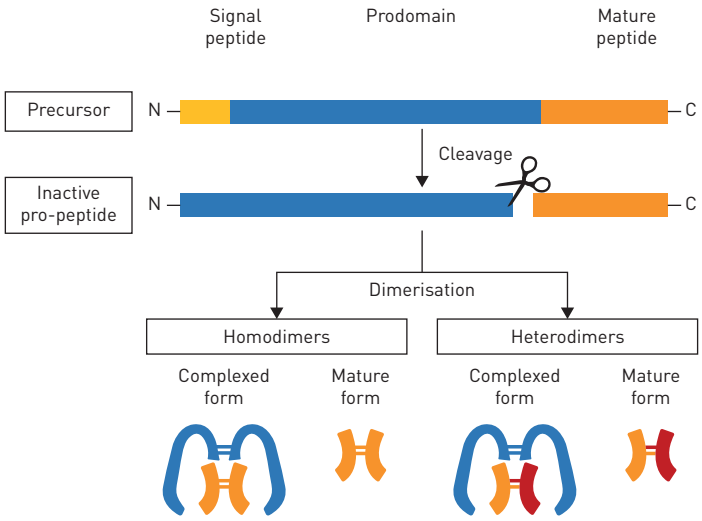
Gene	Evidence level
BMPR2[#]	High
EIF2AK4[¶]	High
ACVRL1[+], *AQP1*[+], *ATP1A3*[+], *CAV1*[+], *ENG*[+], *GDF2*[+], *KCNK3*[+], *SMAD9*[+], *SOX17*[+], *TBX4*[+]	High
ALK6, *KLF2*, *KCNKA5*, *SMAD1*, *SMAD4*	Low
ABCC8, *BMP10*, *CD248*, *EFCAB4B*, *KDR*, *NOTCH3*, *TET2*	Recently identified

[#]: familial PAH (53–86%), sporadic PAH (14–35%); [¶]: familial pulmonary veno-occlusive disease (PVOD)/pulmonary capillary haemangiomatosis (PCH) (~100%); [+]: other genes (<1.5% of sporadic PAH).

a)

	Gene name	Protein name	Alternative name
Group 1	*BMP2*	BMP-2	
	BMP4	BMP-4	
	BMP5	BMP-5	
	BMP6	BMP-6	VGR-1
	BMP7	BMP-7	OP-1
	BMP8A	BMP-8A	OP-2
	BMP8B	BMP-8B	OP-3
	GDF2	BMP-8	GDF-2
	BMP10	BMP-10	
	GDF1	GDF-1	
	GDF3	GDF-3	
	GDF5	GDF-5	BMP-14/CDMP-1
	GDF6	GDF-6	BMP-13/CDMP-2
	GDF7	GDF-7	BMP-12/CDMP-2
	GDF9	GDF-9	
	BMP15	BMP-15	GDF-9B
	BMP3	BMP-3	BMP-3A/osteogenin
	GDF10	GDF-10	BMP-3B/BIP
	MSTN	Myostatin	GDF-8
	GDF11	GDF-11	BMP-11
	GDF15	GDF-15	MIC-1/NSAID-activated protein 1 /PDF/PTGFB/PLAB
Group 2	*INHA*	Inhibin-α	
	INHBA	Inhibin-β_A	Activin beta A chain/inhibin beta A chain /inhibin, beta 1/EDF/FRP
	INHBB	Inhibin-β_B	Activin beta B chain/inhibin beta B chain /inhibin, beta 2
	INHBC	Inhibin-β_C	Activin beta C chain/inhibin beta C chain
	INHBE	Inhibin-β_E	Activin beta E chain/inhibin beta E chain
	LEFTY1	Lefty-B	Lefty-1
	LEFTY2	Lefty-A	Lefty-2/EBAF/TGF-β4
	NODAL	Nodal	BMP-16/HTX-5
Group 3	*TGFB1*	TGF-β1	LAP/DPD-1
	TGFB2	TGF-β2	G-TSF/BSC-1 cell growth inhibitor/ cetermin/polyergin
	TGFB3	TGF-β3	ARVD–1
Group 4	*AMH*	AMH	MIF/MIS
	GDNF	GDNF	ATF
	NRTN	NTN	
	ARTN	ART	NBN/neurotrophic factor
	PSPN	PSP	

b)



c)

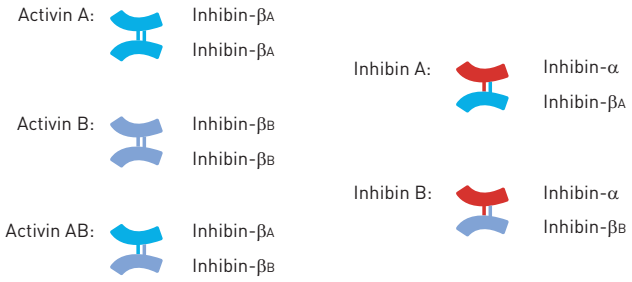


FIGURE 1 Bone morphogenetic protein (BMP) and transforming growth factor-β (TGF-β) signalling in mammals, with a) a listing of protein and gene names for the BMP and TGF-β family proteins; b) a schematic illustration of the different forms occurring during BMP and TGF-β synthesis, secretion and activation; and c) a schematic illustration of the structure of mature activin and inhibin proteins. VGR: vegetal-related growth factor; OP: osteogenic protein; GDF: growth differentiation factor; CDMP: cartilage-derived morphogenetic protein; MIC: macrophage inhibitory cytokine; NSAID: nonsteroidal anti-inflammatory drug; PDF: prostate differentiation factor; PTGFB: placental TGF-β; PLAB: placental BMP; EDF: erythroid differentiation protein; FSH: follicle-stimulating hormone; FRP: FSH-releasing protein; Lefty: left–right determination factor; EBAF: endometrial bleeding-associated factor; HTX: visceral heterotaxy; LAP: latency-associated peptide; DPD: progressive diaphyseal dysplasia; G-TSF: glioblastoma-derived T-cell suppressor factor; ARVD: arrhythmogenic right-ventricular dysplasia; AMH: anti-Müllerian hormone; MIF: Müllerian-inhibiting factor; MIS: Müllerian-inhibiting substance; GDNF: glial cell derived neurotrophic factor; ATF: activating transcription factor; NTN: neurturin; ART: artemin; NBN: neublastin; PSP: persephin.

pro-peptide and its mature dimer plays an important role in the biosynthesis, transportation, stabilisation and signalling of these complexes. For the three isoforms of TGF-β, myostatin and GDF-11, this interaction between the pro-peptide and its mature dimer confers latency to the complex, although this is not the case for other family members (*e.g.* activins or several BMPs such as BMP-7 or BMP-9) [10, 11]. For activin A (β_A–β_A) (figure 1c), this interaction with the pro-peptide is essential for interaction with extracellular matrix (ECM) proteins (*e.g.* perlecan and agrin), thereby facilitating localisation and storage within tissue for later activation [12]. The activity of these different ligands is also tightly regulated by various mechanisms, including through post-translational modifications that increases stability, half-life or specificity of receptor

coupling. The bioavailability of these different ligands is not only dependent of their different specific spatial and temporal patterns of expression, but is also under the control of several specific antagonists (*e.g.* follistatin, gremlin, inhibin-α, left–right determination factor (lefty) and noggin) (figure 2).

The active dimers transduce a signal by binding to a transmembrane heteromeric complex with serine (Ser)/threonine (Thr) kinase activity consisting of two Type I and two Type II receptors. The interaction of active dimers with their binding sites allows the kinase domains of Type II receptors to phosphorylate those of Type I receptors and initiate specific signal transduction cascades. Two distinct main branches can be distinguished. On the one hand, the TGF-β–activin–nodal branch that signals through small mothers against decapentaplegic (Smad) 2/3 and, on the other, the BMP–GDF branch that signals through Smad 1/5/8. Subsequently, pSmad 2/3 or pSmad 1/5/8 oligomerises with the common Smad (co-Smad; Smad 4) and translocates into the nucleus, regulating transcription of target genes. Inhibitory Smads (Smad 6 and Smad 7, also known as I-Smads), several accessory receptors (also known as Type III receptors, such as betaglycan or endoglin (ENG)) and modulators (*e.g.* BMP and activin membrane-bound inhibitor (BAMBI), FK-binding protein-12 (FKBP12), Smurf and Smad 7), as well as various miRNAs, can antagonise or facilitate the signalling mediated by pSmad 2/3–Smad 4 or pSmad 1/5/8–Smad 4. Upon binding to DNA and to their transcriptional partners, these Smad complexes recruit co-activators (*e.g.* p300 and CREB-binding protein (CBP)) or co-repressors (*e.g.* C-Ski and Ski-related novel protein N (SnoN)) to facilitate or impede the initiation of transcription (figure 2). Importantly, noncanonical (Smad-independent or non-Smad) pathways are also initiated by the activated ligand–receptor complex

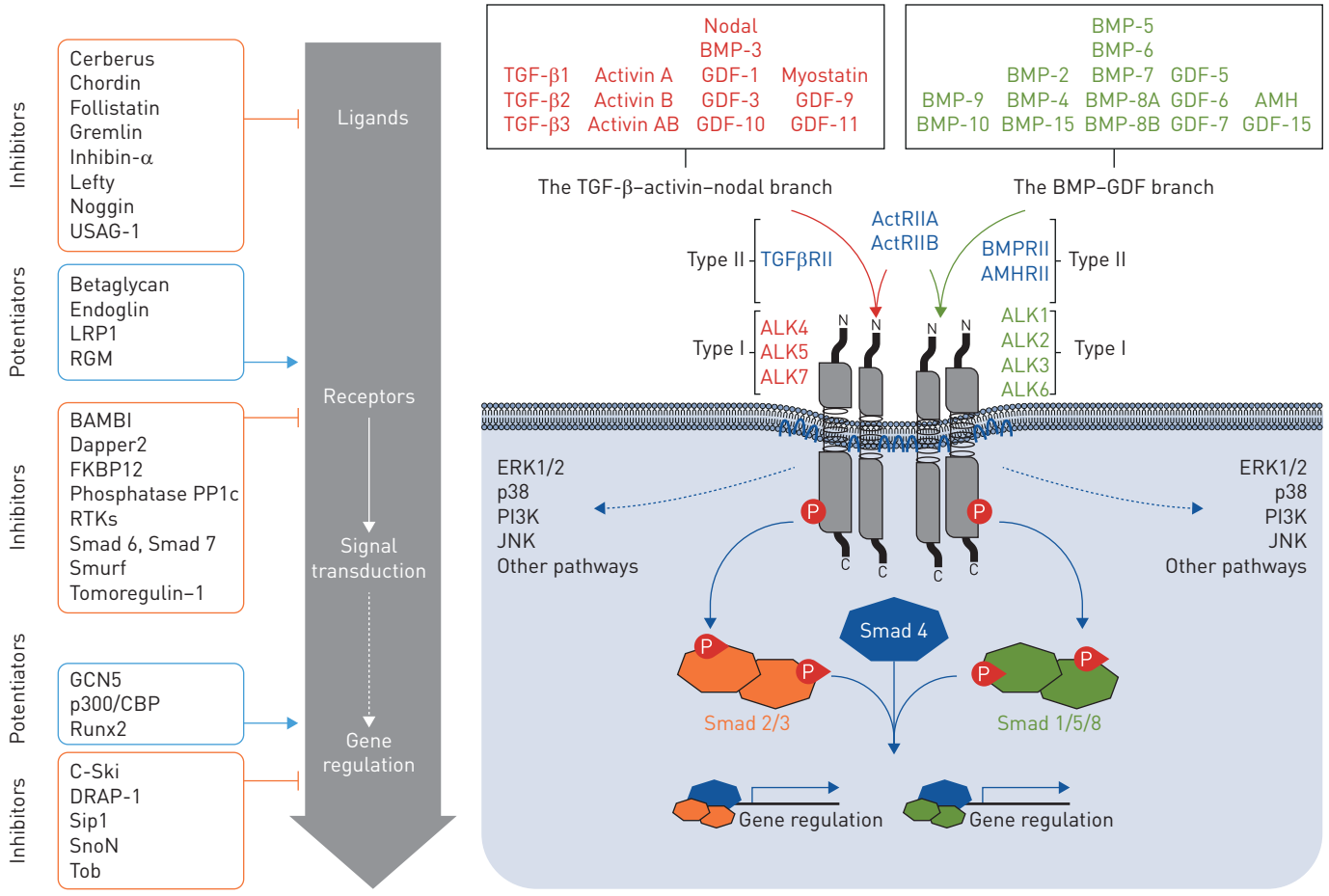


FIGURE 2 Signalling by bone morphogenetic protein (BMP) and transforming growth factor-β (TGF-β). Lefty: left–right determination factor; USAG: uterine sensitisation-associated gene; GDF: growth differentiation factor; AMH: anti-Müllerian hormone; LRP: low-density lipoprotein receptor-related protein; RGM: repulsive guidance molecule A; TGFβRII: TGF-β receptor Type II; ALK: activin receptor-like kinase; ActRII: activin receptor Type II; BMPRII: BMP receptor Type II; AMHRII: AMH receptor Type II; FKBP: FK-binding protein; PP1c: protein phosphatase 1c; RTK: receptor tyrosine kinase; Smad: small mothers against decapentaplegic; ERK: extracellular signal-regulated kinase; PI3K: phosphatidylinositol-3 kinase; JNK: C-Jun N-terminal kinase; GCN5: general control non-repressed 5 protein; CBP: CREB-binding protein; DRAP-1: DR1-associated protein 1; SnoN: Ski-related novel protein N; Sip1: Smad interacting protein 1; runx2: runt-related transcription factor 2; BAMBI: BMP and activin membrane-bound inhibitor.

including, among others, the extracellular signal-regulated kinase (ERK) 1/2 and p38 mitogen-activated protein kinase (MAPK) pathways, C-Jun N-terminal kinase (JNK), phosphatidylinositol-3 kinase (PI3K), peroxisome proliferator-activated receptor gamma (PPARγ), signal transducer and activator of transcription 3 (STAT3), Hippo and nuclear factor-κB (NF-κB), as well as Rho family GTPases (figure 2). Amplitude and duration of both Smad-dependent and non-Smad signalling pathways are finely tuned to generate a specific response programme in a single cell, in its environmental context, at a specific moment in time [9, 10, 11].

In the plasma membrane, distinct functional combinations of Type I and Type II receptor complexes allow for selectivity in ligand binding and diversity of signal transduction cascades and responses. As membrane-embedded Ser/Thr kinase receptors, their cell surface abundance is dynamically regulated by membrane physical and chemical properties (*e.g.* cholesterol content). They are thus partitioned between non-raft clathrin-coated pits and caveolin-1 positive, cholesterol-rich lipid raft microdomains known as caveolae. It is also well established that these functional heterocomplexes are internalised *via* clathrin- and caveolae-mediated endocytic mechanisms and that several soluble forms of these Type I, Type II and Type III receptors can be generated by proteolysis, exerting their ability to sequester ligands and modulating both TGF-β–activin–nodal and BMP–GDF signalling. These intracellular signalling pathways cross-interact with other signalling pathways, such as the Wnt–β-catenin, Hedeghog, estrogen, Hippo and Notch pathways, to ultimately dictate the biological output of pathway activity. Therefore, it is essential to improve our understanding of signal integration between TGF-β–activin–nodal and BMP–GDF signalling and other pathways in the regulation of the different organs under physiological and pathophysiological conditions.

Taken together, these elements illustrate how the signalling induced by the TGF-β–activin–nodal and BMP–GDF branches, and the dynamic equilibrium between these two branches, is highly regulated at multiple levels in order to ensure proper interpretation of these stimuli in different cellular settings.

Genetics of heritable pulmonary arterial hypertension

Several mutations causing heritable PAH involve key members of the activin receptor-like kinase 1 (ALK1)/BMPRII axis, supporting the concept that loss of activity in the BMP–GDF branch predisposes for PAH [8] (table 1). Heterozygous *BMPR2* germline mutations represent the main susceptibility gene for PAH. Several genetic studies have established that *BMPR2* mutations can be detected in 53–86% of patients with a familial history of PAH and in 14–35% of patients with sporadic IPAH [13–15]. In PAH, the female:male sex ratio ranges from 2:1 to 4:1 [13] and the lifetime risk of developing PAH in *BMPR2* mutation carriers is three-times higher in females as compared to males (42% and 14%, respectively) [8], suggesting that other genetic, epigenetic, environmental and hormonal risk factors are likely to influence the development of PAH. The mutations are widely distributed across the 13 exons of the *BMPR2* gene (with the exception of exon 13) and include missense mutations, nonsense mutations, splice defects, deletions and duplications [13]. A total of 486 distinct independent pathogenic variants have been recently identified in a cohort of 806 patients, corresponding to 27% nonsense mutations, 23% frameshift mutations arising from small-nucleotide insertions or deletions, 14% gene rearrangements and 10% splice-site mutations [15]. Thus, a large proportion of these pathogenic variants (*e.g.* nonsense mutations and large rearrangements) predict premature termination of the transcript, with loss likely through nonsense-mediated decay (an mRNA surveillance mechanism that detects and degrades transcripts containing premature termination codons). Therefore, haploinsufficiency through reduction in the available BMPRII protein level is more likely involved in the molecular mechanisms underlying PAH predisposition, even if several mutated alleles (due to missense mutations or deletions/duplications) are suspected of having a dominant negative effect on the wild-type (WT) BMPRII protein. PAH patients carrying a *BMPR2* mutation are younger at diagnosis and have a worse prognosis when compared to noncarriers [13, 16]. *BMPR2* mutation carriers are also less likely to show an acute vasodilator response and their diffusing capacity of the lung for carbon monoxide (D_{LCO}) values are relatively preserved [8, 13]. A similar degree of pulmonary arterial remodelling was recently noted in explanted lungs of PAH patients either carrying or not carrying a *BMPR2* mutation [17]. However, *BMPR2* mutation status was reported to be associated with more pronounced bronchial vascular changes (*e.g.* bronchial arterial hypertrophy/dilatation and bronchial microvessel density) and the formation of large fibrous vascular structures that appeared to connect the systemic vasculature to pulmonary veins [17]. These observations, taken with the fact that plexiform lesions appear to represent anastomosing structures between bronchial microvessels and pulmonary arteries and veins, suggest that BMPRII may influence the interaction between the pulmonary and bronchial circulations. Decreased right-ventricular function that is not attenuated by currently available PAH drugs has been reported in PAH patients carrying a *BMPR2* mutation as compared to non-carriers [18].

Mutations in *ACVRL1* (encoding the ALK1 Type I receptor of the TGF-β superfamily) and *ENG* (encoding the accessory receptor ENG) usually cause hereditary haemorrhagic telangiectasia. These mutations less frequently cause PAH, sometimes early in life [8, 19]. *ACVRL1* mutations are mostly missense mutations and are localised in the catalytic domain, leading to reduced Smad 1/5 phosphorylation [19]. Recently, mutations in *GDF2* and *BMP10* (encoding BMP-9 and BMP-10, respectively) have been described. Several variants in the *GDF2* gene have been found to cluster at the interface between the pro-domain and the growth factor domain, altering ligand stability or its secretion [20]. Sequencing of genes encoding other members of the BMPRII signalling pathway have also allowed identification of rare sequence variants in *SMAD 1*, *SMAD 4* and *SMAD 9*. Mutations in *CAV1*, which encodes caveolin-1, have been detected in some PAH patients (table 1) [8].

BMPRII and ALK1 are highly expressed on the pulmonary vascular endothelium, where they form an ALK1–BMPRII receptor complex that signals specifically in response to circulating BMP-9 and BMP-10 (utilising ENG as a co-receptor) [9, 21]. Notably, the requirement for high levels of BMPRII–ALK1 signalling in the pulmonary endothelium might contribute to the lung-specific effects of *BMPR2* mutations [8]. Decreased BMPRII also favours endothelial cell dysfunction and promotes endothelial to mesenchymal transition, a process involved in PAH [22].

TGF-β–activin–nodal and BMP–GDF imbalance in pulmonary hypertension

Accumulating evidence supports the notion that a shift of balance in favour of TGF-β–activin–nodal signalling is occurring in human PAH, even in the absence of mutations in members of the TGF-β superfamily. This shift increases the risk of developing PAH and is strongly suspected to also contribute to disease pathogenesis by modulating cell survival, metabolism, inflammation, genome instability, migration and cell differentiation (figure 3). Plasma levels of BMP-9 and BMP-10 are reduced in female PAH patients carrying a pathogenic *GDF2* variant, as compared to healthy age-matched females [23]. However, there are no statistical differences in the abundance of circulating BMP-9 and BMP-10 in patients with IPAH or heritable PAH, as compared to control subjects, even if a subset exhibits reduced plasma BMP-9 and BMP-10 levels [23, 24]. Among PAH patients, only those with portopulmonary hypertension have a

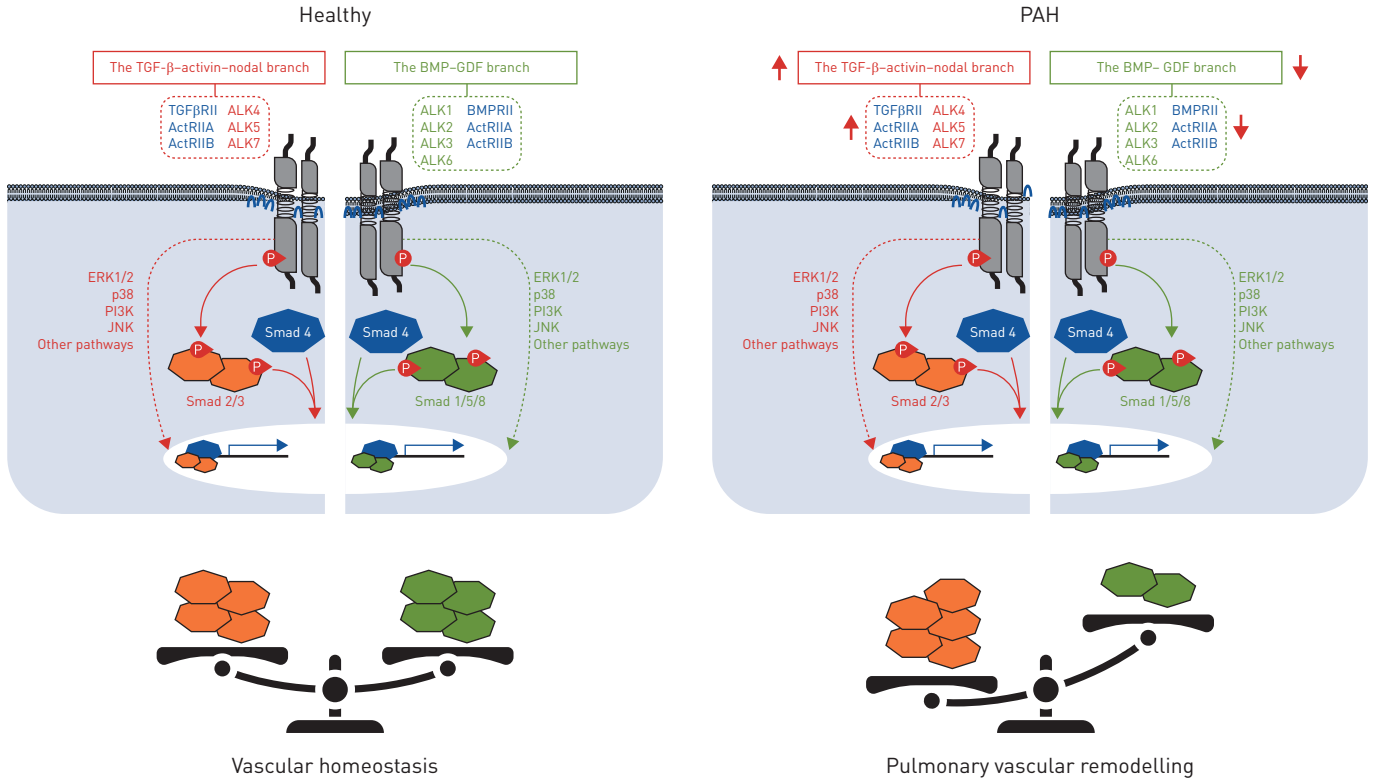


FIGURE 3 Dysregulation of the transforming growth factor-β (TGF-β)–activin–nodal and bone morphogenetic protein (BMP)–growth differentiation factor (GDF) branches in pulmonary arterial hypertension (PAH). TGFβRII: TGF-β receptor Type II; ActRII: activin receptor Type II; ALK: activin receptor-like kinase; BMPRII: BMP receptor Type II; ERK: extracellular signal-regulated kinase; PI3K: phosphatidylinositol-3 kinase; JNK: C-Jun N-terminal kinase; Smad: small mothers against decapentaplegic.

marked decrease in concentrations of circulating BMP-9 and BMP-10 *versus* healthy control subjects or *versus* cirrhotic controls [24, 25]. Notably, decreased concentrations of circulating BMP-9 and BMP-10 are also found in patients with hepatopulmonary syndrome, another pulmonary vascular disease associated with portal hypertension. By contrast, detection of elevated levels of circulating activin A (β_A-β_A) [26, 27], follistatin [26], GDF-15 [28–32], TGF-β [33–35] and soluble endoglin (sENG) [36] has been reported in PAH patients.

Decreased immunoreactivity for ALK3, BMPRII, DNA-binding protein inhibitor-3 (ID-3), Smad 3 and pSmad 1/5/8 has been described in the pulmonary vessel wall of patients with PAH and other forms of PH, as compared to lungs from controls [37–43]. Conversely, increased immunoreactivity for activin A, GDF-8, GDF-11, GDF-15, pSmad 2/3 and TGF-β3 has been reported in remodelled vessels from PAH explanted lungs [27, 39, 44, 45]. Although no changes have been noted in the relative protein levels of ALK5 and TGF-β receptor Type II (TGFβRII), specific TGFβRII upregulation in pericytes has recently been identified in explanted lung tissues from PAH patients [46]. These *in situ* observations have been replicated *in vitro*, with cultured pulmonary endothelial cells and SMCs from patients with IPAH exhibiting decreased capacity to activate Smad and non-Smad pathways, as compared with control cells [9, 47]. PPARγ was recently identified as a molecular link between the BMP-2 and TGF-β1 pathways in vascular SMCs through direct interaction with STAT3 and Smad 3 [48]. Despite this, the reasons for this imbalance in the absence of a compensatory decrease in the signalling of the TGF-β–activin–nodal branch remain unknown and more studies are required. Similarly, further work is needed to dissect the role of cooperation between the Smad and non-Smad signalling pathways in PAH, especially in how these dysregulated TGF-β–activin–nodal and BMP–GDF branches activate ERK1/2, JNK/p38, RhoA, Rac and Cdc42 and influence the activity of other related pathways.

Even if none of the genetically modified animals developed to date have reproduced characteristic findings of human PAH, they have helped greatly in investigating the potential contributions of various receptors and ligands of the TGF-β superfamily to the pulmonary vascular remodelling associated with PH. Mice deficient in ALK1, BMPRII or ENG, or in Smad 1 or Smad 8, are more susceptible to remodelling of the pulmonary vessels, as compared with WT littermates (table 2). However, some of these results are still unclear and much remains to be learned. In addition to the fact that mice are generally less prone to developing remodelling of the pulmonary vasculature, several factors could contribute to the discrepancies in some of these results, including genetic background, sex and age. Due to the small size of the mice, it is also technically challenging to obtain a complete invasive haemodynamic characterisation, as compared with larger animals. A shift of balance in favour of TGF-β–activin–nodal signalling has been observed in multiple PH models, including those induced by monocrotaline (MCT) injection, infection with schistosomiasis, or the combination of Sugen 5416 (SU5416), a vascular endothelial growth factor (VEGF) antagonist and exposure to chronic hypoxia (SuHx). In these animal models of PH, this shift was associated with accumulation of different pulmonary vascular cells and infiltration of inflammatory cells. Consequently, various small molecule and ligand-trap approaches have been tested in these animal models to identify the most adapted and powerful therapeutic agents that could help to restore the TGF-β–activin–nodal and BMP–GDF branch balance during PH progression (table 3).

Targeting the TGF-β superfamily in pulmonary hypertension

To restore the TGF-β–activin–nodal and BMP–GDF balance, several direct and indirect strategies have been tested *in vivo* and/or *in vitro* (table 3). Among these strategies, several pharmacologic inhibitors of ALK5 (such as IN-1233, LDN-193189, SB-525334 and SD-208) have been found to prevent or reverse pulmonary vascular remodelling in MCT-induced PH in rats [66–69]. However, these small molecules are often associated with cardiovascular toxicity that currently limits their use in humans [76]. A promising alternative could be the use of ligand-trap approaches, even if further studies are needed, especially as the exact role of the TGF-β–activin–nodal and BMP–GDF balance in cardiac homeostasis remains to be better understood [77].

Enhancement of BMP–GDF signalling in pulmonary vascular cells could offer a novel approach for the treatment of PAH. A variety of challenges still remain however, including the lack of pulmonary vascular selectivity and associated systemic adverse effects. Therefore, a complete analysis of the overall risk–benefit ratio (with long-term follow-up) remains to be performed in patients with severe PAH in order to know whether these different strategies could be of interest in combination with currently approved PAH therapies. BMPRII activators, such as FK506 (tacrolimus), FK520 (ascomycin) and rapamycin, which remove FKBP12 from BMP Type I receptors, have been investigated in PH [78]. Based on the fact that low-dose FK506 can reverse experimental PH [78], a phase 2 randomised, placebo-controlled safety and tolerability trial has been performed that shows low-level FK506 is well-tolerated and increases BMPRII expression in peripheral blood mononuclear cells (PBMCs) in a small group of PAH patients (identifier:

TABLE 2 Susceptibility of various transgenic animals to the development of pulmonary vessel remodelling

Susceptibility	Animal	Ref
Remodelling of lung vessels under unstressed conditions	*Alk1*$^{+/-}$ mice	[51]
	Bmpr2$^{+/-}$ mice$^{\#}$	[52]
	Bmpr2$^{+/R899X}$ mice	[49]
	Bmpr2$^{+/\Delta 71}$ rats, *Bmpr2*$^{+/\Delta 140}$ rats	[53]
	Eng$^{+/-}$ mice	[54]
	L1cre^{+} mice, *Bmpr2*$^{f/f}$ mice, *L1cre*$^{+}$ mice, *Bmpr2* $^{f/+}$ mice	[55]
	L1Cre^{+} mice, *Smad1*$^{f/f}$ mice	[56]
	SM22-rtTA×TetO^{7}*-BMPR2*^{R899X} mice	[57]
	Smad8$^{lacZ+/-}$ mice, *Smad8*$^{lacZ+/-}$ mice	[58]
	Tagln-Cre^{+} mice, *Smad1*$^{f/f}$ mice	[56]
	TG-TGF-β1 mice	[48]
Increased sensitivity to hypoxia-induced pulmonary vascular remodelling	*Bmp2*$^{+/-}$ mice	[59]
	Eng$^{+/-}$ mice	[54]
Protected against pulmonary vascular remodelling induced by chronic hypoxia	*Bmp4*$^{LacZ/-}$ mice	[60]
	Bmp9$^{-/-}$ mice	[47]
	Bmpr2$^{+/-}$ mice$^{\#}$	[52]
	Dn-Tgfbr2 mice	[61]
	Eng$^{+/-}$ mice	[33]
	Gdf11$^{f/f}$*-Tie2*^{cre} mice	[50]
	Grem1$^{+/-}$ mice	[62]
Similar susceptibility to hypoxia-induced pulmonary vascular remodelling as WT mice/rats	*Bmpr2*$^{\Delta Ex2/+}$ mice	[63]
	Bmpr2$^{+/-}$ mice	[64]
	Bmpr2$^{+/\Delta 527bp}$ rats	[65]
	Bmpr2$^{+/\Delta 16bp}$ rats	[65]
Early embryonic lethality (homozygous KO mice)	*Acvrl1*, *Acvr1*, *Bmpr1a*, *Bmp2*, *Bmp4*, *Bmp10*, *Gdf11*, *Smad1*, *Smad4*, *Smad5*, *Smad7*, *Bmpr2*, *Tgfbr1*, *Tgfbr2*	–
Perinatal lethality (homozygous KO mice)	*Acvr2b*, *Bmp1*, *Bmp7*, *Bmp11*, *Smad6*, *Tgfb1*, *Tgfb2*, *Tgfb3*	–

WT: wild-type; KO: knock-out; ALK: activin receptor-like kinase; ActRII: activin receptor Type II. $^{\#}$: carrying a mutant allele lacking exons 4 and 5.

NCT01647945 at https://clinicaltrials.gov) [79]. A strategy seeking to use a chemical chaperone, such as 4-phenylbutyrate (4PBA), to limit the retention of misfolded BMPRII mutants in the endoplasmic reticulum could also be used in specific subsets of patients, as supported by studies performed in primary cells and in knock-in *Bmpr2*^{C118W} mice [80, 81]. *In vitro*, PTC124 (ataluren), which permits ribosomal read-through of premature stop codons, can restore BMP signalling in cells carrying *BMPR2* and *SMAD9* nonsense mutations [82]. As haploinsufficiency is the most likely molecular mechanism underlying PAH, it remains an open question as to whether this strategy would be sufficient to stop or even reverse pulmonary vascular remodelling in PAH patients.

Administration of recombinant BMP-9 protein could represent another promising approach, as already shown in experimental PH [49]. Notably, mice lacking BMP-9 do not develop remodelling of the pulmonary vessels or PH under unstressed conditions [47] and only a subset of PAH patients exhibits reduced plasma BMP-9 and BMP-10 levels, underscoring the complexity of the pathway and the need for careful preclinical and clinical development [23, 24]. Moreover, the pleiotropic effects of BMP-9, such as the promotion of vascular calcification, vascular tone and inflammation, need to be considered in drug development.

Based on clinical findings which show that distinct ligands of the TGF-β superfamily (including activin A (β_A-β_A) [26, 27], follistatin [26], GDF-15 [28–32] and TGF-β [33–35]) are upregulated in PAH patients and on results obtained in several preclinical studies, ligand-trap molecules and neutralising therapeutic antibodies (such as bimagrumab [83]) which target activin receptor Type II (ActRII)A and/or ActRIIB receptors represent interesting approaches to restoration of the TGF-β–activin–nodal and BMP–GDF balance in PAH. A fusion protein consisting of the extracellular domain of ActRIIA linked to the IgG Fc domain has recently been tested in animal models of PH [27] to sequester activin A, activin B, GDF-8 and GDF-11. In MCT and SuHx rat models, treatment with ActRIIA-Fc attenuated pSmad 2 levels in lung

TABLE 3 List of agents directly targeting the transforming growth factor-β (TGF-β) superfamily tested in animal models of pulmonary hypertension (PH)

Agent	Target	Animal model	Effect on PH	Ref
Small molecules				
BUR1	↑ BMP-2	MCT, SuHx	Positive	[70]
IN-1233	↓ ALK5	MCT	Positive	[66]
IN-1233	↓ ALK5	Chronic hypoxia	None	[66]
LDN-193189	↓ ALK2, ALK3	MCT	Negative	[68]
SB-525334	↓ ALK5	MCT	Positive	[67]
SD-208	↓ ALK5	MCT	Positive	[69]
Ligand-trap approaches				
ActRIIA-Fc ligand trap	↓ ActRIIA ligands	MCT, SuHx	Positive	[27]
ALK1-Fc ligand trap	↓ ALK1 ligands	MCT, SuHx	Positive	[47]
ALK1-Fc ligand trap	↓ ALK1 ligands	Chronic hypoxia	Negative	[24]
ALK3-Fc ligand trap	↓ ALK3 ligands	Chronic hypoxia	None	[24]
sENG-Fc ligand trap	↓ sENG ligands	Chronic hypoxia	None	[24]
TGFβRII-Fc ligand trap	↓ TGFβRII ligands	MCT, SuHx	Positive	[71]
Recombinant proteins				
BMP-9	↑ BMP-9	*Bmpr2*$^{+/R899X}$ mice, MCT, SuHx	Positive	[49]
Neutralising antibodies				
Anti-BMP-9	↓ BMP-9	Chronic hypoxia	Positive	[72]
Anti-Gremlin	↓ Gremlin	Murine SuHx	Positive	[73]
Adenovirus				
AdBMPR2+Fab-9B9	↑ BMPRII	Chronic hypoxia, MCT	Positive	[74]
AdCMVBMPR2myc+Fab9B9	↑ BMPRII	Chronic hypoxia	Positive	[75]

BMP: bone morphogenetic protein; BUR: BMP upregulator; MCT: monocrotaline; SuHx: combination of Sugen 5461 (SU5416) and exposure to chronic hypoxia; ALK: activin receptor-like kinase; ActRII: activin receptor Type II; ENG: endoglin; sENG: soluble ENG; TGFβRII: TGF-β receptor Type II; BMPRII: BMP receptor Type II.

lysates and decreased pulmonary vascular remodelling and PH severity [27]. The pleiotropic effects of activins, GDF-8 and GDF-11, as well as the risk of immunogenicity, must be considered carefully in future drug development. In this context, the safety and efficacy of human ActRIIA-Fc (sotatercept) have been tested in a recently completed 24-week, phase 2 trial evaluating its addition to background therapy in patients with PAH (identifier: NCT03496207 at https://clinicaltrials.gov).

Both Type I and Type II receptors interact independently with a large panel of overlapping ligands (at overlapping epitopes) with different affinities and then cross-interact with each other and form a heteromeric complex [84–86] (table 4). However, ligand–receptor binding affinities are complex to analyse and these interactions could vary in different environmental contexts and in the presence of Type III receptors (such as betaglycan or ENG). Activin A, activin B, myostatin (GDF-8), GDF-11 and BMP-10 are among the ligands preferentially bound by ActRIIA and ActRIIB, while BMP-2, and BMP-4 are among those preferentially bound by ALK3 and ALK6 [84–86]. In this context, the identification of the most adapted ligand-trap or of specific neutralising antibodies to reestablish the balance in PAH is challenging.

Even if their exact mechanisms of action remain to be fully elucidated, several ligand-trap molecules have recently demonstrated efficacy in other BMP-related disorders, such as myelodysplastic syndromes and β-thalassemia. It has been established that these beneficial effects can mainly be explained by the capacity of these therapeutic agents to sequester high affinity ligands and to correct the exaggerated Smad 2/3 signalling impairing erythroid maturation, thus alleviating ineffective erythropoiesis [87]. However, they could also favour the signalling of low affinity ligands [84], or reinforce or even make accessible different inhibitors of these high affinity ligands. In PH, a better availability of gremlin, inhibin-A, follistatin and/or noggin could help activate VEGF receptor 2 (VEGFR2) signalling, inhibit the action of BMP-9 in mesenchymal stem cells [88], suppress RhoA activation [89] and/or reduce vascular inflammation [90–92]. The sequestration of specific ligands of the TGF-β superfamily could also favour specific transmembrane heteromeric complexes, or eventually make accessible different accessory receptors (*e.g.* betaglycan or ENG) or other modulators (*e.g.* BAMBI, FKBP12, Smurf or Smad 7) of these heteromeric complexes [84]. The relative abundance of certain ligands indeed determines the outcome of signalling. For example, high levels of activin A can block the BMP signalling by binding ActRIIA and ActRIIB [93].

TABLE 4 List of high-affinity ligands identified as binding to four Type II receptors using a high-throughput surface plasmon resonance (SPR) based binding assay. Aykul *et al.* [84] captured purified ActRIIA-Fc, ActRIIB-Fc, BMPRII-Fc and TGFβRII-Fc proteins (250 response units each) on a BIAcore sensor chip (Michigan State University, Michigan, USA) and injected 16 different ligands (*i.e.* activin A, activin B, GDF-1, GDF-8, GDF-11, TGF-β1, TGF-β2, TGF-β3, BMP-2, BMP-3, BMP-4, BMP-6, BMP-7, BMP-9, BMP-10 and nodal) at a concentration of 80 nM.

ActRIIA-Fc	ActRIIB-Fc	BMPRII-Fc	TGFβRII-Fc
Activin B[#]	Activin B[#]	Nodal[#]	TGF-β3[#]
BMP-7[#]	Activin A[#]	Activin B[#]	TGF-β1[#]
GDF-11[#]	GDF-11[¶]	BMP-10[¶]	GDF-11[+]
Activin A[#]	BMP-4[¶]	BMP-9[+]	TGF-β2[+]
BMP-10[#]	Myostatin (GDF-8)[¶]	Activin A[+]	BMP-7[+]
BMP-4[#]	BMP-6[¶]	BMP-6[+]	BMP-6[+]
BMP-6[#]	BMP-10[¶]	BMP-4[+]	–
Myostatin (GDF-8)[#]	BMP-9[¶]	BMP-7[+]	–
BMP-9[+]	BMP-7[+]	GDF-11[+]	–
Nodal[+]	BMP-3[+]	–	–

ActRII: activin receptor Type II; BMP: bone morphogenetic protein; BMPRII: BMP receptor Type II; TGF-β: transforming growth factor-β; TGFβRII: TGF-β receptor Type II; GDF: growth differentiation factor. [#]: ligands that bind Type II receptors with very high affinity; [¶]: ligands that bind Type II receptors with high affinity; [+]: ligands that bind Type II receptors with moderate affinity.

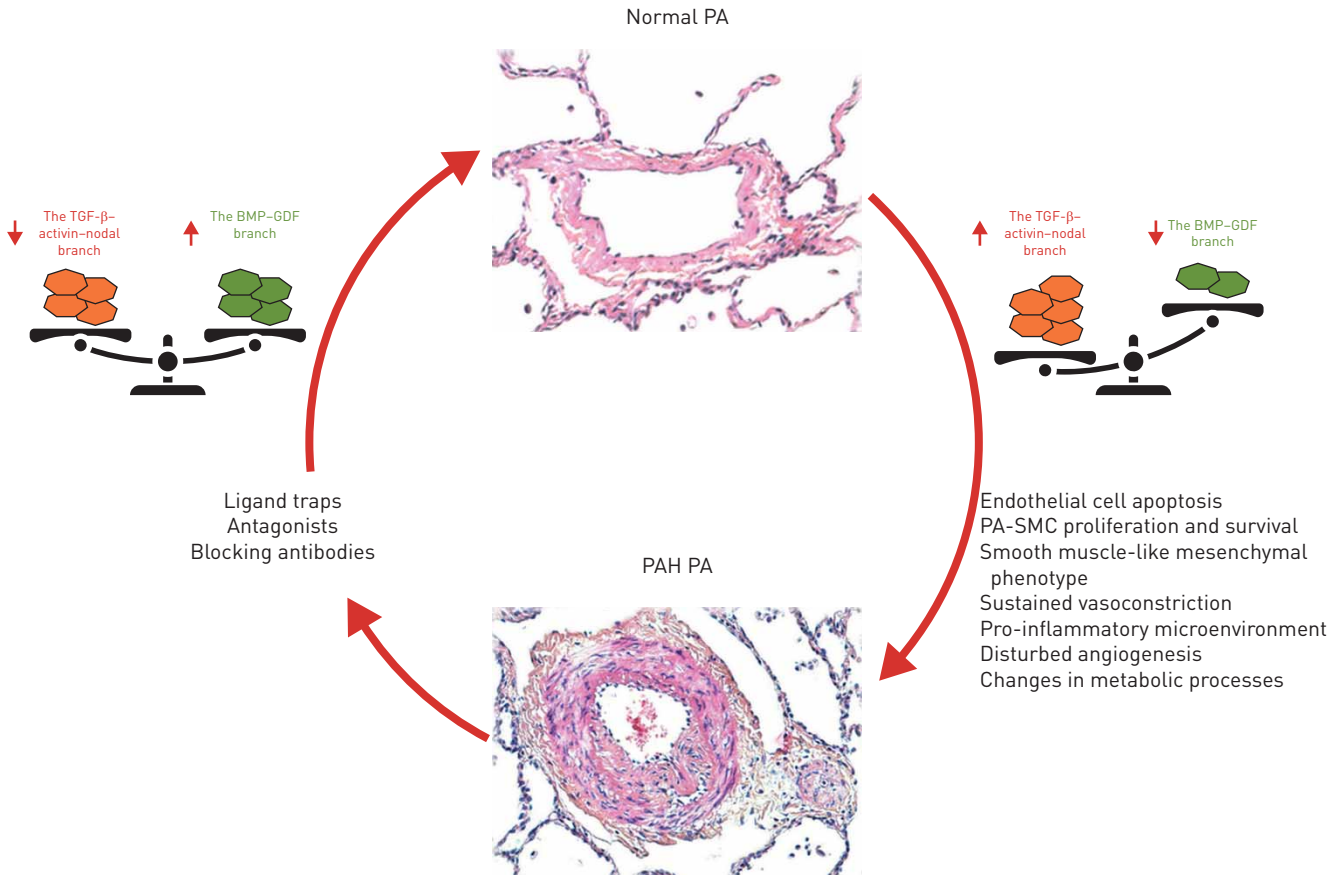


FIGURE 4 Modulation of transforming growth factor-β (TGF-β) superfamily signalling and its functional impact on the pulmonary vascular remodelling associated with pulmonary arterial hypertension (PAH). BMP: bone morphogenetic protein; GDF: growth differentiation factor; PA: pulmonary artery; SMC: smooth muscle cell.

Since GDF-11 has been shown to be involved in PAH, its sequestration in the context of PH/PAH could be of interest [50]. However, the GDF-11/ActRIIB axis is involved in controlling the spatiotemporal expression of multiple *Hox* genes along the anteroposterior axis and their deletion causes anterior transformation of the vertebrae [94]. It is also known that about 70% of ActRIIB-deficient mice die shortly after birth and exhibit multiple patterning defects, including anterior transformation of the vertebrae, kidney agenesis and complex cardiac malformations associated with left–right asymmetrical defects [95]. Even if ActRIIA-deficient mice grow normally, they also exhibit mandibular hypoplasia, reduced fertility and gastrulation defects [96]. Furthermore, myostatin is a critical regulator of muscle mass through its main receptor ActRIIB but also through ActRIIA. Myostatin mutation or inhibition by neutralising antibodies or antagonists results in increased muscle mass, mainly *via* promotion of the proliferation and differentiation of both myoblasts and satellite cells, but also leads to decreased muscle vascularisation and exercise intolerance [97, 98]. Other studies have shown that myostatin neutralisation can prevent diet-induced obesity and insulin resistance [99]. The sequestration of activin A could also limit its potent proinflammatory action through inhibition of the production of different inflammatory mediators (such as interleukin-1 (IL-1), IL-6, tumour necrosis factor-α (TNF-α), nitric oxide, prostaglandin E2 and thromboxane) by monocytes/macrophages and through decreased activation and proliferation of mast cells, T-lymphocytes and B-lymphocytes [90, 91]. Activin A also favours the development of regulatory T-cells [92].

As new ligand-trap molecules are being developed, the long-term risk–benefit ratio of these agents in both sexes should be evaluated. In this context, encouraging preliminary data have been reported in multiple models of heart failure induced by aging, sarcomere mutation, or pressure overload [100], as well as in animal models of PH [27]. Nevertheless, weekly intramuscular administration of ActRIIB-Fc (10 $mg \cdot kg^{-1}$ for 12 weeks) to juvenile rhesus macaques infected with simian immunodeficiency virus has been found to cause mild myocardial hypertrophy, as characterised by an increase in some measures of myocardial mass in the absence of obvious myocardial dysfunction [101]. Furthermore, as myostatin/ActRIIB signalling limits skeletal muscle size, promotes its oxidative properties and promotes the balance between glucose–fat utilisation, its inhibition might trigger muscle fatigability and metabolic myopathy [98].

Conclusions

TGF-β superfamily signalling has emerged as a central actor in PAH pathogenesis (figure 4). It is therefore logical to investigate whether strategies targeting the TGF-β–activin–nodal and BMP–GDF imbalance could be used in combination with currently approved PAH therapies. Further studies in large cohorts of PAH patients are needed to evaluate the long-term efficacy and safety of these treatments. Understanding the context-specific nature of BMP signalling will also help to guide the development of novel drugs to treat PAH and other BMP-related diseases.

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References

1 Boucly A, Weatherald J, Savale L, *et al.* Risk assessment, prognosis and guideline implementation in pulmonary arterial hypertension. *Eur Respir J* 2017; 50: 1700889.
2 Simonneau G, Montani D, Celermajer DS, *et al.* Haemodynamic definitions and updated clinical classification of pulmonary hypertension. *Eur Respir J* 2019; 53: 1801913.
3 Farber HW, Miller DP, Poms AD, *et al.* Five-year outcomes of patients enrolled in the REVEAL Registry. *Chest* 2015; 148: 1043–1054.
4 Humbert M, Lau EM, Montani D, *et al.* Advances in therapeutic interventions for patients with pulmonary arterial hypertension. *Circulation* 2014; 130: 2189–2208.
5 Galie N, Humbert M, Vachiery JL, *et al.* 2015 ESC/ERS guidelines for the diagnosis and treatment of pulmonary hypertension. *Eur Respir J* 2015; 46: 903–975.
6 Humbert M, Sitbon O, Simonneau G. Treatment of pulmonary arterial hypertension. *N Engl J Med* 2004; 351: 1425–1436.
7 Humbert M, Guignabert C, Bonnet S, *et al.* Pathology and pathobiology of pulmonary hypertension: state of the art and research perspectives. *Eur Respir J* 2019; 53; 1801887.
8 Morrell NW, Aldred MA, Chung WK, *et al.* Genetics and genomics of pulmonary arterial hypertension. *Eur Respir J* 2019; 53: 1801899.
9 Guignabert C, Bailly S, Humbert M. Restoring BMPRII functions in pulmonary arterial hypertension: opportunities, challenges and limitations. *Expert Opin Ther Targets* 2017; 21: 181–190.
10 Goumans MJ, Zwijsen A, Ten Dijke P, *et al.* Bone morphogenetic proteins in vascular homeostasis and disease. *Cold Spring Harb Perspect Biol* 2018; 10: a031989.
11 Morikawa M, Derynck R, Miyazono K. TGF-β and the TGF-β family: context-dependent roles in cell and tissue physiology. *Cold Spring Harb Perspect Biol* 2016; 8: a021873.

12 Li S, Shimono C, Norioka N, *et al.* Activin A binds to perlecan through its pro-region that has heparin/heparan sulfate binding activity. *J Biol Chem* 2010; 285: 36645–36655.
13 Evans JD, Girerd B, Montani D, *et al.* BMPR2 mutations and survival in pulmonary arterial hypertension: an individual participant data meta-analysis. *Lancet Respir Med* 2016; 4: 129–137.
14 Machado RD, Southgate L, Eichstaedt CA, *et al.* Pulmonary arterial hypertension: a current perspective on established and emerging molecular genetic defects. *Hum Mutat* 2015; 36: 1113–1127.
15 Southgate L, Machado RD, Graf S, *et al.* Molecular genetic framework underlying pulmonary arterial hypertension. *Nat Rev Cardiol* 2020; 17: 85–95.
16 Sztrymf B, Coulet F, Girerd B, *et al.* Clinical outcomes of pulmonary arterial hypertension in carriers of BMPR2 mutation. *Am J Respir Crit Care Med* 2008; 177: 1377–1383.
17 Ghigna MR, Guignabert C, Montani D, *et al.* BMPR2 mutation status influences bronchial vascular changes in pulmonary arterial hypertension. *Eur Respir J* 2016; 48: 1668–1681.
18 van der Bruggen CE, Happe CM, Dorfmuller P, *et al.* Bone morphogenetic protein receptor type 2 mutation in pulmonary arterial hypertension: a view on the right ventricle. *Circulation* 2016; 133: 1747–1760.
19 Girerd B, Montani D, Coulet F, *et al.* Clinical outcomes of pulmonary arterial hypertension in patients carrying an ACVRL1 (ALK1) mutation. *Am J Respir Crit Care Med* 2010; 181: 851–861.
20 Graf S, Haimel M, Bleda M, *et al.* Identification of rare sequence variation underlying heritable pulmonary arterial hypertension. *Nat Commun* 2018; 9: 1416.
21 David L, Mallet C, Mazerbourg S, *et al.* Identification of BMP9 and BMP10 as functional activators of the orphan activin receptor-like kinase 1 (ALK1) in endothelial cells. *Blood* 2007; 109: 1953–1961.
22 Hopper RK, Moonen JR, Diebold I, *et al.* In pulmonary arterial hypertension, reduced BMPR2 promotes endothelial-to-mesenchymal transition via HMGA1 and its target slug. *Circulation* 2016; 133: 1783–1794.
23 Hodgson J, Swietlik EM, Salmon RM, *et al.* Characterization of GDF2 mutations and levels of BMP9 and BMP10 in pulmonary arterial hypertension. *Am J Respir Crit Care Med* 2020; 201: 575–585.
24 Nikolic I, Yung LM, Yang P, *et al.* Bone morphogenetic protein 9 is a mechanistic biomarker of portopulmonary hypertension. *Am J Respir Crit Care Med* 2019; 199: 891–902.
25 Rochon ER, Krowka MJ, Bartolome S, *et al.* BMP 9/10 in pulmonary vascular complications of liver disease. *Am J Respir Crit Care Med* 2020; 201: 1575–1578.
26 Yndestad A, Larsen KO, Oie E, *et al.* Elevated levels of activin A in clinical and experimental pulmonary hypertension. *J Appl Physiol* 2009; 106: 1356–1364.
27 Yung LM, Yang P, Joshi S, *et al.* ACTRIIA-Fc rebalances activin/GDF versus BMP signaling in pulmonary hypertension. *Sci Transl Med* 2020; 12: eaaz5660.
28 Nickel N, Kempf T, Tapken H, *et al.* Growth differentiation factor-15 in idiopathic pulmonary arterial hypertension. *Am J Respir Crit Care Med* 2008; 178: 534–541.
29 Meadows CA, Risbano MG, Zhang L, *et al.* Increased expression of growth differentiation factor-15 in systemic sclerosis-associated pulmonary arterial hypertension. *Chest* 2011; 139: 994–1002.
30 Geenen LW, Baggen VJM, Kauling RM, *et al.* Growth differentiation factor-15 as candidate predictor for mortality in adults with pulmonary hypertension. *Heart* 2020; 106: 467–473.
31 Mirna M, Rohm I, Jirak P, *et al.* Analysis of novel cardiovascular biomarkers in patients with pulmonary hypertension (PH). *Heart Lung Circ* 2020; 29: 337–344.
32 Li G, Li Y, Tan XQ, *et al.* Plasma growth differentiation factor-15 is a potential biomarker for pediatric pulmonary arterial hypertension associated with congenital heart disease. *Pediatr Cardiol* 2017; 38: 1620–1626.
33 Gore B, Izikki M, Mercier O, *et al.* Key role of the endothelial TGF-β/ALK1/endoglin signaling pathway in humans and rodents pulmonary hypertension. *PLoS One* 2014; 9: e100310.
34 Yan Y, Wang XJ, Li SQ, *et al.* Elevated levels of plasma transforming growth factor-β1 in idiopathic and heritable pulmonary arterial hypertension. *Int J Cardiol* 2016; 222: 368–374.
35 Selimovic N, Bergh CH, Andersson B, *et al.* Growth factors and interleukin-6 across the lung circulation in pulmonary hypertension. *Eur Respir J* 2009; 34: 662–668.
36 Malhotra R, Paskin-Flerlage S, Zamanian RT, *et al.* Circulating angiogenic modulatory factors predict survival and functional class in pulmonary arterial hypertension. *Pulm Circ* 2013; 3: 369–380.
37 Atkinson C, Stewart S, Upton PD, *et al.* Primary pulmonary hypertension is associated with reduced pulmonary vascular expression of type II bone morphogenetic protein receptor. *Circulation* 2002; 105: 1672–1678.
38 Du L, Sullivan CC, Chu D, *et al.* Signaling molecules in nonfamilial pulmonary hypertension. *N Engl J Med* 2003; 348: 500–509.
39 Richter A, Yeager ME, Zaiman A, *et al.* Impaired transforming growth factor-β signaling in idiopathic pulmonary arterial hypertension. *Am J Respir Crit Care Med* 2004; 170: 1340–1348.
40 Yang X, Long L, Southwood M, *et al.* Dysfunctional Smad signaling contributes to abnormal smooth muscle cell proliferation in familial pulmonary arterial hypertension. *Circ Res* 2005; 96: 1053–1063.
41 Yang J, Li X, Li Y, *et al.* Id proteins are critical downstream effectors of BMP signaling in human pulmonary arterial smooth muscle cells. *Am J Physiol Lung Cell Mol Physiol* 2013; 305: L312–L321.
42 Zabini D, Granton E, Hu Y, *et al.* Loss of SMAD3 promotes vascular remodeling in pulmonary arterial hypertension via MRTF disinhibition. *Am J Respir Crit Care Med* 2018; 197: 244–260.
43 Botney MD, Bahadori L, Gold LI. Vascular remodeling in primary pulmonary hypertension. Potential role for transforming growth factor-β. *Am J Pathol* 1994; 144: 286–295.
44 Ricard N, Tu L, Le Hiress M, *et al.* Increased pericyte coverage mediated by endothelial-derived fibroblast growth factor-2 and interleukin-6 is a source of smooth muscle-like cells in pulmonary hypertension. *Circulation* 2014; 129: 1586–1597.
45 Nickel N, Jonigk D, Kempf T, *et al.* GDF-15 is abundantly expressed in plexiform lesions in patients with pulmonary arterial hypertension and affects proliferation and apoptosis of pulmonary endothelial cells. *Respir Res* 2011; 12: 62.
46 Bordenave J, Tu L, Berrebeh N, *et al.* Lineage tracing reveals the dynamic contribution of pericytes to the blood vessel remodeling in pulmonary hypertension. *Arterioscler Thromb Vasc Biol* 2020; 40: 766–782.
47 Tu L, Desroches-Castan A, Mallet C, *et al.* Selective BMP-9 inhibition partially protects against experimental pulmonary hypertension. *Circ Res* 2019; 124: 846–855.

48 Calvier L, Chouvarine P, Legchenko E, *et al.* PPARγ links BMP2 and TGFβ1 pathways in vascular smooth muscle cells, regulating cell proliferation and glucose metabolism. *Cell Metab* 2017; 25: 1118–1134.
49 Long L, Ormiston ML, Yang X, *et al.* Selective enhancement of endothelial BMPR-II with BMP9 reverses pulmonary arterial hypertension. *Nat Med* 2015; 21: 777–785.
50 Yu X, Chen X, Zheng XD, *et al.* Growth differentiation factor 11 promotes abnormal proliferation and angiogenesis of pulmonary artery endothelial cells. *Hypertension* 2018; 71: 729–741.
51 Jerkic M, Kabir MG, Davies A, *et al.* Pulmonary hypertension in adult Alk1 heterozygous mice due to oxidative stress. *Cardiovasc Res* 2011; 92: 375–384.
52 Beppu H, Ichinose F, Kawai N, *et al.* BMPR-II heterozygous mice have mild pulmonary hypertension and an impaired pulmonary vascular remodeling response to prolonged hypoxia. *Am J Physiol Lung Cell Mol Physiol* 2004; 287: L1241–L1247.
53 Hautefort A, Mendes-Ferreira P, Sabourin J, *et al.* Bmpr2 mutant rats develop pulmonary and cardiac characteristics of pulmonary arterial hypertension. *Circulation* 2019; 139: 932–948.
54 Toporsian M, Jerkic M, Zhou YQ, *et al.* Spontaneous adult-onset pulmonary arterial hypertension attributable to increased endothelial oxidative stress in a murine model of hereditary hemorrhagic telangiectasia. *Arterioscler Thromb Vasc Biol* 2010; 30: 509–517.
55 Hong KH, Lee YJ, Lee E, *et al.* Genetic ablation of the BMPR2 gene in pulmonary endothelium is sufficient to predispose to pulmonary arterial hypertension. *Circulation* 2008; 118: 722–730.
56 Han C, Hong KH, Kim YH, *et al.* SMAD1 deficiency in either endothelial or smooth muscle cells can predispose mice to pulmonary hypertension. *Hypertension* 2013; 61: 1044–1052.
57 West J, Harral J, Lane K, *et al.* Mice expressing BMPR2R899X transgene in smooth muscle develop pulmonary vascular lesions. *Am J Physiol Lung Cell Mol Physiol* 2008; 295: L744–L755.
58 Huang Z, Wang D, Ihida-Stansbury K, *et al.* Defective pulmonary vascular remodeling in Smad8 mutant mice. *Hum Mol Genet* 2009; 18: 2791–2801.
59 Anderson L, Lowery JW, Frank DB, *et al.* Bmp2 and Bmp4 exert opposing effects in hypoxic pulmonary hypertension. *Am J Physiol Regul Integr Comp Physiol* 2010; 298: R833–R842.
60 Frank DB, Abtahi A, Yamaguchi DJ, *et al.* Bone morphogenetic protein 4 promotes pulmonary vascular remodeling in hypoxic pulmonary hypertension. *Circ Res* 2005; 97: 496–504.
61 Chen YF, Feng JA, Li P, *et al.* Dominant negative mutation of the TGF-β receptor blocks hypoxia-induced pulmonary vascular remodeling. *J Appl Physiol* 2006; 100: 564–571.
62 Cahill E, Costello CM, Rowan SC, *et al.* Gremlin plays a key role in the pathogenesis of pulmonary hypertension. *Circulation* 2012; 125: 920–930.
63 Frank DB, Lowery J, Anderson L, *et al.* Increased susceptibility to hypoxic pulmonary hypertension in Bmpr2 mutant mice is associated with endothelial dysfunction in the pulmonary vasculature. *Am J Physiol Lung Cell Mol Physiol* 2008; 294: L98–L109.
64 Song Y, Jones JE, Beppu H, *et al.* Increased susceptibility to pulmonary hypertension in heterozygous BMPR2-mutant mice. *Circulation* 2005; 112: 553–562.
65 Tian W, Jiang X, Sung YK, *et al.* Phenotypically silent bone morphogenetic protein receptor 2 mutations predispose rats to inflammation-induced pulmonary arterial hypertension by enhancing the risk for neointimal transformation. *Circulation* 2019; 140: 1409–1425.
66 Long L, Crosby A, Yang X, *et al.* Altered bone morphogenetic protein and transforming growth factor-β signaling in rat models of pulmonary hypertension: potential for activin receptor-like kinase-5 inhibition in prevention and progression of disease. *Circulation* 2009; 119: 566–576.
67 Thomas M, Docx C, Holmes AM, *et al.* Activin-like kinase 5 (ALK5) mediates abnormal proliferation of vascular smooth muscle cells from patients with familial pulmonary arterial hypertension and is involved in the progression of experimental pulmonary arterial hypertension induced by monocrotaline. *Am J Pathol* 2009; 174: 380–389.
68 Yang L, Yin N, Hu L, *et al.* Sildenefil increases connexin 40 in smooth muscle cells through activation of BMP pathways in pulmonary arterial hypertension. *Int J Clin Exp Pathol* 2014; 7: 4674–4684.
69 Zaiman AL, Podowski M, Medicherla S, *et al.* Role of the TGF-β/Alk5 signaling pathway in monocrotaline-induced pulmonary hypertension. *Am J Respir Crit Care Med* 2008; 177: 896–905.
70 Xing Y, Zhao S, Wei Q, *et al.* A novel piperidine identified by stem cell-based screening attenuates pulmonary arterial hypertension by regulating BMP2 and PTGS2 levels. *Eur Respir J* 2018; 51: 1702229.
71 Yung LM, Nikolic I, Paskin-Flerlage SD, *et al.* A selective transforming growth factor-β ligand trap attenuates pulmonary hypertension. *Am J Respir Crit Care Med* 2016; 194: 1140–1151.
72 Tu L, Desroches-Castan A, Mallet C, *et al.* Selective BMP-9 inhibition partially protects against experimental pulmonary hypertension. *Circ Res* 2019; 124: 846–855.
73 Ciuclan L, Sheppard K, Dong L, *et al.* Treatment with anti-gremlin 1 antibody ameliorates chronic hypoxia/SU5416-induced pulmonary arterial hypertension in mice. *Am J Pathol* 2013; 183: 1461–1473.
74 Reynolds AM, Holmes MD, Danilov SM, *et al.* Targeted gene delivery of BMPR2 attenuates pulmonary hypertension. *Eur Respir J* 2012; 39: 329–343.
75 Reynolds AM, Xia W, Holmes MD, *et al.* Bone morphogenetic protein type 2 receptor gene therapy attenuates hypoxic pulmonary hypertension. *Am J Physiol Lung Cell Mol Physiol* 2007; 292: L1182–L1192.
76 Anderton MJ, Mellor HR, Bell A, *et al.* Induction of heart valve lesions by small-molecule ALK5 inhibitors. *Toxicol Pathol* 2011; 39: 916–924.
77 Umbarkar P, Singh AP, Gupte M, *et al.* Cardiomyocyte SMAD4-dependent TGF-β signaling is essential to maintain adult heart homeostasis. *JACC Basic Transl Sci* 2019; 4: 41–53.
78 Spiekerkoetter E, Tian X, Cai J, *et al.* FK506 activates BMPR2, rescues endothelial dysfunction, and reverses pulmonary hypertension. *J Clin Invest* 2013; 123: 3600–3613.
79 Spiekerkoetter E, Sung YK, Sudheendra D, *et al.* Randomised placebo-controlled safety and tolerability trial of FK506 (tacrolimus) for pulmonary arterial hypertension. *Eur Respir J* 2017; 50: 1602449.
80 Wu Y, Adi D, Long M, *et al.* 4-Phenylbutyric acid induces protection against pulmonary arterial hypertension in rats. *PLoS One* 2016; 11: e0157538.

81 Dunmore BJ, Yang X, Crosby A, *et al.* 4PBA restores signalling of a cysteine-substituted mutant BMPR2 receptor found in patients with PAH. *Am J Respir Cell Mol Biol* 2020; 63: 160–171.
82 Drake KM, Dunmore BJ, McNelly LN, *et al.* Correction of nonsense BMPR2 and SMAD9 mutations by ataluren in pulmonary arterial hypertension. *Am J Respir Cell Mol Biol* 2013; 49: 403–409.
83 Polkey MI, Praestgaard J, Berwick A, *et al.* Activin type II receptor blockade for treatment of muscle depletion in chronic obstructive pulmonary disease. A randomized trial. *Am J Respir Crit Care Med* 2019; 199: 313–320.
84 Aykul S, Martinez-Hackert E. Transforming growth factor-β family ligands can function as antagonists by competing for type II receptor binding. *J Biol Chem* 2016; 291: 10792–10804.
85 Souza TA, Chen X, Guo Y, *et al.* Proteomic identification and functional validation of activins and bone morphogenetic protein 11 as candidate novel muscle mass regulators. *Mol Endocrinol* 2008; 22: 2689–2702.
86 Townson SA, Martinez-Hackert E, Greppi C, *et al.* Specificity and structure of a high affinity activin receptor-like kinase 1 (ALK1) signaling complex. *J Biol Chem* 2012; 287: 27313–27325.
87 Verma A, Suragani RN, Aluri S, *et al.* Biological basis for efficacy of activin receptor ligand traps in myelodysplastic syndromes. *J Clin Invest* 2020; 130: 582–589.
88 Huang M, Cheng YL, Zeng JT, *et al.* Inhibin α-subunit inhibits BMP9-induced osteogenic differentiation through blocking BMP/Smad signal and activating NF-κB signal in mesenchymal stem cells. *J Cell Biochem* 2018; 119: 8271–8281.
89 Zhang M, Liu NY, Wang XE, *et al.* Activin B promotes epithelial wound healing *in vivo* through RhoA-JNK signaling pathway. *PLoS One* 2011; 6: e25143.
90 Koga M, Engberding N, Dikalova AE, *et al.* The bone morphogenic protein inhibitor, noggin, reduces glycemia and vascular inflammation in db/db mice. *Am J Physiol Heart Circ Physiol* 2013; 305: H747–H755.
91 Mayer K, Buchbinder A, Morty RE. Activin A: a mediator governing inflammation, immunity, and repair. *Am J Respir Crit Care Med* 2012; 185: 350–352.
92 Huber S, Stahl FR, Schrader J, *et al.* Activin a promotes the TGF-β-induced conversion of $CD4^{+}CD25^{-}$ T cells into $Foxp3^{+}$ induced regulatory T cells. *J Immunol* 2009; 182: 4633–4640.
93 Olsen OE, Wader KF, Hella H, *et al.* Activin A inhibits BMP-signaling by binding ACVR2A and ACVR2B. *Cell Commun Signal* 2015; 13: 27.
94 Oh SP, Yeo CY, Lee Y, *et al.* Activin type IIA and IIB receptors mediate Gdf11 signaling in axial vertebral patterning. *Genes Dev* 2002; 16: 2749–2754.
95 Oh SP, Li E. The signaling pathway mediated by the type IIB activin receptor controls axial patterning and lateral asymmetry in the mouse. *Genes Dev* 1997; 11: 1812–1826.
96 Song J, Oh SP, Schrewe H, *et al.* The type II activin receptors are essential for egg cylinder growth, gastrulation, and rostral head development in mice. *Dev Biol* 1999; 213: 157–169.
97 McCroskery S, Thomas M, Maxwell L, *et al.* Myostatin negatively regulates satellite cell activation and self-renewal. *J Cell Biol* 2003; 162: 1135–1147.
98 Relizani K, Mouisel E, Giannesini B, *et al.* Blockade of ActRIIB signaling triggers muscle fatigability and metabolic myopathy. *Mol Ther* 2014; 22: 1423–1433.
99 Zhao B, Wall RJ, Yang J. Transgenic expression of myostatin propeptide prevents diet-induced obesity and insulin resistance. *Biochem Biophys Res Commun* 2005; 337: 248–255.
100 Roh JD, Hobson R, Chaudhari V, *et al.* Activin type II receptor signaling in cardiac aging and heart failure. *Sci Transl Med* 2019; 11: eaau8680.
101 Guo W, Pencina KM, Gagliano-Juca T, *et al.* Effects of an ActRIIB.Fc ligand trap on cardiac function in simian immunodeficiency virus-infected male rhesus macaques. *J Endocr Soc* 2018; 2: 817–831.