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Targeting Transforming Growth Factor Beta Receptors in Pulmonary Hypertension

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Abstract:

The transforming growth factor (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 (Smad 2/3 and Smad 1/5/8, respectively) and noncanonical signaling pathways, are diverse and vary amongst 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 that include the regulation of cell proliferation and differentiation, as well as the transduction of signaling cascades for the development and maintenance of different tissues and organs. Importantly, alterations in these pathways that include heterozygous germline mutations and/or alterations in the expression of several constitutive members have been identified in patients with familial/heritable or idiopathic pulmonary arterial hypertension (PAH). Consequently, loss or dysfunctions 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 to understand its pathogenesis, but also paved the way for possible novel therapeutic approaches.

Key words:

Bone morphogenetic protein

Bone morphogenetic protein receptor type II (BMPR-II)

Pulmonary arterial hypertension

Pulmonary hypertension

Signal transduction

TGF- β

TGF- β receptor

1. INTRODUCTION

Pulmonary arterial hypertension (PAH) is a severe cardiopulmonary condition with a median survival of 7 years despite best standard of care [1-3]. In the updated pulmonary hypertension (PH) classification, PAH corresponds to group 1 PH defined by right heart catheterization as precapillary PH, in the absence of other causes, such as chronic lung disease or chronic thrombo-embolic disease². PAH may be idiopathic, 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 schistosomiasis².

Several pulmonary vasodilators have been developed in the past decades for the treatment of PAH, 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]: (1) endothelin receptor antagonists (ERAs) targeting the endothelin pathway; (2) phosphodiesterase type 5 inhibitors and guanylate cyclase stimulators (PDE5i) targeting the nitric oxide (NO) pathway; (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 causing 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 remodeling in PAH. In recent years, the respective roles of endothelial and smooth muscle cell dysfunctions, unresolved inflammation and loss or dysfunction in the bone morphogenetic protein (BMP) and transforming growth factor (TGF)- β signaling pathways have been better understood [7]. Indeed, there is now clear evidence that heterozygous *BMPR2* germline mutations represent the main susceptibility gene for PAH. *BMPR2* encodes bone morphogenetic protein receptor type II (BMPR-

II), 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 the loss and/or dysfunctions in the TGF- β /activin/nodal and BMP/growth and differentiation factor (GDF) signaling contributes to disease pathogenesis is lacking.

Because alterations in the signaling of the TGF- β superfamily are well recognized in PAH, several agents targeting the BMPR-II axis and its balance with activins/inhibins/nodal branch are currently tested [7-9]. This review examines the current state-of-the-art regarding the dysfunctions that affect BMP and TGF- β signaling systems, a critical knowledge that might help designing novel approaches for PAH treatment.

2. SIGNALING 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 affinities for specific receptors: BMPs/GDF (group 1), activins/inhibins/nodal (group 2), TGF- β s (group 3) and others (group 4) (**Figure 1A**). In mammals, the TGF- β superfamily is highly conserved and widely distributed throughout the body [10, 11].

These family protein members 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 pro-peptide and its mature dimer plays important roles in the biosynthesis, transportation, stabilization, and signaling of these complexes. For the three isoforms of TGF- β , myostatin, and GDF-11, this interaction between pro-peptide and its mature dimer confers latency to the complex, but not 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 interactions with the extracellular matrix (ECM) proteins (e.g. perlecan and agrin), thereby facilitating localization and storage within tissues for later activation [12]. The activity of these different ligands is also tightly regulated by various mechanisms, including through post-translational modifications that increases the stability, half-life or the 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 also under the control of several specific antagonists (e.g. follistatin, gremlin, inhibin- α , lefty, and noggin) (**Figure 2**).

The active dimers transduce a signal by binding to a transmembrane heteromeric complex with serine/threonine (Ser/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 one hand, the TGF- β /activin/nodal branch that signals through Smad2/3 and on the other hand, the BMP/GDF branch that signals through Smad1/5/8. Subsequently, pSmad2/3 or pSmad1/5/8 oligomerizes with the common Smad (co-Smad), Smad4, and translocates into the nucleus, regulating transcription of target genes. Inhibitory Smads (Smad6 and Smad7, also known as I-Smads), several accessory receptors (also known as type III receptors such as betaglycan or endoglin), modulators (e.g. BAMBI, FKBP12, Smurf, and Smad7) and various miRNAs can antagonize, or facilitate the signaling mediated by pSmad2/3-Smad4 or pSmad1/5/8-Smad4. Upon binding to DNA and to their transcriptional partners, these Smad complexes recruit coactivators [e.g. p300, C/EBP-binding protein (CBP)] or corepressors (e.g. c-Ski and 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 including among other the extracellular-signal-regulated kinase (ERK)1/2 and p38 mitogen-activated protein kinase (MAPK) pathways, c-Jun amino terminal kinase (JNK), phosphatidylinositol-3 kinase (PI3K), Hippo, nuclear

factor- κ B (NF- κ B) and Rho family GTPases (**Figure 2**). Amplitude and duration of both Smad-dependent and non-Smad signaling pathways are finely tuned to generate a specific response program in a single cell in its environmental context at a specific moment in time [8, 10, 11].

In the plasma membrane, distinct functional combinations of type I/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) and are thus partitioned between non-raft clathrin-coated pits and caveolin-1 positive cholesterol-rich lipid rafts microdomains known as caveolae. It is also well established that these functional heterocomplexes are internalized 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 and exert their ability to sequester ligands, modulating both the TGF- β /activin/nodal and BMP/GDF signaling. These intracellular signaling cross-interact with other signaling pathways, such as the Wnt/ β -catenin, Hedgehog, estrogen, Hippo, and the Notch pathways to ultimately dictate the biological output of pathway activity. Therefore, it is essential to improve our understanding of the signal integration between the TGF- β /activin/nodal and BMP/GDF signaling with the other pathways in the regulation of the different organs under physiological and pathophysiological conditions.

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

3. GENETICS OF HERITABLE PULMONARY ARTERIAL HYPERTENSION

Several mutations causing heritable PAH involve key members of the ALK1/BMPR-II axis supporting the concept that loss of the activity in the BMP/GDF branch predisposes for PAH [9] (**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 idiopathic PAH [13-15]. In PAH, the female-to-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) [9], 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 at 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 patient, corresponding to 27% non-sense 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 rearrangement) predict premature termination of the transcript with likely loss through the process of nonsense-mediated decay, a mRNA surveillance mechanism that detects and degrades transcripts containing premature termination codons. Therefore, haploinsufficiency, through reduction in the available BMPR-II protein level, is more likely involved to the molecular mechanisms underlying PAH predisposition, even if several mutated alleles (due to missense mutations or deletions/duplications) are suspected to have a dominant negative effect on the wild type BMPR-II 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 they have a relatively preserved diffusion capacity for carbon monoxide (DLCO) [9, 13]. A similar degree of pulmonary arterial remodeling was recently noted in explanted lungs of PAH patients carrying or not a *BMPR2* mutation [17]. However, the *BMPR2* mutation status was reported to be associated with more pronounced bronchial vascular changes (e.g. bronchial artery 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 BMPR-II may influence the interactions between the pulmonary and bronchial circulation. A decreased right ventricular function that is not attenuated by the currently available PAH drugs has been reported in PAH patients carrying a *BMPR2* mutation, as compared to non-carriers [18].

Mutations in *ACVRL1* (encoding type I receptor ALK1 of the TGF- β superfamily) and *ENG* (encoding accessory receptor endoglin) usually cause hereditary hemorrhagic telangiectasia. These mutations less frequently cause PAH, sometimes early in life [9, 19]. *ACVRL1* mutations are mostly missense mutations and are localized in the catalytic domain, leading to reduced Smad1/5 phosphorylation [19]. Recently, mutations in *GDF2* and *BMP10* (encoding BMP-9 and BMP-10, respectively) have been described. Several variants in *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 BMPR-II signaling pathway have also allowed identification of rare sequence variants in *SMAD1*, *SMAD4* and *SMAD9*. Mutations in *CAVI* which encodes caveolin-1 have been detected in some PAH patients (**Table 1**) [9].

BMPR-II and ALK1 are highly expressed on the pulmonary vascular endothelium, where they form ALK1/BMPR-II receptor complex signaling specifically in response to circulating BMP-9 and BMP-10, utilizing endoglin as a co-receptor [8, 21]. Of note, requirement for high levels of BMPR-II/ALK1 signaling in the pulmonary endothelium might contribute to the lung-specific effects of *BMPR2* mutations [9]. Decreased BMPR-II also favors endothelial cell dysfunction and promotes endothelial to mesenchymal transition, a process involved in PAH [22].

4. TGF- β /ACTIVIN/NODAL AND BMP/GDF IMBALANCE IN PULMONARY HYPERTENSION

Accumulating evidence supports the notion that a shift of the balance in favor of a TGF- β /activin/nodal signaling is occurring in human PAH, even in the absence of mutations in members of the TGF- β superfamily. This shift increases the risk to develop 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 idiopathic 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 marked decrease in concentrations of circulating BMP-9 and BMP-10 *versus* healthy control subjects or *versus* cirrhotic controls [24, 25]. Of note, decreased circulating concentrations of 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 circulating levels of activin A (β_A - β_A) [26, 27], follistatin [26], GDF-15 [28-32], TGF- β [33-35], and soluble endoglin [36] have been reported in PAH patients.

Decreased immunoreactivity for ALK3, BMPR-II, inhibitor of DNA binding (ID)-3, Smad3, 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- β 3 has been reported in remodeled 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 RII, a specific TGF- β RII upregulation in pericytes has been recently identified in explanted lung tissues from PAH patients [46]. These *in situ* observations have been

replicated *in vitro*, with cultured pulmonary endothelial cells and smooth muscle cells from patients with idiopathic PAH exhibiting decreased capacity to activate Smad and non-Smad pathways, as compared with control cells [8, 47]. However, the reasons of this imbalance in the absence of a compensatory decrease in the signaling of the TGF- β /activin/nodal branch remain unknown and more studies are requested. Similarly, further work is also needed to dissect the role of the cooperation between the Smad and non-Smad signaling pathways in PAH, especially how these dysregulated TGF- β /activin/nodal and the BMP/GDF branches activate the Erk1/2, JNK/p38, RhoA, Rac and Cdc42 and influence the activities 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 to investigate potential contributions of various receptors and ligands of the TGF- β superfamily in the pulmonary vascular remodeling associated with PH. Mice deficient in ALK-1, BMPR-II, endoglin, or in Smad1 or Smad8 are more susceptible to remodel the pulmonary vessels as compared with wild type 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 develop remodeling of the pulmonary vasculature, several factors could contribute to the discrepancies in some of these results including among other the genetic background, sex, and age. Due to the small size of the mice, it is also technically challenging to obtain a complete invasive hemodynamic characterization as compared with larger animals. A shift of the balance in favor of a TGF- β /activin/nodal signaling has been observed in multiple PH models, including those induced by monocrotaline (MCT) injection, or infection with schistosomiasis, or to 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 accumulations of different pulmonary vascular cells and infiltration of inflammatory cells. Consequently, various small molecules 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**).

5. 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 remodeling in MCT-induced PH in rats [48-51]. However, these small molecules are often associated with cardiovascular toxicity that currently limits their use in humans [52]. A promising alternative could be the use of ligand-trap approaches even if further studies are needed, especially because the exact role of the TGF- β /activin/nodal and BMP/GDF balance in cardiac homeostasis remains to be better understood [53].

Enhancement of BMP/GDF signaling in pulmonary vascular cells could offer a novel approach for the treatment of PAH. However, a variety of challenges still remain and include 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 in patients with severe PAH remains to be performed to know whether these different strategies could be of interest in combination with currently approved PAH therapies. BMPR-II activators that remove FK-binding protein-12 (FKBP12) from BMP type I receptors, such as FK506 (tacrolimus), FK520 (ascomycin), and rapamycin, have been investigated in PH [54]. Based on the fact that low-dose FK506 can reverse experimental PH [54], a phase 2 randomized placebo-controlled safety and tolerability trial of FK506 has been performed showing that low-level FK506 is well tolerated and increases BMPR-II expression in peripheral blood mononuclear cells in a small group of PAH patients (NCT01647945) [55]. Strategy seeking to limit the retention of misfolded BMPR-II mutants in the endoplasmic reticulum with chemical chaperone

(such as 4PBA) could also be used in specific subsets of patients as supported by studies performed in primary cells and in knock-in *Bmpr2*^{C118W} mice [56, 57]. *In vitro*, ataluren (PTC124) that permits ribosomal readthrough of premature stop codons can restore BMP signaling in cells carrying nonsense *BMPR2* and *SMAD9* mutations [58]. Because 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 remodeling in PAH patients.

Administration of recombinant BMP-9 protein could represent another promising approach, as already shown in experimental PH [59]. Of note, mice lacking BMP-9 do not develop remodeling of 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, needs to be considered in drug development.

Based on clinical findings showing that distinct ligands of the TGF- β superfamily are upregulated in PAH patients [including of activin A (β_A - β_A) [26, 27], follistatin [26], GDF-15 [28-32], and TGF- β [33-35]] and on results obtained in several preclinical studies, ligand-trap molecules and neutralizing therapeutic antibodies (such as bimagrumab [60]) targeting ActRIIA and/or ActRIIB receptors represent interesting approaches to restore the TGF- β /activin/nodal and BMP/GDF balance in PAH. A fusion protein consisting of the extracellular domain of ActRIIA linked to IgG Fc domain to sequester Activin A, Activin B, GDF-8, GDF-11 has been recently tested in animal models of PH [27]. In MCT and SuHx rat models, treatment with ActRIIA-Fc attenuated pSmad2 levels in lung lysates, and decreased pulmonary vascular remodeling 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 the addition of sotatercept to background therapy in patients with PAH (NCT03496207).

Both type I and type II receptors independently interact with different affinities with a large panel of overlapping ligands at overlapping epitopes and then cross-interact with each other and form an heteromeric complex [61-63] (**Table 4**). However, ligand-receptor binding affinities are complex to analyze and these interactions could vary among different environmental contexts and in presence of type III receptors (such as betaglycan or endoglin). Activin A and 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 the ligands preferentially bound by ALK3 and ALK6 [61-63]. In this context, the identification of the most adapted ligand-trap or specific neutralizing 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 is established that these beneficial effects are mainly explained by the capacity of these therapeutic agents to sequester high affinity ligands and to correct the exaggerated Smad 2/3 signaling impairing erythroid maturation, thus alleviating ineffective erythropoiesis [64]. However, they could also favor signaling of low affinity ligands [61] 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 to activate VEGFR2 signaling, to inhibit the action of BMP-9 in mesenchymal stem cells [65], to suppress RhoA activation [66], and/or to reduce vascular inflammation [67-69]. The sequestration of specific ligands of the TGF- β superfamily could also favor specific transmembrane heteromeric complex, or eventually make accessible different accessory receptors (betaglycan or endoglin) or other modulators (e.g. BAMBI, FKBP12, Smurf, and Smad7) of these heteromeric complex [61]. The relative abundance of certain ligands indeed determines the

outcome of signaling. For example, high levels of activin A can block the BMP-signaling by binding ActRIIA and ActRIIB [70].

Since GDF-11 has been shown to be involved in PAH, its sequestration in the context of PH/PAH could be of interest [71]. 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 [72]. It is also known that about 70% of ActRIIB deficient mice die shortly after birth and exhibit multiple patterning defects, including anterior transformation of vertebrae, kidney agenesis, and complex cardiac malformations associated with left-right asymmetrical defects [73]. Even if ActRIIA deficient mice grow normally, they also exhibit mandibular hypoplasia, reduced fertility, and gastrulation defects [74]. Furthermore, myostatin through its main receptor ActRIIB, but also through ActRIIA, is a critical regulator of muscle mass. Myostatin mutation or inhibition by neutralizing antibodies or antagonists results in increased muscle mass, mainly via promotion of the proliferation and differentiation of both myoblasts and satellites cells, but also leads to decreased muscle vascularization and exercise intolerance [75, 76]. Other studies have shown that myostatin neutralization can prevent diet-induced obesity and insulin resistance [77]. The sequestration of Activin A could also limit its potent proinflammatory actions through inhibition of the production of different inflammatory mediators (such as interleukin (IL)-1, IL-6, tumor necrosis factor- α , NO, prostaglandin E2 and thromboxane) by monocyte/ macrophages and decreased activation and proliferation of mast cells, T and B lymphocytes [67, 68]. Activin A also favors the development of regulatory T cells [69].

Because 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 [78] as well as in animal models of PH [27]. Nevertheless, a weekly intramuscular administration of ActRIIB-

Fc at 10 mg/kg for 12 weeks to juvenile simian immunodeficiency virus-infected rhesus macaques has been found to cause mild myocardial hypertrophy characterized by an increase in some measures of myocardial mass in the absence of obvious myocardial dysfunction [79]. Because the myostatin/ActRIIB signaling limits skeletal muscle size and promotes its oxidative properties and the balance between glucose-fat utilization, its inhibition might trigger muscle fatigability and metabolic myopathy [76].

6. CONCLUSIONS

The TGF- β superfamily signaling 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 signaling will also help to guide the development of novel drugs to treat PAH and other BMP-related diseases.

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Table 1 - List of genes causing heritable pulmonary arterial hypertension (PAH)

Modified from Morrell N. *et al.* [9]

High level of evidence:

BMPR2 53-86 % of familial PAH
 14-35 % of sporadic PAH
EIF2AK4 ~ 100 % of familial PVOD/PCH

Other genes (< 1.5 % of sporadic PAH):

ACVRL1, AQP1, ATP1A3, CAV1, ENG, GDF2, KCNK3, SMAD9, SOX17, TBX4

Low level of evidence:

ALK6, KLF2, KCNK45, SMAD1, SMAD4

Other genes recently identified:

ABCC8, BMP10, CD248, EFCAB4B, KDR, NOTCH3, TET2

Table 2 - Reported susceptibility of various transgenic animals to develop remodeling of pulmonary vessels

Remodeling of lung vessels under unstressed conditions	Refs
<i>Alkl</i> ^{+/-} mice	[80]
<i>Bmpr2</i> ^{+/-} mice carrying a mutant allele lacking exons 4 and 5	[81]
<i>Bmpr2</i> ^{+/<i>R899X</i>} mice	[59]
<i>Bmpr2</i> ^{+/<i>Δ71</i>} and <i>Bmpr2</i> ^{+/<i>Δ140</i>} rats	[82]
<i>Eng</i> ^{+/-} mice	[83]
<i>L1cre</i> ⁺ ; <i>Bmpr2</i> ^{<i>ff</i>} and <i>L1cre</i> ⁺ ; <i>Bmpr2</i> ^{<i>f/+</i>} mice	[84]
<i>L1Cre</i> ⁺ ; <i>Smad1</i> ^{<i>ff</i>} mice	[85]
<i>SM22-rtTA</i> x <i>TetO</i> ⁷ - <i>BMPR2</i> ^{<i>R899X</i>} mice	[86]
<i>Smad8</i> ^{<i>lacZ</i> +/-} and <i>Smad8</i> ^{<i>lacZ</i> +/-} mice	[87]
<i>Tagln-Cre</i> ⁺ ; <i>Smad1</i> ^{<i>ff</i>} mice	[85]
Increased sensitivity to hypoxia-induced pulmonary vascular remodeling	Refs
<i>Bmp2</i> ^{+/-} mice	[88]
<i>Eng</i> ^{+/-} mice	[83]
Protected against pulmonary vascular remodeling induced by chronic hypoxia	Refs
<i>Bmp4</i> ^{<i>LacZ</i> -} mice	[89]
<i>Bmp9</i> ^{-/-} mice	[47]
<i>Bmpr2</i> ^{+/-} mice carrying a mutant allele lacking exons 4 and 5	[81]
<i>Dn-Tgfr2</i> mice	[90]
<i>Eng</i> ^{+/-} mice	[33]
<i>Gdf11</i> ^{<i>ff</i>} - <i>Tie2</i> ^{<i>cre</i>}	[71]
<i>Grem1</i> ^{+/-}	[91]
Similar susceptibility to hypoxia-induced pulmonary vascular remodeling as wild-type mice/rats	Refs
<i>Bmpr2</i> ^{<i>ΔEx2/+</i>} mice	[92]
<i>Bmpr2</i> ^{+/-} mice	[93]
<i>Bmpr2</i> ^{+/<i>Δ527bp</i>} rats	[94]
<i>Bmpr2</i> ^{+/<i>Δ16bp</i>} rats	[94]
Early embryonic lethality in homozygous KO mice:	
<i>Acvr11</i> (encoding ALK1), <i>Acvr1</i> (encoding ALK2), <i>Bmpr1a</i> (encoding ALK3), <i>Bmp2</i> , <i>Bmp4</i> , <i>Bmp10</i> , <i>Gdf11</i> , <i>Smad1</i> , <i>Smad4</i> , <i>Smad5</i> , <i>Smad7</i> , <i>Bmpr1a</i> (encoding ALK3), <i>Bmpr2</i> , <i>Tgfr1</i> (encoding ALK5), <i>Tgfr2</i>	
Perinatal lethality in homozygous KO mice:	
<i>Acvr2b</i> (encoding ActRIIB), <i>Bmp1</i> , <i>Bmp7</i> , <i>Bmp11</i> , <i>Smad6</i> , <i>Tgfb1</i> , <i>Tgfb2</i> , <i>Tgfb3</i>	

Table 3 - List of agents directly targeting the TGF- β superfamily tested in PH animal models

Small molecules	Targets	Animal models	Effects on PH	Refs
BMP upregulator (BUR1)	↑ BMP2	MCT, SuHx	Positive	[95]
IN-1233	↓ ALK5	MCT	Positive	[48]
IN-1233	↓ ALK5	Chronic hypoxia	None	[48]
LDN-193189	↓ ALK2, ALK3	MCT	Negative	[50]
SB-525334	↓ ALK5	MCT	Positive	[49]
SD-208	↓ ALK5	MCT	Positive	[51]
Ligand-trap approaches	Targets	Animal models	Effects on PH	Refs
ActRIIA-Fc ligand trap	↓ ActRIIA ligands	MCT, SuHx	Positive	[27]
AKL1-Fc ligand trap	↓ ALK1 ligands	MCT, SuHx	Positive	[47]
AKL1-Fc ligand trap	↓ ALK1 ligands	Chronic hypoxia	Negative	[24]
AKL3-Fc ligand trap	↓ ALK3 ligands	Chronic hypoxia	None	[24]
sEng-Fc ligand trap	↓ sEng ligands	Chronic hypoxia	None	[24]
TGFBRII-Fc ligand trap	↓ TGF β RII ligands	MCT, SuHx	Positive	[96]
Recombinant proteins	Targets	Animal models	Effects on PH	Refs
BMP-9	↑ BMP-9	<i>Bmpr2</i> ^{+/-R899X} mice, MCT, SuHx	Positive	[59]
Neutralizing antibodies	Targets	Animal models	Effects on PH	Refs
Anti-BMP-9	↓ BMP-9	Chronic hypoxia	Positive	[97]
Anti-Gremlin	↓ Gremlin	Murine SuHx	Positive	[98]
Adenovirus	Targets	Animal models	Effects on PH	Refs
AdBMPR2+Fab-9B9	↑ BMPRII	Chronic hypoxia, MCT	Positive	[99]
AdCMVBMPR2myc+Fab9B9	↑ BMPRII	Chronic hypoxia	Positive	[100]

Table 4 – List of high affinity-ligands identified to bind to the 4 type II receptors using a high throughput surface plasmon resonance (SPR)-based binding assay ^[61]

Briefly, Aykul S. *et al.* captured purified ActRIIA-Fc, ActRIIB, BMPRII-Fc, and TGFβRII-Fc proteins (250 response unit each) on a BIAcore sensor chip, and injected 16 different ligands at a concentration of 80nM (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). This table ranks the different ligands that bound type II receptors with very high (red), high (orange) and moderate (blue) affinity.

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		

Figure 1 – The BMP and TGF- β signaling in mammals: (A) Names and genes of the BMP and TGF- β family proteins. (B) Schematic illustration of the different forms occurring during the BMP and TGF- β synthesis, secretion and activation. (C) Structure of mature activin and inhibin proteins. *BMP: bone morphogenetic protein; GDF: growth differentiation factor; TGF- β : transforming growth factor, beta.*

Figure 2 – Signaling by the BMP and TGF- β signaling: *ActRII indicates activin type II receptor; ALK: type I BMP receptor activin-like kinase; AMH: anti-Müllerian hormone; AMHRII: anti-Müllerian hormone type II receptor; BMP: bone morphogenetic protein; BMPRII: bone morphogenetic protein receptor type II; ERK1/2: extracellular signal-regulated kinase 1/2; GDF: growth differentiation factor; JNK: c-Jun N-terminal kinase; LRP-1: Low density lipoprotein receptor-related protein-1; PI3K: phosphoinositide 3-kinase; PP1c: protein phosphatase 1c; RGM: Repulsive guidance molecule A; RTKs: receptor tyrosine kinases; Smad: small mothers against decapentaplegic; TGF- β : transforming growth factor- β ; TGF β RII: transforming growth factor, beta receptor II; USAG-1: uterine sensitization-associated gene-1.*

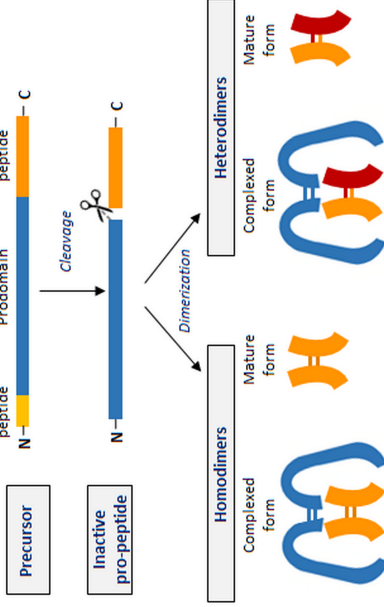
Figure 3 – Dysregulation of the TGF- β –ACTIVIN–NODAL and BMP–GDF branches in PAH: *ActRII indicates activin type II receptor; ALK: type I BMP receptor activin-like kinase; BMP: bone morphogenetic protein; BMPRII: bone morphogenetic protein receptor type II; ERK1/2: extracellular signal-regulated kinase 1/2; GDF: growth differentiation factor; JNK: c-Jun N-terminal kinase; PI3K: phosphoinositide 3-kinase; Smad: small mothers against decapentaplegic; TGF- β : transforming growth factor- β ; TGF β RII: transforming growth factor, beta receptor II.*

Figure 4 – Modulation of the TGF- β superfamily signaling and its functional impact on the pulmonary vascular remodeling associated to PAH: *BMP: bone morphogenetic protein; GDF: growth differentiation factor; PA-SMC: pulmonary artery smooth muscle cell; TGF- β : transforming growth factor- β .*

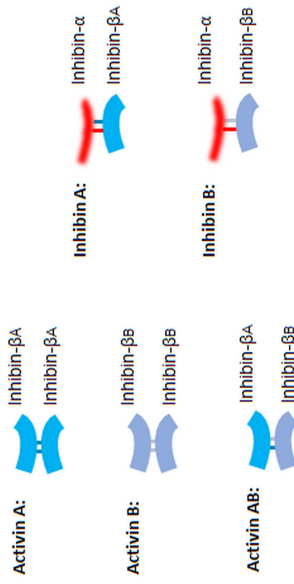
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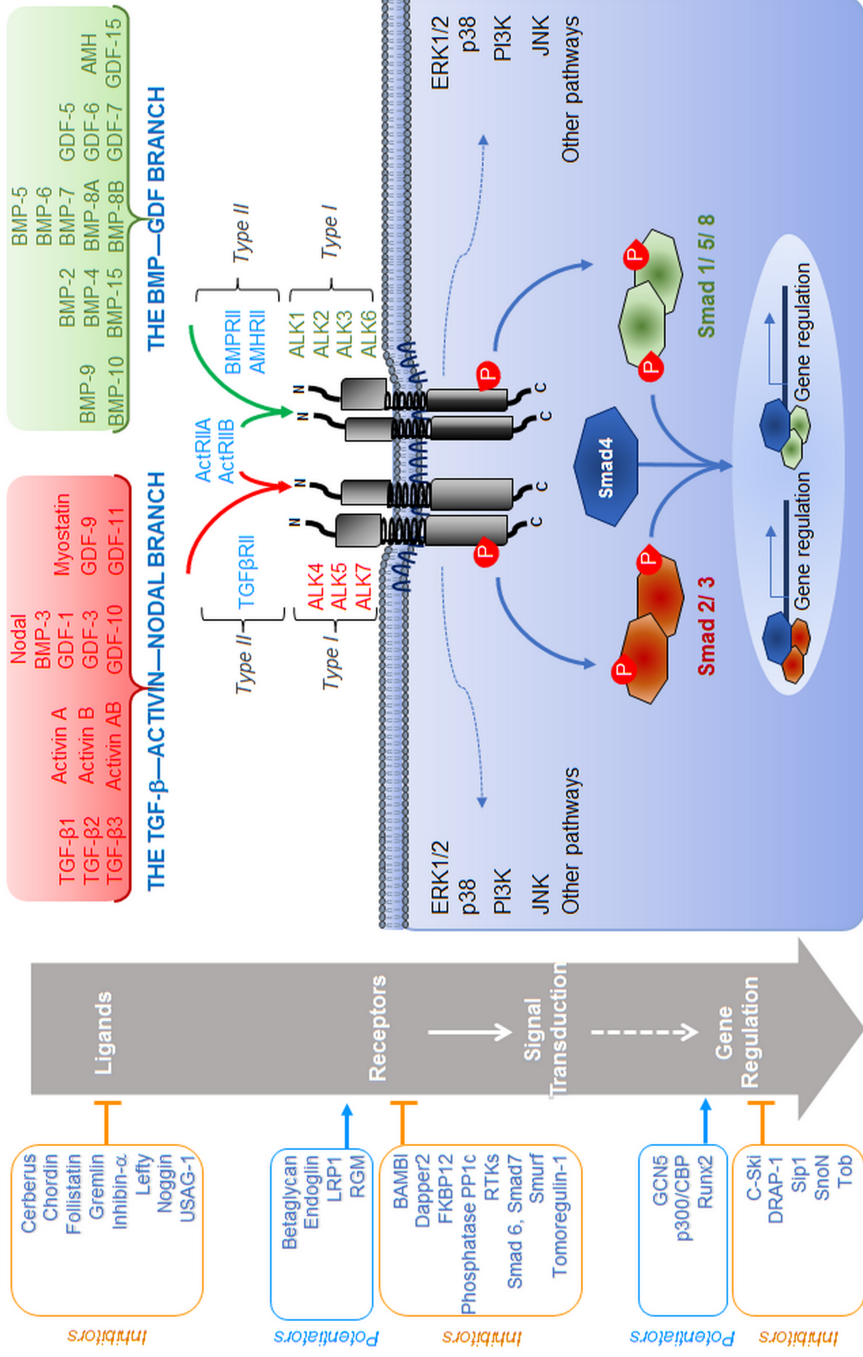
	Gene Name	Protein Name	Alternative Name
Group 1	<i>BMP2</i>	BMP-2	
	<i>BMP4</i>	BMP-4	
	<i>BMP5</i>	BMP-5	
	<i>BMP6</i>	BMP-6	VGR-1, vegetal related growth factor 1
	<i>BMP7</i>	BMP-7	OP-1, osteogenic protein 1
	<i>BMP8A</i>	BMP-8A	OP-2, osteogenic protein 2
	<i>BMP8B</i>	BMP-8B	OP-3, osteogenic protein 3
	<i>GDF2</i>	BMP-9	GDF-2, growth differentiation factor 2
	<i>BMP10</i>	BMP-10	
	<i>GDF1</i>	GDF-1	
	<i>GDF3</i>	GDF-3	
	<i>GDF5</i>	GDF-5	BMP-14 / CDMP-1, cartilage-derived morphogenetic protein-1
	<i>GDF6</i>	GDF-6	BMP-13 / CDMP-2, cartilage-derived morphogenetic protein-2
	<i>GDF7</i>	GDF-7	BMP-12 / CDMP-2, cartilage-derived morphogenetic protein-3
	<i>GDF9</i>	GDF-9	
	<i>BMP15</i>	BMP-15	GDF-3B, growth differentiation factor 3B
	<i>BMP3</i>	BMP-3	BMP-3A / Osteogenin
	<i>GDF10</i>	GDF-10	BMP-3B / BP, bone-inducing protein
Group 2	<i>ILSTN</i>	Myostatin	
	<i>GDF11</i>	GDF-11	BMP-11
	<i>GDF15</i>	GDF-15	MIC-1, macrophage inhibitory cytokine 1 / NSAID-Activated Protein 1, non-steroidal anti-inflammatory drug-activated protein 1 / PDF, prostate differentiation factor / PTGFB, placental TGF-beta / PLAB, placental BMP
	<i>INH4</i>	Inhibin-α	
	<i>INH6A</i>	Inhibin-βA	
Group 3	<i>INH8B</i>	Inhibin-βB	Activin Beta A Chain / Inhibin Beta A Chain / Inhibin, Beta 1 / EDF, erythroid differentiation protein / FRP, follicle-stimulating hormone (FSH)-releasing protein /
	<i>INH9B</i>	Inhibin-βB	Activin Beta B Chain / Inhibin Beta B Chain / Inhibin, Beta 2
	<i>INH9C</i>	Inhibin-βC	Activin Beta C Chain / Inhibin Beta C Chain
	<i>INH9E</i>	Inhibin-βE	Activin Beta E Chain / Inhibin Beta E Chain
	<i>LEFTY1</i>	Lefty-B	Lefty-1, left-right determination factor 1 / EBAF, endometrial bleeding-associated factor / TGF-β4
	<i>LEFTY2</i>	Lefty-A	Lefty-2, left-right determination factor 2 / TGF-β4
	<i>NODAL</i>	Nodal	BMP-16 / HTX-5, visceral heterotaxy-5
	<i>TGFβ1</i>	TGF-β1	LAP, latency-associated peptide / DPD-1, progressive dysplasia 1
Group 4	<i>TGFβ2</i>	TGF-β2	G-TSF, glioblastoma-derived T-cell suppressor factor / BSC-1 cell growth inhibitor / cetermin / polyargin
	<i>TGFβ3</i>	TGF-β3	ARVD-1, arrhythmogenic right ventricular dysplasia 1
	<i>AMH</i>	AMH	Anti-müllerian hormone / MF, Müllerian-inhibiting factor / MIS, Müllerian inhibiting substance
Group 5	<i>GDNF</i>	GDNF	Glial cell derived neurotrophic factor / ATF, astrocyte-derived
	<i>NTN</i>	NTN	Neurturin
	<i>ARTN</i>	ARTN	Artemin / NBN, neublastin / neurotrophic factor
	<i>PSPN</i>	PSPN	Persephin

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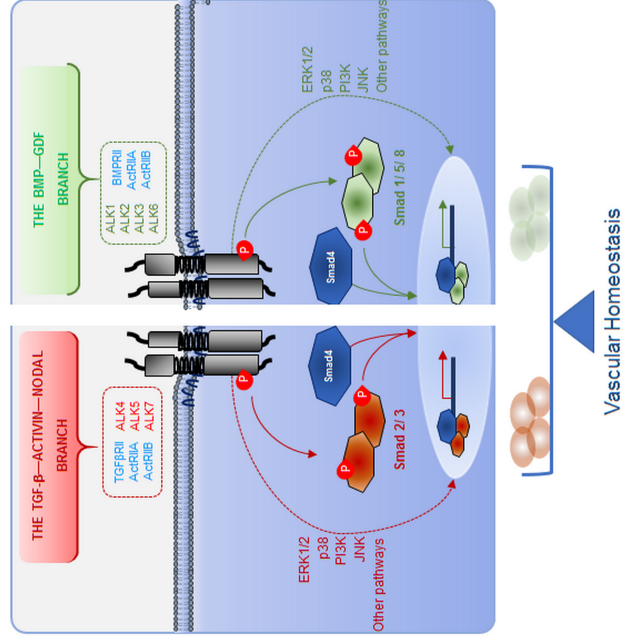


C





Healthy:



PAH:

