



REVIEW ARTICLE

Transforming Growth Factor-Beta Superfamily Members in the Pathogenesis of Pulmonary Arterial Hypertension

ABSTRACT

Pulmonary arterial hypertension (PAH) is a devastating and rapidly progressing disease that induces substantial pulmonary vascular remodeling. The pathologic changes especially in pulmonary microvasculature result in progressive increases in the mean pulmonary artery pressure and pulmonary vascular resistance, which, if untreated leads to right-ventricular failure and death. Although it is clear that PAH has a multifactorial pathobiology, recent discoveries pointed out crucial role of Transforming Growth Factor (TGF)-beta family members in the pathophysiology of PAH. The TGF-beta superfamily is composed of multifunctional mediators, including the TGF-beta isoforms and the Bone Morphogenetic Proteins (BMPs). Germline mutations in the gene coding for BMP receptor 2 (*BMPR2*) have been identified in 60% of familial and 10-30% of idiopathic PAH. Mutations in the TGF-beta receptors, ALK-1 and endoglin, have been found in PAH patients with a personal or family history of hereditary hemorrhagic telangiectasia. Non-canonical TGF-beta pathways as well as TGF-beta receptor ligands (i.e. BMP9) are also involved in PAH development. Our improved understanding of TGF-beta pathway regulation will have important implications for the development of novel therapeutic strategies for this complex and serious disease. Animal models will undoubtedly have an important role in this process; however human studies will give the final answer about the efficacy and safety of the novel treatments for PAH. This review provides an overview of the TGF-beta and BMPs potential role in PAH.

KEY WORDS

Transforming growth factor-beta, pulmonary arterial hypertension, bone morphogenetic proteins, endoglin, ALK1.

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Pulmonary arterial hypertension (PAH) is either due to cardiac, pulmonary or extrathoracic disorders (secondary PAH) or without any detectable cause (primary or idiopathic PAH; IPAH).¹ In some cases, primary PAH occurs in young patients with a mitochondrial disorder,² but whether there is a causal relation between them, remains to be elucidated. Regardless of the etiology, unrelieved pulmonary hypertension advance to right-sided heart failure, progressive debilitation and death, often within 2 to 3 years after its initial diagnosis. Lung specimens from patients with PAH and from experimental models underline

the importance of vascular cell proliferation and obliteration of small pulmonary arteries by smooth muscle cells and myofibroblasts in the pathogenesis of this disease. In addition, plexiform lesions comprising endothelial cells and myofibroblasts are found in about 50% of cases.

Over the last decade, some major advances have led to substantial improvements in the management of PAH. Much of this progress was pioneered by work in animal models. Although none of the current animal models of PAH completely recapitulate the human disease, they do provide

insight into the cellular pathways contributing to its development and progression. Genetic studies have revealed heterozygous mutations in the *BMPR2* gene encoding the type II bone morphogenetic protein receptor (Bmpr2), a member of the TGF-beta superfamily of receptors, underlying the familial form of the disease.³ Subsequently, *BMPR2* mutations were found in about 25% of apparently sporadic cases of IPAH, many of which are examples of familial transmission with low disease gene penetrance. These studies pointed toward a critical role for the TGF-beta superfamily in the pathogenesis of PAH through the molecular mechanisms that include regulation of pulmonary vascular cell growth and differentiation.⁴

TGF-beta signalling pathways

TGF-beta superfamily has over 35 structurally related pleiotropic cytokines that also includes TGF-beta1, TGF-beta3, Activins, Nodals and bone morphogenetic proteins (BMPs). Signalling by TGF-beta family members occurs through type II serine/threonine kinase receptors and

type I receptors, also termed Activin-receptor-like kinases (ALKs).⁵ Each member of the TGF-beta superfamily binds to a characteristic combination of type I and type II receptors. Ligand binding induces the assembly of type I and type II receptors into complexes, within which type II phosphorylates type I receptor and this phosphorylation is both essential and sufficient for TGF-beta signalling (Figure 1). Endoglin, an accessory receptor, modulates TGF-beta signaling by regulating surface TGF-beta receptors and their activation.⁶ Once activated, the type I receptor can induce several signaling outcomes which include phosphorylation of cytoplasmic proteins called regulatory Smads (Smad2/3 or Smad1/5/8) that trimerize with Smad4 leading to nuclear shuttling and gene transcription regulation. In addition, non-Smad signaling pathways can also be induced (Figure 1) including members of the mitogen activated protein kinase (MAPK) pathway, JNK, p38, p42/44 (ERK), PI3-kinase and Rho pathways.⁷ Involvement of non-Smad pathways in pathogenesis of PAH is well known, especially the ras homolog family member A (RhoA)-Rho kinase (ROCK) axis which specific inhibition is currently the most promising therapeutic approach for PAH.⁸ Also,

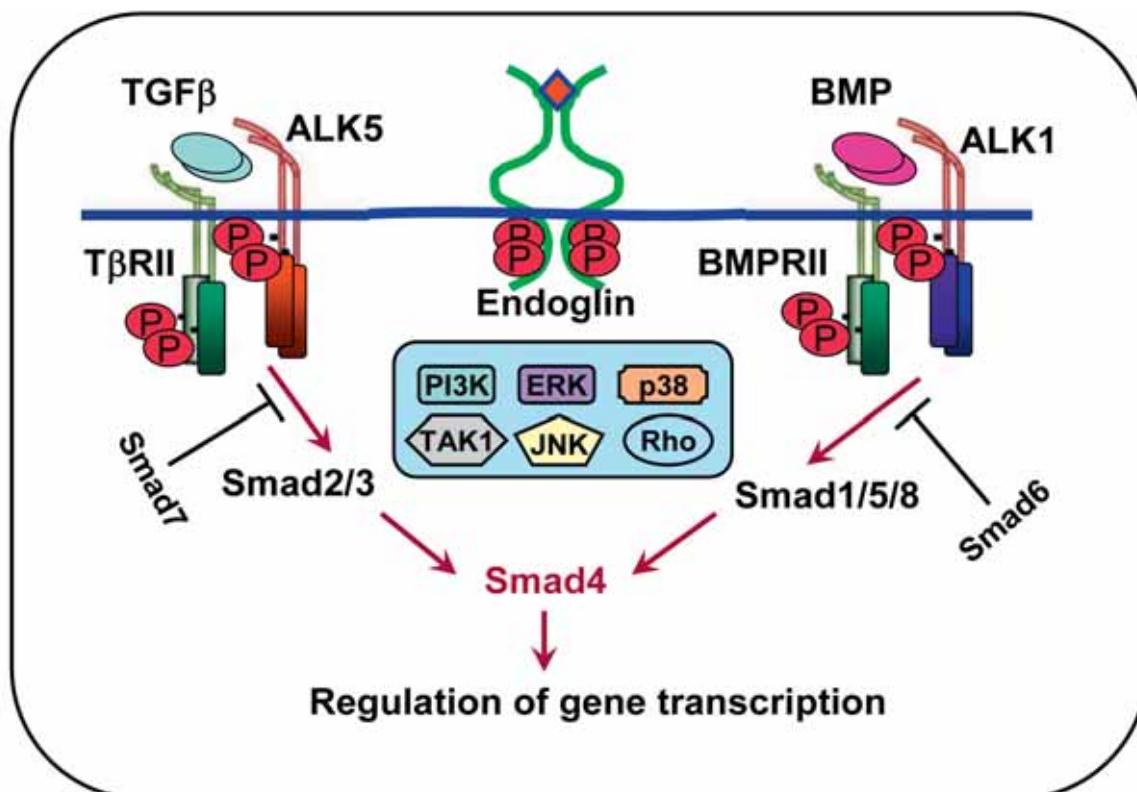


Figure 1. TGF-beta family signalling pathways. Ligands (TGF-beta and BMPs) bind to the receptors and induce heteromeric complex formation between specific type II and type I receptors. Endoglin, an auxiliary receptor, is able to modulate and regulate both TGF-beta and BMPs effects. The type II receptors phosphorylate the type I receptors, leading to their activation. Subsequently, phosphorylation of receptor-regulated (R) Smads occur, which form complexes with Smad4 (common Smad) and translocate into the nucleus where interact with transcription factors and regulate gene responses (canonical Smad signaling pathway). Inhibitory Smads 6 and 7 inhibit receptor activation of R-Smads. In addition, the activated type I receptors can signal through non-Smad pathways (PI3 kinase, mitogen activated protein kinases (MAPK) ERK, p38, TAK1, and Jun kinase, and GTP-ase Rho).

it was recently reported that *BMPR2* deficiency promotes pro-proliferative and anti-apoptotic responses in pulmonary arterial smooth muscle cells through the activation of TGF β -TAK1-MAPK pathways in PAH.⁹

TGF-beta receptor mutations in PAH

Although mutations in the *BMPR2* gene are the most frequent germline mutations identified in familial (~60%) and idiopathic PAH (10-30%), they are absent in some families and in the majority of sporadic and associated cases of PAH. This fact led to the investigation of other gene involvement from TGF-beta family in PAH. Indeed, mutations in the TGF-beta receptors, ALK-1 and endoglin, have been identified in PAH patients with a personal or family history of hereditary hemorrhagic telangiectasia (HHT).¹⁰

It is important to stress out that TGF-beta signalling is essential for regulation of vasculogenesis and angiogenesis. Mutations in at least five genes result in HHT (Rendu Osler-Weber-syndrome), an autosomal dominant disorder with a prevalence of about 1 in 5-8,000 individuals, but mutations in two genes (*Endoglin*, *ENG* causing HHT1 and *ALK1* causing HHT2) account for approximately 85% of cases. HHT is characterized by epistaxis, mucocutaneous and gastrointestinal telangiectases and arteriovenous malformations (AVMs) in pulmonary, hepatic and cerebral circulation.¹¹ Deficiency in endoglin or *Alk1* causes cardiovascular defects leading to embryonic lethality. Mouse heterozygous models for HHT1 and HHT2 has been developed allowing further insight in the role of TGF-beta family in blood vessel and heart development and vascular homeostasis.^{12, 13} Our and other studies have revealed a marked deficiency in nitric oxide (NO) mediated vasodilation in endoglin-haploinsufficient mice (*Eng*^{+/-}), as well as that endoglin has an important role in endothelial NO synthase (eNOS) activation.^{14, 15} Moreover, endoglin and *Alk1* associate with and stabilize the eNOS activation complex leading to NO production. In heterozygous conditions for either endoglin or *Alk1*, eNOS becomes uncoupled and produces more superoxide than NO which leads to tissue damage and impaired vascular tone control. Interestingly, our recent work also revealed that adult *Eng* and *Alk1* heterozygous mice have signs of pulmonary arterial hypertension including increased right ventricular (RV) systolic pressure, RV hypertrophy, degeneration of the distal pulmonary vasculature, and muscularization of small arteries. PAH that heterozygous mice develop is attributable to uncoupled eNOS activity and increased superoxide ($\bullet\text{O}_2$) production, which can be prevented by antioxidant treatment.^{16, 17}

On the other hand, *Bmpr2* heterozygous mice had no or mildly elevated pulmonary pressure.^{18, 19} However, the infusion of serotonin caused several signs of PAH in *Bmpr2*^{+/-} mice, further increased by hypoxic conditions.

Authors showed that *BMPR2* haploinsufficiency increased susceptibility to PAH and pulmonary vascular remodeling *in vivo* providing a link between two key systems widely implicated in the pathogenesis of PAH.¹⁹ Also, a transgenic dominant-negative form of *Bmpr2* specifically expressed and activated after birth in smooth muscle cells led to increased pulmonary arterial pressure suggesting that the mutation need to be expressed in smooth muscle to produce the phenotype.²⁰ More recently, Hong et al. have used conditional knockout mice in which *BMPR2* gene was deleted in pulmonary endothelial cells. They showed that endothelial *BMPR2* deletion is in itself not sufficient to cause PAH but can increase the susceptibility to PAH.²¹

It is therefore clear that PAH is very complex disease with initial stages likely involving the interaction between genetic predisposition (i.e. *BMPR2*, *ALK1*, and *ENG* mutations) and environmental risk factors. Genetic mutations of TGF-beta family members are not sufficient to induce PAH, but are serious predisposing factors. To add even more complexity to the topic, mutations of *SMAD4* (common Smad; Figure 1) do not predispose to PAH, but are seen in patients with juvenile polyposis (JP), colorectal cancer, and with the combined syndrome of JP and HHT (JP-HHT).²² Finally, one report showed patient with *BMPR2* mutation exhibiting PAH with HHT features, particularly pulmonary AVMs.²³ That pointed out the possibility that PAH and HHT have a common molecular pathogenesis.

Not only the TGF-beta family receptors but also their ligands could have important role in PAH pathobiology. The recent finding of circulating BMP9 as the true physiological ligand for ALK1, acting in combination with *Bmpr2* (Figure 1), supports a role for BMP9 in PAH. Endoglin potentiates the effects of BMP9 and can therefore affect these pathways. Moreover, BMP9 can stimulate endothelin 1 (ET-1) release from pulmonary endothelial cells.²⁴ ET-1 involvements in the pathogenesis of PAH is well known and endothelin receptor antagonists have been a therapeutic mainstay in PAH, providing benefit to many patients.²⁵

Epilogue

TGF-beta superfamily has a critical role in the development and maintenance of the pulmonary vasculature. Genetic defects and imbalance of TGF-beta and BMP pathways contribute significantly to the development of pulmonary arterial hypertension. Pharmacological approach and identification of small inhibitory molecules to these pathways will allow modulation of the TGF-beta superfamily signaling and assist in development of future therapeutics for PAH.

However, the complex regulation of these pathways and their involvement in many fundamental biological processes such as cell growth, differentiation, embryonic de-

velopment and tissue homeostasis, may raise many safety issues and slow the pace of progress. Nevertheless, our growing knowledge of the TGF-beta pathway regulation should teach us how to restore the balance that is lost in patients with PAH. It should assure the progress in this field and open new avenues and possibilities of gene replacement therapy and personalized medicine in the treatment of this life threatening disease.

Financial disclosure

I declare that I have no conflicts of interest.

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Uloga transformišućeg faktora rasta-beta i srodnih molekula u patogenezi plućne arterijske hipertenzije

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APSTRAKT

Plućna arterijska hipertenzija (PAH) je veoma teška i progresivna bolest koja za kratko vrijeme dovodi do anatomskih promjena na krvnim sudovima pluća. Patološke promjene, koje su naročito izražene u plućnoj mikrocirkulaciji, dovode do progresivnog porasta srednjeg plućnog arterijskog pritiska i vaskularnog otpora. Netretirane, ove promjene dovode do insuficijencije desnog srca i često imaju fatalan ishod. Široka lepeza faktora je uključena u patofiziologiju nastanka PAH. Međutim, najnovija naučna istraživanja ističu ključnu ulogu transformišućeg faktora rasta-beta (TGF-beta) i njemu srodnih molekula u nastanku PAH.

Familija ovih proteina obuhvata različite forme liganda i receptora TGF-beta, kao i koštane morfogene proteine (BMP), koji imaju višestruke i složene funkcije. Mutacije gena za BMP receptor 2 (BMP2) identifikovane su u 60% slučajeva porodične PAH i u oko 10-30% slučajeva idiopatske PAH. Mutacije receptora TGF-beta, kao što su ALK1 i endoglin, nađene su kod pacijenata sa PAH i ličnom ili porodičnom istorijom hereditarne hemoragične teleangijektazije. U patogenezu PAH uključeni su i brojni ligandi receptora TGF-beta (npr. BMP9), kao i faktori koji aktiviraju ne samo glavne nego i sporedne signalne puteve. Brojni napori, usmjereni ka boljem razumijevanju funkcije članova porodice TGF-beta imaće direktan uticaj na razvoj novih terapijskih strategija za liječenje ove teške bolesti. Početnu fazu takvih napora predstavljaju eksperimentalna istraživanja na životinjama. Međutim, predklinička i klinička istraživanja će dati konačan odgovor o primjenljivosti i efikasnosti novih terapijskih strategija u liječenju PAH. Ovaj reviski članak sažeto prikazuje naša sadašnja znanja o ulozi članova porodice TGF-beta u PAH.

KLJUČNE RIJEČI

Transformišući faktor rasta-beta, plućna arterijska hipertenzija, koštani morfogeni proteini, endoglin, ALK1.