
Transforming Growth Factor β /Bone Morphogenic Protein Signaling in Pulmonary Arterial Hypertension: Remodeling Revisited

Oliver Eickelberg* and Rory E. Morty*

*Growth factors of the transforming growth factor (TGF) β superfamily have emerged as important regulators of normal cardiovascular development, as well as modulators of the onset or progression of vascular diseases. Recently, familial and idiopathic pulmonary arterial hypertension (IPAH) has been causally linked to somatic and genetic perturbations to the TGF- β /bone morphogenic protein (BMP) system, particularly because heterogeneous germline mutations in *bmpr2* (encoding the type II BMP receptor) have been detected in IPAH patients. Transgenic animal models and functional genomic studies have begun investigating TGF- β /BMP-induced effects in the pulmonary vasculature, as well as the cellular effects of *bmpr2* mutations on vascular cell phenotypes. While these studies have significantly increased our knowledge about the biologic effects of TGF- β /BMP signaling in the lung vasculature, the molecular mechanisms leading to pulmonary vasculopathy in the context of *bmpr2* mutations in IPAH remain largely unknown. (Trends Cardiovasc Med 2007;17:263–269) © 2007, Elsevier Inc.*

• Introduction

Growth factors of the transforming growth factor (TGF)- β superfamily have emerged as important regulators of normal cardiovascular development, as well as modulators of the onset or progression of vascular diseases including atherosclerosis, myocardial infarction, and pulmonary hypertension. The TGF- β

superfamily comprises a multitude of pleiotropic and pluripotent growth factors, including the TGF- β ligands themselves, activins, and bone morphogenic proteins (BMPs). All ligands of this superfamily induce their biologic effects through a similar signal transduction cascade, characterized by the sequential activation of two serine-threonine kinase receptors (a type I and a type II receptor) and receptor-specific transcription factors (Smads). Recently, idiopathic pulmonary arterial hypertension (IPAH) has been causally linked to somatic and genetic perturbations to the TGF- β /BMP system, particularly because heterogeneous germline mutations in *bmpr2* (encoding the type II BMP receptor) have been detected in IPAH patients. Transgenic animal models and functional genomic studies have begun investigating TGF- β /BMP-induced effects in the pulmonary vasculature, as well as the cellular effects of *bmpr2* mutations on vascular cell phenotypes.

Although these studies have significantly increased our knowledge about the biologic effects of TGF- β /BMP signaling in the lung vasculature, the molecular mechanisms leading to pulmonary vasculopathy in the context of *bmpr2* mutations in IPAH remain largely unknown.

Diseases of the cardiovascular system represent a major cause of morbidity and mortality in the Western world. Most cardiovascular abnormalities are associated with molecular, cellular, or histologic alterations to the vasculature: a system ultimately defined by a single cell layer of endothelial cells, surrounded by vascular smooth muscle cells, and varying amounts of adventitial tissue. Vessel architecture and function is tightly controlled by a network of soluble mediators, cell–cell, and cell–matrix interactions (Owens et al. 2004). In particular, growth factors can be secreted locally or can be delivered directly to the vessel wall from the circulation. In this respect, platelet-derived growth factor, basic fibroblast growth factor, vascular endothelial growth factor, endothelin, transforming growth factor (TGF) β , and BMPs are essential for the maintenance of vascular integrity (Topper 2000). Among these, the TGF- β superfamily, including TGF- β and BMP isoforms, are pleiotropic mediators known to affect all cells within the vascular system, owing to ubiquitous expression of their receptors and signaling components. In recent years, our knowledge about the signal transduction mechanisms of TGF- β superfamily members, and the diseases associated with perturbations to it, has expanded exponentially (Massague et al. 2005). Both TGF- β and BMP isoforms essentially control endothelial and smooth muscle cell proliferation and apoptosis, as well as extracellular matrix (ECM) secretion and deposition. Knockout animals of most members of the TGF- β superfamily are embryonically or perinatally lethal, as most of these animal models exhibit severe defects in vasculogenesis (Chang et al. 2002).

• The TGF- β Superfamily

TGF- β represents the prototypic member of a large family of polypeptide growth factors, including (1) TGF- β isoforms themselves, (2) activins, and (3) a complex

Oliver Eickelberg and Rory E. Morty are at the Department of Medicine II, University of Giessen Lung Center, University of Giessen School of Medicine, 35392 Giessen, Germany.

* Address correspondences to: Oliver Eickelberg, MD or Rory Edward Morty, PhD, Department of Medicine II, University of Giessen Lung Center, Aulweg 123, 35392 Giessen, Germany. Tel.: (+49) 641 9942300; fax: (+49) 641 9942309; e-mails: oliver.eickelberg@innere.med.uni-giessen.de, rory.morty@innere.med.uni-giessen.de.

© 2007, Elsevier Inc. All rights reserved.
1050-1738/07/\$-see front matter

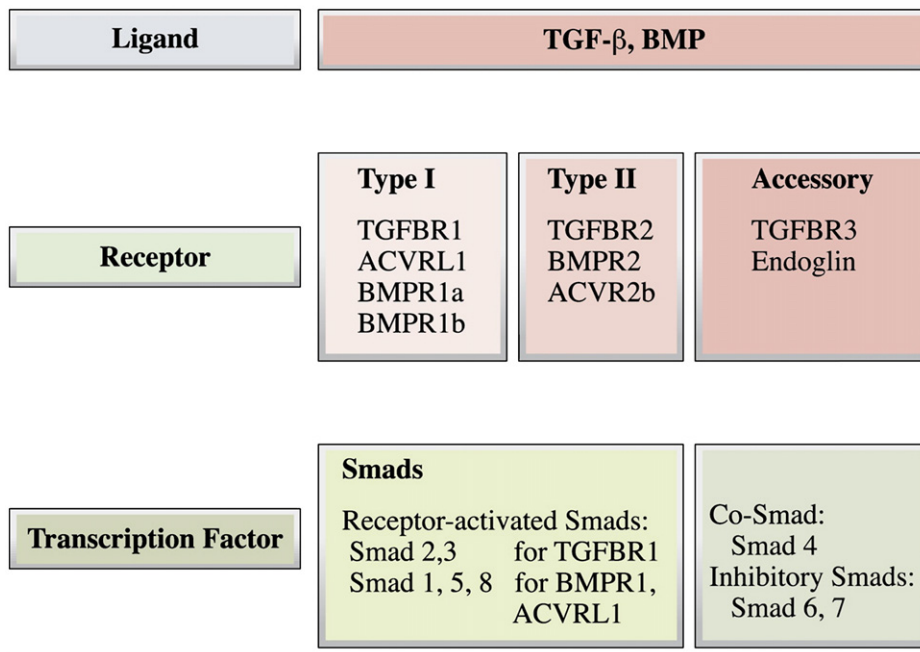


Figure 1. The components of the TGF- β /BMP signaling pathway. Members of each group are presented according to their function indicated on the left column.

third subfamily consisting of morphogenic proteins (BMP, nodal, *Xenopus Vg-1*, *Drosophila dpp*, and *screw*) (Massague et al. 2005, Roberts 1998). The TGF- β system is one of the most evolutionarily conserved signal transduction pathways across the animal kingdom, consisting of several ligands, receptors, transcription factors, and interacting factors (Figure 1). Biologic effects of TGF- β family members are induced after binding of TGF- β to the ligand-binding receptor isotype, transforming growth factor beta receptor (TGFBR)2 (also called “T β RII”). This leads to formation and stabilization of a heterotetrameric complex of TGFBR1 (also called “ALK5”) or activin A receptor, type I (ACVRI; also called “ALK1”) with TGFBR2, followed by transphosphorylation of TGFBR1 by the constitutively phosphorylated TGFBR2 kinase. Subsequent phosphorylation of the receptor-associated cytoplasmic effector molecules Smad2 and Smad3 by TGFBR1 then leads to heterooligomerization of phosphorylated Smad2/3 with the common Smad4, and modulation of gene transcription in the nucleus (Figure 2).

The BMP family of the TGF- β superfamily exhibits similar characteristics and signal transduction mechanisms to TGF- β (Miyazono et al. 2005). The extracellular ligand, in this case, a BMP isoform, binds to a heteromeric receptor complex of BMPR1a (also called ALK3)

or BMPR1b (also called ALK6) and BMPR2, thereby initiating intracellular signaling. Although TGF- β is unable to bind to TGFBR1 in the absence of TGFBR2, BMP isoforms can bind to BMPR1a or BMPR1b even in the absence of Bmpr2. Furthermore, oligomerization patterns of BMP receptors are more flexible and susceptible to modulation by BMP ligands than are the TGF- β receptors, leading to more multifaceted cross-talk between BMP receptors. Pathway specificity between TGF- β and BMP is granted in part through use of specific Smad isoforms, depending on the type I receptor involved. Although TGFBR1 activation leads to phosphorylation and activation of receptor-specific Smad2 and Smad3, BMPR1a/b and ACVRL1 activation leads to activation of Smads 1, 5, and possibly 8 (Miyazono et al. 2005) (Figure 2).

• Bmpr2 and Pulmonary Hypertension

Recently, the TGF- β and BMP signaling systems have attracted significant medical attention because mutations in genes encoding members of either system have been associated with pulmonary arterial hypertension (PAH). Pulmonary arterial hypertension is a heterogeneous and fatal disease characterized by an elevated blood pressure in the pulmonary circulation of up to 30 mm Hg at rest and >30 mm Hg

during exercise (Newman 2005). This increased pressure is due to complex alterations to the pulmonary vasculature, leading to an increased vascular resistance of pulmonary arterioles. Patients presenting with PAH include individuals with IPAH or secondary PAH due to systemic pathologies such as congenital heart disease, connective tissue disease, liver disease, or hypoxic conditions. Several pathogenic mechanisms, such as initial vasoconstriction, in situ thrombosis, and remodeling of the pulmonary arterial vessel wall contribute to the increased vascular resistance observed in patients with PAH (Pietra et al. 2004, Yuan and Rubin 2005). During the course of the disease, initial augmented vasoconstriction has also been linked to endothelial dysfunction, which may trigger subsequent remodeling processes that involve all layers of the vessel wall, including pulmonary arterial endothelial cells (PAEC), pulmonary arterial smooth muscle cells (PASMC), and adventitial fibroblasts.

In 2000, positional cloning revealed that patients affected with familial PAH (FPAH) exhibited germline mutations within the *bmpr2* locus (Deng et al. 2000, Lane et al. 2000; Newman et al. 2001). Direct sequence analysis has subsequently identified multiple heterogeneous germline mutations in *bmpr2* exons in ~75% of FPAH cases and 25% of IPAH cases, most of which are predicted to lead to missense, nonsense, or frameshift mutations (Humbert et al. 2004, Machado et al. 2006, Newman et al. 2004). In addition, PAH in patients with hereditary hemorrhagic telangiectasia has been linked to mutations in the *acvr1l* gene, encoding a type I TGF- β receptor (Trembath 2001). Although these genetic studies have assigned an unambiguous causative role for TGF- β /BMP receptors in the development of PAH, our knowledge about the functional contribution and the expression of this system in the lung is still evolving.

Several recent studies have begun to address the expression of TGF- β /BMP system components in the human lung. BMPR2 is highly expressed in the endothelium and smooth muscle layer of the pulmonary vasculature (Atkinson et al. 2001, Richter et al. 2004). Atkinson et al. (2002) have reported reduced pulmonary vascular expression of BMPR2, as assessed by in situ hybridization and immunostaining, whereas

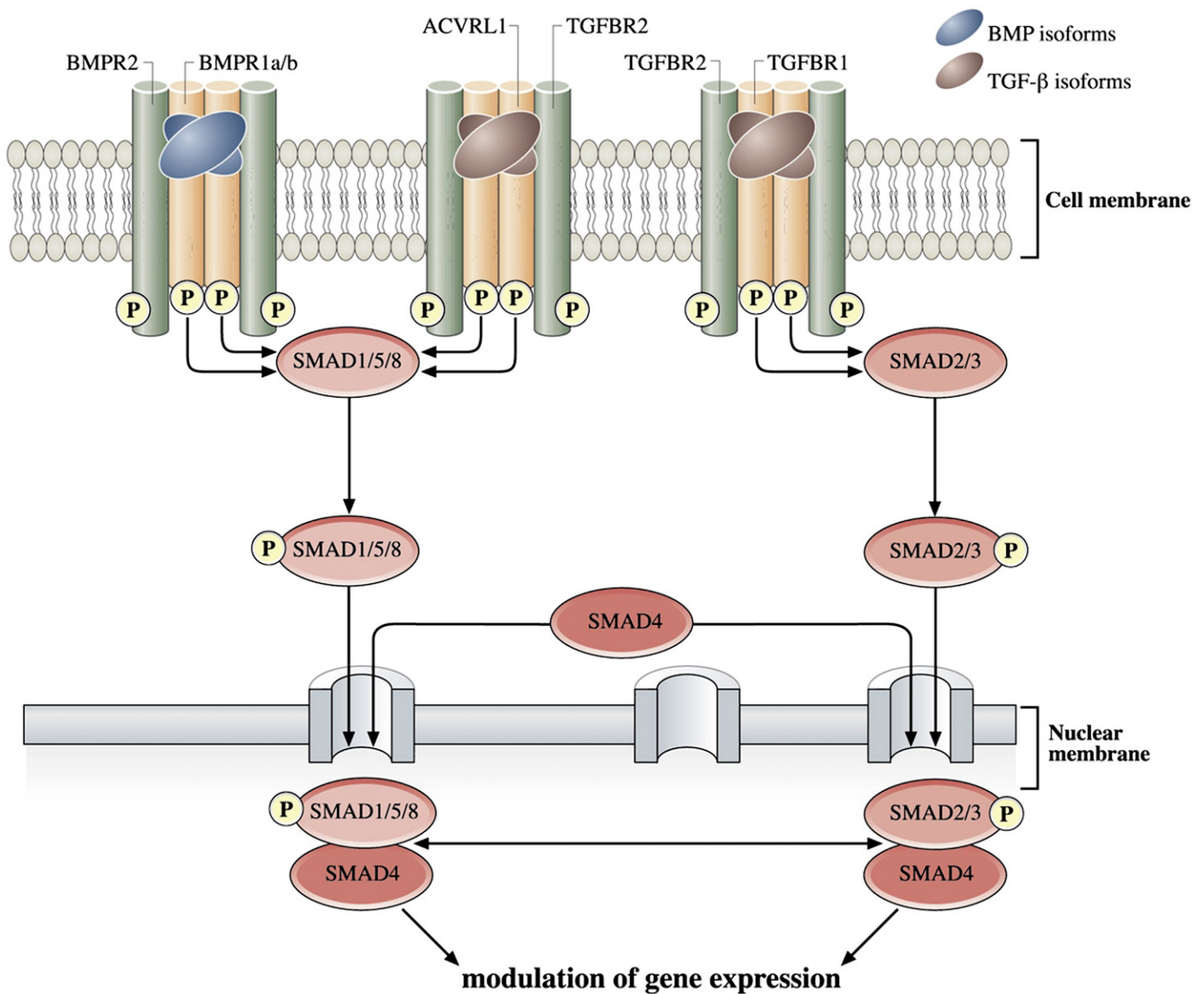


Figure 2. The TGF- β /BMP signaling pathway. Signaling is initiated after formation of a heterotetrameric complex of type I and type II receptors on the cell membrane. Note that TGF- β signaling can activate either Smad1/5/8 or Smad2/3 via phosphorylation of ACVRL1 or TGFBR1, respectively, whereas BMP signaling induces Smad1/5/8 activation via phosphorylation of BMPR1a/b. P indicates phosphorylation.

two other studies did not observe any differences in BMPR2 expression comparing primary and secondary PAH with control patients (Du et al. 2003, Richter et al. 2004). Richter et al. (2004) have demonstrated a loss of TGF- β receptor and Smad expression in endothelial cells at the core of plexiform lesions in IPAH, in the absence of overall expression differences compared with control lungs.

The available data obtained from transgenic animal models with modifications to BMPR2 expression suggest a similarly complex, yet somewhat discordant, scenario. Mice with a heterozygous deletion of *bmpr2*, or overexpression of a

dominant-negative form of BMPR2, are more likely to develop PAH (Beppu et al. 2004, Song et al. 2005, Tada et al. 2007, West et al. 2004). In contrast, other investigators were unable to detect significant differences in pulmonary arterial pressures between heterozygous *Bmpr2*-deficient and control mice (by genomic deletion or stable overexpression of siRNA), at baseline conditions or in response to chronic hypoxia (Liu et al. 2007, Long et al. 2006). Furthermore, it is unclear at this point whether reconstitution or overexpression of BMPR2 by gene therapy impacts the development of PAH in animal models (McMurtry et al. 2007, Reynolds et al. 2007).

Initial functional genomic studies have also yielded opposing data in that a loss of BMPR2 expression leads to an attenuation of BMP signaling (Morrell et al. 2001, Rudarakanchana et al. 2002, Yang et al. 2005) but may also increase BMP-6 or -7 signaling (Yu et al. 2005). In this respect, a closer look at TGF- β - and BMP-induced effects on relevant cell populations of the pulmonary vasculature will enable a fuller appreciation of the role played by these growth factors in PAH. For the purpose of this review, we will focus on the effects of TGF- β /BMP on proliferation, apoptosis, migration, and ECM synthesis by PASC and PAEC.

The pulmonary vascular pathology observed in advanced PAH is characterized by abnormal muscularization of small-resistance pulmonary arterioles, intimal thickening, fibrosis, and the obstruction of the vascular lumen by plexiform and concentric lesions (Pietra et al. 2004). Two of these features, increased vessel muscularization and lesion formation, have been attributed to dysregulated PSMC and PAEC phenotypes, respectively (Humbert et al. 2004, Mandegar et al. 2004, Pietra et al. 2004). Given that members of the TGF- β superfamily of growth factors are important mediators of cell growth, proliferation, migration, and ECM synthesis in the vasculature (Bobik 2006) and that TGF- β /BMP signaling is dysregulated in PAH (Humbert et al. 2004), it is likely that aberrant TGF- β /BMP signaling underlies some of the vascular pathology associated with PAH.

Smooth Muscle Cells

Smooth muscle cell mass is an important determinant of pulmonary artery wall thickness and is controlled by a balance between proproliferative and antiproliferative signaling pathways (Mandegar et al. 2004). The increased muscularization of small pulmonary arteries observed in PAH is thought to result from the aberrant growth of PSMC, which have impaired responses to injurious stimuli that control proliferation and apoptosis (Humbert et al. 2004). The injury-repair response includes PSMC proliferation, with the subsequent secretion and deposition of a provisional ECM that facilitates cell motility, growth, and survival. Such stimuli include BMP and TGF- β , which promote apoptosis of PSMC from healthy donors and patients with PAH (Lagna et al. 2006, Zhang et al. 2003) and inhibit the proliferation of healthy PSMC and PSMC from patients with PAH (Morrell et al. 2001, Takeda et al. 2004, Zhang et al. 2003). The PSMC from patients with IPAH or FPAH, however, are resistant to the antiproliferative effects of BMP and TGF- β (Morrell et al. 2001), suggesting that the failure of BMP and TGF- β to suppress PSMC growth in PAH may, in part, underlie the increased muscularization of normally nonmuscularized small pulmonary arteries of patients with FPAH or IPAH.

Growth of PSMC is also controlled by mitogen-activated protein kinase (MAPK) signaling, undertaken by extracellular regulated kinase (ERK) and p38^{MAPK}, among others. These signals are countered by antiproliferative or proapoptotic Smad-mediated signaling, in response to TGF- β or BMP ligands. The pulmonary vasculature of patients with both FPAH and IPAH is deficient in the activated forms of Smad1, suggesting that defective Smad signaling and unopposed ERK/p38^{MAPK} signaling contribute to abnormal PSMC proliferation in PAH (Yang et al. 2005). In addition, PSMC proliferation is preceded by cytoskeletal reorganization, which is also required for the deposition of a provisional ECM and cell migration. Although this coordinated interplay of ECM synthesis and degradation supports an antiproliferative PSMC phenotype in vessel homeostasis, the pulmonary vasculature in IPAH is characterized by enhanced expression of proteases, such as members of the matrix metalloproteinases (Lepetit et al. 2005), and exaggerated ECM remodeling (Humbert et al. 2004). It is of particular interest that tenascin-C, an ECM glycoprotein, has been shown to facilitate PSMC migration, proliferation, and survival (Jones and Rabinovitch 1996) and is expressed at higher levels in patients with PAH (Ihida-Stansbury et al. 2006, Jones et al. 1997). These observations may link defective BMP/Smad1/5 signaling with vascular lesion development, as inhibition of BMP signaling-induced tenascin-C expression, which is higher in PSMC from patients with *bmpr2* mutations compared with controls (Ihida-Stansbury et al. 2006). The BMP-induced Id1 expression inhibits PSMC migration in response to platelet-derived growth factor (Wu et al. 2006), while Frank et al. (2005) have reported increased PSMC migration in response to BMP-4. In summary, the results described above, obtained from in vitro studies, suggest an inhibitory effect of BMP signaling on PSMC ECM synthesis, migration, and proliferation.

These ideas have been further explored in two animal models of pulmonary hypertension (PH), with the use of exposure to the pyrrolizidine alkaloid monocrotaline (MCT) or to chronic hypoxia. Although early studies have demonstrated increased TGF- β 1, TGF- β 2, and TGF- β 3 ligand expression in MCT-induced PH (Arcot

et al. 1993, Tanaka et al. 1996), recent studies have revealed a dramatic reduction in TGF- β and BMP signaling in this model (McMurtry et al. 2007, Morty et al. 2007, Zakrzewicz et al. 2007). A significant down-regulation of BMPR2 expression has been detected in MCT-treated rats, along with a significant reduction in phosphorylated Smad1, transcription of the BMP/Smad1-responsive genes *id1* and *id3*, and BMP-dependent apoptosis of PSMC (McMurtry et al. 2007, Morty et al. 2007). Similarly, TGF- β signaling was perturbed in this model, documented by decreased expression of TGFBR2, ACVRL1, Smad3, and Smad4, leading to reduced phosphorylation of Smad2 and *ctgf* transcription (Zakrzewicz et al. 2007). In contrast, only a slight reduction in ERK1/2 phosphorylation, and no change in p38^{MAPK} phosphorylation, were observed, suggesting specific perturbation to TGF- β /BMP/Smad signaling in MCT-induced PH. Exclusive overexpression of BMPR2 by a gene therapy approach did not ameliorate MCT-induced PH in rats (McMurtry et al. 2007), illustrating that reconstitution of a single component of this pathway (i.e., *Bmpr2*) was unable to restore TGF- β /BMP signaling and, thus, did not prevent disease onset or progression. Interestingly, animals with MCT-induced PH exhibit an increased expression of elastase, and treatment with a serine elastase inhibitor completely reverses vascular remodeling in PAH (Cowan et al. 2000), underscoring the importance of ECM remodeling in this disease.

The available data concerning hypoxia-induced PAH suggest a more complex scenario. Although increased BMP ligand expression and signaling (assessed by Smad1/5/8 phosphorylation and *id1* expression) and increased MAPK phosphorylation have been reported by several independent investigators in hypoxia-induced PAH (Frank et al. 2005, Long et al. 2006), Takahashi et al. (2005, 2007) have reported decreased BMPR2, BMP-2, and Id1 expression, as well as decreased Smad1/5/8 phosphorylation in this chronic model. In addition, the in vitro exposure of rat PAEC to hypoxia down-regulated mRNA transcripts for *bmpr2* and *smad8* (Takahashi et al. 2007). In contrast to MCT-induced PH, overexpression of BMPR2 alone, as well as functional knockdown of TGFBR2, attenuated chronic hypoxia-induced PH (Chen et al. 2005, Reynolds et al. 2007). This suggests

that, at least in the mouse exposed to chronic hypoxia, restoration of BMPR2 (leading to increased BMP/Smad1/5 signaling) or ablation of TGFBR2 (leading to decreased TGF- β /Smad2/3 signaling) attenuated vascular remodeling, implying a functional imbalance of TGF- β and BMP signaling in favor of TGF- β in chronic hypoxia-induced PH.

Endothelial Cells

In addition to PASMC, dysregulated BMP/TGF- β signaling also impacts the endothelium. *Bmpr2* appears to play an opposite role in PAEC, compared with PASMC, because *Bmpr2* promotes PAEC survival and protects PAEC against apoptosis (Teichert-Kuliszewska et al. 2006). In addition, silencing the *bmpr2* gene increased the susceptibility of PAEC to apoptosis (Teichert-Kuliszewska et al. 2006), and overexpression of inhibitory Smad6 and Smad7, which antagonize BMP signaling, protected arterial endothelial cells from apoptosis induced by BMP-4 (Kiyono and Shibuya 2006). Given the prevalence of predicted *bmpr2* loss-of-function mutations in PAH patients (Lane et al. 2000, Thomson et al. 2000), these data suggest that loss of BMP function in the vascular endothelium increases the susceptibility of the endothelium to damage.

In contrast to these data, it has recently been reported that PAEC isolated from patients with IPAH exhibit an atypical hyperproliferative potential and increased bioenergetics in vitro, with decreased susceptibility to apoptosis (Masri et al. 2007, Xu et al. 2007). It has not yet been resolved whether these hyperproliferating PAEC cells are derived from a resident progenitor cell that is enriched in patients with IPAH, or whether these cells arise by epigenetic modification or from spontaneous somatic mutations or combinations thereof. Given that TGFBR2 is reportedly not expressed in PAEC in the core of plexiform lesions in IPAH (Richter et al. 2004, Yeager et al. 2002) and that TGF- β signaling is impaired in these core PAEC (irrespective of *bmpr2* status) (Richter et al. 2004), it is tempting to speculate that these hyperproliferative PAEC arise, in part, from resistance to TGF- β -regulated growth control. Taken together, impaired BMPR2 function in IPAH patients promotes PAEC loss via

increased susceptibility to apoptosis, causing obliteration of the microvasculature, which is thought to be the initiating lesion in IPAH. On the other hand, reduced TGFBR2 expression observed in the core PAEC of plexiform lesions would prevent TGF- β growth inhibition of PAEC and generate a hyperproliferative phenotype. It may well be that the vasculature reacts to an initial loss of PAEC by driving secondary PAEC proliferation, which, in turn, selects for and enriches apoptosis-resistant hyperproliferative PAEC, giving rise to plexiform lesion formation.

• Conclusions and Outlook

It appears that BMP/TGF- β signaling plays important but opposite roles in the maintenance and growth of PASMC vs. PAEC, with important consequences for the onset and development of PAH. Loss of BMP/TGF- β responsiveness in PAEC would yield a cell population that is more susceptible to apoptosis and may lead to significant endothelial damage, the improper repair of which may generate concentric and/or plexiform lesion formation. A similar defect of BMP/TGF- β responsiveness in PASMC would result in the loss of growth restriction and aberrant proliferation, causing increased vessel wall thickness, promoting concentric obliteration of the vessel lumen. Most likely, aberrant vascular remodeling in PAH is not secondary to a global loss of BMP/TGF- β responsiveness but, rather, to changes in the balance of different heteromeric BMP/TGF- β receptor complexes, which shift the balance from a growth inhibitory to a mitogenic response (Frank et al. 2005). This idea has emerged from observations that BMPR2 ablation in PASMC can diminish or augment cellular responses to different BMP ligands by promoting the formation of atypical type I-type II receptor complexes. For example, BMPR1a can form complexes with an alternative type II receptor, the activin receptor IIa (Yu et al. 2005), and BMP signals can be transmitted by activin A receptor, type I-IIa complexes. Neither complex forms in the presence of BMPR2, and these atypical complexes exhibit peculiar BMP signaling (Yu et al. 2005). This suggests that, if BMPR2 levels are reduced, BMPR1a is free to form alternative, atypical signaling complexes with other receptors, and aberrant BMP

signaling may thus be undertaken by these atypical complexes. As BMP/Smad1/5 and TGF- β /Smad2/3 signaling both require the common Smad4 for proper function, a functional antagonism of both signaling pathways may further perpetuate unopposed proliferation and ECM deposition in the vascular wall, a process that has been shown to occur during *Xenopus* embryogenesis (Candia et al. 1997).

Alternatively, the common pathogenic effect of heterogeneous *bmpr2* mutations in IPAH may be found in a yet underappreciated molecular system, the nonsense-mediated decay (NMD) system. Presumably, the diverse transcripts generated by transcription from mutated *bmpr2* alleles are degraded by the NMD system (Cogan et al. 2005, Machado et al. 2006), which constitutes an mRNA surveillance system that has evolved to degrade mRNA transcripts containing premature termination codons to prevent translation of aberrant transcripts (Kuzmiak and Maquat 2006). It is currently unclear whether cells with *bmpr2* mutations exhibit lower levels of BMPR2 translated from the normal allele compared with cells without mutations or whether mutated *bmpr2* transcripts result in properly processed but functionally altered BMPR2 proteins. As NMD does not work with a 100% efficiency (Kuzmiak and Maquat 2006), the relative translation efficiency of different mutated *bmpr2* transcripts may partly explain the different penetrance of disease in IPAH families. Detailed investigations of the expression patterns of BMPR2 protein variants in lungs from patients with IPAH, and possibly other tissues, will undoubtedly enrich our understanding of how *bmpr2* mutations lead to the dramatic phenotype of vascular remodeling in IPAH and possibly explain why this disease, in the presence of germline mutations occurring in all cells throughout the body, selectively affects only the pulmonary vasculature.

References

- Arcot SS, Lipke DW, Gillespie MN, Olson JW: 1993. Alterations of growth factor transcripts in rat lungs during development of monocrotaline-induced pulmonary hypertension. *Biochem Pharmacol* 46: 1086–1091.
- Atkinson C, Stewart S, Imamura T, et al: 2001. Immunolocalization of BMPR-II and

- TGF- β type I and II receptors in primary plexogenic pulmonary hypertension. *J Heart Lung Transplant* 20:149.
- Atkinson C, Stewart S, Upton PD, et al: 2002. Primary pulmonary hypertension is associated with reduced pulmonary vascular expression of type II bone morphogenetic protein receptor. *Circulation* 105:1672-1678.
- Beppu H, Ichinose F, Kawai N, et al: 2004. 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* 287:L1241-L1247.
- Bobik A: 2006. Transforming growth factor-betas and vascular disorders. *Arterioscler Thromb Vasc Biol* 26:1712-1720.
- Candia AF, Watabe T, Hawley SH, et al: 1997. Cellular interpretation of multiple TGF-beta signals: intracellular antagonism between activin/BVg1 and BMP-2/4 signaling mediated by Smads. *Development* 124:4467-4480.
- Chang H, Brown CW, Matzuk MM: 2002. Genetic analysis of the mammalian transforming growth factor-beta superfamily. *Endocr Rev* 23:787-823.
- Chen YF, Feng JA, Li P, et al: 2005. Dominant negative mutation of the TGF-beta receptor blocks hypoxia-induced pulmonary vascular remodeling. *J Appl Physiol* 100:564-571.
- Cogan JD, Vnencak-Jones CL, Phillips JA, III, et al: 2005. Gross BMPR2 gene rearrangements constitute a new cause for primary pulmonary hypertension. *Genet Med* 7:169-174.
- Cowan KN, Heilbut A, Humpl T, et al: 2000. Complete reversal of fatal pulmonary hypertension in rats by a serine elastase inhibitor. *Nat Med* 6:698-702.
- Deng Z, Morse JH, Slager SL, et al: 2000. Familial primary pulmonary hypertension (gene PPH1) is caused by mutations in the bone morphogenetic protein receptor-II gene. *Am J Hum Genet* 67:737-744.
- Du L, Sullivan CC, Chu D, et al: 2003. Signaling molecules in nonfamilial pulmonary hypertension. *N Engl J Med* 348:500-509.
- Frank DB, Abtahi A, Yamaguchi DJ, et al: 2005. Bone morphogenetic protein 4 promotes pulmonary vascular remodeling in hypoxic pulmonary hypertension. *Circ Res* 97:496-504.
- Humbert M, Morrell NW, Archer SL, et al: 2004. Cellular and molecular pathobiology of pulmonary arterial hypertension. *J Am Coll Cardiol* 43:13S-24S.
- Ihida-Stansbury K, McKean D, Lane K, et al: 2006. Tenascin-C is induced by mutated BMP type II receptors in familial forms of pulmonary arterial hypertension. *Am J Physiol Lung Cell Mol Physiol* 291:L694-L702.
- Jones PL, Rabinovitch M: 1996. Tenascin-C is induced with progressive pulmonary vascular disease in rats and is functionally related to increased smooth muscle cell proliferation. *Circ Res* 79:1131-1142.
- Jones PL, Cowan KN, Rabinovitch M: 1997. Tenascin-C, proliferation and subendothelial fibronectin in progressive pulmonary vascular disease. *Am J Pathol* 150:1349-1360.
- Kiyono M, Shibuya M: 2006. Inhibitory Smad transcription factors protect arterial endothelial cells from apoptosis induced by BMP4. *Oncogene* 25:7131-7137.
- Kuzmiak HA, Maquat LE: 2006. Applying nonsense-mediated mRNA decay research to the clinic: progress and challenges. *Trends Mol Med* 12:306-316.
- Lagna G, Nguyen PH, Ni W, Hata A: 2006. BMP-dependent activation of caspase-9 and caspase-8 mediates apoptosis in pulmonary artery smooth muscle cells. *Am J Physiol Lung Cell Mol Physiol* 291:L1059-L1067.
- Lane KB, Machado RD, Pauculo MW, et al: 2000. Heterozygous germline mutations in BMPR2, encoding a TGF-beta receptor, cause familial primary pulmonary hypertension. *Nat Genet* 26:81-84.
- Lepelet H, Eddahibi S, Fadel E, et al: 2005. Smooth muscle cell matrix metalloproteinases in idiopathic pulmonary arterial hypertension. *Eur Respir J* 25:834-842.
- Liu D, Wang J, Kinzel B, et al: 2007. Dosage-dependent requirement of BMP type II receptor for maintenance of vascular integrity. *Blood* 110:1502-1510.
- Long L, MacLean MR, Jeffery TK, et al: 2006. Serotonin increases susceptibility to pulmonary hypertension in BMPR2-deficient mice. *Circ Res* 98:818-827.
- Machado RD, Aldred MA, James V, et al: 2006. Mutations of the TGF-beta type II receptor BMPR2 in pulmonary arterial hypertension. *Hum Mutat* 27:121-132.
- Mandegar M, Fung YC, Huang W, et al: 2004. Cellular and molecular mechanisms of pulmonary vascular remodeling: role in the development of pulmonary hypertension. *Microvasc Res* 68:75-103.
- Masri FA, Xu W, Comhair SA, et al: 2007. Hyperproliferative apoptosis-resistant endothelial cells in idiopathic pulmonary arterial hypertension. *Am J Physiol Lung Cell Mol Physiol* 239:L548-L554.
- Massague J, Seoane J, Wotton D: 2005. Smad transcription factors. *Genes Dev* 19:2783-2810.
- McMurtry MS, Moudgil R, Hashimoto K, et al: 2007. Overexpression of human bone morphogenetic protein receptor 2 does not ameliorate monocrotaline pulmonary arterial hypertension. *Am J Physiol Lung Cell Mol Physiol* 292:L872-L878.
- Miyazono K, Maeda S, Imamura T: 2005. BMP receptor signaling: transcriptional targets, regulation of signals, and signaling cross-talk. *Cytokine Growth Factor Rev* 16:251-263.
- Morrell NW, Yang X, Upton PD, et al: 2001. Altered growth responses of pulmonary artery smooth muscle cells from patients with primary pulmonary hypertension to transforming growth factor-beta(1) and bone morphogenetic proteins. *Circulation* 104:790-795.
- Morty RE, Nejman B, Kwapiszewska G, et al: 2007. Dysregulated bone morphogenetic protein signaling in monocrotaline-induced pulmonary arterial hypertension. *Arterioscler Thromb Vasc Biol* 27:1072-1078.
- Newman JH: 2005. Pulmonary hypertension. *Am J Respir Crit Care Med* 172:072-077.
- Newman JH, Trembath RC, Morse JA, et al: 2004. Genetic basis of pulmonary arterial hypertension: current understanding and future directions. *J Am Coll Cardiol* 43:33S-39S.
- Newman JH, Wheeler L, Lane KB, et al: 2001. Mutation in the gene for bone morphogenetic protein receptor II as a cause of primary pulmonary hypertension in a large kindred. *N Engl J Med* 345:319-324.
- Owens GK, Kumar MS, Wamhoff BR: 2004. Molecular regulation of vascular smooth muscle cell differentiation in development and disease. *Physiol Rev* 84:767-801.
- Pietra GG, Capron F, Stewart S, et al: 2004. Pathologic assessment of vasculopathies in pulmonary hypertension. *J Am Coll Cardiol* 43:25S-32S.
- Reynolds AM, Xia W, Holmes MD, et al: 2007. Bone morphogenetic protein type 2 receptor gene therapy attenuates hypoxic pulmonary hypertension. *Am J Physiol Lung Cell Mol Physiol* 292:L1182-L1192.
- Richter A, Yeager ME, Zaiman A, et al: 2004. Impaired transforming growth factor-beta signaling in idiopathic pulmonary arterial hypertension. *Am J Respir Crit Care Med* 170:1340-1348.
- Roberts AB: 1998. Molecular and cell biology of TGF-beta. *Miner Electrolyte Metab* 24:111-119.
- Rudarakanchana N, Flanagan JA, Chen H, et al: 2002. Functional analysis of bone morphogenetic protein type II receptor mutations underlying primary pulmonary hypertension. *Hum Mol Genet* 11:1517-1525.
- Song Y, Jones JE, Beppu H, et al: 2005. Increased susceptibility to pulmonary hypertension in heterozygous BMPR2-mutant mice. *Circulation* 112:553-562.
- Tada Y, Majka S, Carr M, et al: 2007. Molecular effects of loss of BMPR2 signaling in smooth muscle in a transgenic mouse model of PAH. *Am J Physiol Lung Cell Mol Physiol* 292:L1556-L1563.
- Takahashi H, Goto N, Kojima Y, et al: 2005. Down-regulation of type II bone morphogenetic protein receptor in hypoxic pulmonary hypertension. *Am J Physiol Lung Cell Mol Physiol* 290:L450-L458.
- Takahashi K, Kogaki S, Matsushita T, et al: 2007. Hypoxia induces alteration of bone

morphogenetic protein receptor signaling in pulmonary artery endothelial cell. *Pediatr Res* 61:392–397.

Takeda M, Otsuka F, Nakamura K, et al: 2004. Characterization of the bone morphogenetic protein (BMP) system in human pulmonary arterial smooth muscle cells isolated from a sporadic case of primary pulmonary hypertension: roles of BMP type IB receptor (activin receptor-like kinase-6) in the mitotic action. *Endocrinology* 145: 4344–4354.

Tanaka Y, Schuster DP, Davis EC, et al: 1996. The role of vascular injury and hemodynamics in rat pulmonary artery remodeling. *J Clin Invest* 98:434–442.

Teichert-Kuliszewska K, Kutryk MJ, Kuliszewska MA, et al: 2006. Bone morphogenetic protein receptor-2 signaling promotes pulmonary arterial endothelial cell survival: implications for loss-of-function mutations in the pathogenesis of pulmonary hypertension. *Circ Res* 98:209–217.

Thomson JR, Machado RD, Pauciulo MW, et al: 2000. Sporadic primary pulmonary hypertension is associated with germline mutations of the gene encoding BMPRII, a receptor member of the TGF- β family. *J Med Genet* 37:741–745.

Topper JN: 2000. TGF- β in the cardiovascular system: molecular mechanisms of a context-specific growth factor. *Trends Cardiovasc Med* 10:132–137.

Trembath RC: 2001. Mutations in the TGF- β type 1 receptor, ALK1, in combined primary pulmonary hypertension and hereditary haemorrhagic telangiectasia, implies pathway specificity. *J Heart Lung Transplant* 20:175.

West J, Fagan K, Steudel W, et al: 2004. Pulmonary hypertension in transgenic mice expressing a dominant-negative BMPRII gene in smooth muscle. *Circ Res* 94:1109–1114.

Wu X, Chang MS, Mitsialis SA, Kourembanas S: 2006. Hypoxia regulates bone morphogenetic protein signaling through C-terminal-binding protein 1. *Circ Res* 99:240–247.

Xu W, Koeck T, Lara AR, et al: 2007. Alterations of cellular bioenergetics in pulmonary artery endothelial cells. *Proc Natl Acad Sci U S A* 104:1342–1347.

Yang X, Long L, Southwood M, et al: 2005. Dysfunctional Smad signaling contributes to abnormal smooth muscle cell proliferation in familial pulmonary arterial hypertension. *Circ Res* 96:1053–1063.

Yeager ME, Golpon HA, Voelkel NF, Tuder RM: 2002. Microsatellite mutational analysis of endothelial cells within plexiform lesions from patients with familial, pediatric, and sporadic pulmonary hypertension. *Chest* 121:61S.

Yu PB, Beppu H, Kawai N, et al: 2005. Bone morphogenetic protein (BMP) type II recep-

tor deletion reveals BMP ligand-specific gain of signaling in pulmonary artery smooth muscle cells. *J Biol Chem* 280: 24443–24450.

Yuan JX, Rubin LJ: 2005. Pathogenesis of pulmonary arterial hypertension: the need for multiple hits. *Circulation* 111:534–538.

Zakrzewicz A, Kouri FM, Nejman B, et al: 2007. The transforming growth factor- β /Smad2,3 signalling axis is impaired in

experimental pulmonary hypertension. *Eur Respir J* 29:1094–1104.

Zhang S, Fantozzi I, Tigno DD, et al: 2003. Bone morphogenetic proteins induce apoptosis in human pulmonary vascular smooth muscle cells. *Am J Physiol Lung Cell Mol Physiol* 285:L740–L754.

PII S1050-1738(07)00180-6

TCM

An Oxidized Lipid–Peroxisome Proliferator-Activated Receptor γ -Chemokine Pathway in the Regulation of Macrophage-Vascular Smooth Muscle Cell Adhesion

Jana Barlic and Philip M. Murphy*

Recent genetic studies have implicated pro-inflammatory chemokines and chemokine receptors in atherogenesis. Studies at the molecular and cellular levels have suggested specific atherogenic mechanisms for two chemokine-chemokine receptor pairs, CCL2-CCR2 and CX3CL1-CX3CR1, involving differential receptor regulation by the transcription factor peroxisome proliferator-activated receptor γ . This pathway is triggered by oxidized proatherogenic lipids, such as oxidized low-density lipoprotein and linoleic acid derivatives, which promote differentiation of CCR2^{hi}CX3CR1^{lo} human monocytes to CCR2^{lo}CX3CR1^{hi} macrophages that adhere to coronary artery smooth muscle cells in a CX3CR1- and peroxisome proliferator-activated receptor γ -dependent manner. Switching CX3CR1 on and CCR2 off in vivo may result in cessation of CCR2-dependent migration and activation of CX3CR1-dependent retention that together may promote foam cell accumulation in the vessel wall. (Trends Cardiovasc Med 2007;17:269–274) © 2007, Elsevier Inc.

• Introduction

Atherogenesis is now widely accepted to involve not only progressive buildup of oxidized lipids in the arterial wall but also chronic inflammation in which progres-

sion toward a rupture-prone, unstable atherosclerotic plaque is driven in part by leukocytes infiltrating the vascular subendothelium (Ross 1999). The molecular basis for leukocyte accumulation in the vessel wall has not been clearly delineated; however, there is increasing

Jana Barlic is at the Leukocyte Biology Section, National Heart and Lung Institute, Faculty of Medicine, Imperial College London, South Kensington Campus, SW7 2AZ London, United Kingdom. Philip M. Murphy is at the Molecular Signaling Section, Laboratory of Molecular Immunology, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD 20892.

* Address correspondence to: Philip M. Murphy, MD, Bldg 10, Rm 11N113, National Institutes of Health, Bethesda, MD 20892, USA. Tel.: (+1) 301 496 8616; fax: (+1) 301 402 4369; e-mail: pmm@nih.gov.

© 2007, Elsevier Inc. All rights reserved. 1050-1738/07/\$-see front matter