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Authors

Morrell, Nicholas W
Bloch, Donald B
ten Dijke, Peter
et al.

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Targeting BMP signalling in cardiovascular disease and anaemia

Nicholas W. Morrell, Donald B. Bloch, Peter ten Dijke, Marie-Jose T. H. Goumans, Akiko Hata, Jim Smith, Paul B. Yu and Kenneth D. Bloch

Abstract | Bone morphogenetic proteins (BMPs) and their receptors, known to be essential regulators of embryonic patterning and organogenesis, are also critical for the regulation of cardiovascular structure and function. In addition to their contributions to syndromic disorders including heart and vascular development, BMP signalling is increasingly recognized for its influence on endocrine-like functions in postnatal cardiovascular and metabolic homeostasis. In this Review, we discuss several critical and novel aspects of BMP signalling in cardiovascular health and disease, which highlight the cell-specific and context-specific nature of BMP signalling. Based on advancing knowledge of the physiological roles and regulation of BMP signalling, we indicate opportunities for therapeutic intervention in a range of cardiovascular conditions including atherosclerosis and pulmonary arterial hypertension, as well as for anaemia of inflammation. Depending on the context and the repertoire of ligands and receptors involved in specific disease processes, the selective inhibition or enhancement of signalling via particular BMP ligands (such as in atherosclerosis and pulmonary arterial hypertension, respectively) might be beneficial. The development of selective small molecule antagonists of BMP receptors, and the identification of ligands selective for BMP receptor complexes expressed in the vasculature provide the most immediate opportunities for new therapies.

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Introduction

Bone morphogenetic proteins (BMPs) were originally discovered because of their capacity to induce the formation of bone and cartilage in ectopic sites. Marshall Urist, a pioneer in this field, first observed that demineralized bone matrix induced bone growth when implanted into rabbit muscle, suggesting that the matrix contained bone morphogenetic activity.¹ Subsequent efforts to purify BMP and to elucidate its amino acid sequence revealed several closely related proteins of the transforming growth factor β (TGF- β) family of secreted ligands.² BMPs account for 20 of the 33 known members of the TGF- β superfamily in humans. They are highly conserved throughout evolution, possessing orthologues in vertebrates and invertebrates, including Cnidaria and sponges. BMPs are usually secreted as active dimeric complexes, and some are bound to a prodomain (such as BMP9 and BMP10). BMPs communicate with neighbouring cells primarily in a paracrine or autocrine fashion, and local concentration gradients of BMPs are thus critical during early development and organogenesis. Several BMPs, including BMP6, BMP9, and BMP10 also circulate in blood,^{3–6} and have the potential to exert effects on distant tissues and organs. BMPs thereby function as important endocrine regulators of cardiovascular, metabolic, and haematopoietic function (Table 1).

The past 15 years has witnessed an increasing awareness of the fundamental role of BMPs in the development and maintenance of homeostasis in the heart and circulation, and the perturbation of BMP signalling in cardiovascular disease, with evidence of elevated and reduced BMP signalling activity in different disease states. Increased activity of BMP is associated with vascular inflammation, atherosclerosis, and calcification, as well as anaemia of inflammation. Conversely, reduced BMP signalling is associated with pulmonary arterial hypertension (PAH) and hereditary haemorrhagic telangiectasia (HHT). Promising strategies have now been developed to inhibit or enhance BMP signalling in pre-clinical models of cardiovascular disease and anaemia, and in future might hold promise as a novel therapy for these disorders. In this Review, we discuss the role of BMP ligands, receptors, and signalling pathways in cardiovascular development and disease. In addition, we indicate how this knowledge might be exploited to treat specific cardiovascular diseases and anaemia.

Structural diversity of BMPs

Like other members of the TGF- β family, BMPs bind to serine–threonine kinase receptors, known as type I and type II receptors. Similar to TGF- β ligands, BMPs form heterotetrameric signalling complexes with two type I and two type II receptors, but unlike TGF- β ligands, BMPs bind with greater affinity to the type I rather than type II receptors. Based on their structural homology,

Department of Medicine, University of Cambridge School of Clinical Medicine, UK (N.W.M.). Center for Immunology and Inflammatory Diseases, Massachusetts General Hospital and Harvard Medical School, USA (D.B.B.). Department of Molecular Cell Biology and Cancer Genomics Centre Netherlands, Leiden University Medicine Centre, Netherlands (P.t.D., M.J.T.H.G.). Cardiovascular Research Institute, University of California, USA (A.H.). MRC National Institute for Medical Research, UK (J.S.). Department of Medicine, Brigham and Women's Hospital, USA (P.B.Y.). Anaesthesia Centre for Critical Care Research, Department of Anaesthesia, Critical Care and Pain Medicine, 55 Fruit Street, Boston, MA 02114, USA (K.D.B.).

Correspondence to: N.W.M. nwm23@cam.ac.uk

Competing interests

The authors declare no competing interests.

Key points

- Bone morphogenetic proteins (BMPs) have important roles in cardiovascular growth, homeostasis, and disease development
- The wide repertoire of BMP ligands and receptors, coupled with numerous modifiers of BMP signalling, confer marked tissue-specific and cell-specific responses to BMPs
- Understanding the context-specific nature of BMP signalling can help to guide the development of novel therapeutics to treat cardiovascular disease and anaemia
- Small molecule inhibition of BMP signalling is efficacious in preclinical models of atherosclerosis, vascular calcification, and anaemia
- Enhancement of BMP receptor-II (BMPR-II) signalling by increasing cell-surface levels of BMPR-II or by endothelial-selective BMPR-II agonists such as BMP9 shows promise in preclinical models of pulmonary arterial hypertension

BMP type I receptors can be divided into two groups: the BMP type-IA receptor (BMPR-IA; also known as activin receptor-like kinase-3, or ALK3) and BMP type-IB receptor (BMPR-IB; also known as ALK6) group, and the serine/threonine-protein kinase receptor R3 (SKR3, also known as ALK1) and activin receptor type-1 (ACTR-I; also known as ALK2) group. Whereas ALK2, ALK3, and ALK6 are widely expressed by diverse cell types, ALK1 is more restricted to endothelial cells. Type II receptors of the BMP family include the BMP type II receptor (BMPR-II), activin receptor type-2A (ACTRIIA) and activin receptor type-2B (ACTRIIB). BMPR-II is selective for BMPs, whereas ACTRIIA and ACTRIIB are activated in response to activins, growth differentiation factors, and Nodal. Both BMPR-II and ACTRIIA are expressed broadly in mesenchyme-derived tissues, but only BMPR-II is expressed at high levels in endothelial and endocardial tissues. Upon complex formation, the type II receptors transphosphorylate specific residues within type I receptor intracellular glycine-serine-rich juxtamembrane regions, causing type I receptor activation and phosphorylation of receptor-regulated SMAD (R-SMAD) effector proteins. BMP ligands can activate the BMP-responsive SMADs 1, 5, and 8, but exert diverse effects on target tissues, owing in part to their structural and receptor complementarity. BMPs form subgroups based on their affinities for specific receptors. BMP2 and BMP4 bind preferentially to BMPR-II in a complex with ALK3 or ALK6, whereas BMP6 and BMP7 bind to ACTRIIA with ALK2, and BMP9 and BMP10 bind to BMPR-II in combination with ALK1 or ALK2 (Table 1).^{7–10} While BMPs have been most extensively studied as homodimeric ligands, the interaction of heterodimeric ligands such as BMP2 and BMP7, and their respective heteromeric ALK2 and ALK3 receptor complexes has become an increasingly appreciated signalling motif in vertebrate development.¹¹

Regulation of BMP signalling

Given that BMPs are potent modulators of cell growth and fate, each component of the BMP signalling pathway is subjected to extensive positive and negative regulation in order to maintain the integrity of tissue development and repair. The expression of BMP antagonists is especially important during development, when BMP signalling gradients are established by diffusion to

fine-tune the magnitude and duration of BMP signalling.¹² A large number of extracellular (chordin, noggin, and gremlin) and membrane-bound (BMP-binding endothelial regulator protein [BMPER], Twisted gastrulation, matrix Gla protein, and neogenin) antagonist molecules sequester BMP ligands to reduce signalling. Noggin is the prototypical secreted antagonist, and possesses high affinity for BMP2 and BMP4, but low affinity for BMP6 and BMP9, whereas BMPER, a transmembrane protein, potently inhibits BMP9 in the context of endothelial signalling.^{13,14} After signalling activation, phosphorylated R-SMADs form heteromeric complexes with a common-mediator SMAD (SMAD4) and translocate into the nucleus and directly regulate target gene expression by binding to SMAD-binding elements. This complex can also indirectly regulate gene expression by interacting with DNA-binding transcription factors, or by associating with co-activators or co-repressors, and histone-modifying factors. Targets of BMP/SMAD transcriptional activity include the inhibitory-SMADs (SMAD6 and SMAD7) and the BMP and activin membrane-bound inhibitor homologue (BAMBI). SMAD6 and SMAD7 provide feedback inhibition to antagonize BMP and TGF- β signalling by inducing degradation of their respective receptors and R-SMADs, whereas BAMBI sequesters ligands away from receptors, thereby inhibiting BMP signalling. In addition, several classes of BMP co-receptors that lack enzymatic activity, but modulate BMP ligand-receptor interactions, have also been identified. These co-receptors include the so-called type III receptors endoglin and betaglycan, as well as the glycosylphosphatidylinositol-anchored repulsive guidance molecule proteins, all of which potentiate ligand-specific signalling to modify the cellular consequences of signalling.^{15–17} These BMP co-receptors can be produced via pro-protein convertases or can be shed from the cell surface by matrix metalloproteases, resulting in soluble receptor fragments that function as BMP ligand traps.¹⁸

The repertoire of BMP receptors, co-receptors, and modulators is highly specialized in the cardiovascular system, helping to confer spatiotemporal specificity to BMP signalling, and particularly for sensitizing endothelial and endocardial tissues to BMP9 and BMP10. BMPR-II, ALK1, and endoglin are abundant in vascular endothelium, as well as in embryonic ventricular and atrioventricular endocardium. Indeed, the depletion of ALK1¹⁹ or endoglin^{20,21} in endothelial cells from diverse vascular beds significantly impairs signalling via BMP9 and BMP10, and diminishes BMP9-mediated modulation of angiogenic activity. Whereas the depletion of BMPR-II does not abolish BMP9 or BMP10 signalling, the loss of BMPR-II in endothelium substantially impairs the downstream antimitotic and cytokine regulatory functions of BMP9.²² ALK2 is also expressed in the endothelium, and its signalling seems to modulate the expression of ALK1 to respond dynamically to various stimuli.²³ BMPR-II, ACTRIIA, ALK2, and ALK3 are expressed in cardiomyocytes and vascular smooth muscle, and signalling is activated in response to their canonical ligands to modulate cell phenotype, growth,

Table 1 | BMP ligand expression in cardiovascular biology

Ligand	Source	Activity	Target(s)	Receptor complementarity			Effector(s)
				Type I	Type II	Type III	
BMP2/4	ECs, SMCs Cardiomyocytes	Autocrine	ECs, SMCs	ALK3	BMPR-II	—	SMAD1/5/8
		Paracrine	Cardiomyocytes	ALK2	ACTRIIA		
				ALK6			
BMP6	Hepatocytes	Autocrine	Hepatocytes	ALK3	BMPR-II	Haemojuvelin	SMAD1/5/8
		Paracrine	ECs, SMCs	ALK2	ACTRIIA		
		Endocrine					
BMP9	Hepatocytes ECs	Endocrine	ECs	ALK1	BMPR-II	Endoglin	SMAD1/5/8 SMAD2/3
		Autocrine	ECs	ALK2	ACTRIIA		
BMP10	Cardiomyocytes	Autocrine	Cardiomyocytes	ALK1	BMPR-II	Endoglin	SMAD1/5/8 SMAD2/3
		Endocrine	ECs	ALK2	ACTRIIA		

Abbreviations: ACTRIIA, activin receptor type 2a; ALK1, activin receptor-like kinase 1 (also known as serine/threonine-protein kinase receptor R3); ALK2, activin receptor-like kinase 2 (also known as activin receptor type-1); ALK3, activin receptor-like kinase 3 (also known as BMP type-IA receptor); ALK6, activin receptor-like kinase 6 (also known as BMP type-IB receptor); BMP, bone morphogenetic protein; BMPR-II, BMP type II receptor; EC, endothelial cell; SMC, smooth muscle cell.

and survival.^{24–29} In addition, ALK2 and ALK3 contribute to the calcification of vascular smooth muscle cells.^{30,31} BMPER and Twisted gastrulation modulate BMP9 signalling to regulate these functions in the endothelium and thereby maintain vascular homeostasis.³² As a group, these molecules have essential roles in vasculogenesis, cardiomyogenesis, ventricular compaction, septation, valve formation, and maintenance of the integrity of the pulmonary and lymphatic circulation.³³

Cross-talk with other signalling pathways

BMPs and their signalling effector molecules exhibit various degrees of cross-talk with other pathways essential to vascular development and homeostasis, including vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF), and Notch and Wnt signalling.^{16,34–36} In the cardiovascular system, the recruitment or modulation of these pathways by BMP signalling can be critical for proper function; BMP signalling via SMAD1/5 activation regulates the expression of Jagged 1 in endothelial cells to transactivate Notch signalling in neighbouring cells.³⁷ BMPs also coregulate the Notch transcriptional targets *HES1* and *HEY1* in mesenchymal lineages via a Notch intracellular domain–SMAD interaction to modulate cellular plasticity.^{38–40} Similarly, the interplay between BMP and delta-ligand 4/Notch pathways determines the identity of tip versus stalk cells during angiogenesis.⁴¹ BMP9 has been documented to suppress VEGF expression and VEGF-induced angiogenesis via ALK1 and BMPR-II signalling,^{16,35} whereas in the developing outflow tract of the heart, BMP4 and BMP7 repress VEGFA expression via the miRNA-17-92 cluster to stimulate outflow tract cushion formation.⁴² Furthermore, during cardiac cushion formation, the coordination of the endothelial-to-mesenchymal transformation response is mediated by the interplay of Notch, BMP, and TGF- β signalling.^{43,44} The antagonism of FGF signalling by BMP ligands is necessary for specifying cardiomyocyte fate in the early embryo or in embryonic stem cells.⁴⁵ Taken together, these findings show that the BMP pathway provides essential gating, amplifying, and damping effects on Notch, Wnt, VEGF, and FGF signalling in vascular development and homeostasis.

Experimental evaluation of BMP signalling

Early BMP genetic ablation procedures that resulted in severe defects with prenatal or perinatal lethality revealed the importance of many receptors of the BMP signalling pathway for embryogenesis and organogenesis (Table 2). Subsequent studies to address postnatal and physiological functions of BMP ligands have utilized Cre–Lox technology for postnatal ablation of BMP receptors, or have targeted the individual ligands themselves (Table 2). Genetic and pharmacological epistasis has been accomplished using several methods: transgenic expression or administration of recombinant BMPs or endogenous BMP signalling inhibitors (such as noggin or gremlin); antibodies directed against specific BMP ligands; and recombinant ligand traps derived from receptor extracellular domains that scavenge subsets of BMP ligands (such as ALK1-Fc, ALK3-Fc, and Haemojuvelin-Fc).^{31,46,47} Small molecule inhibitors of BMP type I receptors also allow temporally restricted modulation of BMP signalling (Table 3). Together, these experimental approaches to evaluate BMP signalling have helped to elucidate their roles in diverse biological processes such as iron metabolism and inflammation, and in cardiovascular diseases such as atherosclerosis, PAH, and vascular calcification.

A small molecule inhibitor of BMP signalling, dorsomorphin, was identified by screening a library of small molecules for their capacity to induce dorsalization in embryonic zebrafish.⁴⁸ This compound mimicked a mild form of dorsalization seen in zebrafish deficient in BMP type I receptor orthologues, or fish overexpressing noggin or chordin.⁴⁹ Dorsomorphin inhibited the expression of hepcidin, a master iron regulatory hormone, in zebrafish and mice via the antagonism of BMP signalling in the liver. Given that dorsomorphin is a broad acting multi-kinase inhibitor identified originally for its capacity to inhibit AMP-activated protein kinase (compound C), further potent and selective inhibitors of BMP type I receptors were generated, including pyrazolopyrimidine analogues,⁵⁰ and other scaffolds such as 2-aminopyridine compound K02288 and its derivatives.^{51,52} These small molecules have been used extensively to study the role of BMP signalling *in vitro*, as well as the postnatal roles of BMP signalling *in vivo*.

Table 2 | Cardiovascular phenotypes of BMP mutant models

Gene/Protein	Mutation	Tissue	Phenotype	Analogous human syndrome
<i>BMPR2/BMPR-II</i>	Null	Global	Embryonic lethality owing to gastrulation defect ¹⁸⁹	—
	Heterozygous	Global	Mild susceptibility to PAH ¹⁹⁰	Preclinical HPAH
	N-terminal exon 2 deletion	Global	Outflow tract and septation defects ¹⁹¹ and susceptibility to hypoxic PAH ¹⁹²	Congenital heart disease with PAH ¹⁹³
	Dominant negative transgene	Smooth muscle	PAH and pulmonary vascular remodelling ¹³⁰	HPAH ^{121,194}
	R899X (premature truncation)	Smooth muscle or global	PAH and pulmonary vascular lesions ^{195,196}	HPAH ^{121,194}
<i>ACVRL1/ALK1</i>	Null	Global	Midgestational lethality owing to cavernous vessel defects, arterial dilatation, smooth muscle recruitment defects ^{155,156}	—
	Heterozygous	Global	Arteriovenous malformations with HHT-like features ^{155,197}	HHT-2 ¹⁹⁸ with or without PAH ¹⁹⁹
<i>ENG/Endoglin</i>	Null	Global	Embryonic lethality owing to yolk-sac angiogenesis defect ^{154,200}	—
	Heterozygous	Global	Protection against RV hypertrophy in RV pressure overload ^{178,179}	HHT-1 ²⁰¹
<i>GDF2/BMP9</i>	Null	Global	Viable with abnormal lymphatic development and drainage ²⁰²	HHT-5 ¹⁶⁰
<i>BMP10/BMP10</i>	Null	Global	Embryonic lethality with diminished cardiomyocyte proliferation ⁹⁶	—
<i>BMP6/BMP6</i>	Null	Global	Massive iron overload owing to defective hepcidin expression ²⁰³	Haemochromatosis owing to mutations at other loci
<i>BMP2/BMP2</i>	Null	Global	Embryonic lethality with defects in extra-embryonic and cardiac development ²⁰⁴	—
<i>BMP4/BMP4</i>	Null	Myocardium	Defects in atrioventricular septation ²⁰⁵	Atrioventricular canal defect
<i>ACVR1/ALK2</i>	Null	Endocardial cells	Defects in atrioventricular septa and valves ⁹⁵	Congenital septal and valvular defects
<i>BMPR1A/ALK3</i>	Null	Global	Early embryonic lethality ²⁰⁶	—
	Null	Smooth muscle	Cardiac structural and vasculogenesis defect ²⁰⁷	—
	Heterozygous or null	Patchy smooth muscle	Decreased susceptibility to hypoxic PAH and increased proximal pulmonary arterial stiffness ^{208,209}	Familial juvenile polyposis ^{210,211}
<i>BMPR1B/ALK6</i>	Null	Global	Chondrogenesis defects ²¹²	Associated mutations in paediatric PAH ²¹³

Abbreviations: ALK1, activin receptor-like kinase 1 (also known as serine/threonine-protein kinase receptor R3); ALK2, activin receptor-like kinase 2 (also known as activin receptor type-1); ALK3, activin receptor-like kinase 3 (also known as BMP type-IA receptor); ALK6, activin receptor-like kinase 6 (also known as BMP type-IB receptor); BMP, bone morphogenetic protein; BMPR-II, BMP type II receptor; HHT, hereditary haemorrhagic telangiectasia; HPAH, heritable pulmonary arterial hypertension; PAH, pulmonary arterial hypertension; RV, right ventricular.

BMPs and the cardiovascular system Vasculature

The critical role of BMP signalling in the vasculature is evident from both clinical and experimental observations. Genetic lesions in this pathway result in the development of congenital vascular syndromes, and corresponding phenotypes are also observed in loss-of-function animal models (Table 2). Consistent with these roles in disease, BMPs exert potent effects on all vascular cell lineages, affecting the capacity of endothelial cells to migrate, proliferate, and form basic tubular structures, and establishing the fates of surrounding pericytes, vascular smooth muscle, and adventitial cells that contribute to the structural integrity and function

of the vessel.⁵³ Similar to other developmental processes, BMP signalling is tightly regulated in a spatiotemporal manner, and is thus lineage-sensitive, tissue-sensitive, and context-sensitive (Figure 1).

BMPs can function in a proangiogenic or antiangiogenic manner, depending on its ligand, concentration, cellular target, and the context.⁵⁴ Although a number of BMPs have been reported to modulate angiogenesis, BMP9 is the most important BMP ligand in the vasculature owing to its presence in the circulation and the high expression of its cognate receptors BMPR-II, ALK1, and endoglin in endothelial and smooth muscle cells. In human pulmonary artery endothelial cells, BMP9 signals through distinct type II receptors to stimulate SMAD1

Table 3 | Cardiovascular disease models probed with BMP inhibitors

Inhibitor	Model	Route	Effect	Application
Dorsomorphin	TNF- α induced vascular inflammation in mice ²¹⁴	Systemic	Inhibited leucocyte adhesion after challenge with TNF- α	Vascular inflammation
Dorsomorphin and LDN-193189	Zebrafish vasculogenesis ²¹⁵	Embryonic	Dorsalization without intersomitic vessel disruption using selective inhibitor LDN-193189	—
LDN-193189	Subtotal nephrectomy model in mice ²¹⁶	Systemic	Attenuation of chronic kidney disease-induced endothelial dysfunction and smooth muscle osteogenic differentiation	Chronic kidney disease and associated vascular calcification
LDN-193189 and ALK3-Fc	<i>Ldlr</i> -deficient hyperlipidaemic mice ³¹	Systemic	Attenuation of vascular calcification, vascular inflammation, and atherosclerosis	Atherosclerosis and vascular calcification
LDN-193189 and ALK3-Fc	<i>Mgp</i> -deficient mice ³⁰	Systemic	Attenuation of vascular calcification and improved survival	Vascular medial calcification (Monckeberg arteriosclerosis)
LDN-193189	<i>ApoE</i> -deficient hyperlipidaemic mice ¹⁷³	Systemic	Reduced foam cell formation, atherosclerotic plaque formation, and vascular inflammation via inhibition of hepcidin to reduce macrophage iron stores	Atherosclerosis and vascular inflammation
Anti-ALK1	<i>In vitro</i> endothelial cell sprouting ²¹⁷	<i>In vitro</i>	Inhibited <i>in vitro</i> angiogenic activity in a VEGF-independent manner	Solid tumour angiogenesis
sEng-Fc	Angiogenesis and tumour xenograft vascularization ¹⁸⁰	Systemic	Inhibited tumour xenograft vascularization in a BMP9-dependent and BMP10-dependent manner	Solid tumour angiogenesis
ALK1-Fc	Angiogenesis and tumour xenograft vascularization ⁵⁶	Systemic	Inhibited tumour xenograft vascularization	Solid tumour angiogenesis
LDN-193189 and HJV-Fc	Rats with anaemia of inflammation ¹⁸⁷	Systemic	Blockade of BMP signalling mobilized iron from reticuloendothelial cells and reversed anaemia	Anaemia of inflammation
LDN-193189 and ALK3-Fc	Mice with anaemia of inflammation ¹⁸⁶	Systemic	Blockade of BMP signalling restores iron bioavailability, decreases hepcidin, prevents development of anaemia, and reverses established anaemia owing to chronic inflammation	Anaemia of inflammation
LDN-193189	Wild-type mice ¹⁸⁸	Oral	Oral administration of BMP inhibitor prevents anaemia associated with inflammation	Anaemia of inflammation

Abbreviations: ALK1, activin receptor-like kinase 1 (also known as serine/threonine-protein kinase receptor R3); ALK3, activin receptor-like kinase 3 (also known as BMP type-IA receptor); BMP, bone morphogenetic protein; HJV, haemojuvelin; sEng, soluble endoglin; TNF- α , tumour necrosis factor- α ; VEGF, vascular endothelial growth factor.

and SMAD2 phosphorylation and to induce distinct target genes.²² Under normal conditions, BMP9 primarily has an angiostatic effect. BMP9 inhibits the formation of vessel-like tubular networks *in vitro*, which requires the function of BMPR-II and ALK1. In addition, BMP9 inhibits VEGF and FGF-induced angiogenic responses. Furthermore, BMP9 has been shown to inhibit lymphangiogenesis.³³ Interestingly, low BMP9 concentrations were reported to have proangiogenic properties,⁵⁵ and inhibiting BMP9 levels using an ALK1-Fc ligand trap was shown to inhibit tumour angiogenesis.^{56,57} In addition, other BMPs, including BMP2, BMP4, BMP6, and BMP7, have been shown to induce endothelial cell proliferation, migration, and formation of tube-like structures, and to promote angiogenesis *in vitro* and *in vivo* in mouse models.⁵⁸ The induction of inhibitor of DNA binding protein (Id) expression by BMP contributes to its proangiogenic response.^{59,60} The indirect induction of alpha-crystallin B chain signalling via Id1 by BMP further contributes to the survival of pulmonary microvascular endothelial cells.⁶¹ Id proteins also have an important effector role in the regulation of smooth muscle cell function by BMPs. Id1 and Id3 are potently regulated by BMP signalling in pulmonary arterial smooth muscle cells *in vitro*,⁶² are jointly upregulated by hypoxia in pulmonary vascular smooth muscle *in vivo*,⁶³ and might have a complementary and partially

redundant role in regulating cell cycling in vascular and other tissues.⁶⁴ BMPs also function to maintain the contractile phenotype of vascular smooth muscle cells by inducing the expression of miRNA-21,⁶⁵ either through suppression of miRNA-96,⁶⁶ or via the induction of myocardin-related transcription factors.⁶⁷

Finally, physiological stimuli including shear stress can influence endothelial cell behaviour in a BMP-dependent manner. For example, in response to oscillatory shear stress, BMPR-II mediates a physical association between BMPR1B and integrin $\alpha V\beta 3$ in endothelial cells, thereby inducing endothelial proliferation.⁶⁸ BMP receptor activation under conditions of shear stress might involve the cAMP-dependent transcription factor ATF-2 pathway, and can stimulate the secretion of BMP ligands during early haematopoietic stem cell differentiation.⁶⁹ In zebrafish, BMP10 induces vascular quiescence via shear stress in an ALK1-dependent manner.^{5,70} Thus, whether BMP has positive or negative effects on the vasculature is highly dependent on the BMP isoform, concentration, and cellular context.⁷¹

Cardiovascular development

A critical event during embryonic development is the establishment of a vascular network and a beating heart. The growth of the heart is initiated by specification of the cardiac mesoderm, bilaterally distributed on both sides

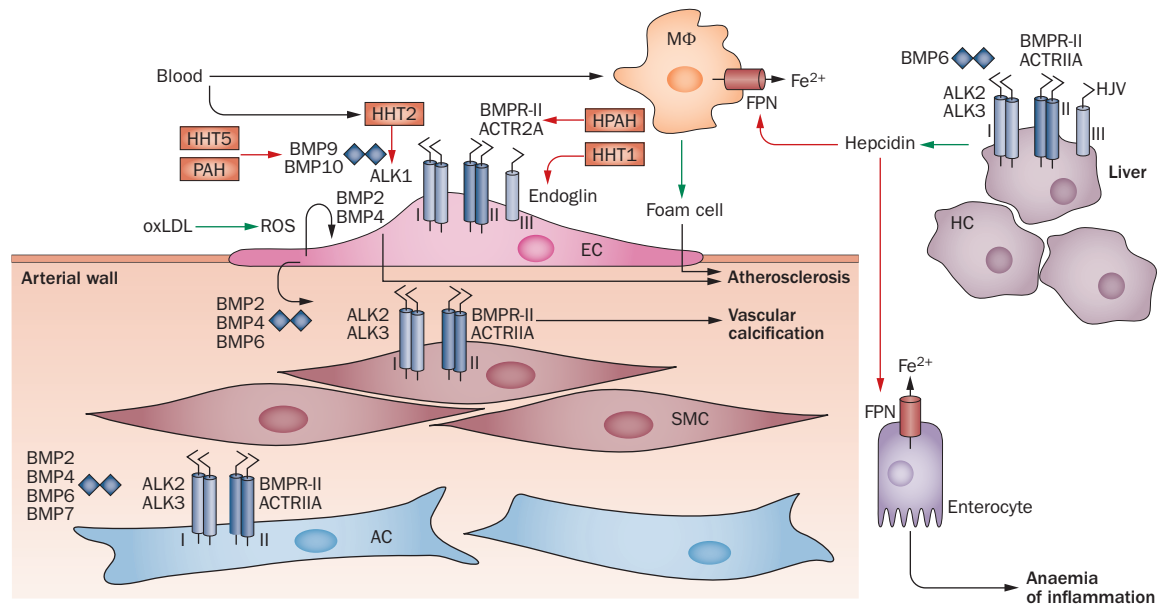


Figure 1 | The repertoire of receptor complexes and ligands involved in cardiovascular and related metabolic responses to BMP signalling. A variety of BMP ligands (BMP6, BMP9, and BMP10) are present in the circulation at biologically active concentrations. ECs, SMCs, and ACs express receptors, co-receptors, and antagonists in a tissue-specific manner to allow complementarity and precise regulation of signalling. Congenital vascular syndromes are shown in red to depict the loss of function of several of these signalling molecules, resulting in human HHT syndromes HHT-1, HHT-2, HHT-5, and HPAH. Positive regulatory effects downstream of BMP signalling are depicted by green arrows, whereas negative effects are depicted with red arrows. Hepatocytes respond to BMP6 signalling to express hepcidin, which subsequently downregulates the expression of FPN to increase the retention of Fe²⁺ in macrophages and duodenal enterocytes, consequently enhancing macrophage activation and reducing serum iron. This activity contributes to iron-deficiency anaemia associated with chronic inflammatory states, and potentiates the activity of macrophages and foam cells in atherosclerosis. Whereas BMP9 signalling seems to be protective in ECs, signalling by BMP2, BMP4, and BMP6 seems to be osteogenic and proatherogenic in SMCs and ECs, acting downstream of atherogenic stimuli including oxLDL via the induction of ROS to promote atherosclerosis and vascular calcification. Abbreviations: AC, adventitial myofibroblast cell; ACTRIIA, activin receptor type 2a; ALK1, activin receptor-like kinase 1 (also known as serine/threonine-protein kinase receptor R3; SKR3); ALK2, activin receptor-like kinase 2 (also known as activin receptor type-1; ACTR-I); ALK3, activin receptor-like kinase 3 (also known as BMP type-IA receptor; BMPR-IA); BMP, bone morphogenetic protein; BMPR-II, BMP type II receptor; EC, endothelial cell; FPN, ferroportin; HHT, hereditary haemorrhagic telangiectasia; HJV, haemojuvelin; HPAH, heritable pulmonary arterial hypertension; MΦ, macrophages; oxLDL, oxidized LDL; PAH, pulmonary arterial hypertension; ROS, reactive oxygen species; SMC, smooth muscle cell.

of the primitive streak.^{72,73} These mesodermal patches migrate to the midline, fuse, and form the cardiac crescent. On day 7 of murine embryonic development, the cardiac crescent develops into the primitive heart tube with an inner endocardial layer and an outer myocardial layer, separated by a layer of extracellular matrix, known as the cardiac jelly.^{74,75} The heart then undergoes 'looping' to form the atrioventricular canal, the hallmark of the chambered heart. In the atrioventricular canal, the cardiac cushions form the valve primordia. The endocardial layer at the site of the atrioventricular canal undergoes endocardial-to-mesenchymal transition (EMT) and migrates as mesenchymal cells to populate the cardiac jelly.⁷⁶

The differentiation of pluripotent stem cells and embryonic stem cells into the cardiac lineage is partly regulated by BMP signalling in a tightly controlled spatiotemporal sequence, as demonstrated by *in vivo*⁷⁷ and *in vitro* studies.^{78,79} BMP4 cooperates with FGF2 via the activation of ERK signalling to induce cardiac mesoderm formation by increasing expression of Brachyury protein, homeobox

protein CDX-2, and homeobox protein NANOG⁸⁰ in a SMAD1-dependent manner,⁸¹ while inhibiting endoderm differentiation. BMP4 signalling again has a critical role in the terminal differentiation of cardiomyocyte progenitors into cardiomyocytes, but its effect at commitment stages is dependent on a precise balance, with activin A, Nodal, and Wnt signals in combinations that are specific not only to lineages, but also to progenitor clones.⁸²⁻⁸⁴ Importantly, if BMP signalling persists for 3 days beyond when cardiac mesoderm is formed, cardiac progenitor cells differentiate into epicardial lineages instead of cardiomyocytes.^{85,86} The epicardium has been shown to serve as an important reservoir of cardiac fibroblasts and vascular smooth muscle progenitors in the developing heart, and has an important role in cardiac repair after injury.⁸⁷ BMP signal transduction has been demonstrated in the atrioventricular canal,^{88,89} and to a lesser extent in the cardiac crescent⁸⁸⁻⁹⁰ using BMP reporter mice, which harbour a BMP responsive element that regulates the reporter genes *lacZ* or *GFP*. BMP2 and BMPR-1A are both expressed in the cardiac crescent; deletion of BMPR-1A in the cardiac mesoderm

results in embryos that lack the cardiac crescent and cardiomyocytes, suggesting a role for BMP in early cardiomyocyte formation.^{91,92} BMPs 2, 4, 5, 6, and 7, as well as ALK2 and BMPR-1A, are expressed in the atrioventricular canal.⁷⁶ Studies using mouse and chicken models have shown that only BMP2 is required for EMT and cushion formation in the atrioventricular canal. Furthermore, endocardial-specific deletion studies involving ALK2 and BMPR-1A have demonstrated that they are both involved in EMT, albeit for different processes.^{93–95} Whereas deletion of either ALK2 or BMPR-1A led to a reduction in SMAD1/5/8 phosphorylation, deletion of ALK2 also diminished phosphorylation of SMAD2/3. Only myocardial-specific deletion of BMPR-1A resulted in cardiac defects, namely reduced atrioventricular cushion size and myocardial thinning.^{93–95}

BMP10 is transiently expressed in the ventricular trabeculae during midgestation when the ventricles grow and mature.⁹⁶ BMP10 levels are tightly controlled because any fluctuations in expression can result in abnormalities in trabecular development and maturation of the ventricular wall. Myocardin, a muscle-specific transcription factor, induces the expression of BMP10 by binding to its promoter.⁹⁷ In the adult heart, expression of BMP10 is restricted to the right atrium.

In addition to their role in cardiac development, BMPs are also involved in the development of the vascular network that is crucial for the embryo after the heart begins to beat. Vascularization is a two-step process: firstly, vasculogenesis—the *de novo* formation of vessels from angioblasts—takes place, followed by angiogenesis, which involves the sprouting of new vessels from pre-existing vessels.⁹⁸ During vasculogenesis, a new vessel forms when endothelial cells start to proliferate, migrate, and form a primitive tube. The primitive tube becomes stabilized by the deposition of extracellular matrix proteins and coverage by mural cells (pericytes or smooth muscle cells).⁹⁹ BMP signalling is required for both endothelial cell and mural cell development and function. BMPs 2, 4, 6, and 7 stimulate the growth of endothelial cells and induce their migration.^{59,100} BMP9 has a dual effect on angiogenesis, depending partly on its concentration. BMP9 and ALK1 signalling at low concentrations induces proliferation and migration of endothelial cells,⁵⁵ whereas complete inhibition of BMP9 and ALK1 signalling using a recombinant ligand trap (ALK1-Fc) prevents tumour angiogenesis.⁵⁶ However, other studies showed that BMP9 and ALK1 signalling inhibited VEGF-induced and FGF-induced angiogenesis^{3,16} in cooperation with Notch signalling,⁴¹ thereby inhibiting the activation phase of angiogenesis. BMP9 and ALK1 signalling can also inhibit lymphangiogenesis.³³

During vascular development, BMP signalling can also influence the differentiation of smooth muscle cells. BMP2 and BMP7 inhibit the growth and migration of smooth muscle cells, and promote their differentiation into a contractile phenotype.^{101–104} Mutations in ALK1 were associated with reduced mural cell coverage in brain vessels.¹⁰⁵ Interestingly, TGF- β was shown to reduce BMP4 signalling in smooth muscle cells,

suggesting a cross-talk between the two signalling pathways.¹⁰⁶ Therefore, the effect of BMP signalling on angiogenesis is context-dependent; appropriate signalling in both endothelial cells and mural cells is necessary for normal development of the vasculature.

Cardiovascular disease

The essential role of BMP signalling in cardiovascular homeostasis and function is underscored by numerous genetic studies in humans. Mutations in the genes that encode transducers of the BMP signalling pathway have been associated with cardiovascular diseases such as PAH and HHT. Other vascular abnormalities, such as those contributing to Marfan syndrome and Loeys–Dietz syndrome have been linked to exaggerated TGF- β activity and reduced responsiveness to BMPs.^{107,108} BMPs have also been implicated in the promotion of vascular calcification and tumour angiogenesis. Furthermore, studies conducted in the past 5 years support a protective role of BMP signalling during post-infarction remodelling of the heart,^{109–112} and patients with coronary artery disease also have aberrant expression of BMP signal transducers.^{113,114} Finally, the level of BMP receptor expression correlates with anaemia and the degree of iron deficiency, which is prevalent in patients with heart failure.

Pulmonary arterial hypertension

PAH is defined as an increase in mean pulmonary artery pressure >25 mmHg at rest in the setting of normal left atrial pressure and increased pulmonary vascular resistance.¹¹⁵ The increase in pulmonary vascular resistance is attributable to a reduction in the cross-sectional area of the pulmonary vascular bed, which occurs as a result of obliteration of the vascular lumen in small pulmonary arterioles.¹¹⁶ Affected individuals present with dyspnoea on exertion and are at increased risk of death from right heart failure.¹¹⁷ Although a number of systemic conditions are associated with PAH,¹¹⁸ such as congenital heart disease, liver cirrhosis, connective tissue diseases, and HIV infection, a rare, idiopathic form of PAH has been observed, with an annual incidence of 1–2 cases per million people.¹¹⁷ Approximately 6–10% of patients with PAH have more than one affected family member, and have inherited the disease via an autosomal dominant pattern of inheritance (though with reduced penetrance of approximately 20–30%).¹¹⁹ Data from two studies published in 2000 established that this familial form of PAH was caused by heterozygous germline mutations in BMPR-II.^{120,121} A subsequent study has shown that >70% of heritable cases of PAH are caused by mutations in BMPR-II,¹²² and approximately 20% of apparently sporadic cases of idiopathic PAH are also caused by BMPR-II mutations, either as a result of *de novo* mutations or owing to the combination of reduced disease penetrance and small family size.¹²³ Approximately 70% of mutations in BMPR-II result in premature termination codons; the remaining 30% are missense mutations that might lead to loss of function by a variety of mechanisms, including loss of kinase activity and aberrant trafficking of misfolded BMPR-II in the endoplasmic reticulum.¹²²

Although our understanding of the pathogenesis of PAH has progressed since the seminal papers published in 2000, major questions still remain. For example, how loss-of-function mutations in BMPR-II cause a disease that seems to be confined to the pulmonary circulation remains uncertain, as do the mechanisms that influence variable penetrance. Notably, BMPR-II has a very long C-terminal extension and might also regulate and modify intracellular signalling by recruitment of factors other than SMADs, such as protein kinase G, in the vasculature.^{25,124,125} The high prevalence of asymptomatic carriers of disease-associated mutations in families with PAH suggest that additional factors are required to trigger the onset and progression of the disease. Mice that are heterozygous for BMPR-II deficiency develop minimal signs of pulmonary hypertension in the absence of additional stimuli.¹²⁶ However, mice with a heterozygous knock-in of a human disease-causing mutation in BMPR-II (R899X) do show susceptibility to spontaneous pulmonary hypertension by 6 months of age,¹²⁷ highlighting potential important differences between heterozygous null and knock-in mouse models. Additional genetic factors, including polymorphisms in the *CYP1B1* gene (linked to oestrogen metabolite levels)¹²⁸ have been suggested to have a role in the development of PAH, but these require confirmation in prospective cohorts. Environmental factors, particularly inflammation, have also been shown to increase disease penetrance in BMPR-II heterozygous mice.¹²⁹ Interestingly, overexpression of dominant negative kinase deficient BMPR-II in pulmonary artery smooth muscle cells is sufficient to promote pulmonary hypertension in transgenic mice.¹³⁰ These observations suggest that a critical reduction in the expression or function of BMPR-II is required to trigger the development of PAH.

In patients with PAH and BMPR-II mutations, pulmonary arterial smooth muscle cells have a loss of the normal BMP/SMAD-mediated growth suppression,^{131,132} and demonstrate heightened proliferation to growth factors such as platelet-derived growth factor, TGF- β , and 5-hydroxytryptamine.¹²⁶ Therefore, loss-of-function mutations of BMPR-II promotes pulmonary arterial smooth muscle cell proliferation and resistance to apoptosis, key factors in the occlusion of small pulmonary arteries. Downstream of BMP/SMAD signalling, the induction of Id genes has a critical role in smooth muscle growth suppression.¹³³ Interestingly, approaches currently employed to treat PAH, such as phosphodiesterase type 5 inhibition and prostanoids, have been shown to partly rescue SMAD/Id gene signalling *in vitro* and *in vivo* via mechanisms involving cAMP and cGMP.^{134,135}

Studies from the past 8 years have demonstrated that BMPR-II forms a signalling complex with ALK1, which is almost exclusively expressed in endothelial cells, and signals specifically in response to BMP9 and BMP10 activation.^{22,136} Although mutations in ALK1 usually cause HHT, there are well-documented cases of severe PAH in families affected by HHT that are indistinguishable from idiopathic PAH.¹³⁷ Therefore, BMP9 and BMP10 signalling via BMPR-II/ALK1 is likely to have a central role in

the pathobiology of PAH as well as HHT, and implicates endothelial cells as an important cell type involved in the initiation of both diseases. Indeed, endothelial selective deletion of BMPR-II¹³⁸ or ALK1 deficiency¹³⁹ can also promote the development of pulmonary hypertension in mice.

Loss of BMPR-II in endothelial cells promotes endothelial apoptosis and increases endothelial permeability *in vitro* and *in vivo*,^{140,141} whereas BMP9 protects endothelial cells from apoptosis and reduces agonist-induced endothelial permeability.¹⁴¹ Loss of BMPR-II function, therefore, leads to changes in the function of vascular wall cells that promote the development of PAH, particularly in the presence of an injurious trigger. An increased understanding of the role of BMPR-II in these processes suggests that approaches to restore BMPR-II expression, function, or signalling might have a role in disease treatment or prevention. Proof-of-concept studies *in vitro* have shown the potential for rescue of translational readthrough of mutations, which result in premature termination codons by small molecules,¹⁴² and for the use of chemical chaperones to rescue misfolded BMPR-II mutants.¹⁴³ In addition, targeted delivery of BMPR-II gene therapy to the pulmonary vascular endothelium has been shown to prevent and reverse PAH in the monocrotaline rat model.¹⁴⁴

Another potential approach to the treatment and prevention of PAH stems from the identification of the rapid turnover of cell surface BMPR-II and its subsequent degradation by the lysosome.¹⁴⁵ This mechanism seems to involve an E3 ubiquitin protein ligase called Itch. Inhibition of the lysosomal degradation of BMPR-II by chloroquine prolongs the half-life of BMPR-II at the cell surface and restores signalling in pulmonary artery smooth muscle cells and endothelial cells that harbour haploinsufficiency-inducing mutations in BMPR-II.¹⁴⁶ In nongenetic models of pulmonary hypertension, such as those caused by the plant alkaloid toxin monocrotaline, protein levels of BMPR-II are reduced. In these rodent models of pulmonary hypertension the 4-aminoquinoline chloroquine, and the better-tolerated hydroxychloroquine, have shown preclinical efficacy in preventing the development and progression of pulmonary hypertension, and can be shown to restore BMPR-II protein levels. In addition to lowering pulmonary blood pressure, chloroquine also inhibits autophagy, which promotes apoptosis of pulmonary artery smooth muscle cells.^{146–148} Thus, as the use of chloroquine and hydroxychloroquine has already been well-established for the treatment of malaria, sarcoidosis, rheumatoid arthritis, and systemic lupus erythematosus, repurposing these agents for the treatment of PAH might prove a novel and readily testable approach. While the mechanism of action of these drugs should aid in the normalization of BMPR-II expression levels in mutation-carrying individuals with PAH, they might also augment BMPR-II expression and signalling and possibly benefit individuals without PAH-causing mutations.

A further approach to restoring BMP signalling was identified by screening 3,756 FDA-approved drugs

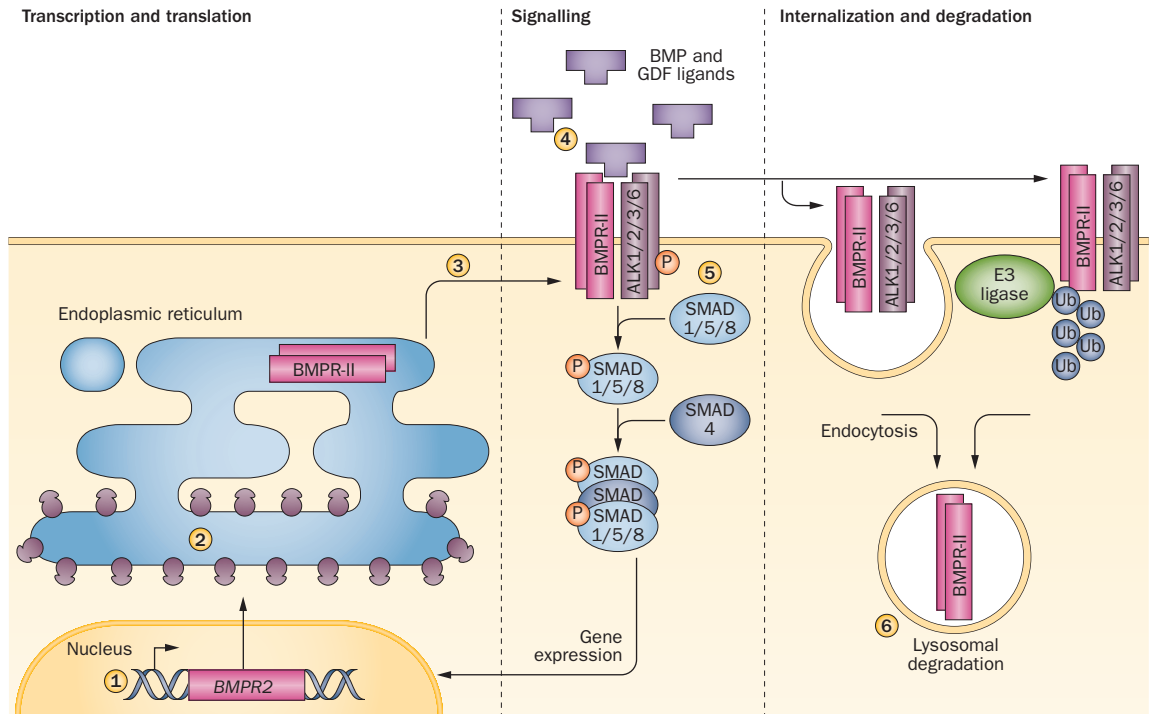


Figure 2 | Potential approaches to target and enhance signalling or expression of BMPR-II. BMPR-II cell surface expression can be increased by endothelial gene therapy targeted to the pulmonary circulation (1). An increase in BMPR-II level can be achieved using small molecules that increase translational readthrough of BMPR-II mutations encoding premature termination codons (2), and chemical chaperones that increase trafficking of BMPR-II misfolded mutations that are held up within the endoplasmic reticulum (3). In addition, BMPR-II signalling can be restored by ligands (BMP9 and BMP10) that target the BMPR-II/ALK1 receptor complex on endothelial cells (4), and agents that enhance downstream SMAD phosphorylation, such as tacrolimus, sildenafil, and prostanoids (5). Finally, inhibition of BMPR-II turnover by the lysosome, using agents such as hydroxychloroquine, can enhance cell surface expression of BMPR-II protein (6). Abbreviations: ALK1, activin receptor-like kinase 1 (also known as serine/threonine-protein kinase receptor R3; SKR3); ALK2, activin receptor-like kinase 2 (also known as activin receptor type-1; ACTR-1); ALK3, activin receptor-like kinase 3 (also known as BMP type-IA receptor; BMPR-IA); ALK6, activin receptor-like kinase 6 (also known as BMP type-IB receptor; BMPR-IB); BMP, bone morphogenetic protein; BMPR-II, BMP type II receptor; GDF, growth differentiation factor; Ub, ubiquitin.

and bioactive compounds in a transcriptional high-throughput luciferase reporter assay.¹⁴⁹ The best response was achieved with the drug tacrolimus, which although is recognized as a calcineurin inhibitor, also binds to the peptidyl-prolyl cis-trans isomerase FKBP12, a repressor of BMP signalling. Tacrolimus releases FKBP12 from type I receptors ALK1, ALK2, and ALK3, which are activated downstream of SMAD1/5 and mitogen-activated protein kinase signalling and *ID1* gene regulation. In pulmonary artery endothelial cells from patients with idiopathic PAH, low-dose tacrolimus therapy reversed the reduction in BMP signalling.¹⁴⁹ Low-dose tacrolimus therapy reversed severe monocrotaline-induced PAH in rats with medial hypertrophy and in rats with neointima formation caused by VEGF receptor blockade and chronic hypoxia.¹⁴⁹ A clinical trial examining low-dose tacrolimus for the treatment of PAH is currently underway.¹⁵⁰

In a study published in 2015, systemic administration of BMP9 was shown to prevent and reverse PAH in various genetic and nongenetic preclinical models.¹⁴¹ BMP9 is highly selective for endothelial cells, owing to its specificity for the BMPR-II/ALK1 receptor complex. The growth of angioproliferative lesions observed in rats exposed to chronic hypoxia and a VEGF receptor 2

antagonist, which are thought closely to resemble the lesions observed in patients with severe PAH, was suppressed by systemic administration of BMP9.¹²⁷ BMP9 administration also increased the expression of BMPR-II in these models in a SMAD-dependent manner. Although BMP9 has been shown to be a potent stimulus of ossification at high concentrations, no ossification was observed in animals treated with BMP9 daily for 4 weeks at the site of injection, either via the peritoneal or the intramuscular route.¹⁴¹ These studies raise the possibility that BMP9, BMP10, or their analogues might be promising candidates for novel therapies in patients with PAH. Potential approaches to restore BMPR-II expression or function in PAH are summarized in Figure 2.

Hereditary haemorrhagic telangiectasia

HHT is a genetic autosomal dominant vascular disorder, with a prevalence of 1 in 5,000–10,000 people worldwide.^{56,151} Clinical manifestations of HHT include spontaneous and recurrent epistaxis, telangiectasias of the lips, hands, and nasal and oral mucosa, and the development of arteriovenous malformations in visceral organs, in particular the lungs, brain, and liver.¹⁵² These presentations suggest that HHT predominantly involves vascular cells,

although a contribution of dysfunctional mononuclear cells cannot be excluded. Subtypes of HHT have been recognized based on their underlying mutation. HHT-1 and HHT-2 are caused by mutations in *ENG*^{153,154} and *ACVRL1*,^{155–157} respectively, and together account for 80% of all patients with HHT. Juvenile polyposis-HHT (JP-HHT) refers to a group of patients with HHT who have a mutation in *SMAD4* and features of juvenile polyposis, such as the development of benign polyps.^{158,159} Mutations in BMP9 have been associated with a syndrome resembling HHT, in which patients develop nose bleeds and dermal telangiectasia, although not limited to the hands, face, and mouth, as typically found in HHT.¹⁶⁰ Furthermore, impaired BMP signalling is also central to primary PAH, and several families have been identified with both pathologies.¹⁵⁸ The tortuous and fragile HHT vessels, caused by abnormal endothelial cell proliferation and aberrant recruitment of perivascular cells, can be linked to impaired BMP9 and BMP10 signalling.³¹⁶ Given that BMP9 and BMP10 are present in the circulation, bind to ALK1 and endoglin with high affinity, and are highly expressed in endothelial cells, dysfunctional BMP signalling is likely to be central to HHT pathology.

Given that the BMP9/ALK1/endoglin pathway suppresses VEGF expression and inhibits angiogenesis, patients with HHT have elevated levels of VEGF and dysregulated angiogenesis. Accordingly, inhibition of VEGF with anti-VEGF-A antibody attenuated the vascular dysplasia in a heterozygous *Acvrl1*^{+/-} mouse model of HHT-2.^{19,161,162} Furthermore, treatment of HHT-1 mice with thalidomide¹⁶³ and HHT-2 mice with an anti-VEGF-A antibody¹⁶⁴ stabilized vascular sprouts and reduced arteriovenous malformation expansion, respectively. Given these positive *in vivo* findings, antiangiogenic agents are currently being tested in clinical trials as therapy for HHT.^{165–169} Early reports indicate that treating patients with HHT and severe liver vascular malformations with the anti-VEGF antibody bevacizumab decreases recurrent nose bleedings and cardiac index.^{169,170}

Park and colleagues demonstrated that a homozygous deletion of both *ACVRL1* alleles was by itself insufficient to recapitulate the formation of arteriovenous malformations in a mouse model for HHT-2, but required an additional local wound injury.¹⁷¹ The formation of the telangiectasia and arteriovenous malformations in this mouse model also required the complete loss of *ACVRL1* expression in endothelial cells. Depletion of ALK1 expression in endothelial cells results in increased angiogenesis, which is characterized by faster migration, increased sprouting, and resistance to BMP9-induced inhibition of angiogenesis.¹⁹ To date, loss of heterozygosity or epigenetic modification of endoglin have not been reported in HHT-1, suggesting that the additional insult required for the disease phenotype to manifest is not a second genetic event at this locus.

Atherosclerosis and vascular calcification

BMP ligands have been detected in arteries from patients with advanced atherosclerosis associated with vascular calcification.¹⁷² The role of BMP signalling in atherosclerosis

has been explored using a variety of BMP signalling inhibitors. Saeed and colleagues showed that administration of LDN-193189 could reduce vascular inflammation and inhibit the development of atherosclerosis in *ApoE*^{-/-} mice.¹⁷³ Subsequently, Derwall and colleagues demonstrated that both LDN-193189 and ALK3-Fc could inhibit atherosclerosis in LDL-receptor-deficient (*Ldlr*^{-/-}) mice fed a Western diet.³¹ Moreover, they found that LDN-193189 reduces the vascular calcification seen in *Ldlr*^{-/-} mice with advanced atherosclerosis.

In vitro and *in vivo* studies suggest that there are many potential mechanisms by which BMP signalling can promote atherosclerosis and, perhaps, secondary vascular calcification. These mechanisms include BMP-mediated endothelial cell activation¹⁷⁴ and reactive oxygen species generation.³¹ A compelling potential anti-inflammatory mechanism for the antiatherogenic effects of LDN-193189 was proposed by Saeed and co-workers,¹⁷³ after observations that BMP inhibition modified iron-loading in macrophages.¹⁷⁵ They also reported that LDN-193189 reduced hepatic hepcidin expression, increased ferroportin expression in peritoneal macrophages, and reduced macrophage iron levels and hydrogen peroxide production. Furthermore, peritoneal macrophages from mice treated with LDN-193189 showed enhanced gene expression of *Abca1* and *Abcg1*, two cholesterol efflux transporters, suggesting an increased capacity to export cholesterol. All the effects of *in vivo* treatment with LDN-193189 on peritoneal macrophage function could be reversed by *ex vivo* administration with hepcidin to induce ferroportin degradation.¹⁷³ Together, these observations suggest that inhibiting BMP signalling can modulate macrophage phenotypes by altering hepcidin–ferroportin signalling and intracellular iron levels. Whether BMP inhibition modulates macrophage function in atherosclerotic vessels via hepcidin–ferroportin signalling remains to be determined.

Myocardial remodelling

BMP4 expression can be acutely or chronically elevated in pressure overload-induced pathological hypertrophy; importantly, an increase in BMP4 expression is not observed in exercise-induced physiologic hypertrophy.^{29,176,177} BMP4 expression is similarly upregulated in acute infarction and ischaemia–reperfusion injury, as well as in chronic ischaemic heart disease.^{28,29} BMP4-induced hypertrophy in isolated cardiomyocytes can be inhibited upon treatment with BMP inhibitors noggin, dorsomorphin, or DMH1,^{28,177} or interestingly, antagonized by the co-administration of BMP2.¹¹⁰ The direct effect of BMP4 signalling on the development of cardiac hypertrophy was demonstrated in *in vivo* studies showing that pressure overload-induced left ventricular hypertrophy¹⁷⁷ and cardiac ischaemia–reperfusion injury²⁸ were attenuated using BMP inhibitors or in BMP4-deficient animals, respectively.

The expression of endoglin is elevated in both human failing hearts and in mice with pressure overload-induced heart failure.¹⁷⁸ Both endoglin heterozygosity and the expression of a soluble form of endoglin limited cardiac

fibrosis and improved survival in an experimental model of left ventricular pressure overload.¹⁷⁸ In follow-up studies, haploinsufficiency of endoglin, and inhibition of endoglin in the myocardium using a specific neutralizing antibody, reduced calcineurin activation in pressure overload-induced right heart failure, limited myofibroblast transformation and cardiac fibrosis, and improved survival.¹⁷⁹ These findings were attributed to the capacity of endoglin to modulate TGF- β signalling. Given that soluble endoglin selectively binds to and inhibits BMP9 and BMP10 to exert antiangiogenic effects *in vivo*,¹⁸⁰ the reduction in cardiac fibrosis as a result of treatment with soluble endoglin or endoglin-neutralizing antibody might also result from attenuated signalling of those ligands or their downstream effects. However, BMP10 has also been reported to reduce cardiac fibrosis and improve cardiac function after experimental myocardial infarction in mice.¹¹¹

Anaemia of inflammation

Anaemia of chronic disease, or anaemia of inflammation, deserves special consideration in this discussion of cardiovascular disease for several reasons. The reduction in oxygen-carrying capacity associated with anaemia is an important exacerbating factor in cardiovascular disease, aggravating symptoms of hypoxic lung and pulmonary vascular disease, heart failure, as well as coronary and systemic atherosclerotic disease. In moderate-to-severe states, all these conditions can be linked to a clinically relevant degree of anaemia that is caused by a disease-associated systemic inflammatory state, through a mechanism that is BMP-dependent. BMP signal transduction regulates the expression of hepcidin, the central hormonal regulator of iron metabolism.¹⁸¹ Ferroportin is the sole molecule responsible for exporting iron from intracellular stores within intestinal epithelial cells, hepatocytes, and macrophages into the circulation.¹⁸² Ferroportin is targeted for internalization and degradation by hepcidin to reduce circulating iron levels. Hepcidin synthesis in the liver is regulated by BMP6, numerous inflammatory stimuli including IL-6, as well as increased iron levels themselves (Figure 1).

The important role of BMP signalling in the regulation of hepcidin expression was identified by Babitt and colleagues, who observed that haemojuvelin, a protein defective in severe forms of haemochromatosis, is a BMP co-receptor and that soluble haemojuvelin could inhibit the capacity of BMP ligands to induce hepatocyte hepcidin gene expression.¹⁸¹ Moreover, these investigators observed that soluble haemojuvelin prevents the induction of hepcidin gene expression by IL-6, suggesting that inhibition of BMP signalling could be used to treat anaemia of inflammation. The role of BMP signal transduction in the regulation of iron transport and potentially anaemia of inflammation was subsequently confirmed by the use of BMP inhibitor dorsomorphin to reduce hepcidin gene expression and increase serum iron levels in zebrafish and mice.⁴⁸

Anaemia of inflammation is attributable to a reduction in red blood cell production by the bone marrow owing

to reduced systemic iron bioavailability, yet frequently occurs in healthy individuals with an iron-replete diet. Several factors contribute to reduced red blood cell production: sequestration of iron in macrophages leading to a reduction in plasma iron levels; reduced absorption of iron from the duodenum; impaired erythropoiesis despite an increase in erythropoietin levels; and in some individuals, a reduction of red blood cell life-span.^{183,184} Anaemia of inflammation is associated with a wide variety of disorders including infection, chronic kidney disease, cancer, connective tissue diseases such as rheumatoid arthritis and systemic lupus erythematosus, inflammatory bowel disease, and congestive heart failure. All these disorders share acute or chronic immune activation pathways leading to the production of inflammatory cytokines, including IL-6. Although haemoglobin levels are in general only moderately reduced in anaemia of inflammation, the decreased oxygen delivery associated with this condition is sufficient to exacerbate symptoms in patients with cardiovascular comorbidities.¹⁸⁵ Treatment in these individuals is directed towards alleviating symptoms such as angina, fatigue, or functional limitation.

Steinbicker and colleagues assessed whether inhibiting BMP signalling could prevent the induction of hepatic hepcidin gene expression by IL-6.¹⁸⁶ Intraperitoneal injection of LDN-193189 or a soluble ALK3 receptor inhibited the capacity of IL-6 to increase hepatic hepcidin mRNA levels. This group and others subsequently showed that administration of LDN-193189, or a BMP ligand trap using recombinant haemojuvelin expressed as an IgG-Fc domain fusion protein, could prevent and reverse established anaemia in several rodent models of anaemia of inflammation.^{186,187} Mayeur and colleagues subsequently showed that oral administration of LDN-193189 was sufficient to prevent anaemia in this model of chronic inflammation.¹⁸⁸ Taken together, these observations suggest that inhibiting BMP signalling might be a novel and practical therapeutic target for treating anaemia of inflammation.

Conclusions

Human genetic studies have revealed major, previously unsuspected, roles for the BMP family of ligands and receptors in cardiovascular disease and iron homeostasis. The large BMP family of ligands, receptors, ligand traps, and cell surface modifiers of signalling suggests a finely regulated pathway with complex interactions. Nevertheless, meticulous dissection of the BMP pathway over the past 15 years has led to a greater understanding of the cell-specific nature of signalling and has uncovered potential opportunities for therapeutic intervention. In particular, restoration of BMPRII receptor expression or function, and the use of selective ligands such as BMP9 and BMP10 have shown promise for PAH. Small molecule inhibition of other BMPs and their receptors might provide novel opportunities in conditions as diverse as atherosclerosis and anaemia. Future studies will determine whether this intriguing family of bone-forming proteins can be used safely for the treatment of cardiovascular disease.

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Author contributions

All the authors researched data for the article, discussed its content, wrote the manuscript, and reviewed/edited it before submission.