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Review

BMP signaling in vascular diseases

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ABSTRACT

Bone morphogenetic proteins (BMPs) are members of the transforming growth factor-β (TGF-β) family that signal via type I and type II serine/threonine kinase receptors and intracellular Smad transcription factors. BMPs are multifunctional regulators of development and tissue homeostasis and they were initially characterized as inducers of bone regeneration. Genetic studies in humans and mice showed that perturbations in BMP signaling lead to various diseases, such as skeletal diseases, vascular diseases and cancer. Mutations in BMP type II receptor and BMP type I receptor/activin receptor-like kinase 1 have been linked to pulmonary arterial hypertension and hereditary hemorrhagic telangiectasia, respectively. BMPs have also been implicated in promoting vascular calcification and tumor angiogenesis. In this review we discuss the role of BMP signaling in vascular diseases and the value of BMP signaling as a vascular disease marker or a therapeutic target.

1. Introduction

Bone morphogenetic proteins (BMPs) are members of the transforming growth factor-β (TGF-β) family, which also includes TGF-βs, growth and differentiation factors (GDFs), anti-müllerian hormone (AMH), activins and nodal. BMPs were first identified as potent inducers of ectopic bone formation when implanted subcutaneously in rats [1,2]. Subsequent studies demonstrated that BMPs, as is the case for other TGF-β family members, are multifunctional regulators in development that regulate cell proliferation, differentiation, and apoptosis in different tissues [3,4]. BMPs exert their signals via type I and type II transmembrane serine/threonine kinase receptors. Inside the cell, Smad proteins play an important role in the transduction of the signal from the active receptor complex to the nucleus. Interestingly, misregulated BMP signaling has been shown to be involved in the pathogenesis of skeletal and (cardio) vascular disorders as well as cancer. Despite the recent advances in therapeutic interventions, cardiovascular disease remains the largest health problem worldwide causing morbidity and mortality. This review will focus on the role of BMP signaling in the pathology of vascular diseases and potential clinical applications.

2. BMPs

Among the 33 members of the TGF-β superfamily, over 20 molecules form the BMP subfamily. The BMP subfamily can be further subdivided into several subgroups, including BMP-2/4, BMP-5/6/7/8, GDF-5/6/7 and BMP-9/10 [4,5]. BMPs are synthesized as large precursor proteins consisting of an amino (N)-terminal signal peptide, a prodomain for folding and secretion, and a bioactive carboxy (C)-terminal mature peptide. BMP precursor proteins are produced

Abbreviations: ALK, activin receptor-like kinase; AMH, anti-müllerian hormone; Apo, apolipoprotein; AV, aortic valve; AVMs, arteriovenous malformation; BAECs, bovine aortic endothelial cells; BAMBI, BMP and activin membrane-bound inhibitor; bFGF, basic fibroblast growth factor; BMPs, bone morphogenetic proteins; BMPER, BMP endothelial cell precursor derived regulator; BMPR2, BMP Type II receptor; CV2, cross-veinless 2; DAN, differential screening-selected gene aberrative in neuroblastoma; Dll4, delta-like 4; ECs, endothelial cells; EndoMT, endothelial to mesenchymal transition; eNOS, endothelial nitric-oxide synthase; EPCs, endothelial progenitor cells; ER, endoplasmic reticulum; FOP, fibrodysplasia ossificans progressiva; FPAH, hereditary or familial PAH; GDFs, growth and differentiation factors; HHT, hereditary hemorrhagic telangiectasia; HUVECs, human umbilical vein endothelial cells; IPAH, sporadic or idiopathic PAH; iPSCs, induced pluripotent stem cells; I-Smads, inhibitory Smads; LAP, latency associated peptide; LDL, low-density lipoprotein; LTBP, latent TGF-β binding protein; MGP, matrix GLA protein; miR, micro RNAs; PAEC, pulmonary artery endothelial cells; PDGF-BB, platelet-derived growth factor subunit BB; PKC, protein kinase C; RGM, repulsive guidance molecule; PRDC, protein related to DAN and Cerberus; PTPN14, tyrosine-protein phosphatase non-receptor type 14; ROS, reactive oxygen species; R-Smads, receptor-regulated Smads; SBE, Smad-binding elements; SMCs, smooth muscle cells; USAG-1, uterine sensitization-associated gene-1; TGF-β, transforming growth factor-β; VEGF, vascular endothelial growth factor

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in the cytoplasm as dimeric pro-protein complexes, which are cleaved by serine endoproteases (e.g. BMP-4 is cleaved by furin, PC6 and PC7 [6]) to generate N-terminal and C-terminal fragments, of which the latter is capable of binding to its receptor [7]. Whereas the secretion of BMPs in a latent inactive form is not common [7], TGF-β is secreted as a latent form in which the N-terminal remnant, also known as latency associated peptide (LAP), sequesters and prevents the bioactive mature part from binding to its receptors. This complex is also associated with the latent TGF-β binding proteins (LTBP). Thus, proteolytic cleavage of latent TGF-β by different activators is required for the release of the mature, active TGF-β [8].

BMP activity is also regulated by several intracellular and extracellular modulators (Fig. 1). A large number of extracellular soluble antagonists bind BMPs and block their interaction with signaling receptors, thus dampening BMP signaling [9]. These antagonists can be divided into three subgroups based on their structure similarity: the CAN (Cerberus/DAN) family, twisted gastrulation, chordin and noggin. The CAN family includes gremlin and cerberus, differential screening-selected gene aberrative in neuroblastoma (DAN), protein related to DAN and cerberus (PRDC), coco, uterine sensitization-associated gene-1 (USAG-1) and sclerostin [10]. Several additional BMP regulators have been identified, such as cross-veinless 2 [CV2, also referred to as BMP endothelial cell precursor derived regulator (BMPER)], matrix GLA protein (MGP) and neogenin [11–14]. MGP is a small, carboxyglutamic acid modified protein, which can bind and inhibit BMP-2 and BMP-4 by direct protein interaction [12,15,16]. It is highly expressed in kidneys and lungs, where excessive MGP in *MGP*-transgenic mice altered pulmonary BMP-4 distribution and resulted in significant morphological defects in the pulmonary artery tree [17]. Neogenin was identified as a receptor for netrins and proteins of the repulsive guidance molecule (RGM) family. The interaction of netrins-neogenin or RGM-neogenin stimulated or repelled neuronal axon guidance depending on the developmental context [18,19]. Recent research suggested that neogenin is a regulator of BMP signaling during chondrogenesis and skeletal development, since there is reduced expression levels of BMP target genes and intracellular BMP signaling mediators in chondrocytes from neogenin mutant mice, and the neogenin-deficient mice is retarded in digit/limb development and endochondral ossification [13]. However, others reported that neogenin acts as a repressor of BMP signaling and knockdown of neogenin in C2C12 cells leads to increased BMP-2-induced phosphorylation of Smad1, Smad5, and Smad8 and osteoblast differentiation [14]. The expression pattern of BMP antagonists is important for embryonic development, as an aberrant expression pattern can lead to defects in bone, limb and kidney formation [20].

3. BMP receptors

Like other members of the TGF-β family, BMPs bind to two types of serine-threonine kinase receptors, known as type I and

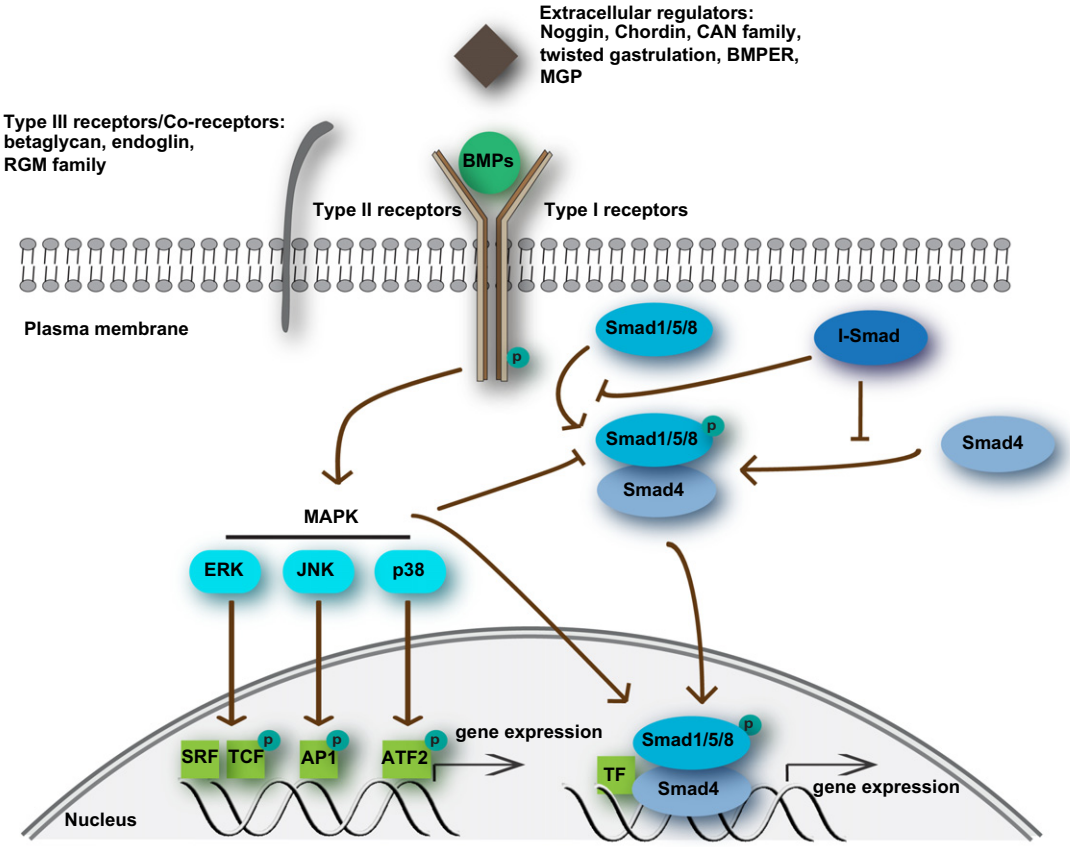

Fig. 1. Schematic overview of the BMP signaling pathway. BMPs interaction with surface receptors induces heteromeric complex formation between specific type II and type I receptors. This activity is regulated by extracellular regulators and type III receptors/co-receptors. After being activated by type II receptors, the type I receptors phosphorylate Smad1/5/8 (R-Smads) to propagate the signal into the cell. Smad1/5/8 form heteromeric complexes with Smad4 (Co-Smad) and translocate to the nucleus where, by interacting with other transcription factors, they regulate target gene expression (canonical Smad signaling pathway). I-Smads (Smad6/7) inhibit receptor activation of R-Smads. Besides Smad-depend signaling, non-Smad pathways are involved. Activated MAPKs can regulate R-Smad activation by a direct phosphorylation or through their downstream effectors molecules. Activated MAPKs can translocate to the nucleus to phosphorylate a number of transcription factors (TF), such as serum response factor (SRF), ternary complex factor (TCF) family members, activator protein 1 (AP1) complexes and activating transcription factor 2 (ATF2), thereby changing target gene transcription.

type II receptors [21,22] (Fig. 1). Both receptors share a similar structure and are comprised of a short extracellular domain, a single transmembrane domain and an intracellular domain with serine-threonine kinase activity. The affinity of BMPs for type I receptors is higher than for type II receptors and its affinity is increased by the formation of a heterotetrameric receptor complex [23]. The type II receptor kinase is constitutively active in the absence of ligand. BMP type II receptor (BMPR2) has a long C-terminal tail rich in serine and threonine residues [23]. Besides BMPR2, BMPs can signal also via the activin type II receptors ACVR2A, and ACVR2B [4,24], which are expressed in various tissues. Whereas BMPR2 is a specific receptor for BMPs, ACVR2A and ACVR2B also can be used by activins, myostatin and nodal. Based on the structural similarity, BMP type I receptors can be divided into two subgroups: activin receptor-like kinase 3 (ALK3, or BMPR-IA) and ALK6 (BMPR-IB) group, and the ALK1 and ALK2 group. While ALK2 and ALK3/6 are widely expressed in various cell types, ALK1 has a more selective expression pattern being mainly restricted to endothelial cells and few other cell types.

A number of BMP co-receptors have been identified (Fig. 1). These co-receptors modulate the interactions between BMP ligands and receptors. There are two co-receptors, endoglin and betaglycan, which play important roles in vascular development and disease, although they lack a signaling domain [25]. Endoglin and betaglycan can potentiate BMP signaling [26,27]. BMPs can also bind to the decoy receptor BMP and activin membrane-bound inhibitor (BAMBI). BAMBI resembles the type I receptors but lacks an active kinase domain and consequently sequesters ligands from the active receptors and inhibits BMP signaling [28]. Family members of RGM, RGMa, RGMb (DRAGON) and RGMc, were shown to be implicated in BMP signaling [29–31]. DRAGON was the first RGM family member identified as a BMP co-receptor [30]. Cell surface GPI-anchored DRAGON directly binds to BMPs enhancing BMP signaling, but not TGF-β. Moreover, this effect can be reduced by noggin [30]. Interestingly, DRAGON interacted directly with all BMP type I receptors as well as BMPR2, ActRII and ActRIIB [30]. Furthermore a soluble form of DRAGON fused to Fc (DRAGON-Fc) inhibited BMP signaling in vitro [30,32]. It is possible that RGM proteins modulate the ability of cells responding to a low concentration of BMP ligands by altering the sensitivity of BMPR2 to BMP ligands. However, the precise mechanism by which RGM proteins regulate different physiological processes is still not known [33].

4. Smad and non-Smad signaling pathways

After BMP ligand-induced heteromeric complex formation, the type II receptor kinase phosphorylates the type I receptor. Subsequently, the activated type I receptor initiates intracellular signaling by activating the Smad proteins (Fig. 1). Smads can be divided into three groups: receptor-regulated Smads (R-Smads), inhibitory Smads (I-Smads), and a common-mediator Smad (i.e. Smad4) [21]. Upon type I receptor-mediated phosphorylation/activation of R-Smads, they form heteromeric complexes with Smad4. These heteromeric R-Smad/Smad4 complexes translocate into the nucleus, where they regulate target gene expression by directly binding to Smad-binding elements (SBE), or indirectly through interactions with DNA-binding transcription factors, and by associating with co-activators/co-repressors and histone-modifying factors [34]. Inhibitory Smads (I-Smad6 and 7) antagonize BMP and TGF-β receptor-initiated Smad signaling by mediating the degradation of receptors and R-Smads. Smad7 inhibits all TGF-β family members, while Smad6 is more selective towards BMP family members. Smad ubiquitin ligases Smurf1 and Smurf2 are recruited by I-Smads to promote the proteasomal degradation of receptors and Smads [35–37].

Besides canonical BMP receptor/Smad signaling, activated BMP receptors can initiate non-Smad signaling pathways. MAP kinases (ERK, JNK and p38 MAPK), phosphoinositide (PI)3 kinase/Akt and protein kinase C (PKC) signaling pathways, and Rho-GTPases can also be activated by BMPs and TGF-βs in various cells [38] (Fig. 1). These non-Smad pathways are also important in creating diversity and fine-tuning of signals generated by the TGF-β family ligands [39,40]. Smad-independent pathways can also be involved in the pathogenesis of vascular diseases, such as pulmonary arterial hypertension (PAH), which will be discussed later.

5. BMP signaling during vessel development

The establishment of the vascular system is an important event during embryonic development. Neovascularization involves two mechanisms: first the de novo formation of vessels termed vasculogenesis, and second, the sprouting and growth of new vessels from pre-existing ones, known as angiogenesis [41]. Angiogenesis is a crucial process, which occurs primarily during embryonic development, and it is almost absent during adulthood besides wound healing, inflammation and the female reproductive cycle. In healthy tissues, blood vessels are formed by a combination of several mechanisms, such as sprouting angiogenesis, bone-marrow derived and/or vascular-wall-resident endothelial progenitor cells (EPCs) differentiation, and vessel splitting [41]. Main players in the process of angiogenesis are the endothelial cells (ECs) as well as smooth muscle cells (SMCs) and pericytes. EC proliferation, migration and tube formation are critical in the process of angiogenesis. Sprouting angiogenesis involves the selection of a leading migrating tip EC that invades the surrounding tissue by extending numerous filopodia. VEGF/VEGFR2 signaling triggers single EC to switch into a tip cell phenotype; these cells thereby express Delta-like 4 (Dll4), a Notch ligand, which instructs neighbor ECs to become so-called stalk cells [42]. Stalk cells trail behind the tip cells proliferate and form tubes; stalk cell proliferation ensures elongation of sprouting vessel [43,44]. Ultimately ECs stop proliferating, acquire a quiescent phenotype and become phalanx ECs. Finally, the new formed vessel is stabilized by deposition of basement membrane and recruitment of pericytes/SMCs [45]. Interestingly, it has been reported that besides ECs, tumor cells can also contribute to angiogenesis. It has been suggested that cancer cells with stem cell features can dedifferentiate and acquire an EC-like phenotype. These cells can incorporate in the blood vessels and contribute to angiogenesis [41,46].

The role of BMP signaling in vascular development has been illustrated by studies in knockout animal models [47]. Table 1 (see below and references therein) shows a list of mouse knockout models for BMP signaling components, including ligands, receptors and Smads. Genetic deletion or misexpression of different components of BMP signaling leads to embryonic death due to cardiovascular malformations and defects in vascular remodeling. Moreover, proper BMP signaling in both ECs and mural/SMCs has been shown to be required for appropriate vasculogenesis and angiogenesis. Interestingly, deletion of the BMP target genes Id1 and Id3 in mice leads to impaired angiogenesis both in brain and tumor xenografts [48].

It has been reported that BMP-2, -4, -6 and -7 induce angiogenesis, EC proliferation and migration [49,50]. Capillary tube formation is increased upon activation of the BMP signaling pathway by overexpression of BMPs or Id1 [51,52]. In contrast, BMP-9 inhibits basic fibroblast growth factor (bFGF)-stimulated proliferation and migration of bovine aortic endothelial cells (BAECs) and blocks VEGF-induced angiogenesis [36]. BMP-9 has also been reported to inhibit the migration and growth of human dermal microvascular ECs [37]. Although (high dose) BMP-9 seems to have inhibitory

Table 1
Deregulated BMP signaling leads to (cardio) vascular abnormalities.

Gene	Animal model	Human disease	References
Bmp-2	KO: Embryonic lethal with defect in cardiac development; Het: Susceptible to hypoxic pulmonary hypertension associated with reduced endothelial nitric oxide synthase (eNOS) expression	Unknown	[155,156]
Bmp-4	Het: Less severe hypoxic pulmonary hypertension and vascular smooth muscle cell proliferation, impaired vascular remodeling	Unknown	[63]
Bmpr2	Het: Pulmonary hypertension	PAH	[69,70,157,158]
Alk1	KO: Embryonic lethal (E10.5), severe vascular abnormalities; Het: Models HHT type 2; EC conditional KO: Severe vascular malformations mimicking all pathologic features of HHT.	HHT	[159–162]
Alk3	Mesoderm conditional KO: Embryonic lethal (E10.5–E11.5), hemorrhage, impaired vessel remodeling; SMC (embryo): Embryonic lethal (E11) due to vascular and pericardial hemorrhage, impaired vascular remodeling; SMC (adult): Impaired vascular remodeling	unknown	[163–165]
Endoglin	KO: Embryonic lethal (E10.5) due to impaired mature vessel formation; Conditional mutation: AVM	HHT	[166,167]
Smad1	KO: Embryonic lethal (E9.5) due to defects in allantois formation; with impaired embryonic circulation system	Unknown	[168]
Smad4	EC conditional KO: Embryonic lethal (E10.5) due to cardiovascular defects	HHT (with or without JP)	[169–171]
Smad5	KO: Embryonic lethal (E9.5–E11.5) due to cardiac and angiogenesis defects	Unknown	[172,173]
Smad6	KO: Cardiovascular defects, vascular calcification, hypertension	CVM	[121,174]
Smad7	KO: Embryonic lethal due to cardiovascular defects	Unknown	[175]
Smad8	Smad8 mutation mice: Defective pulmonary vascular remodeling	PAH	[77]

Abbreviations: KO, knockout; het, heterozygous; JP, juvenile polyposis; CVM, congenital cardiovascular malformation.

effects on ECs, another report demonstrated that (low dose) BMP-9 induces proliferation of various types of ECs in vitro and promoted angiogenesis in matrigel plug assays and human pancreatic cancer xenografts in vivo [53]. It is likely that BMP-9 has disparate effects on ECs depending on the cellular context and concentration of BMP-9. The effects of BMPs on ECs can be regulated by various BMP antagonists and modulators as well. For example, BMPER is an extracellular matrix protein expressed by ECs, which was shown to modulate BMP-4 activity in a concentration-dependent manner, and to exert proangiogenic effects in vascular ECs [54]. Interestingly, *MGP* gene deletion in mice leads to misregulated BMP signaling and as a result in arteriovenous malformation (AVMs) in lungs and kidneys [55]. Thus, selective BMP family members can stimulate and/or inhibit angiogenesis. Besides, BMP-induced signaling in ECs response can switch from stimulation to inhibition when co-stimulated with other signals, e.g. Notch [56]. As mentioned earlier, Notch was shown to have an important role in stalk cell determination. Recently, Moya et al. reported that endothelium-specific inactivation of Smad1/Smad5 in mouse embryos decreased Notch signaling and increased numbers of tip cells. In HUVECs downregulation of Smad1/5 reduced the expression of Notch target genes Hes1 and Hey1, and other stalk cell specific transcripts [57], In addition, Larrivée et al. showed that ALK1-dependent SMAD signaling collaborated with Notch signal to induce expression of HEY1 and HEY2 in stalk cells, which would limit the response of stalk cells to VEGF and thus reduce endothelial tip cell formation and sprouting [58].

A lot of research has focused on ECs due to their role in the formation of new vessels. However, research showed that SMCs are also involved in the maturation of the new-formed vessels, as well as in vascular diseases. In addition to their effects on EC function, BMPs were also shown to play key roles in SMC differentiation and function. BMPs have been shown to inhibit the proliferation of vascular SMC while enhancing the differentiation of these cells [59–61]. BMP-2 inhibits the proliferation of cultured rat arterial SMCs in the presence of serum and injury-induced intimal hyperplasia in the in vivo rat carotid artery balloon injury model by inhibiting SMC proliferation without stimulating extracellular matrix synthesis [61]. BMP-7 inhibits primary human aortic SMC proliferation in serum-stimulated conditions, as well as upon induction with platelet-derived growth factor subunit BB (PDGF-BB) and TGF-β1, and maintains the expression of the vascular SMC phenotype. Furthermore, anti-inflammatory activities have been attributed to BMP-7 suggesting that BMP-7 may play an important role in maintaining vascular integrity [59,62]. BMP-4, however, is expressed by ECs in response to hypoxia and it promotes vascular SMC proliferation [63]. It has been demonstrated that vascular SMCs isolated from different parts of the pulmonary vasculature have different proliferation responses to BMP-4. Whereas the proliferation ability of human pulmonary arterial SMCs isolated from proximal pulmonary arteries is inhibited by BMP-4, the proliferation of human pulmonary artery SMCs from peripheral arteries is increased by BMP-4 [64]. In summary, similarly to ECs, the effects of BMPs on vascular SMCs depend on the source of cells and the culture condition.

6. BMP signaling pathway in vascular diseases

The critical role of BMP signaling in vascular function was further corroborated by genetic studies in human [65]. Genetic analysis revealed that mutations in genes of the BMP signaling or genes which affect BMP signaling function lead to vascular dysfunction and disease such as hereditary hemorrhagic telangiectasia (HHT) and pulmonary arterial hypertension (PAH), vascular calcification, and tumor angiogenesis. In addition, disturbance of vascular homeostasis due to vascular injury, hypertension or atherosclerosis was shown to affect the expression of BMPs, thereby suggesting a role of BMPs in abnormal vascular responses [65].

6.1. Pulmonary arterial hypertension

PAH is a disease characterized by elevated pulmonary artery pressure leading to heart failure. Processes underlying PAH include abnormal remodeling of small peripheral vessels in the lung, due to aberrant proliferation and migration of vascular SMCs, ECs and fibroblasts [66]. Two types of PAH have been described: sporadic or idiopathic PAH (IPAH) and hereditary or familial PAH (FPAH). Heterozygous germ line mutations in *BMPR2* are found in more than 70% of patients with FPAH and 20% of patients with IPAH [67,68]. Mutations have been found in various regions of *BMPR2*, including the ligand-binding domain, the kinase domain, or the long cytoplasmic tail. Mice expressing a *BMPR2* tail domain

mutation in pulmonary SMCs develop vascular lesions similar to PAH [69]. Non-sense mutations in the C-terminal tail of *BMPR2* were identified also in some FPAH patients, suggesting that this region might play an important role in BMP signaling [67,68]. Heterozygous and homozygous BMPR2 deletion specifically in pulmonary ECs and pulmonary SMCs mimicked the PAH phenotype [69,70]. Endothelial injury and enhanced inflammatory responses may contribute together with BMPR2 heterozygosity to the development of PAH [71]. Interestingly it was shown that disruption of BMPR2 expression in PASMCs leads to reduced BMP-2 and BMP-4 signaling, while signaling by BMP-6 and BMP-7 is enhanced [72]. It was shown that reduced BMP/Smad signaling resulted in activation of the p38 MAPK pathway, leading to aberrant PASMC proliferation [64,73,74]. A recent report suggested that lack of endothelial nitric-oxide synthase (eNOS) due to BMPR2 mutations in pulmonary artery ECs (PAEC) may contribute to the pathogenesis of PAH. BMP-2 and BMP-4 cannot activate eNOS in *BMPR2* knockdown cell lines or in PAEC from *BMPR2* gene mutations patients and inhibition of NOS activity inhibited BMP-2 and BMP-4 stimulated PAEC migration [75].

Mutations in *Smad8* have also been reported in PAH patients [76]. In addition, loss of Smad8 function in mice results in abnormal vascular remodeling and increased vascular inflammation [77]. It was demonstrated that *Smad8* mutation leads to vascular cell proliferation in HPAH, due to decreased expression of specific micro RNAs (miR) miR-21 and miR-27a in pulmonary artery ECs and pulmonary artery SMCs from tissues of PAH patients [78]. Additionally, overexpression of Smad8 resulted in increased expression of miRs and reversed the hyper-proliferative phenotype [78]. Interestingly, certain HHT2 patients develop PPH-like syndromes, suggesting that ALK1 mutations can also be involved in PPH [79,80]. Moreover *alk1*$^{+/-}$ mice display increased pulmonary vascular remodeling which may lead to signs of PAH. This was shown to be associated with eNOS-dependent reactive oxygen species (ROS) production and it could be averted by anti-oxidant treatment [81].

6.2. Hereditary hemorrhagic telangiectasia

Mutations in the ALK1 gene have been reported in some PAH patients [79]. ALK1 mediates both TGF-β and BMP-9 signaling in ECs. Interestingly, mutations in ALK1 lead to another vascular disease related to deregulated BMP signaling, HHT. HHT is an autosomal dominant disease and is associated with telangiectases in skin and mucosa, frequent epistaxis, and the presence of AVMs in the lung, liver or brain [82]. HHT type 1 (HHT1) results from pathogenic mutations in ENG that lead to haploinsufficiency of endoglin [83], while HHT type 2 (HHT2) is caused by loss of function or dominant negative mutations in ALK1 [84,85]. Interestingly mice heterozygous for *acvrl1* (*alk1*), *tβr1* (*alk*5), *tβr2* and *eng* develop vascular abnormalities highly reminiscent of those described in patients with HHT [25,50]. Several studies have provided evidence that haploinsufficiency of the HHT genes both in ECs and SMCs leads to abnormal EC proliferation and SMC recruitment. As a result, vascular abnormalities and fragile leaky vessels occur, together with the generation of telangiectasias and AVMs [86,87]. In addition, disrupted Notch signaling has been reported to correlate with AVMs [88], and ChIP-seq analyses on human umbilical vein ECs (HUVECs) and pulmonary arterial SMCs pretreated with BMPs have demonstrated JAG1 as a direct target of Smad1/5 [89]. Another report showed that human polymorphic variants of tyrosine-protein phosphatase non-receptor type 14 (PTPN14) influences the severity of pulmonary arteriovenous malformation acting via ALK1 and EphrinB2, which suggested that PTPN14 may also be involve in the pathogenesis of HHT [90].

6.3. Atherosclerosis and vascular calcification

Atherosclerosis is a chronic arterial wall disease that is characterized by chronic inflammation and the accumulation of atheromatous lesions in the inner layer of arteries. BMPs have been implicated in atherosclerosis progression by regulating endothelial inflammation and cell differentiation. BMP-2 and -4 have been shown to induce proinflammatory effects in the ECs [91,92]. Besides, inhibiting BMP signaling pathway by MGP resulted in reduced atherosclerotic lesions formation in apolipoprotein (Apo) E knockout mice, while enhanced BMP activity led to increased atherosclerotic lesions formation in Apo E knockout mice [91,93]. Atherosclerosis is the most common cause of aortic aneurysms, a vascular disease which attributes to misregulation of TGF-β signaling [94,95]. However, Jones et al. showed that in 2-week post thoracic aortic aneurysms induction mice, the expression level of BMP signal components and BMP regulators were elevated in mRNA level, indicating that activation of BMP signaling may also be involved in the pathogenesis of aortic aneurysms [96].

One key histological and clinical event of atherosclerosis is vascular calcification, which is known as the abnormal deposition of calcium phosphate salts in blood vessels, myocardium, and cardiac valves. Vascular calcification is a tightly regulated process which leads to differentiation of cells such as SMCs or pericytes into osteoblast-like cells, and the mineralization of the extracellular matrix [97]. It is speculated that the course of vascular calcification shares many similarities with that of bone mineralization [98]. Pericytes, mesenchymal stem cells, multipotent cells from the adventitia, resident cells in the media or intima and trans-differentiated SMCs, are the possible cells which transdifferentiate into osteoblast-like cells in blood vessels [99–102]. It has been suggested that vascular endothelial cells may contribute to osteogenic differentiation [103]; ECs can transdifferentiate into mesenchymal stem cells through a process termed endothelial to mesenchymal transition (EndoMT) [104–106]. Interestingly, in fibrodysplasia ossificans progressiva (FOP), a disease characterized by overactive osteoblasts and ectopic bone formation and linked to a point mutation in BMP type I receptor ALK2 [107], it was shown that ECs can acquire a progenitor-like phenotype and differentiate into bone forming osteoblastic cells [103].

BMPs expression is increased at vascular calcification sites; in addition BMPs can trigger the differentiation of multipotential cells into the osteogenic lineage. This raises the possibility that BMPs may be involved in the process of vascular calcification [108–112]. Indeed it was shown that BMPs can direct osteogenic programming of vascular mesenchymal progenitors of the pericyte lineage [110] and that they can promote expression of osteoblast lineage markers such as alkaline phosphatase in cultured vascular SMCs [93,97,98,113–115]. Cheng et al. showed that BMP-2 and the osteoblast homeoprotein Msx2 were expressed during the osteogenic process in the aorta of diabetic patients. The BMP-2-Msx2 signaling pathway may enhance vascular calcification by promoting the differentiation of myofibroblasts into the osteogenic lineage [116]. In addition BMP-2 enhances the expression of Runx2, a core transcription factor that is known to regulate osteoblast and chondrocyte differentiation and promote vascular SMCs calcification by increasing oxidative stress and endoplasmic reticulum (ER) stress in human coronary artery SMCs. Interestingly, the inhibition of oxidant stress or ER stress reversed this gene expression pattern and mineralization process [117]. Moreover, recent research showed that BMPs are involved in vascular calcification in low-density lipoprotein (LDL) receptor-deficient (LDLR$^{-/-}$) mice. Blockade of BMP type I receptor function by using either the small molecule inhibitor LDN-193189 or ALK3-Fc in LDLR$^{-/-}$ mice inhibited high-fat diet-induced vascular inflammation as well as osteogenic activity and

calcification, thus suggesting BMP inhibition as a potential treatment for vascular calcification.

BMP signaling antagonists have been also implicated in vascular calcification. Research suggested that MGP might influence vascular calcification by modulating the effect of BMP-2. In C3H10T1/2 cells, MGP overexpression inhibited BMP-2 induced osteogenic and chondrogenic differentiation, whereas lack of MGP enhanced these differentiation processes [118]. Notably, it was shown that transgenic expression of MGP in ApoE$^{-/-}$ mice results in diminished Smad1/5/8 signaling and reduced inflammation, lesion formation, and calcification after fat feeding [93]. On the other hand MGP deficient ApoE$^{-/-}$ mice displayed enhanced Smad1/5/8 signaling and extensive medial calcification [93]. However, recent research showed that MGP can inhibit calcification in a BMP-2 independent manner in intact vessels and lack of GlaMGP (carboxylated MGP) was not the reason for medial calcification in rat renal failure model [119].

As mentioned earlier the inhibitory Smad6 interferes specifically with the BMP pathway. Interestingly, perturbation of Smad6 expression was found to be associated with calcification of the aortic valve. In human aortic valve (AV), high levels of BMP antagonists (noggin and CV-2/BMPER) and Smad6 were detected in the ventricular endothelium, while low levels of such inhibitors were found in the fibrosa endothelium. This uneven distribution was shown to be responsible for the side-dependent calcification of human AVs [120]. In addition, mutations in the *Smad6* gene were found to predispose to congenital cardiovascular malformation. The capacity of Smad6 to inhibit BMP-induced osteogenic differentiation was significantly decreased by a C484F mutation in Smad6 [121]. Thus, BMPs may be important in the pathology of vascular calcification, even though definitive evidence supporting this is still lacking.

6.4. Tumor angiogenesis

Tumor growth beyond 2–3 mm in size makes diffusion insufficient to supply tumor cells with oxygen and nutrients and for the removal of the waste products [122]. Angiogenesis, i.e. the formation of new blood vessels from pre-existing ones, is then needed for the tumors to grow. In addition, blood vessels provide the main route for metastatic spread [122]. Several inhibitors of angiogenesis, such as bevacizumab (monoclonal antibody targeting VEGF) and sorafenib and sunitinib (tyrosine kinase inhibitors) have been used for the treatment of solid tumors [123,124].

BMPs have been found misexpressed in gastric, ovarian, prostate, pancreatic breast, lung and colon tumors [125–131]. BMP-2 and BMP-4 were shown to favor angiogenesis by stimulating the secretion of pro-angiogenic growth factors, such as VEGF [52,132]. In the case of lung cancer, BMP-2 is highly expressed in the majority of patient-derived lung carcinomas [133] and recombinant BMP-2 potently increases the size and number of blood vessels in tumors formed by A549 cells in nude mice [134]. Moreover, either recombinant noggin or an anti-BMP-2 antibody could inhibit the activity of BMP-2, resulting in a significant reduction in tumor growth [133]. Besides BMP-2, other BMPs have also been reported to be involved in tumor angiogenesis. Rothhammer et al. showed that BMP-2 and BMP-4 are highly expressed in malignant melanomas, and they promoted cell invasion and migration of microvascular endothelial cells. Moreover, ECs have a reduced tube formation capacity when BMPs activities were inhibited [135]. BMP antagonist chordin has been reported to inhibit in vitro BMP-4 induced tube formation in malignant melanoma cells [135].

ALK1, a type I receptor for TGF-β, BMP-9 and BMP-10 have received a lot of attention recently as an anti-angiogenesis target. A recent study indicated that ALK1 is widely expressed on prostate, skin, thyroid, kidney, ovary, lung, pancreas, and liver tumor blood vessels [136,137]. ALK1 is mainly expressed in developing arterial endothelial cells and is greatly reduced in adult arteries. However, ALK1 expression can be induced during tumor angiogenesis [137,138]. It has been suggested that ALK1 signaling and function in ECs may depend on multiple proangiogenic factors (including VEGF and bFGF), and BMP-9-induced (tumor) angiogenesis can be specifically inhibited by an ALK1 antibody (anti-ALK1) [136]. Besides, anti-ALK1 can decrease tumor growth and angiogenesis when combined with VEGF receptor inhibitor in human/mouse chimera tumor model [136]. Other research described that a soluble chimeric protein (ALK1-Fc) which serves as BMP-9 (and -10) ligand trap, can inhibit (tumor) angiogenesis by interfering with ALK1 signaling both in vitro and in vivo [137,139]. Therefore, targeting ALK1 may effectively inhibit tumor angiogenesis and it is therefore a promising therapeutic strategy for cancer patients.

Endoglin plays a crucial role in EC function. Studies in mice revealed that tumor growth and angiogenesis is reduced in endoglin-haploinsufficient mice [140]. In addition endoglin neutralizing antibodies have been used for vascular targeting and it was shown that they can inhibit both endothelial cell proliferation and tumor growth in mouse cancer models [25]. It is known that a soluble form of endoglin (sol Eng) contributes to the pathogenesis of preeclampsia [25]. Research showed that a fusion protein, which combined the endoglin extracellular domain (ECD) and immunoglobulin Fc domain, can significantly reduce VEGF induced angiogenesis in vitro and ex vivo [141], presumably by specifically binding to pro-angiogenic BMP-9 with a high affinity. These results suggest that endoglin-Fc may be used as a potential anti-angiogenesis therapeutic agent [142]. Since the process of angiogenesis is tightly regulated by BMPs, a further understanding of their molecular mechanisms will provide opportunities for better diagnosis and development of new therapies targeting angiogenesis, tumor growth, and metastatic spread of disease.

7. Conclusions and perspective

BMP signaling plays a crucial role in cardiovascular homeostasis and disease. Genetic studies in mice indicate that components of BMP signaling are involved in EC and SMC interactions, EC function and angiogenesis. The knowledge regarding the role of BMP signaling in vascular diseases and cancer has mainly come from mouse models and clinical investigations. However, definitive evidences from functional studies in human tissues are still rare. Genetic mouse model studies showed that BMP function might depend on cell type and environment, but the availability of human tissues and the limited life span of patient-derived somatic cells limit the development of this research area. The use of induced pluripotent stem cells (iPSCs) technology could help to overcome these limitations [143–145]. Generated iPSCs from human skin fibroblasts, keratinocytes, adipose stem cells and lymphocytes [146–148], can be differentiated into various cell types [149], including ECs and SMCs [150]. It is possible to utilize this new technology to generate ECs and SMCs from patients with vascular disorders (and from healthy volunteers) in order to investigate the pathology of vascular diseases and perhaps transplant cells to cure patients [151], or perform screens to identify small chemical compounds to rescue disease phenotypes. Of interest, the BMP receptor antagonist dorsomorphin and its more selective derivative LDN-193189 have recently been reported to inhibit BMP signaling [152,153]. Yu et al. found that dorsomorphin selectively inhibited the BMP type I receptors ALK2, ALK3 and ALK6 and blocked BMP-mediated SMAD1/5/8 phosphorylation [152]. In addition, an optimized compound (LDN-193189 or DM-3189) with higher activity and specificity for BMP type I receptors has been developed from a structure–activity relationship study on dorsomorphin [153]. The

ongoing development of small molecule inhibitors/activators of BMP signaling will offer new opportunities for manipulating BMP signaling in therapeutic means. This will benefit future therapy of BMP related diseases caused by insufficient BMP signaling, such as PAH and overactive BMP signaling, such as tumor angiogenesis and FOP [154].

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