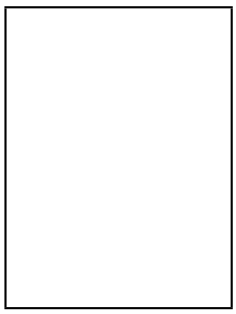
Accepted Manuscript



Title: BMP signaling in vascular biology and dysfunction

Author: Amaya García de Vinuesa Salim Abdelilah-Seyfried Petra Knaus An Zwijsen Sabine Bailly

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

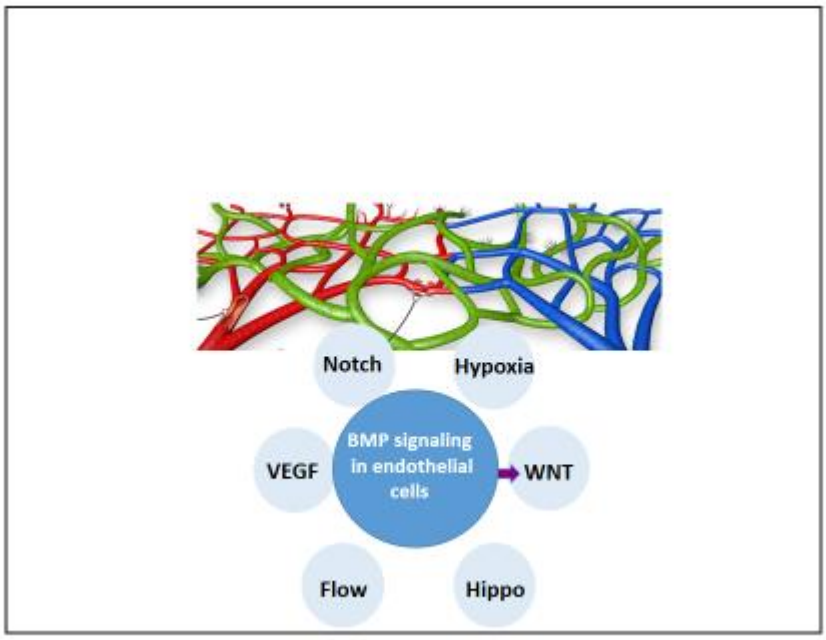
BMP signaling in vascular biology and dysfunction

Amaya García de Vinuesa[1], Salim Abdelilah-Seyfried[2,3], Petra Knaus[4], An Zwijsen[5,6*], Sabine Bailly[7,8,9*]

[1]Department of Molecular Cell Biology, Cancer Genomics Centre Netherlands, Leiden University Medical Center, Leiden, the Netherlands.
[2]Institute of Biochemistry and Biology, Potsdam University, Karl-Liebknecht-Straße 24-25, D-14476 Potsdam, Germany.
[3]Institute of Molecular Biology, Hannover Medical School, Carl-Neuberg Straße 1, D-30625 Hannover, Germany.
[4]Institute for Chemistry and Biochemistry, Freie Universitaet Berlin, Berlin, Germany.
[5]VIB Center for the Biology of Disease, Leuven, Belgium
[6]KU Leuven, Dept Human Genetics, Leuven, Belgium
[7]Institut National de la Santé et de la Recherche Médicale (INSERM, U1036), Grenoble, France F-38000
[8]Commissariat à l'Énergie Atomique et aux Energies Alternatives, Institut de Recherches en Technologies et Sciences pour le Vivant, Laboratoire Biologie du Cancer et de l'Infection, Grenoble, France F-38000
[9]Université Grenoble-Alpes, Grenoble, France F-38000

*Co-Last authors

Graphical Abstract



Highlights

- BMP signaling is a core signaling cascade in endothelium during vascular development and homeostasis
- Unbalanced BMP signaling is causative for several vascular dysfunctions
- BMP signaling is highly context-dependent in the vasculature
- Signaling interplay between BMPs and Notch, WNT, and Hippo signaling cascades co-regulates endothelial cell biology
- BMP signaling is implicated in interpretation of mechanosensitive responses derived from blood flow
- BMP and anti-BMP treatments are progressively been considered for the treatment of vascular disorders

Abstract

The vascular system is critical for developmental growth, tissue homeostasis and repair but also for tumor development. Bone morphogenetic protein (BMP) signaling has recently emerged as a fundamental pathway of the endothelium by regulating cardiovascular and lymphatic development and by being causative for several vascular dysfunctions. Two vascular disorders have been directly linked to impaired BMP signaling, pulmonary arterial hypertension and hereditary hemorrhagic telangiectasia. Endothelial BMP signaling critically depends on the cellular context, which includes amongst others vascular heterogeneity, exposure to flow, and the intertwining with other signaling cascades (Notch, WNT, Hippo and hypoxia). The purpose of this review is to highlight the most recent findings illustrating the clear need for reconsidering the role of BMPs in vascular biology.

Keywords: Bone morphogenetic proteins (BMP); Signaling; Vasculature; Development; Disease

1. Introduction

The vascular system is critical for developmental growth as well as for tissue homeostasis and repair. It allows an adequate supply of oxygen and nutrients, removal of waste products, and transports liquid and cells through blood and lymphatic vessels. These vessels consist of endothelial cells (ECs) lining the interior surface, which – depending on the vessel type - are covered by pericytes or vascular smooth muscle cells (VSMCs). The vasculature is constantly adapting to meet demands from tissues, which undergo growth, repair or regression. During embryonic development, vessel formation first depends on vasculogenesis, which refers to the *de novo* formation of blood vessels from locally differentiating ECs. Subsequently, angiogenesis or lymphangiogenesis produces new blood vessels or lymphatic vessels from pre-existing ones; all these processes can also occur during adult life. Vascular development via sprouting angiogenesis and vascular pruning requires the integration of multiple signaling cascades, including vascular endothelial growth factor (VEGF), Notch/Dll4 (delta-like protein 4) and the TGFβ (transforming growth factor)/BMP (Bone morphogenetic protein) pathways [1, 2].

An extensive review has recently been dedicated on the role of BMP signaling in cardiovascular disease [3]. In this review, we discuss the role of the different BMP subgroups in angiogenesis, lymphangiogenesis, cardiovascular development, and their function in vascular diseases. In addition, the intertwining between BMPs and other signaling pathways that impact vascular biology will be highlighted. We will conclude by asking pertinent questions in this emerging field e.g. on how mutations in the same pathway can result in different diseases in different vascular beds, and how non-SMAD versus SMAD pathways or BMP9/10 contribute to endothelial heterogeneity.

2. BMP signaling

The BMP family consists of about 20 ligands, which are subdivided into at least four groups based on sequence similarity, and affinities for specific receptors [4]; the BMP2/4 subgroup; the BMP5-7 subgroup; the BMP9/10 subgroup and the GDF5-7 (growth and differentiation factor) subgroup. The different subgroups have been implicated in vascular biology, with BMP signaling in the endothelium being triggered mostly by BMP2/4/6/9/10 [5]. BMPs are secreted dimeric ligands that signal via binding to heteromeric combinations of type 1 [ALK1 (also known as SKR3), ALK2 (ACTRIA), ALK3 (BMPRIA) and ALK6 (BMPRIB)] and type 2 (BMPRII, ActRIIA, ActRIIB) transmembrane serine/threonine kinase type receptors (Fig.

1) [6]. BMP-receptor binding triggers phosphorylation of the receptor-regulated SMAD1, -5 and -8, in their C-terminal segment. Once phosphorylated these SMADs form hetero-oligomeric complexes with SMAD4 that bind to DNA together with other transcription factors, initiating the SMAD pathway (Fig. 1). In pulmonary and some other ECs, BMPs also activate SMAD2 though less potently [7]. Furthermore, in epithelial cells, mixed complexes of pSMAD2 and pSMAD1 have been reported which can activate different sets of target genes [8]. Whether or not this is valid in ECs has not yet been documented. BMPs can trigger non-SMAD-signaling pathways involving different MAPK kinases (ERK, p38 and JNK), PI3 kinase/AKT, PKC and others [9] (Fig. 1). BMP signaling can be modulated by co-receptors such as Endoglin, which is strongly expressed on ECs [10], and agonistic or antagonistic extracellular proteins such as BMPER (BMP-binding endothelial cell precursor-derived regulator), which play an important role in vascular systems [11]. BMPs regulate (Fig. 1) and are also regulated by inputs from other pathways that fine-tune BMP signaling activity according to context (see section 7, Fig. 4).

3. BMP signaling subgroups in angiogenesis

3.1.The BMP2/4 subgroup

Ligands of the BMP2/4 subgroup bind to ALK3 and ALK6 and the corresponding type II receptors (BMPRII, ActRIIA or ActRIIB) (Fig. 1) to initiate distinct signaling pathways (SMAD or non-SMAD) depending on the mode of receptor oligomerization [12]. While SMAD signaling primarily leads to transcriptional regulation of SMAD target genes, non-SMAD pathways include also direct remodeling of the cytoskeleton or the plasma membrane which leads to the polarization and migration of cells [13] or to the induction and/or crosstalk regulation of pathways such as MAPK, JNK or PI3K (Fig. 1). *Bmp2*- and *Bmp4*-knockout mice are early embryonic lethal due to extraembryonic malformations, impaired cardiac development, and massive mesoderm defects, respectively [14]. These early and severe defects have precluded the analysis of potential vascular phenotypes.

BMP2 and BMP4 have been described to mediate pro-angiogenic effects both *in vitro* as well as *in vivo* [15, 16]. There have been contradictory reports regarding the proliferative effect of BMP2 in pulmonary arteries versus human umbilical vein ECs (HUVECs) [17, 18]. BMP4 was also shown to induce the proliferation of mouse embryonic stem cell (ESC)-derived endothelial cells (MESECs) and human microvascular endothelial cells (HMECs)

[19]. BMP2 and BMP4 both induce migration and tube formation in human microvascular ECs and HUVECs [16, 18, 19]. BMP4 promotes the proliferation and migration of ECs via stimulation of VEGF-A/VEGFR2 and angiopoietin-1/TIE2 signaling [19]. BMP4, but also BMP7 and BMP9, decrease the expression of Apelin in ECs, the ligand for the G protein coupled receptor APJ, which is enriched in tips cells [20].

3.2.The BMP5/6/7 subgroup

The ligands of the BMP5-7 subgroup can bind to ALK3, ALK6 or ALK2 [12], thereby offering a wider range of response (Fig. 1). *Bmp7* deficient mice display kidney failure, and defects in skeletogenesis, eye and heart development, and in neurogenesis [21]. However, deletion of *Bmp5* or *Bmp6* is not lethal suggesting that functional compensation by other family members takes place, at least in part. These studies did not allow conclusions on their potential implication in vascular development. However, interestingly, analysis of *Bmp6;Bmp7* double mutants showed that BMP6/7 were required for cardiac cushion formation [22].

In vitro, BMP6 induces migration and tube formation in bovine aortic ECs [23]. BMP6 induced migration of ECs was shown to be MyoX dependent, and both MyoX and ALK6 co-localize in filopodia of polarized cells [24]. Cyclooxygenase-2, which catalyzes the conversion of arachidonic acid to prostaglandins, was also shown to play a key role in BMP6-mediated angiogenesis [25]. A Chip-Seq analysis of SMAD1/5 binding sites in HUVECs showed that most of the sites identified with BMP6 overlapped with those of BMP9. The target genes include *ID1* (*Inhibitor of differentiation proteins*), *HEY1* (*hes-related family bHLH transcription factor with YPRW motif 1*), *BMPRII*, *Endoglin,* and *JAG1* (*Jagged1*) supporting the notion that these BMPs share some molecular mechanisms in ECs [26]. BMP7 increased *VEGF receptor* expression, proliferation and tube formation in ECs [27]. However, it was also recently published that a variant form of BMP7 represses tube formation in a SMAD4-independent manner, and decreases *VEGFR2* and *FGR1* expression [28].

3.3. The BMP9/10 subgroup

BMP9 and BMP10 bind to the endothelial-specific receptor ALK1 with very high affinity (EC_{50} = 50 pg/mL, 2 pM) [29-31] (Fig. 1). BMP9 can also bind to ALK2 but only in the presence of a type II receptor [31], while BMP10 can also bind ALK3 [32]. BMP9 and BMP10 bind to the three BMP type II receptors though apparently with different affinities [29,

33]. The direct binding of BMP9 and -10 to the co-receptor Endoglin contrasts other TGFß family members that require the presence of a type 1 or type 2 receptor for Endoglin binding [30, 31, 34, 35]. So, despite apparent sequence homology it is likely that BMP9 and BMP10 do not always use the same receptor complex.

The main source of BMP9 and BMP10 are the liver and the heart, respectively [36-38]. The onset of *Bmp10* expression precedes *Bmp9* expression in the mouse (E8.5 versus E9.75–10) [39]. BMP9 and BMP10 are present in the circulation (0.5-15 ng/mL [30, 39]). However, plasma from *Bmp9*-knockout mice is not able to activate a BMP-SMAD signaling reporter (BRE, BMP Responsive Element [40]) [41], and most of the BRE-activity is inhibited by a neutralizing anti-BMP9 antibody [30, 39, 41] suggesting that BMP9 is the bioactive form within the circulation.

The role of ALK1 and Endoglin in vascular development has been clearly demonstrated by gene deletion in mice. Both *Acvrl1* (ALK1) and *Eng* deletions lead to embryonic lethality due to severe vascular abnormalities, including excessive capillary fusion [arteriovenous malformations (AVMs)], hyperdilation of vessels and deficiency of differentiation and recruitment of VSMCs [42-45]. *Bmp10*-deficient mice die between E9.5 and E10.5, due to cardiac development defects [46], but recently vascular defects in the yolk sac and in the embryo have also been found [39]. On the other hand, *Bmp9* inactivation results in viable mice. Analysis of the retinal vascularization in these mice, as a model of physiological angiogenesis, does not show any significant defect [39, 41]. However, injection of a neutralizing anti-BMP10 antibody in *Bmp9* knockout mice strongly inhibits vascular expansion of the retina and induces an increase in vessel density, demonstrating a redundancy between these two BMPs in vascular development [39, 41]. Similar results were obtained by injecting the extracellular domain of ALK1 ($ALK1_{ECD}$), which traps all ligands of ALK1 [41, 47]. BMP9 overproduction inversely inhibits retinal sprouting [47]. Redundancy between BMP9 and BMP10 has recently also been shown in the closure of the ductus arteriosus [48]. The role of ALK1, BMP9, and BMP-10 in vascular development has been confirmed in the zebrafish [49]. Injection of *bmp9* morpholinos had no effect on cranial vasculature but generated a venous remodeling defect in the tail [50]. On the other hand, *bmp10* morphants present a phenotype that is indistinguishable from *alk1* morphants [51]; arteries are enlarged, contain supernumerary ECs and AVMs connect the arterial system underlying the midbrain and hindbrain to adjacent veins. Therefore, BMP10, which is expressed first in mice and zebrafish, apparently plays a key role during early embryonic development and may control

EC numbers in nascent arteries [39, 51]. With the onset of BMP9 synthesis, both BMPs are involved in blood vascular development in an interchangeable manner and ensure vascular quiescence. Interestingly, this does not seem to account for lymphatic development, as *Bmp9* knockout mice present lymphatic defects [52] (see section 4).

Although *in vivo* data clearly indicate major roles for BMP9 and BMP10 in vascular remodeling, the cellular mechanisms mediated by BMP9 and BMP10 signaling in ECs are still not fully understood. Pro- and anti-angiogenic roles have been proposed for BMP9/BMP10 [7, 30, 31, 53-61]. These studies used different ECs, different doses of BMP9 and different experimental models, which likely explains the observed differences. *Ex vivo*, BMP9 inhibits EC sprouting in the metatarsal culture model and in angiogenic pancreatic islet cultures [31, 62] and it inhibits neo-angiogenesis in the sponge assay [63]. Concerning cellular mechanisms, BMP9 and BMP10 regulate the expression of many genes involved in vascular remodeling. They increase the synthesis of *ID1*, *ID2*, *SMAD6*, *SMAD7*, *ENG*, *BMPRII*, *Interleukin-8* (*IL-8*), *E-selectin*, *Endothelin-1*, *CXCR4* (the SDF1 (stromal-derived factor 1) receptor) [7, 30, 53, 55, 56, 64], while they reduce *APLN* (*Apelin*) expression [20, 41, 64]. BMP9 and BMP10 activate the expression of transcription factors (*HEY1*, *HEY2*, *HES1*) as well as ligands *JAG1* (*Jagged1*) of the Notch signaling pathway [26, 41, 47, 65-67], but also *ephrinB2* [56], *FGFR1* and *2* [68] and *VEGFR1* [47] supporting important crosstalks between the BMP signaling pathways and other pathways (see further). Interestingly, BMP9 also strongly induces the expression of *ALK3*, *ALK6* and *BMPR2* allowing ECs to respond to other BMPs [55] in a positive feed forward loop as previously proposed [69]. Conversely, BMP9 also increases the expression of *BMPER*, which negatively regulates the activity of many BMPs [11, 70] (Fig. 1).

Altogether, these data show that the circulating BMP9 and BMP10, owing to the high expression of their receptors BMPRII, ALK1 and Endoglin in ECs, are two fundamental players in vascular development. Their cellular roles are still not completely understood but they are likely to function in a dose- and context-dependent manner within normal and dysregulated vascular beds. Importantly, BMP9 has recently been tested with success in pre-clinical mouse trials to treat vascular dysfunction like PAH [59] and ischemic neovascularization [60] (see section 6.5).

4. BMPs and lymphangiogenesis

The lymphatic vascular system drains interstitial fluids into the circulation, and regulates fat uptake and immune control. It is involved in numerous pathologies such as lymphedema,

lymph stasis, inflammatory diseases and tumor metastasis [71]. BMP signaling has recently been shown to be essential for lymphatic development in mice and zebrafish. Niessen et al. [72] showed that injection of $ALK1_{ECD}$ or of a blocking anti-ALK1 antibody into newborn mice disrupted lymphatic development within the tail and intestine. An anti-proliferative role of ALK1 in postnatal lymphatic development was proposed because of the presence of enlarged lymphatic vessels within the cornea, intestine, and diaphragm of mice with an induced deletion of *Alk1* [73]. Enlarged lymphatic capillaries and collecting lymphatic vessels in mesentery, ear skin and back skin are also present in *Bmp9*-deficient mice, compatible with BMP9 being the ALK1 ligand involved in lymphatic vascular development [52, 73]. Furthermore, *Bmp9*-knockout mice also have a reduced number of lymphatic valves and decreased drainage efficiency [52]. Further, adenovirus-mediated overexpression of *BMP9* diminishes inflammatory lymphangiogenesis in mice [73]. *In vitro*, lymphatic endothelial cell (LEC) sprouting is induced by the addition of $ALK1_{ECD}$, $BMPRII_{ECD,}$ or $ACVRIIB_{ECD}$ [72]. In accordance with this result, BMP9 and BMP10 inhibit LEC proliferation, migration, and tube formation [73, 74]. In LEC, BMP9 inhibits *LYVE-1* expression in agreement with a role in lymphatic maturation and induces the expression of several genes known to be involved in valve formation (*Foxc2, Connexin37, EphrinB2* and *neuropilin1*) [52]. BMP9 also transiently inhibits the expression of the lymphatic transcription factor *Prox1* [52, 73] and induces *SMAD6* expression [72]. BMP9 is also proposed to induce the reprograming of LEC to blood ECs (BECs) as it inhibits the expression of *VEGFR3, Podoplanin, Neuropilin2* expression and increases *VEGFR2*, *Endoglin* and *TIE2* expression [73]. Taken together, these data are in support of a role of BMP9 in inhibiting lymphangiogenesis *in vitro* and in promoting lymphatic maturation and valve formation *in vivo*.

BMP2 also regulates lymphatic development: *Bmp2b* overexpression inhibits the differentiation of LECs in zebrafish embryos and addition of BMP2 attenuated LEC differentiation in mouse embryoid body cultures [75]. Mechanistically, BMP2 promotes the expression of miRNAs, including *miR-31* and *miR-181a*, which in turn negatively regulate the stability of *Prox-1* mRNA [75]. On the other hand, morpholinos against the receptors *Alk3, Alk3b*, *Bmpr2a* and *-b* or *Smad5* decreased the number of LECs in zebrafish [76]. These results are in contrast with the anti-lymphangiogenic effects of BMP2 signaling which also signals via Alk3 [75] and suggest that other BMPs can bind to ALK3 and play a pro-lymphangiogenic role.

BMPs have also been involved in tumor lymphangiogenesis. *BMP9* expression in the tumor mammary cell line 4T1 inhibits tumor lymphangiogenesis [73]. In contrast, blockade of

ALK1 reduced tumor lymphangiogenesis in the MDA-MB-231 mouse breast cancer cell model [77]. Taken together, these results illustrate that - like in the case of blood vessels - BMP signaling may modulate development and/or maintenance of lymphatic vessels in a context-dependent manner.

5. BMPs in cardiovascular development

BMPs play well-established roles in the regulation of early heart development and morphogenesis (reviewed in [78]). Recent studies, mainly in zebrafish but also in mice, show that BMPs also mediate the accommodation of the vasculature to altered hemodynamics and vascular remodeling, a new emerging domain for BMP signaling.

5.1. BMPs in the formation of axial and caudal veins in zebrafish

In tune with cell culture experiments, striking molecular differences for the roles of BMPs were also discovered in the zebrafish vasculature. ECs of the embryonic axial and caudal veins require BMP signaling to initiate sprouting morphogenesis, whereas those of the neighboring dorsal aorta are not responsive to this molecular cue [79]. These differences in sensitivity can be explained by differences in the expression of essential components of BMP signaling between both arterial and venous cell types. The higher expression levels of BMP type II receptors [79, 80], cargo-adaptor protein Dab2 [80], a factor that is required for the propagation of BMP signaling via SMADs [81], and LDL receptor-related protein 1, a component of the endocytic machinery required for BMP receptor signaling [82], may make venous beds more sensitive to BMPs. Two reviews have covered the main discoveries of these studies and, hence, this work will not be further discussed here [83].

Bmp2b-induced sprouting from the caudal vein was recently shown to be mediated through a regulatory cascade involving Arhgef9b, Cdc42, and Formin-like 3, an Actin-regulatory protein important for filopodia formation [84].

BMPs also play important roles in the stabilization of caudal vein EC differentiation by activating β-catenin-dependent transcriptional activity in a BMP receptor-dependent manner, which leads to the transcription of venous-specific genes including *nr2f2*/*coup-tfII*, a key regulator of caudal vein differentiation in the zebrafish embryo [85]. The activation of β-catenin by Bmps involves a Bmp-dependent upregulation of Angiogenic factor with G patch and FHA domains 1 (aggf1), a chromatin-associated nuclear protein that is required for venous specification of ECs in the zebrafish embryo that has been implicated as the causative

factor in a human venous endothelial malformation that is part of the Klippel-Trenaunay syndrome [86]. Recent studies have implicated *miR-26a* as a negative regulator of Bmp signaling during formation of the zebrafish caudal vein. *miR-26a* targets *Smad1* in ECs and the overexpression of *miR-26a* in zebrafish inhibited caudal vein formation [87].

However, a number of findings also point at different context-dependent molecular and cellular roles for BMP signaling in arteries and veins in mouse and zebrafish. There is prominent BMP-SMAD signaling in arteries and veins in the mouse embryo, as indicated by nuclear pSMAD1/5/8 expression and activity from a transgenic BRE:GFP reporter [65, 88]. Also, the primarily arterial defects observed in mice with endothelial deficiency of *Acvrl1*/*Eng*, or with an endothelial-specific double knockout of *Smad1;Smad5* contrast with the exclusive venous phenotypes upon loss of BMP signaling in the zebrafish. The phenotypes observed under these conditions included ectopic sprouting of intersomitic vessels from the dorsal aorta [65]. Yet, the different approaches used to interfere with BMP signaling in zebrafish and mouse may also contribute to some of the observed differences [89]. Also, the role of BMP within arteries might only be very temporal and, hence, a combination of sophisticated inducible cell-type specific gain- and loss-of-function approaches and their crucial rescue experiments are required to resolve this issue.

5.2. BMPs and the endocardium

The endocardium is a specialized endothelial bed that is lining the interior of the myocardium. Studies in the mouse demonstrate that myocardial expression of BMPs (reviewed in [78, 90, 91]), and especially BMP2 [91-93], are critical for induction of endocardial cushion differentiation and endothelial-to-mesenchymal transition (EndMT) in the atrioventricular canal, which precedes valve formation. BMP4 is also required for endocardial cell proliferation in the outflow tract [94] while compound mutants for *Bmp6/7* show distinct valvulogenesis defects [22].

Recent studies in zebrafish confirm that BMP signaling plays also important roles in early endocardial morphogenesis and differentiation [95, 96]. During cardiac ballooning, when the two cardiac chambers of zebrafish approximately double to triple in volume, the inhibition of BMP signaling almost completely blocks endocardial cell proliferation [95]. The myocardium is a rich source of BMPs (reviewed in [78]) and among the BMPs that are expressed within the myocardium are BMP 2/4/5/6/7/10/16 [97, 98]. While the activity of BMP signaling is dampened within the myocardium (in the early zebrafish this is in part due

to the strong activation of inhibitory Smad6a [99]), endocardial cells in zebrafish and mouse strongly express the BMP receptor genes *alk3/6/8* and *alk2/6* respectively, and are highly responsive to BMPs as indicated by high levels of nuclear pSMAD-1/5/8 [78, 95]. In mouse, ectopic production of Noggin within the cushion endocardium only, results in undersized bradycardic hearts with immature cardiomyocyte contractile apparatus, hypoplastic endocardial cushions and altered expression profiles of *Alk1/Endoglin* and BMP/TGFβ effectors [100]. In line with this is the observation that BMPER and Smad6 dampen the BMP-SMAD pathway in cardiac cushions to control cushion mesenchyme thickness [101, 102]. This illustrates that balanced levels of BMP activity are necessary for endocardial-cardiomyocyte crosstalk, and that suppression of BMP signaling results in both heart valve and myocardial trabeculae defects.

Depletion of myocardial cells in zebrafish by chemical inhibition using the genetically-encoded nitroreductase system prevents normal endocardial differentiation as indicated by the lack of expression of the endocardial-specific marker gene *nfatc1* [96]. The endocardial phenotype was rescued by expression of *Bmp2b*, which complemented the lack of myocardium and restored the expression of *nfatc1*. Hence, myocardium-derived Bmp2/4 signaling is pivotal for endocardial proliferation and differentiation.

5.3. BMP signaling and blood flow responses

Intriguingly, endocardial proliferation during these developmental stages was also triggered by the mechanical stimulus of blood flow [95]. Hemodynamic alterations induced by shear stress are sensed in the endothelium by PECAM/VE-cadherin/VEGFR2, proteoglycans embedded in the glycocalyx, the primary cilium and ion channels and are transmitted into biochemical signaling cues. Crosstalk between biomechanical and BMP signaling has been described before in several cell systems including osteoblasts and ECs [51, 103-105]. However the molecular mechanisms of how distinct mechanosensitive target genes are regulated synergistically with the BMP pathway in the vasculature are still largely unclear.

In particular the fraction of retrograde flow is a hemodynamic parameter with a strong impact on endocardial cell proliferation. These observations support the involvement of BMP signaling in mechanosensitive responses to blood flow and circulatory BMP ligands. One example is the BMP receptor ALK1, which is activated in response to blood flow [106, 107] (Fig. 2). Conversely, the occlusion of blood vessels or a lack of blood flow will result in the activation of angiogenesis or adaptive arteriogenesis in the adult. In zebrafish, blood flow

promotes Alk1 activity by concomitantly inducing *alk1* expression and distributing Bmp10, thereby reinforcing this pathway, which is proposed to limit arterial caliber at the onset of flow [51].

Altered hemodynamics in the adult aortic valve leaflets in mice upregulate the levels of the endothelial adhesion molecules ICAM-1 and VCAM-1 in a BMP4-dependent manner [108], while transient and persistent SMAD1/5/8 phosphorylation was reported upon exposure of endothelium to laminar shear stress (LSS) or oscillatory shear stress (OSS), respectively [104] (Fig. 2). Remarkably, the pro-inflammatory role of BMP4 was only shown for the ligand in systemic but not in pulmonary circulation [109]. BMP2, in contrast to BMP4, was not shown to be shear stress responsive, yet, it also elicits pro-inflammatory responses [110]. Force-specific activation of SMAD1/5 has been reported to cause endothelial proliferation in stenosed rat aorta, which involves BMP receptor-integrin interactions (ALK6-αvβ3) [111]. This pathway subsequently activates Runx2/mTOR/p70S6K pathways that regulate the cell cycle [111] (Fig. 2). The authors concluded that BMP-SMAD activation occurred ligand-independently because overexpression of the BMP-antagonist Noggin could not rescue the effect; however such an approach would not rule out the involvement of BMP9/10 and ALK1-mediated activation of SMAD1/5 because BMP9/10 are Noggin insensitive [63]. Recently, MSX1 was identified in mice as an arterial-specific transcriptional mediator of SMAD1/5 dependent transduction of extrinsic arterial shear after femoral artery ligation. MSX1 induces an inflammation-mediated vascular remodeling response by direct induction and production of ICAM-1 and VCAM-1 [112] (Fig. 2).

Flow is reported to regulate cilia in ECs, with cilia being present when flow is low and disturbed, while being absent in regions with high shear stress [113]. Flow-induced loss of cilium sensitizes the endothelium for EndMT in cardiac valve forming regions in the embryo. Moreover, lack of primary cilium sensitizes ECs for BMP-induced mineralization in the adult vessel wall in atherosclerotic plaques [113, 114]. The primary cilium in ECs is thought to arrest the cells in a quiescent stage by controlling β-catenin availability. The lack or the disruption of the primary cilium leads to nuclear accumulation of β-catenin and activation of expression of *Slug* (Fig. 2). BMP-SMAD signaling potentiates expression of *Slug* in a β-catenin-dependent manner, thereby contributing to the atherosclerotic calcification of ECs [114]. Whether pSMAD1/5 triggers potentiation of β-catenin through aggf1, like in venous identity specification [85], has remained unaddressed. Recently, it was shown that BMP6, but not BMP9, synergizes with pro-atherogenic oxidized low-density-lipoproteins on transcription of different osteogenic and chondrogenic proteins in

human aortic ECs [115], linking BMP signaling and oxidative stress, and the above described flow and inflammation in recruiting vascular calcification associated with atherosclerosis.

6. BMPs in vascular diseases

Disruption of BMP signaling has been associated with the development of various vascular disorders and vascular dysfunctions. We will present here the major ones (Fig. 3).

6.1. Pulmonary Arterial Hypertension

Pulmonary Arterial Hypertension (PAH) is a rare vascular disorder (25 cases/million) defined as an increase in mean pulmonary arterial pressure >25 mmHg at rest [116]. The increase in pulmonary vascular resistance is attributable to constriction of small pulmonary arteries caused by profound vascular remodeling. Aberrant proliferation and apoptosis resistance of ECs, VSMCs and fibroblasts lead to a reduction in the luminal area, increased blood pressure and ultimately, death from right ventricular failure. An association between mutations in BMPRII (*BMPR2)*, ALK1 (*ACVRL1*), or more recently SMAD8 (*SMAD8*) and PAH has been described [117-119].

Several BMP ligands play a key role in pulmonary hypertension. BMP2/4 stimulate BMPRII and induce endothelial nitric oxide synthase (eNOS) phosphorylation and activity leading to pulmonary artery EC (PAEC) proliferation, survival, and migration. However, in the presence of *BMPR2* mutations, BMP2/4 cannot induce *eNOS* expression in ECs, contributing to the phenotype of PAH [120]. Recent studies have demonstrated that BMPRII forms a signaling complex with ALK1 in EC in response to BMP9 and BMP10 [29-31], supporting that this pathway has a central role in the pathophysiology of PAH. BMP9 induces *BMPRII* expression that mediates the *IL-8* and *E-selectin* induction and growth inhibition observed in PAECs [7, 30]. Interestingly, PAECs from *Bmpr2 (+/-)* mice have a constitutively activated SRC kinase, an increased numbers of caveolae, and impaired endothelial barrier function supporting that non-SMAD signaling pathways are involved in the physiopathology of BMP-related vascular diseases [121].

Sildenafil and prostacyclin analogues are currently used to treat PAH and they were shown to partly restore deficient BMP signaling [122, 123]. Sildenafil is a PDE5 (phosphodiesterase 5) inhibitor, thereby blocking the enzyme for cGMP degradation. Of

interest here is that the cGMP kinase (cGKI) was shown to interact with BMPRII and to support SMAD-signaling [124] Recently, screening of FDA-approved drugs identified tacrolimus, a potent immunosuppressor, which when injected into mice with conditional deletion of *Bmpr2* prevented the development of PAH [125]. More recently, administration of BMP9 *in vivo* prevented and also reversed established pulmonary hypertension in two rat models of PAH, as well as in a new mouse model bearing a knock-in allele of a common human disease-causing allele (*Bmpr2*$^{+/R899X}$), highlighting the therapeutic potential of BMP9 as a possible treatment for PAH patients [59].

6.2. Hereditary Hemorrhagic Telangiectasia

Hereditary Hemorrhagic Telangiectasia **(**HHT) or Rendu–Osler–Weber syndrome is a rare (1/8000) autosomal dominant disorder characterized by frequent epistaxis, telangiectasia in skin and mucosa, and AVMs in lung, liver or brain, and hemorrhages associated with these vascular lesions [126]. Mutations in the genes encoding endoglin (*ENG),* ALK1 (*ACVRL1*), SMAD4 (*SMAD4)* and more recently BMP9 (*GDF2*) have been associated with the development of HHT or related HHT [127].

The cellular role of ALK1 is still not completely understood and the most frequent hypothesis is that the pathogenesis of HHT could be due to an enhanced response to angiogenic cues in ALK1- or Endoglin-deficient ECs [57]. In accordance, therapeutic approaches blocking pathological angiogenesis (such as anti-VEGF antibodies or thalidomide) have yielded beneficial effects to the patients [128, 129]. In the future, administration of BMP9 or BMP10, as for PAH [59], or additional approaches directed to stimulate the deficient pathway, could result in an effective therapy for HHT patients (discussed in [130]). In this sense, the immunosuppressant sirolimus, when given to an HHT patient to prevent liver transplantation rejection, resulted in loss of telangiectases, epistaxes, and anemia [131] suggesting that a similar approach for PAH [125] and HHT could potentially be intended in the future.

6.3. Cerebral cavernous malformation

Cerebral cavernous malformation (CCM) is a vascular dysplasia, mainly localized within the brain. CCM lesions are formed by enlarged and irregular blood vessels that often result in cerebral hemorrhages. CCM is caused by *loss-of-function* mutations in one of three genes, namely *CCM1*, *CCM2* and *CCM3*, and occurs in both sporadic and familial forms [132]. It was recently described that EndMT in CCM1-abblated ECs is mediated by upregulation of *Bmp6* and inhibitors of the TGFß and BMP pathways reduced the numbers and sizes of vascular lesions in *Ccm1*-deficient mice [133].

6.4. Vascular calcification

Vascular calcification is a common feature encountered in several pathologies such as atherosclerosis, chronic kidney disease, diabetes and hypertension. Vascular calcification is a tightly regulated process that involves differentiation of ECs into chondrocyte- and osteoblast-like cells followed by mineralization of the surrounding matrix. It was shown that BMP2 and -4 s are upregulated in atherosclerotic sites potentiating the calcification [134, 135], whereas BMP inhibition by BMP antagonist (matrix Gla protein, MGP) or treatment with pharmacological inhibitors of BMP signaling reduces vascular inflammation and calcification [136]. In this sense, overexpression of MGP is shown to attenuate vascular calcification in *ApoE*$^{-/-}$ mice [137, 138]. Another study showed that treatment with LDN-193189, a small molecule BMPRI kinase inhibitor, or $ALK3_{ECD}$ led to reduction of vascular inflammation and calcification in *Ldlr*$^{-/-}$ mice [139]. A recent study showed that oxidized-LDL (oxLDL) and BMP6 synergistically recruit osteogenic differentiation in ECs providing a potential mechanism for the interactions of BMP signaling, oxidative stress, and inflammation in recruiting vascular calcification associated with atherosclerosis [115].

6.5. Ischemic neovascularization

Vasculogenesis and angiogenesis play also a crucial role in adults in various ischemic disorders. Several therapeutic approaches are highlighting the potential of using endothelial progenitor cells (EPCs) to promote re-endothelialization of damaged vessels and to enhance neovascularization after ischemic diseases such as heart and limb ischemia. A very recent

report showed that BMP9 in a mouse hindlimb ischemia model enhances blood flow recovery *in vivo* [60].

6.7. Tumor angiogenesis

Angiogenesis plays also a key role in tumor growth, invasion and metastasis. Current anti-angiogenic therapies in cancer are mainly focusing on targeting VEGF signaling. However, the development of anti-VEGF therapy resistance as well as compensatory angiogenic mechanisms by the tumors, has urged the search of alternative approaches, including modulation of BMP signaling [136]. Altered expression of BMPs has been found in several types of cancer (ovarian, gastric, lung, colon, breast) [136]. Among them, BMP2 and -4 have been shown to promote tumor angiogenesis via different mechanisms. Blockade by BMP antagonists like Noggin or Chordin, or specific antibodies resulted in reduced tube formation in a melanoma model, or decreased tumor growth *in vivo* in a lung carcinoma mouse model (reviewed in [136]).

Since ALK1 and endoglin are specifically expressed on ECs, several studies have focused on ALK1 or Endoglin as promising targets to interfere with tumor angiogenesis [10, 140]. Targeting ALK1 directly by using anti-ALK1 antibodies or by sequestering BMP9 and 10 with the ligand trap $ALK1_{ECD}$, has been performed in mouse preclinical models and phase 2 clinical trials; Two studies published in 2015, using mouse models and $ALK1_{ECD}$, show slightly different results; one shows that $ALK1_{ECD}$ reduces tumor growth, vessel density and metastasis [141] while the other shows no effect on tumor growth but a normalization of the vessels and a beneficial effect combining $ALK1_{ECD}$ with cisplatin [142]. The conclusions of the first two clinical trials are that blocking ALK1 as a monotherapy is insufficient and that combinatory therapy should be investigated [143, 144]. Inhibition of EC proliferation and tumor growth has also been achieved in mouse cancer models by targeting Endoglin [10]. Injection of $Endoglin_{ECD}$ results in inhibition of VEGF-induced angiogenesis and tumor growth, highlighting its potential as a therapeutic target in tumor angiogenesis [145, 146].

7. BMP signaling interplay in the vasculature

BMP pathways are intensely regulated by inputs from other pathways that fine-tune BMP signaling activity according to contextual status (Notch, WNT, Hippo, FGF, VEGF, Ephrins, apelin). We will present here the most relevant signaling interplay in the vasculature.

7.1. BMP and Notch signaling

Notch signaling is critical to balance angiogenic sprouting by limiting the tip cell and promoting the stalk cell phenotype [1](Fig. 4A), to trigger EndMT in endocardium during cardiac valve formation and to regulate context dependently LEC specification and lymphatic valve formation. In contrast to BMPs, Notch mediated signaling elicits binary types of responses. Interaction between membrane-embedded Delta-like (DLL) and Jagged (JAG) ligands and the Notch-receptor requires cell-cell contact. The Notch receptor becomes proteolytically processed upon ligand-receptor interaction, its intracellular domain (NICD) translocates into the nucleus where it complexes with RBP-J and Mastermind to activate expression of target genes [147].

Different interactions between the BMP and Notch cascade have been reported in vascular cell types (reviewed in [78, 148]). BMP9 and -6 signaling directly regulate expression of *JAG1* and *HEY2* in ECs through binding of SMAD1/5 to GC-SBE binding sites in their promoter [26]. JAG1 dampens DLL4-Notch signaling in tip cells [149]. BMP-SMADs and SMAD4 can also form a complex with NICD and RBP-J resulting in BMP enhanced recruitment of the complex to the RBP-J binding site to transactivate target genes of Notch signaling like *Hey1/2* and *Cdh2* [150, 151] (Fig. 4A).

Interestingly, several studies indicate that pSMAD1/5/8 can activate Notch target genes like *Hey-1/2* and *Ephb2* in a Notch/RBPJ-independent manner [41, 47, 66, 152]. *Loss-of-function* of *Notch* and/or *Alk1* signaling in zebrafish shows that both exhibit context-specific and target-specific interactions in controlling Notch target gene expression *in vivo* e.g. in the dorsal aorta, but also that AVMs associated with *Alk1* deficiency do not result from perturbations in Notch activity [152]. This illustrates that care should be given when generalizing findings to different vascular beds and setting.

BMP and Notch signaling seem to synergize and to antagonize each other often in the same cell type to dynamically control cellular signaling responses (Fig. 4A). HEY and HES1 are basic helix-loop-helix (bHLH) transcriptional repressors, while ID proteins are HLH factors that can dimerize with bHLH proteins. The ID-HES1 interaction releases the negative autoregulation of HES1, which results in increased expression of *Hes1* [153].

However, upon increased production of HEY2, the latter can compete with HES1 for ID-binding, and HEY2-ID complexes are targeted for proteosomal degradation [65, 150]. Relative abundance of HES1, ID and HEY-components may thus pivot BMP and Notch signaling modes between synergy and antagonism [148].

Reciprocal BMP and Notch signaling between the endocardium and the myocardium is required for initiation of EndMT in the valve forming site: with *Jag1* being induced in endocardium by BMP signaling, while JAG1-Notch1 signaling regulates on its turn *Bmp2* expression in myocardium [92, 154], a process that has extensively been reviewed [78].

Recently, Neuropilin 1 (NRP1) has been linked in ECs to Notch and TGFβ signaling [155]. NRP1, a transmembrane co-receptor for several unrelated ligands, limits activation of SMAD2/3, and perhaps also SMAD1/5/8, through ALK1 and -5. Doing so, NRP1 represses actively stalk cell phenotype in the tip cell; while Notch mediated signaling represses *Nrp1* in the stalk cell. It is yet unclear how NRP1 interferes with phosphorylation of SMAD2/3; whether Notch-mediated repression of *Nrp1* involves SMADs directly or involves SMAD- and Notch-effectors. This work supports a paradigm launched by Moya et al. [65], that the tip cell is not the default response, but requires active suppression of the stalk cell phenotype. These studies show that cells transfected with siRNAs against *ALK1, ALK5, SMAD2/3* or *SMAD1/5* preferentially occupy the tip position in mosaic HUVEC tip cell competition assays. Hence, pSMAD1/5 and now also pSMAD2/3 appear critical for stalk cell competence and phenotype, likely prior tip cell selection. Interestingly, deletion of a single copy of *Nrp1* normalizes the hypervascular phenotype observed in *Alk1*-deficient retinas raising the possibility of targeting NRP1 as a therapeutic approach for HHT patients or other vascular disorders [155].

7.2. BMP and WNT signaling

WNT signaling pathways play a key role in angiogenesis, cardiac development and disease [156]. WNTs are a family of 19 secreted glycoproteins that signal through distinct pathways, referred to as the canonical WNT/β-catenin pathway, the non-canonical WNT/calcium and the planar cell polarity (PCP) pathways depending on the WNTs acting respectively via frizzled (FRZ) receptors, or via a complex composed of FRZ and LRP5/6, or the transmembrane receptor tyrosine kinases ROR2 and RYK.

Reciprocal BMP and Notch signaling is required for EndMT in the valve forming site, and regulates *Wnt4* expression in the endocardium [154]. Inhibition of WNT signaling,

presumably WNT4, in whole embryo mouse cultures has shown that *Bmp2* and *Msx1* expression in myocardium depends on WNT signaling; and either BMP2 and WNT4 can rescue the defective EndMT resulting from Notch inhibition, with WNT4 rescue requiring BMP activity [154]. The BMP/SMAD axis activates directly expression of the T-box transcription factor *Tbx20* [157], which on its turn regulates the WNT pathway to direct endocardial cushion maturation and valve elongation, but is not required for initiation of EndMT [158]. In addition, TBX20 regulates *Lef1*, a key transcriptional mediator for WNT/β-catenin signaling, in this developmental process [159] (Fig. 4B). As discussed in section 5.1 BMP signaling induces the expression of *aggf1* in ECs, which promotes β-catenin-mediated transcription of *Nr2f2/CouptfII* important for cardinal vein identity [85] (Fig. 4B).

Finally, it has been described – although not yet in ECs - that the BMP signaling duration becomes prolonged when levels of canonical WNT signaling are high. This can be explained by the fact that WNT signaling inhibits GSK3 and thus prevents GSK3-mediated inhibitory linker phosphorylation of SMAD1 and SMAD4 [160, 161] (Fig. 4B).

7.3. BMP and Hippo signaling

The Hippo cascade is a critical pathway for mechanotransduction and the regulation of cell-cell contacts; this pathway seems not to have a classical ligand-receptor interaction but to be activated by cell polarity, cell-cell contact, cellular stress and mechanotransduction (Fig. 4C). Activation of LATS1/2 (large tumor suppressor) kinases results in phosphorylation and cytoplasmic retention of amongst others YAP (yes-associated protein) and TAZ (transcriptional co-activator with a PDZ-binding domain). In the absence of Hippo signaling, YAP and TAZ act in the nucleus and regulate transcription via interaction with cognate transcription factors, like TEADs (reviewed in [162]). YAP mediates endothelial junctional stability and vascular remodeling via Angiopoietin 2 [163].

Recently, BMP-SMADs have also been implicated in Hippo crosstalk in ECs. BMP9 has been shown to induce YAP1 nuclear localization in an Endoglin-dependent manner [164]. Furthermore, the YAP target genes *CCN1* (cysteine-rich 61, CYR61) and *CCN2*, also known as connective tissue growth factor (CTGF), as well as the chemokine *CCL2* (monocyte chemotactic protein 1, MCP-1) are regulated by BMP9 [164].

It was recently shown in epithelial cells that SMAD1 linker phosphorylation mediates binding of YAP to this region resulting in full activation of SMAD-dependent transcription [165]. It is not known yet whether this will also occur in the endothelium.

YAP has recently been shown to regulate EndMT in endocardium [166]; given the pivotal role of BMP2 in this event it is tempting to speculate that Hippo and BMP pathways may cooperate in the process of atrioventricular valve formation as well.

7.4. BMP and FGF signaling

FGF signaling has been implicated in early vascular development. The FGF family consists of 18 FGFs that activate via interaction with tyrosine kinase receptors intracellular RAS-MAPK, PI3K-Akt, PLC-γ and STAT intracellular signaling pathways [167]. Many of these pathways intersect with BMP elicited non-SMAD cascades. High levels of FGF signaling will also shorten the BMP signaling duration because of increased MAPK activity and MAPK-mediated inhibitory linker phosphorylation of SMADs [160, 161], however this has not been described in ECs yet. Enhanced expression and activation of the FGF signaling pathway results in increased levels of BMPER, which has been described to dampen the BMP response and to promote angiogenesis [82, 168]. BMP was recently shown to induce FGFR1 and 2 in ECs [68].

7.5. BMP and VEGF signaling and the hypoxic environment

BMP4, BMP7 and BMP9 repress *VEGF* expression [169, 170] (Fig. 4D). BMP9 also represses the expression of *VEGFR2* and induces the expression of *VEGFR1* [19, 47, 56], which is described as a receptor trap for VEGF, further supporting that BMPs decrease VEGF signaling in ECs (Fig. 4D). Interestingly, AVM formation in *Eng* or *Alk1* loss-of-function is strongly potentiated by VEGF and reciprocally interference with the VEGF pathway has shown beneficial effects in the treatment of HHT [171, 172].

Although hypoxia is not a classical signaling pathway, it is a major trigger for angiogenesis; hence we discuss here the consequences of hypoxia on expression of BMP signaling components. BMPs secreted under hypoxic conditions – remotely from the vessel - may diffuse and contribute in concert with other pro-angiogenic signals like VEGF in the activation of tip cells in nearby vessels; this may trigger activation of distinct receptor complexes and cascades than the circulatory BMPs that activate ECs at the luminal side. Several studies have reported that expression of *BMP4* is induced by hypoxia [173-175] (Fig. 4D). Hypoxia and ischemic reperfusion in intestinal epithelium causes upregulation of *Bmp2/4* and *Alk3* and *Bmpr2* [176]. In cartilage and osteoblasts, hypoxia induced expression of *Bmp2* is partially mediated through HIF1α induced ILK/mTOR/AKT pathways [177, 178]. Conversely, BMP9/SMAD1/5/8 mediated signaling induces expression of *HIF1α* in

mesenchymal stromal cells [179], while BMP2 downregulates *HIF1α* in GBM cells [180]. It remains whether similar regulations occur in ECs.

8. Conclusions and perspectives

In this review we have zoomed in on recent discoveries on how BMP signaling co-regulates vascular biology and functions, and how impaired BMP signaling can underlie disease. One of the emerging trends is the pro-inflammatory and atheriogenic, remodeling role of BMP signaling in the vessel wall and the valvular endocardium, which seems tightly linked to the interpretation of mechanosensitive responses derived from blood flow. In addition to the crucial crosstalks of BMP signaling and Notch, WNT, or Hippo signaling cascades it becomes very clear that the BMP-pathway is a core signaling cascade in vascular cells. Exciting progress has also been made towards the development of agents for the treatment of vascular disorders.

The recent studies have opened the door for several important unanswered questions with respect to BMP signaling within endothelial beds. We have listed a few of them:

- What are the differences in ALK1-BMP9/10 signaling versus other branches of the BMP signaling pathway? In other words what determines the specificity of the type-1 receptor signaling? Which roles play non-SMAD versus SMAD signaling in explaining EC plasticity and heterogeneity?
- Are there specific targets and functions for SMAD1, SMAD5 and SMAD8? SMAD8 being linked to vascular disease suggests no redundancy in the adult vasculature.
- How widespread or scattered and dynamic is signaling in different vascular beds?
- What is the impact of miRNA or lncRNA mediated silencing of BMP signaling components on vascular integrity of different vascular beds?
- Could somatic loss-of-heterozygosity of *ALK1* or *ENG* trigger lesion development in HHT or PAH patients, as was shown for CCM [181]?
- How can mutations in the same *ACVRL1/BMPR2* pathway cause different diseases, PAH versus HHT? Can we imagine similar therapeutically approach for these 2 diseases? How do mutations of *BMPR2*, *ACVRL1* and *ENG* cause diseases that are restricted to pulmonary circulation or liver, lungs, and brain, respectively [182]?

The many levels of modulation of the BMP signaling cascade (see also review by others in this issue) by e.g. the ECM, co-receptors, competition for the receptors or the SMADs between BMPs, endocytosis, post-translational modifications and degradation, or miRNAs all

contribute to the highly context-dependent physiological roles and activities of BMPs in different vascular beds. So does the intersection between BMP and other signaling cascades; and the formation of different receptor complexes with different ligand affinities. This complexity often confounds results obtained in cell culture experiments using different cell types and model organisms. A deeper understanding of the context-dependency and the different effects of BMP signaling on arteries, veins and lymphatic vessels, on capillary beds of different organs or on tip, stalk and phalanx ECs may give insight in vessel-type restricted disorders and lead to refinement of (anti)-angiogenic therapies.

Acknowledgements

We apologize to those whose work was not cited owing to lack of space. We thank Andreas Benn and Somersault 1824 for graphic contributions. A.G.V. was supported by the Dutch Arthritis Association (Reumafonds), S.A.-S. by the excellence cluster REBIRTH and DFG grant SE 2016/8-1, A.Z. by FWO-V G.0542.13 and GOA011/012. The work was supported by the DFG funded Focus Area "DynAge" at FUB to P.K. S.B. was supported by INSERM, CEA, Université Grenoble-Alpes (UGA), Association pour la Recherche sur le Cancer, the Ligue Contre le Cancer de la Loire et de la Savoie, and the Association Maladie de Rendu-Osler (AMRO).

References

[1] Eilken HM, Adams RH. Dynamics of endothelial cell behavior in sprouting angiogenesis. Current opinion in cell biology. 2010;22:617-25.

[2] Korn C, Augustin HG. Mechanisms of Vessel Pruning and Regression. Developmental cell. 2015;34:5-17.

[3] Morrell NW, Bloch DB, Ten Dijke P, Goumans MT, Hata A, Smith J, et al. Targeting BMP signalling in cardiovascular disease and anaemia. Nature reviews Cardiology. 2015.

[4] Bragdon B, Moseychuk O, Saldanha S, King D, Julian J, Nohe A. Bone Morphogenetic Proteins: A critical review. Cell Signal. 2011;23:609-20.

[5] David L, Feige JJ, Bailly S. Emerging role of bone morphogenetic proteins in angiogenesis. Cytokine & growth factor reviews. 2009;20:203-12.

[6] Lowery JW, de Caestecker MP. BMP signaling in vascular development and disease. Cytokine & growth factor reviews. 2010;21:287-98.

[7] Upton PD, Davies RJ, Trembath RC, Morrell NW. BMP and activin type-II receptors balance BMP9 signals mediated by activin receptor-like kinase-1 in human pulmonary artery endothelial cells. The Journal of biological chemistry. 2009.

[8] Daly AC, Randall RA, Hill CS. Transforming growth factor beta-induced Smad1/5 phosphorylation in epithelial cells is mediated by novel receptor complexes and is essential for anchorage-independent growth. Molecular and cellular biology. 2008;28:6889-902.

[9] Zhang YE. Non-Smad pathways in TGF-beta signaling. Cell research. 2009;19:128-39.

[10] Paauwe M, ten Dijke P, Hawinkels LJ. Endoglin for tumor imaging and targeted cancer therapy. Expert opinion on therapeutic targets. 2013;17:421-35.

[11] Moser M, Binder O, Wu Y, Aitsebaomo J, Ren R, Bode C, et al. BMPER, a novel endothelial cell precursor-derived protein, antagonizes bone morphogenetic protein signaling and endothelial cell differentiation. Molecular and cellular biology. 2003;23:5664-79.

[12] Nohe A, Hassel S, Ehrlich M, Neubauer F, Sebald W, Henis YI, et al. The mode of bone morphogenetic protein (BMP) receptor oligomerization determines different BMP-2 signaling pathways. The Journal of biological chemistry. 2002;277:5330-8.

[13] Hiepen C, Benn A, Denkis A, Lukonin I, Weise C, Boergermann JH, et al. BMP2-induced chemotaxis requires PI3K p55gamma/p110alpha-dependent

phosphatidylinositol (3,4,5)-triphosphate production and LL5beta recruitment at the cytocortex. BMC biology. 2014;12:43.
[14] Zhang H, Bradley A. Mice deficient for BMP2 are nonviable and have defects in amnion/chorion and cardiac development. Development. 1996;122:2977-86.
[15] Langenfeld EM, Langenfeld J. Bone morphogenetic protein-2 stimulates angiogenesis in developing tumors. Mol Cancer Res. 2004;2:141-9.
[16] Rothhammer T, Bataille F, Spruss T, Eissner G, Bosserhoff AK. Functional implication of BMP4 expression on angiogenesis in malignant melanoma. Oncogene. 2007;26:4158-70.
[17] de Jesus Perez VA, Alastalo TP, Wu JC, Axelrod JD, Cooke JP, Amieva M, et al. Bone morphogenetic protein 2 induces pulmonary angiogenesis via Wnt-beta-catenin and Wnt-RhoA-Rac1 pathways. The Journal of cell biology. 2009;184:83-99.
[18] Finkenzeller G, Hager S, Stark GB. Effects of bone morphogenetic protein 2 on human umbilical vein endothelial cells. Microvascular research. 2012;84:81-5.
[19] Suzuki Y, Montagne K, Nishihara A, Watabe T, Miyazono K. BMPs promote proliferation and migration of endothelial cells via stimulation of VEGF-A/VEGFR2 and angiopoietin-1/Tie2 signalling. J Biochem. 2008;143:199-206.
[20] Poirier O, Ciumas M, Eyries M, Montagne K, Nadaud S, Soubrier F. Inhibition of apelin expression by BMP signaling in endothelial cells. Am J Physiol Cell Physiol. 2012;303:C1139-45.
[21] Luo G, Hofmann C, Bronckers AL, Sohocki M, Bradley A, Karsenty G. BMP-7 is an inducer of nephrogenesis, and is also required for eye development and skeletal patterning. Genes & development. 1995;9:2808-20.
[22] Kim RY, Robertson EJ, Solloway MJ. Bmp6 and Bmp7 are required for cushion formation and septation in the developing mouse heart. Developmental biology. 2001;235:449-66.
[23] Valdimarsdottir G, Goumans MJ, Rosendahl A, Brugman M, Itoh S, Lebrin F, et al. Stimulation of Id1 expression by bone morphogenetic protein is sufficient and necessary for bone morphogenetic protein-induced activation of endothelial cells. Circulation. 2002;106:2263-70.
[24] Pi X, Ren R, Kelley R, Zhang C, Moser M, Bohil AB, et al. Sequential roles for myosin-X in BMP6-dependent filopodial extension, migration, and activation of BMP receptors. The Journal of cell biology. 2007;179:1569-82.

[25] Ren R, Charles PC, Zhang C, Wu Y, Wang H, Patterson C. Gene expression profiles identify a role for cyclooxygenase 2-dependent prostanoid generation in BMP6-induced angiogenic responses. Blood. 2007;109:2847-53.
[26] Morikawa M, Koinuma D, Tsutsumi S, Vasilaki E, Kanki Y, Heldin CH, et al. ChIP-seq reveals cell type-specific binding patterns of BMP-specific Smads and a novel binding motif. Nucleic Acids Res. 2011;39:8712-27.
[27] Akiyama I, Yoshino O, Osuga Y, Shi J, Harada M, Koga K, et al. Bone morphogenetic protein 7 increased vascular endothelial growth factor (VEGF)-a expression in human granulosa cells and VEGF receptor expression in endothelial cells. Reproductive sciences (Thousand Oaks, Calif). 2014;21:477-82.
[28] Tate CM, Mc Entire J, Pallini R, Vakana E, Wyss L, Blosser W, et al. A BMP7 Variant Inhibits Tumor Angiogenesis In Vitro and In Vivo through Direct Modulation of Endothelial Cell Biology. Plos One. 2015;10:e0125697.
[29] Brown MA, Zhao Q, Baker KA, Naik C, Chen C, Pukac L, et al. Crystal structure of BMP-9 and functional interactions with pro-region and receptors. The Journal of biological chemistry. 2005;280:25111-8.
[30] David L, Mallet C, Mazerbourg S, Feige JJ, Bailly S. Identification of BMP9 and BMP10 as functional activators of the orphan activin receptor-like kinase 1 (ALK1) in endothelial cells. Blood. 2007;109:1953-61.
[31] Scharpfenecker M, van Dinther M, Liu Z, van Bezooijen RL, Zhao Q, Pukac L, et al. BMP-9 signals via ALK1 and inhibits bFGF-induced endothelial cell proliferation and VEGF-stimulated angiogenesis. Journal of cell science. 2007;120:964-72.
[32] Mazerbourg S, Sangkuhl K, Luo CW, Sudo S, Klein C, Hsueh AJ. Identification of receptors and signaling pathways for orphan bone morphogenetic protein/growth differentiation factor ligands based on genomic analyses. The Journal of biological chemistry. 2005;280:32122-32.
[33] Townson SA, Martinez-Hackert E, Greppi C, Lowden P, Sako D, Liu J, et al. Specificity and structure of a high affinity activin receptor-like kinase 1 (ALK1) signaling complex. The Journal of biological chemistry. 2012;287:27313-25.
[34] Nolan-Stevaux O, Zhong W, Culp S, Shaffer K, Hoover J, Wickramasinghe D, et al. Endoglin Requirement for BMP9 Signaling in Endothelial Cells Reveals New Mechanism of Action for Selective Anti-Endoglin Antibodies. Plos One. 2012;7:e50920.

[35] Barbara NP, Wrana JL, Letarte M. Endoglin is an accessory protein that interacts with the signaling receptor complex of multiple members of the transforming growth factor-beta superfamily. The Journal of biological chemistry. 1999;274:584-94.
[36] Miller AF, Harvey SA, Thies RS, Olson MS. Bone morphogenetic protein-9. An autocrine/paracrine cytokine in the liver. The Journal of biological chemistry. 2000;275:17937-45.
[37] Bidart M, Ricard N, Levet S, Samson M, Mallet C, David L, et al. BMP9 is produced by hepatocytes and circulates mainly in an active mature form complexed to its prodomain. Cellular and molecular life sciences : CMLS. 2012;69:313-24.
[38] Neuhaus H, Rosen V, Thies RS. Heart specific expression of mouse BMP-10 a novel member of the TGF-beta superfamily. Mechanisms of development. 1999;80:181-4.
[39] Chen H, Brady Ridgway J, Sai T, Lai J, Warming S, Chen H, et al. Context-dependent signaling defines roles of BMP9 and BMP10 in embryonic and postnatal development. Proceedings of the National Academy of Sciences of the United States of America. 2013;110:11887-92.
[40] Korchynskyi O, ten Dijke P. Identification and functional characterization of distinct critically important bone morphogenetic protein-specific response elements in the Id1 promoter. The Journal of biological chemistry. 2002;277:4883-91.
[41] Ricard N, Ciais D, Levet S, Subileau M, Mallet C, Zimmers TA, et al. BMP9 and BMP10 are critical for postnatal retinal vascular remodeling. Blood. 2012;119:6162-71.
[42] Urness LD, Sorensen LK, Li DY. Arteriovenous malformations in mice lacking activin receptor-like kinase-1 [In Process Citation]. Nature genetics. 2000;26:328-31.
[43] Oh SP, Seki T, Goss KA, Imamura T, Yi Y, Donahoe PK, et al. Activin receptor-like kinase 1 modulates transforming growth factor- beta 1 signaling in the regulation of angiogenesis. Proceedings of the National Academy of Sciences of the United States of America. 2000;97:2626-31.
[44] Li DY, Sorensen LK, Brooke BS, Urness LD, Davis EC, Taylor DG, et al. Defective angiogenesis in mice lacking endoglin. Science. 1999;284:1534-7.
[45] Arthur HM, Ure J, Smith AJ, Renforth G, Wilson DI, Torsney E, et al. Endoglin, an ancillary TGFbeta receptor, is required for extraembryonic angiogenesis and plays a key role in heart development. Developmental biology. 2000;217:42-53.
[46] Chen H, Shi S, Acosta L, Li W, Lu J, Bao S, et al. BMP10 is essential for maintaining cardiac growth during murine cardiogenesis. Development. 2004;131:2219-31.

[47] Larrivee B, Prahst C, Gordon E, Del Toro R, Mathivet T, Duarte A, et al. ALK1 Signaling Inhibits Angiogenesis by Cooperating with the Notch Pathway. Developmental cell. 2012;22:489-500.
[48] Levet S, Ouarne M, Ciais D, Coutton C, Subileau M, Mallet C, et al. BMP9 and BMP10 are necessary for proper closure of the ductus arteriosus. Proceedings of the National Academy of Sciences of the United States of America. 2015;112:E3207-15.
[49] Roman BL, Pham VN, Lawson ND, Kulik M, Childs S, Lekven AC, et al. Disruption of acvrl1 increases endothelial cell number in zebrafish cranial vessels. Development. 2002;129:3009-19.
[50] Wooderchak-Donahue WL, McDonald J, O'Fallon B, Upton PD, Li W, Roman BL, et al. BMP9 mutations cause a vascular-anomaly syndrome with phenotypic overlap with hereditary hemorrhagic telangiectasia. American journal of human genetics. 2013;93:530-7.
[51] Laux DW, Young S, Donovan JP, Mansfield CJ, Upton PD, Roman BL. Circulating Bmp10 acts through endothelial Alk1 to mediate flow-dependent arterial quiescence. Development. 2013;140:3403-12.
[52] Levet S, Ciais D, Merdzhanova G, Mallet C, Zimmers TA, Lee SJ, et al. Bone morphogenetic protein 9 (BMP9) controls lymphatic vessel maturation and valve formation. Blood. 2013;122:598-607.
[53] Park JE, Shao D, Upton PD, Desouza P, Adcock IM, Davies RJ, et al. BMP-9 induced endothelial cell tubule formation and inhibition of migration involves Smad1 driven endothelin-1 production. Plos One. 2012;7:e30075.
[54] Suzuki Y, Ohga N, Morishita Y, Hida K, Miyazono K, Watabe T. BMP-9 induces proliferation of multiple types of endothelial cells in vitro and in vivo. Journal of cell science. 2010;123:1684-92.
[55] Rhodes CJ, Im H, Cao A, Hennigs JK, Wang L, Sa S, et al. RNA Sequencing Analysis Detection of a Novel Pathway of Endothelial Dysfunction in Pulmonary Arterial Hypertension. American journal of respiratory and critical care medicine. 2015;192:356-66.
[56] Kim JH, Peacock MR, George SC, Hughes CC. BMP9 induces EphrinB2 expression in endothelial cells through an Alk1-BMPRII/ActRII-ID1/ID3-dependent pathway: implications for hereditary hemorrhagic telangiectasia type II. Angiogenesis. 2012;15:497-509.

[57] Choi EJ, Kim YH, Choe SW, Tak YG, Garrido-Martin EM, Chang M, et al. Enhanced responses to angiogenic cues underlie the pathogenesis of hereditary hemorrhagic telangiectasia 2. Plos One. 2013;8:e63138.
[58] van Meeteren LA, Thorikay M, Bergqvist S, Pardali E, Stampino CG, Hu-Lowe D, et al. Anti-human activin receptor-like kinase 1 (ALK1) antibody attenuates bone morphogenetic protein 9 (BMP9)-induced ALK1 signaling and interferes with endothelial cell sprouting. The Journal of biological chemistry. 2012;287:18551-61.
[59] Long L, Ormiston ML, Yang X, Southwood M, Graf S, Machado RD, et al. Selective enhancement of endothelial BMPR-II with BMP9 reverses pulmonary arterial hypertension. Nature medicine. 2015.
[60] Kim J, Kim M, Jeong Y, Lee WB, Park H, Kwon JY, et al. BMP9 Induces Cord Blood-Derived Endothelial Progenitor Cell Differentiation and Ischemic Neovascularization via ALK1. Arteriosclerosis, thrombosis, and vascular biology. 2015;35:2020-31.
[61] Nickel NP, Spiekerkoetter E, Gu M, Li CG, Li H, Kaschwich M, et al. Elafin Reverses Pulmonary Hypertension via Caveolin-1-Dependent Bone Morphogenetic Protein Signaling. American journal of respiratory and critical care medicine. 2015;191:1273-86.
[62] Cunha SI, Pardali E, Thorikay M, Anderberg C, Hawinkels L, Goumans MJ, et al. Genetic and pharmacological targeting of activin receptor-like kinase 1 impairs tumor growth and angiogenesis. The Journal of experimental medicine. 2010;207:85-100, S1-5.
[63] David L, Mallet C, Keramidas M, Lamande N, Gasc JM, Dupuis-Girod S, et al. Bone morphogenetic protein-9 is a circulating vascular quiescence factor. Circulation research. 2008;102:914-22.
[64] Young K, Conley B, Romero D, Tweedie E, O'Neill C, Pinz I, et al. BMP9 regulates endoglin-dependent chemokine responses in endothelial cells. Blood. 2012;120:4263-73.
[65] Moya IM, Umans L, Maas E, Pereira PN, Beets K, Francis A, et al. Stalk cell phenotype depends on integration of notch and smad1/5 signaling cascades. Developmental cell. 2012;22:501-14.
[66] Woltje K, Jabs M, Fischer A. Serum induces transcription of Hey1 and Hey2 genes by Alk1 but not Notch signaling in endothelial cells. Plos One. 2015;10:e0120547.
[67] Kerr G, Sheldon H, Chaikuad A, Alfano I, von Delft F, Bullock AN, et al. A small molecule targeting ALK1 prevents Notch cooperativity and inhibits functional angiogenesis. Angiogenesis. 2015;18:209-17.

[68] Rostama B, Turner JE, Seavey GT, Norton CR, Gridley T, Vary CP, et al. DLL4/Notch1 and BMP9 Interdependent Signaling Induces Human Endothelial Cell Quiescence via P27KIP1 and Thrombospondin-1. Arteriosclerosis, thrombosis, and vascular biology. 2015.

[69] Yao Y, Jumabay M, Wang A, Bostrom KI. Matrix Gla protein deficiency causes arteriovenous malformations in mice. The Journal of clinical investigation. 2011;121:2993-3004.

[70] Yao Y, Jumabay M, Ly A, Radparvar M, Wang AH, Abdmaulen R, et al. Crossveinless 2 regulates bone morphogenetic protein 9 in human and mouse vascular endothelium. Blood. 2012;119:5037-47.

[71] Zheng W, Aspelund A, Alitalo K. Lymphangiogenic factors, mechanisms, and applications. The Journal of clinical investigation. 2014;124:878-87.

[72] Niessen K, Zhang G, Ridgway JB, Chen H, Yan M. ALK1 signaling regulates early postnatal lymphatic vessel development. Blood. 2010;115:1654-61.

[73] Yoshimatsu Y, Lee YG, Akatsu Y, Taguchi L, Suzuki HI, Cunha SI, et al. Bone morphogenetic protein-9 inhibits lymphatic vessel formation via activin receptor-like kinase 1 during development and cancer progression. Proceedings of the National Academy of Sciences of the United States of America. 2013;110:18940-5.

[74] Osada M, Inoue O, Ding G, Shirai T, Ichise H, Hirayama K, et al. Platelet activation receptor CLEC-2 regulates blood/lymphatic vessel separation by inhibiting proliferation, migration, and tube formation of lymphatic endothelial cells. The Journal of biological chemistry. 2012;287:22241-52.

[75] Dunworth WP, Cardona-Costa J, Bozkulak EC, Kim JD, Meadows S, Fischer JC, et al. Bone morphogenetic protein 2 signaling negatively modulates lymphatic development in vertebrate embryos. Circulation research. 2014;114:56-66.

[76] Kim JD, Kim J. Alk3/Alk3b and Smad5 mediate BMP signaling during lymphatic development in zebrafish. Molecules and cells. 2014;37:270-4.

[77] Hu-Lowe DD, Chen E, Zhang L, Watson KD, Mancuso P, Lappin P, et al. Targeting Activin Receptor-Like Kinase 1 Inhibits Angiogenesis and Tumorigenesis through a Mechanism of Action Complementary to Anti-VEGF Therapies. Cancer research. 2011;71:1362-73.

[78] Garside VC, Chang AC, Karsan A, Hoodless PA. Co-ordinating Notch, BMP, and TGF-beta signaling during heart valve development. Cellular and molecular life sciences : CMLS. 2013;70:2899-917.
[79] Wiley DM, Kim JD, Hao J, Hong CC, Bautch VL, Jin SW. Distinct signalling pathways regulate sprouting angiogenesis from the dorsal aorta and the axial vein. Nature cell biology. 2011;13:686-92.
[80] Covassin L, Amigo JD, Suzuki K, Teplyuk V, Straubhaar J, Lawson ND. Global analysis of hematopoietic and vascular endothelial gene expression by tissue specific microarray profiling in zebrafish. Developmental biology. 2006;299:551-62.
[81] Kim JD, Kang H, Larrivee B, Lee MY, Mettlen M, Schmid SL, et al. Context-dependent proangiogenic function of bone morphogenetic protein signaling is mediated by disabled homolog 2. Developmental cell. 2012;23:441-8.
[82] Pi X, Schmitt CE, Xie L, Portbury AL, Wu Y, Lockyer P, et al. LRP1-dependent endocytic mechanism governs the signaling output of the bmp system in endothelial cells and in angiogenesis. Circulation research. 2012;111:564-74.
[83] Kim JD, Lee HW, Jin SW. Diversity is in my veins: role of bone morphogenetic protein signaling during venous morphogenesis in zebrafish illustrates the heterogeneity within endothelial cells. Arteriosclerosis, thrombosis, and vascular biology. 2014;34:1838-45.
[84] Wakayama Y, Fukuhara S, Ando K, Matsuda M, Mochizuki N. Cdc42 mediates Bmp-induced sprouting angiogenesis through Fmnl3-driven assembly of endothelial filopodia in zebrafish. Developmental cell. 2015;32:109-22.
[85] Kashiwada T, Fukuhara S, Terai K, Tanaka T, Wakayama Y, Ando K, et al. beta-Catenin-dependent transcription is central to Bmp-mediated formation of venous vessels. Development. 2015;142:497-509.
[86] Chen D, Li L, Tu X, Yin Z, Wang Q. Functional characterization of Klippel-Trenaunay syndrome gene AGGF1 identifies a novel angiogenic signaling pathway for specification of vein differentiation and angiogenesis during embryogenesis. Hum Mol Genet. 2013;22:963-76.
[87] Icli B, Wara AK, Moslehi J, Sun X, Plovie E, Cahill M, et al. MicroRNA-26a regulates pathological and physiological angiogenesis by targeting BMP/SMAD1 signaling. Circulation research. 2013;113:1231-41.

[88] Monteiro RM, de Sousa Lopes SM, Bialecka M, de Boer S, Zwijsen A, Mummery CL. Real time monitoring of BMP Smads transcriptional activity during mouse development. Genesis. 2008;46:335-46.
[89] Rossi A, Kontarakis Z, Gerri C, Nolte H, Holper S, Kruger M, et al. Genetic compensation induced by deleterious mutations but not gene knockdowns. Nature. 2015;524:230-3.
[90] Kruithof BP, Duim SN, Moerkamp AT, Goumans MJ. TGFbeta and BMP signaling in cardiac cushion formation: lessons from mice and chicken. Differentiation; research in biological diversity. 2012;84:89-102.
[91] Ma L, Lu MF, Schwartz RJ, Martin JF. Bmp2 is essential for cardiac cushion epithelial-mesenchymal transition and myocardial patterning. Development. 2005;132:5601-11.
[92] Luna-Zurita L, Prados B, Grego-Bessa J, Luxan G, del Monte G, Benguria A, et al. Integration of a Notch-dependent mesenchymal gene program and Bmp2-driven cell invasiveness regulates murine cardiac valve formation. The Journal of clinical investigation. 2010;120:3493-507.
[93] Cai X, Nomura-Kitabayashi A, Cai W, Yan J, Christoffels VM, Cai CL. Myocardial Tbx20 regulates early atrioventricular canal formation and endocardial epithelial-mesenchymal transition via Bmp2. Developmental biology. 2011;360:381-90.
[94] McCulley DJ, Kang JO, Martin JF, Black BL. BMP4 is required in the anterior heart field and its derivatives for endocardial cushion remodeling, outflow tract septation, and semilunar valve development. Developmental Dynamics. 2008;237:3200-9.
[95] Dietrich AC, Lombardo VA, Veerkamp J, Priller F, Abdelilah-Seyfried S. Blood flow and Bmp signaling control endocardial chamber morphogenesis. Developmental cell. 2014;30:367-77.
[96] Palencia-Desai S, Rost MS, Schumacher JA, Ton QV, Craig MP, Baltrunaite K, et al. Myocardium and BMP signaling are required for endocardial differentiation. Development. 2015;142:2304-15.
[97] Veerkamp J, Rudolph F, Cseresnyes Z, Priller F, Otten C, Renz M, et al. Unilateral dampening of Bmp activity by nodal generates cardiac left-right asymmetry. Developmental cell. 2013;24:660-7.
[98] Thomas PS, Rajderkar S, Lane J, Mishina Y, Kaartinen V. AcvR1-mediated BMP signaling in second heart field is required for arterial pole development: implications for

myocardial differentiation and regional identity. Developmental biology. 2014;390:191-207.
[99] de Pater E, Ciampricotti M, Priller F, Veerkamp J, Strate I, Smith K, et al. Bmp signaling exerts opposite effects on cardiac differentiation. Circulation research. 2012;110:578-87.
[100] Snider P, Simmons O, Wang J, Hoang CQ, Conway SJ. Ectopic Noggin in a Population of Nfatc1 Lineage Endocardial Progenitors Induces Embryonic Lethality. Journal of cardiovascular development and disease. 2014;1:214-36.
[101] Dyer L, Lockyer P, Wu Y, Saha A, Cyr C, Moser M, et al. BMPER Promotes Epithelial-Mesenchymal Transition in the Developing Cardiac Cushions. Plos One. 2015;10:e0139209.
[102] Galvin KM, Donovan MJ, Lynch CA, Meyer RI, Paul RJ, Lorenz JN, et al. A role for smad6 in development and homeostasis of the cardiovascular system. Nature genetics. 2000;24:171-4.
[103] Kopf J, Petersen A, Duda GN, Knaus P. BMP2 and mechanical loading cooperatively regulate immediate early signalling events in the BMP pathway. BMC biology. 2012;10:37.
[104] Zhou J, Lee PL, Tsai CS, Lee CI, Yang TL, Chuang HS, et al. Force-specific activation of Smad1/5 regulates vascular endothelial cell cycle progression in response to disturbed flow. Proceedings of the National Academy of Sciences of the United States of America. 2012;109:7770-5.
[105] Kopf J, Paarmann P, Hiepen C, Horbelt D, Knaus P. BMP growth factor signaling in a biomechanical context. Biofactors. 2014;40:171-87.
[106] Seki T, Yun J, Oh SP. Arterial endothelium-specific activin receptor-like kinase 1 expression suggests its role in arterialization and vascular remodeling. Circulation research. 2003;93:682-9.
[107] Corti P, Young S, Chen CY, Patrick MJ, Rochon ER, Pekkan K, et al. Interaction between alk1 and blood flow in the development of arteriovenous malformations. Development. 2011;138:1573-82.
[108] Sucosky P, Balachandran K, Elhammali A, Jo H, Yoganathan AP. Altered shear stress stimulates upregulation of endothelial VCAM-1 and ICAM-1 in a BMP-4- and TGF-beta1-dependent pathway. Arteriosclerosis, thrombosis, and vascular biology. 2009;29:254-60.

[109] Csiszar A, Labinskyy N, Smith KE, Rivera A, Bakker EN, Jo H, et al. Downregulation of bone morphogenetic protein 4 expression in coronary arterial endothelial cells: role of shear stress and the cAMP/protein kinase A pathway. Arteriosclerosis, thrombosis, and vascular biology. 2007;27:776-82.
[110] Csiszar A, Ahmad M, Smith KE, Labinskyy N, Gao Q, Kaley G, et al. Bone morphogenetic protein-2 induces proinflammatory endothelial phenotype. The American journal of pathology. 2006;168:629-38.
[111] Zhou J, Lee PL, Lee CI, Wei SY, Lim SH, Lin TE, et al. BMP receptor-integrin interaction mediates responses of vascular endothelial Smad1/5 and proliferation to disturbed flow. Journal of thrombosis and haemostasis : JTH. 2013;11:741-55.
[112] Vandersmissen I, Craps S, Depypere M, Coppiello G, van Gastel N, Maes F, et al. Endothelial Msx1 transduces hemodynamic changes into an arteriogenic remodeling response. The Journal of cell biology. 2015;210:1239-56.
[113] Egorova AD, Khedoe PP, Goumans MJ, Yoder BK, Nauli SM, ten Dijke P, et al. Lack of primary cilia primes shear-induced endothelial-to-mesenchymal transition. Circulation research. 2011;108:1093-101.
[114] Sanchez-Duffhues G, de Vinuesa AG, Lindeman JH, Mulder-Stapel A, DeRuiter MC, Van Munsteren C, et al. SLUG is expressed in endothelial cells lacking primary cilia to promote cellular calcification. Arteriosclerosis, thrombosis, and vascular biology. 2015;35:616-27.
[115] Yung LM, Sanchez-Duffhues G, Ten Dijke P, Yu PB. Bone morphogenetic protein 6 and oxidized low-density lipoprotein synergistically recruit osteogenic differentiation in endothelial cells. Cardiovascular research. 2015;108:278-87.
[116] Simonneau G, Gatzoulis MA, Adatia I, Celermajer D, Denton C, Ghofrani A, et al. Updated clinical classification of pulmonary hypertension. Journal of the American College of Cardiology. 2013;62:D34-41.
[117] Harrison RE, Flanagan JA, Sankelo M, Abdalla SA, Rowell J, Machado RD, et al. Molecular and functional analysis identifies ALK-1 as the predominant cause of pulmonary hypertension related to hereditary haemorrhagic telangiectasia. J Med Genet. 2003;40:865-71.
[118] Shintani M, Yagi H, Nakayama T, Saji T, Matsuoka R. A New Nonsense Mutation of SMAD8 associated with Pulmonary Arterial Hypertension. J Med Genet. 2009.

[119] Deng Z, Morse JH, Slager SL, Cuervo N, Moore KJ, Venetos G, et al. Familial primary pulmonary hypertension (gene PPH1) is caused by mutations in the bone morphogenetic protein receptor-II gene. American journal of human genetics. 2000;67:737-44.
[120] Gangopahyay A, Oran M, Bauer EM, Wertz JW, Comhair SA, Erzurum SC, et al. Bone morphogenetic protein receptor II is a novel mediator of endothelial nitric-oxide synthase activation. The Journal of biological chemistry. 2011;286:33134-40.
[121] Prewitt AR, Ghose S, Frump AL, Datta A, Austin ED, Kenworthy AK, et al. Heterozygous null bone morphogenetic protein receptor type 2 mutations promote SRC kinase-dependent caveolar trafficking defects and endothelial dysfunction in pulmonary arterial hypertension. The Journal of biological chemistry. 2015;290:960-71.
[122] Yang J, Li X, Al-Lamki RS, Wu C, Weiss A, Berk J, et al. Sildenafil potentiates bone morphogenetic protein signaling in pulmonary arterial smooth muscle cells and in experimental pulmonary hypertension. Arteriosclerosis, thrombosis, and vascular biology. 2013;33:34-42.
[123] Yang J, Li X, Al-Lamki RS, Southwood M, Zhao J, Lever AM, et al. Smad-dependent and smad-independent induction of id1 by prostacyclin analogues inhibits proliferation of pulmonary artery smooth muscle cells in vitro and in vivo. Circulation research. 2010;107:252-62.
[124] Schwappacher R, Weiske J, Heining E, Ezerski V, Marom B, Henis YI, et al. Novel crosstalk to BMP signalling: cGMP-dependent kinase I modulates BMP receptor and Smad activity. The EMBO journal. 2009;28:1537-50.
[125] Spiekerkoetter E, Tian X, Cai J, Hopper RK, Sudheendra D, Li CG, et al. FK506 activates BMPR2, rescues endothelial dysfunction, and reverses pulmonary hypertension. The Journal of clinical investigation. 2013;123:3600-13.
[126] Dupuis-Girod S, Bailly S, Plauchu H. Hereditary hemorrhagic telangiectasia (HHT): from molecular biology to patient care. Journal of thrombosis and haemostasis : JTH. 2010;8:1447-56.
[127] McDonald J, Wooderchak-Donahue W, VanSant Webb C, Whitehead K, Stevenson DA, Bayrak-Toydemir P. Hereditary hemorrhagic telangiectasia: genetics and molecular diagnostics in a new era. Front Genet. 2015;6:1.

[128] Dupuis-Girod S, Ginon I, Saurin JC, Marion D, Guillot E, Decullier E, et al. Bevacizumab in patients with hereditary hemorrhagic telangiectasia and severe hepatic vascular malformations and high cardiac output. JAMA. 2012;307:948-55.
[129] Lebrin F, Srun S, Raymond K, Martin S, van den Brink S, Freitas C, et al. Thalidomide stimulates vessel maturation and reduces epistaxis in individuals with hereditary hemorrhagic telangiectasia. Nature medicine. 2010;16:420-8.
[130] Tillet E, Bailly S. Emerging roles of BMP9 and BMP10 in hereditary hemorrhagic telangiectasia. Front Genet. 2014;5:456.
[131] Skaro AI, Marotta PJ, McAlister VC. Regression of cutaneous and gastrointestinal telangiectasia with sirolimus and aspirin in a patient with hereditary hemorrhagic telangiectasia. Annals of internal medicine. 2006;144:226-7.
[132] Fischer A, Zalvide J, Faurobert E, Albiges-Rizo C, Tournier-Lasserve E. Cerebral cavernous malformations: from CCM genes to endothelial cell homeostasis. Trends in molecular medicine. 2013;19:302-8.
[133] Maddaluno L, Rudini N, Cuttano R, Bravi L, Giampietro C, Corada M, et al. EndMT contributes to the onset and progression of cerebral cavernous malformations. Nature. 2013;498:492-6.
[134] Bostrom K, Watson KE, Horn S, Wortham C, Herman IM, Demer LL. Bone morphogenetic protein expression in human atherosclerotic lesions. The Journal of clinical investigation. 1993;91:1800-9.
[135] Dhore CR, Cleutjens JP, Lutgens E, Cleutjens KB, Geusens PP, Kitslaar PJ, et al. Differential expression of bone matrix regulatory proteins in human atherosclerotic plaques. Arteriosclerosis, thrombosis, and vascular biology. 2001;21:1998-2003.
[136] Cai J, Pardali E, Sanchez-Duffhues G, ten Dijke P. BMP signaling in vascular diseases. FEBS letters. 2012;586:1993-2002.
[137] Bostrom K, Tsao D, Shen S, Wang Y, Demer LL. Matrix GLA protein modulates differentiation induced by bone morphogenetic protein-2 in C3H10T1/2 cells. The Journal of biological chemistry. 2001;276:14044-52.
[138] Yao Y, Bennett BJ, Wang X, Rosenfeld ME, Giachelli C, Lusis AJ, et al. Inhibition of bone morphogenetic proteins protects against atherosclerosis and vascular calcification. Circulation research. 2010;107:485-94.

[139] Derwall M, Malhotra R, Lai CS, Beppu Y, Aikawa E, Seehra JS, et al. Inhibition of bone morphogenetic protein signaling reduces vascular calcification and atherosclerosis. Arteriosclerosis, thrombosis, and vascular biology. 2012;32:613-22.
[140] Cunha SI, Pietras K. ALK1 as an emerging target for antiangiogenic therapy of cancer. Blood. 2011;117:6999-7006.
[141] Cunha SI, Bocci M, Lovrot J, Eleftheriou N, Roswall P, Cordero E, et al. Endothelial ALK1 Is a Therapeutic Target to Block Metastatic Dissemination of Breast Cancer. Cancer research. 2015;75:2445-56.
[142] Hawinkels LJ, Garcia de Vinuesa A, Paauwe M, Kruithof-de Julio M, Wiercinska E, Pardali E, et al. Activin Receptor-like Kinase 1 Ligand Trap Reduces Microvascular Density and Improves Chemotherapy efficiency to Various Solid Tumors. Clinical cancer research : an official journal of the American Association for Cancer Research. 2015.
[143] Makker V, Filiaci VL, Chen LM, Darus CJ, Kendrick JE, Sutton G, et al. Phase II evaluation of dalantercept, a soluble recombinant activin receptor-like kinase 1 (ALK1) receptor fusion protein, for the treatment of recurrent or persistent endometrial cancer: an NRG Oncology/Gynecologic Oncology Group Study 0229N. Gynecologic oncology. 2015;138:24-9.
[144] Necchi A, Giannatempo P, Mariani L, Fare E, Raggi D, Pennati M, et al. PF-03446962, a fully-human monoclonal antibody against transforming growth-factor beta (TGFbeta) receptor ALK1, in pre-treated patients with urothelial cancer: an open label, single-group, phase 2 trial. Investigational new drugs. 2014;32:555-60.
[145] Hawinkels LJ, Kuiper P, Wiercinska E, Verspaget HW, Liu Z, Pardali E, et al. Matrix metalloproteinase-14 (MT1-MMP)-mediated endoglin shedding inhibits tumor angiogenesis. Cancer research. 2010;70:4141-50.
[146] Castonguay R, Werner ED, Matthews RG, Presman E, Mulivor AW, Solban N, et al. Soluble endoglin specifically binds BMP9/BMP10 via its orphan domain,inhibits blood vessel formation and suppresses tumor growth. The Journal of biological chemistry. 2011.
[147] Kopan R. Notch signaling. Cold Spring Harbor perspectives in biology. 2012;4.
[148] Beets K, Huylebroeck D, Moya IM, Umans L, Zwijsen A. Robustness in angiogenesis: notch and BMP shaping waves. Trends in Genetics. 2013;29:140-9.
[149] Benedito R, Roca C, Sorensen I, Adams S, Gossler A, Fruttiger M, et al. The notch ligands Dll4 and Jagged1 have opposing effects on angiogenesis. Cell. 2009;137:1124-35.

[150] Itoh F, Itoh S, Goumans MJ, Valdimarsdottir G, Iso T, Dotto GP, et al. Synergy and antagonism between Notch and BMP receptor signaling pathways in endothelial cells. The EMBO journal. 2004;23:541-51.
[151] Li F, Lan Y, Wang Y, Wang J, Yang G, Meng F, et al. Endothelial Smad4 maintains cerebrovascular integrity by activating N-cadherin through cooperation with Notch. Developmental cell. 2011;20:291-302.
[152] Rochon ER, Wright DS, Schubert MM, Roman BL. Context-specific interactions between Notch and ALK1 cannot explain ALK1-associated arteriovenous malformations. Cardiovascular research. 2015;107:143-52.
[153] Bai G, Sheng N, Xie Z, Bian W, Yokota Y, Benezra R, et al. Id sustains Hes1 expression to inhibit precocious neurogenesis by releasing negative autoregulation of Hes1. Developmental cell. 2007;13:283-97.
[154] Wang Y, Wu B, Chamberlain AA, Lui W, Koirala P, Susztak K, et al. Endocardial to myocardial notch-wnt-bmp axis regulates early heart valve development. Plos One. 2013;8:e60244.
[155] Aspalter IM, Gordon E, Dubrac A, Ragab A, Narloch J, Vizan P, et al. Alk1 and Alk5 inhibition by Nrp1 controls vascular sprouting downstream of Notch. Nature communications. 2015;6:7264.
[156] Franco CA, Liebner S, Gerhardt H. Vascular morphogenesis: a Wnt for every vessel? Current opinion in genetics & development. 2009;19:476-83.
[157] Mandel EM, Kaltenbrun E, Callis TE, Zeng XX, Marques SR, Yelon D, et al. The BMP pathway acts to directly regulate Tbx20 in the developing heart. Development. 2010;137:1919-29.
[158] Cai X, Zhang W, Hu J, Zhang L, Sultana N, Wu B, et al. Tbx20 acts upstream of Wnt signaling to regulate endocardial cushion formation and valve remodeling during mouse cardiogenesis. Development. 2013;140:3176-87.
[159] Singh R, Horsthuis T, Farin HF, Grieskamp T, Norden J, Petry M, et al. Tbx20 interacts with smads to confine tbx2 expression to the atrioventricular canal. Circulation research. 2009;105:442-52.
[160] Eivers E, Demagny H, Choi RH, De Robertis EM. Phosphorylation of Mad controls competition between wingless and BMP signaling. Science signaling. 2011;4:ra68.

[161] Demagny H, Araki T, De Robertis EM. The tumor suppressor Smad4/DPC4 is regulated by phosphorylations that integrate FGF, Wnt, and TGF-beta signaling. Cell reports. 2014;9:688-700.
[162] Hansen CG, Moroishi T, Guan KL. YAP and TAZ: a nexus for Hippo signaling and beyond. Trends in cell biology. 2015;25:499-513.
[163] Choi HJ, Zhang H, Park H, Choi KS, Lee HW, Agrawal V, et al. Yes-associated protein regulates endothelial cell contact-mediated expression of angiopoietin-2. Nature communications. 2015;6:6943.
[164] Young K, Tweedie E, Conley B, Ames J, FitzSimons M, Brooks P, et al. BMP9 Crosstalk with the Hippo Pathway Regulates Endothelial Cell Matricellular and Chemokine Responses. Plos One. 2015;10:e0122892.
[165] Alarcon C, Zaromytidou AI, Xi Q, Gao S, Yu J, Fujisawa S, et al. Nuclear CDKs drive Smad transcriptional activation and turnover in BMP and TGF-beta pathways. Cell. 2009;139:757-69.
[166] Zhang H, von Gise A, Liu Q, Hu T, Tian X, He L, et al. Yap1 is required for endothelial to mesenchymal transition of the atrioventricular cushion. The Journal of biological chemistry. 2014;289:18681-92.
[167] Ornitz DM, Itoh N. The Fibroblast Growth Factor signaling pathway. Wiley interdisciplinary reviews Developmental biology. 2015;4:215-66.
[168] Esser JS, Rahner S, Deckler M, Bode C, Patterson C, Moser M. Fibroblast growth factor signaling pathway in endothelial cells is activated by BMPER to promote angiogenesis. Arteriosclerosis, thrombosis, and vascular biology. 2015;35:358-67.
[169] Shao ES, Lin L, Yao Y, Bostrom KI. Expression of vascular endothelial growth factor is coordinately regulated by the activin-like kinase receptors 1 and 5 in endothelial cells. Blood. 2009;114:2197-206.
[170] Bai Y, Wang J, Morikawa Y, Bonilla-Claudio M, Klysik E, Martin JF. Bmp signaling represses Vegfa to promote outflow tract cushion development. Development. 2013;140:3395-402.
[171] Hao Q, Zhu Y, Su H, Shen F, Yang GY, Kim H, et al. VEGF Induces More Severe Cerebrovascular Dysplasia in Endoglin than in Alk1 Mice. Translational stroke research. 2010;1:197-201.
[172] Ardelean DS, Letarte M. Anti-angiogenic therapeutic strategies in hereditary hemorrhagic telangiectasia. Front Genet. 2015;6:35.

[173] Frank DB, Abtahi A, Yamaguchi DJ, Manning S, Shyr Y, Pozzi A, et al. Bone morphogenetic protein 4 promotes pulmonary vascular remodeling in hypoxic pulmonary hypertension. Circulation research. 2005;97:496-504.
[174] Maegdefrau U, Amann T, Winklmeier A, Braig S, Schubert T, Weiss TS, et al. Bone morphogenetic protein 4 is induced in hepatocellular carcinoma by hypoxia and promotes tumour progression. The Journal of pathology. 2009;218:520-9.
[175] Wang J, Fu X, Yang K, Jiang Q, Chen Y, Jia J, et al. Hypoxia inducible factor-1-dependent up-regulation of BMP4 mediates hypoxia-induced increase of TRPC expression in PASMCs. Cardiovascular research. 2015;107:108-18.
[176] Chen K, Xie W, Luo B, Xiao W, Teitelbaum DH, Yang H, et al. Intestinal mucosal barrier is injured by BMP2/4 via activation of NF-kappaB signals after ischemic reperfusion. Mediators of inflammation. 2014;2014:901530.
[177] Tseng WP, Yang SN, Lai CH, Tang CH. Hypoxia induces BMP-2 expression via ILK, Akt, mTOR, and HIF-1 pathways in osteoblasts. Journal of cellular physiology. 2010;223:810-8.
[178] Kamiya N, Shafer S, Oxendine I, Mortlock DP, Chandler RL, Oxburgh L, et al. Acute BMP2 upregulation following induction of ischemic osteonecrosis in immature femoral head. Bone. 2013;53:239-47.
[179] Hu N, Jiang D, Huang E, Liu X, Li R, Liang X, et al. BMP9-regulated angiogenic signaling plays an important role in the osteogenic differentiation of mesenchymal progenitor cells. Journal of cell science. 2013;126:532-41.
[180] Pistollato F, Rampazzo E, Abbadi S, Della Puppa A, Scienza R, D'Avella D, et al. Molecular mechanisms of HIF-1alpha modulation induced by oxygen tension and BMP2 in glioblastoma derived cells. Plos One. 2009;4:e6206.
[181] Chan AC, Drakos SG, Ruiz OE, Smith AC, Gibson CC, Ling J, et al. Mutations in 2 distinct genetic pathways result in cerebral cavernous malformations in mice. The Journal of clinical investigation. 2011;121:1871-81.
[182] Arthur H, Geisthoff U, Gossage JR, Hughes CC, Lacombe P, Meek ME, et al. Executive summary of the 11th HHT international scientific conference. Angiogenesis. 2015;18:511-24.
[183] Sieber C, Kopf J, Hiepen C, Knaus P. Recent advances in BMP receptor signaling. Cytokine & growth factor reviews. 2009;20:343-55.

Legends to Figures

Throughout all figures, genes are italicized and in capitals, while proteins are in regular font irrespective whether data is obtained in human, mouse or zebrafish models. See main text for further details, abbreviations and references.

Figure 1. **Schematic overview of BMP signaling in endothelial cells (ECs).** BMP dimers signaling are triggered by ligand binding to heteromeric surface receptor complexes of specific type II and type I receptors. Different BMP subgroups can signal via different receptor complexes (black and dashed arrows correspond respectively to high and lower affinity receptors). The relative levels of type I and type II (mRNA) in ECs are represented, this however depends on the EC origin as ALK6 has been found highly expressed in HUVECs (asterisk) [18]. Ligand binding triggers activation of the type I receptors by the type II receptors. SMAD- and non-SMAD pathways can become activated. SMAD1/5/8 are the effectors of the BMP SMAD-pathway. Phosphorylated SMAD1/5/8 (pSMAD1/5/8) form heteromeric complexes with SMAD4 and translocate to the nucleus where they regulate target gene expression by interaction with other transcription factors (not illustrated). Non-SMAD pathways (depicted in blue) involve the activation of different kinases that can affect transcriptional regulation or non-transcriptional functions [183]. BMPs regulate many signaling components involved in EC signaling.

Figure 2. **BMP signaling and hemodynamics forces in the vasculature.** BMPs and flow-induced hemodynamic changes co-regulate the remodeling response of ECs. Blood flow increases ALK1 levels. Laminar shear stress (LSS) and oscillatory shear stress (OSS) affect intracellular SMAD1/5/8 signaling differentially and OSS upregulates ICAM-1 and VCAM-1 in a BMP4-dependent manner. OSS induces synergistic interactions between BMP receptors and integrin (αvβ3) to activate SMAD1/5 through the Shc/FAK/ERK pathway, which leads to the activation of the Runx/mTOR/p70S6K pathway to regulate EC cycle. BMP-SMAD signaling induces *Msx1* in arteries during ischemic reperfusion. MSX1 then induces through induction of ICAM-1 and VCAM-1 an inflammatory response and atheriogenic remodeling response. BMP-SMAD signaling and the lack of tethering of β-catenin at the plasma membrane due to absence of the primary cilium cooperatively enhances the β-catenin-mediated induction of *Slug*, which triggers EndMT and mineralization remodeling.

Figure 3. **Defects associated with impaired BMP signaling in blood and lymphatic vasculature.** The blood vasculature consists of a hierarchical network of arteries and arterioles (red), a capillary network, and venules and veins (blue). Blind-ending lymphatic capillaries (green) drain extravasated fluid and cells from the capillary bed into collecting vessels. The lymph fluid is further transported to lymph nodes and ducts, and back into the blood circulation. Peri-endothelial cells cover the ECs; VSMCs are associated with arteries and veins, whereas the capillaries in the capillary bed are covered only sparsely by pericytes (the difference in coverage is not indicated in the scheme). The collecting lymphatic vessels are covered by few VSMCs and these vessels contain valves that prevent backflow of lymph fluid. When BMP signaling is unbalanced in the vasculature, then this can result in different blood and lymphatic vessel defects. So far two diseases have been linked to impaired BMP signaling in human: pulmonary arterial hypertension (PAH, mutations in *BMPR2, ACVRL1, SMAD8*) characterized by aberrant vascular remodeling that can lead to obstruction of small arteries and hereditary hemorrhagic telangiectasia (HHT, mutations in *ENG, ACVRL1, SMAD4, GDF2* (BMP9*)*) characterized by arteriovenous shunts, poor vessel coverage and hemorrhages. Cerebral cavernous malformation (CCM) has been associated to increased BMP6 signaling. Lymphatic defects (hyperproliferation, dilation, lack of valves, poor drainage) have so far only been observed in mouse and zebrafish models with impaired BMP2, BMP9, ALK1 and SMAD5 signaling. BMPs have also been associated with tumor angiogenesis.

Figure 4. **Intersections between BMP signaling cascades and other signaling pathways in endothelium. A.** BMP and Notch signaling synergize and antagonize in ECs. BMP-SMAD signaling induces several genes independently of Notch-mediated signaling, also so-called Notch targets (1), but pSMAD1/5/8 and NICD, the Notch intracellular domain, can interact and can synergistically induce genes (2). HES and HEY are bHLH transcriptional repressors, while ID proteins are HLH factors that can dimerize with bHLH proteins. HES1 autorepresses its own transcription, this repression is released by the interaction between ID and HES1 (3), resulting in HES1 accumulation and HES1 mediated repression of *Dll4* (4) and *Vegfr2*, which is important for stalk cell competence. When protein interactions of IDs shift towards HEY, then IDs is targeted for proteosomal degradation (5), this antagonism results in regained autorepression of HES1. pSMAD1/5/8 induced production of JAG1 dampens Notch signaling in the tip cell (6). Neuropilin 1 (NRP1), a tip cell determinant, has also been reported to be actively repressed by Notch-mediated signaling in the stalk cell. **B.** BMP and WNT signaling

enforce each other in ECs. In brief, the absence of canonical WNT ligand-receptor binding, GSK3 mediates phosphorylation and destabilization of cytoplasmic β-catenin and activated BMP-SMADs. When WNTs bind Frizzled-LRP receptors, then GSK3 activity is blocked resulting in stabilization of activated BMP-SMADs and β-catenin. In zebrafish**,** *aggf1* is induced by BMP-SMAD signaling in veins, which on its turn enforce β-catenin/Tcf/Lef mediated induction of *Nr2f2/CoupTFII*. CoupTFII is an important regulator of venous and lymphatic identity. Also in the heart BMP signaling enforces WNT signaling because LEF1 becomes enriched in endocardium through BMP-SMAD signaling induced production of TBX20. **C.** YAP and TAZ are Hippo effectors. The BMP9/Endoglin axis triggers nuclear localization of YAP, and co-regulates the YAP target genes *Ccn1/Cyr61, Ccn2/Ctgf,* as well as the chemokine *Ccl2/Mcp*-1 and subsequent cell matrix remodeling and local inflammatory responses. **D.** BMP signaling and hypoxia synergize in hypoxia sensitive processes**.** Hypoxia induces via HIF1α the induction of pro-angiogenic cues like *Vegf* but also of *Bmp2* and *-4*. It is tempting to speculate that these BMPs can enhance angiogenic sprouting and migration in nearby vessels. HIF1α also induces expression of the BMP receptors *Alk3* and *BmpRII*. Activation of the BMP-SMAD signaling cascade further boosts the HIF1α-mediated induction of hypoxia sensitive genes through the induction of *Hif1α, Ilk1* and *Ets1 (*172-173), but also down-regulates the VEGF signaling pathway.

Fig. 1

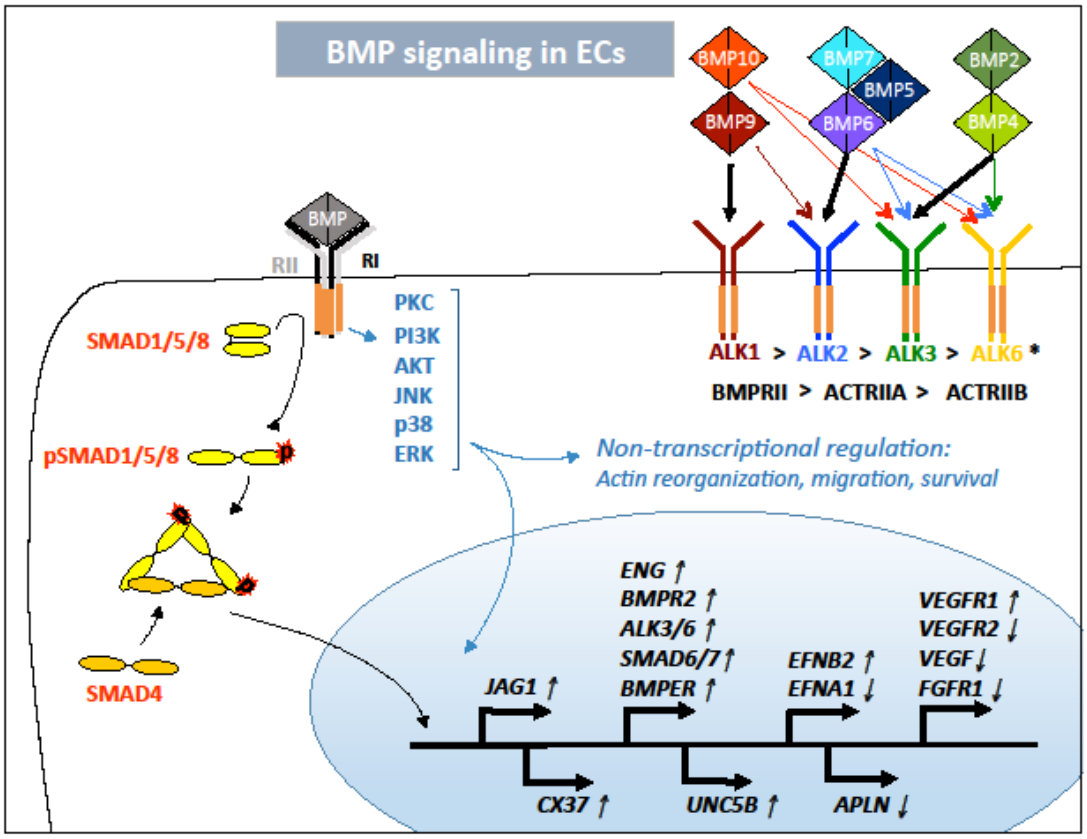
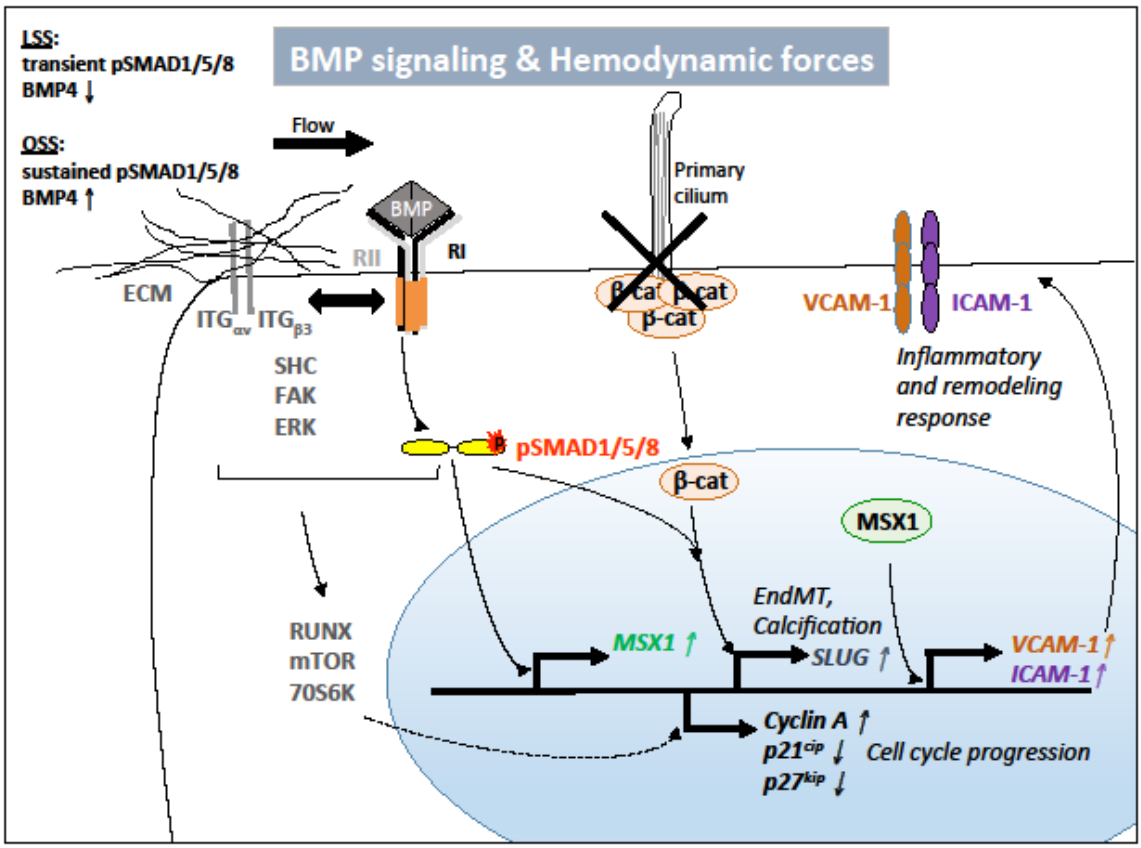

BMP signaling in ECs
BMP10
BMP9
BMP7
BMP6
BMP5
BMP2
BMP4
BMP
RII
RI
SMAD1/5/8
pSMAD1/5/8
SMAD4
PKC
PI3K
AKT
JNK
p38
ERK
ALK1 > ALK2 > ALK3 > ALK6 *
BMPRII > ACTRIIA > ACTRIIB
Non-transcriptional regulation:
Actin reorganization, migration, survival
ENG ↑
BMPR2 ↑
ALK3/6 ↑
SMAD6/7 ↑
BMPER ↑
JAG1 ↑
EFNB2 ↑
EFNA1 ↓
VEGFR1 ↑
VEGFR2 ↓
VEGF ↓
FGFR1 ↓
CX37 ↑
UNC5B ↑
APLN ↓

Fig. 2


LSS:
transient pSMAD1/5/8
BMP4 ↓
OSS:
sustained pSMAD1/5/8
BMP4 ↑
BMP signaling & Hemodynamic forces
Flow
Primary cilium
BMP
RII
RI
ECM
ITGαv ITGβ3
β-cat
β-cat
β-cat
VCAM-1
ICAM-1
Inflammatory and remodeling response
SHC
FAK
ERK
pSMAD1/5/8
β-cat
MSX1
EndMT, Calcification
RUNX
mTOR
70S6K
MSX1 ↑
SLUG ↑
VCAM-1 ↑
ICAM-1 ↑
Cyclin A ↑
p21cip ↓
p27kip ↓
Cell cycle progression

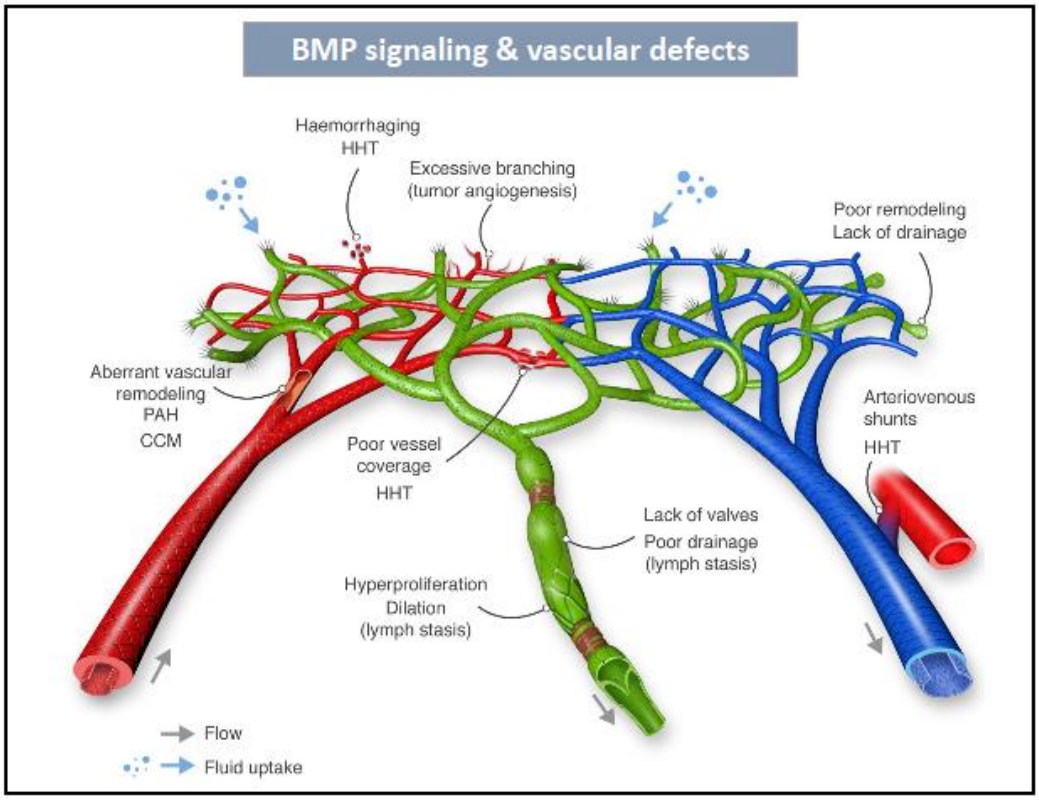
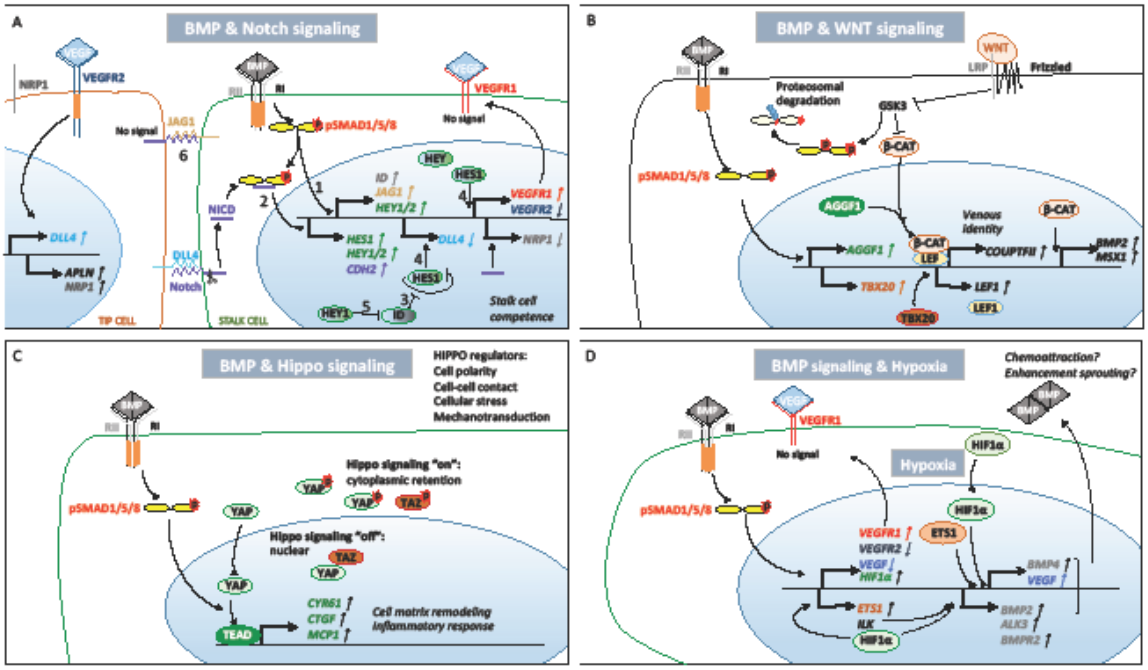

BMP signaling & vascular defects
Haemorrhaging
HHT
Excessive branching
(tumor angiogenesis)
Poor remodeling
Lack of drainage
Aberrant vascular
remodeling
PAH
CCM
Poor vessel
coverage
HHT
Arteriovenous
shunts
HHT
Lack of valves
Poor drainage
(lymph stasis)
Hyperproliferation
Dilation
(lymph stasis)
Flow
Fluid uptake

Fig. 4


A
BMP & Notch signaling
VEGF
VEGFR2
NRP1
No signal
JAG1
6
pSMAD1/5/8
VEGFR1
No signal
HEY
HES1
ID ↑
JAG1 ↑
HEY1/2 ↑
VEGFR1 ↑
VEGFR2 ↓
NICD
2
1
4
DLL4 ↑
APLN ↑
NRP1 ↑
HES1 ↑
HEY1/2 ↑
CDH2 ↑
DLL4 ↓
NRP1 ↓
DLL4
Notch
4
HES1
3
HEY1
5
ID
TIP CELL
STALK CELL
Stalk cell competence
B
BMP & WNT signaling
WNT
LRP
Frizzled
Proteosomal degradation
GSK3
β-CAT
pSMAD1/5/8
AGGF1
Venous identity
β-CAT
AGGF1 ↑
β-CAT
LEF
COUPTFII ↑
BMP2 ↑
MSX1
TBX20 ↑
LEF1 ↑
TBX20
LEF1
C
BMP & Hippo signaling
HIPPO regulators:
Cell polarity
Cell-cell contact
Cellular stress
Mechanotransduction
pSMAD1/5/8
YAP
Hippo signaling "on": cytoplasmic retention
YAP
YAP
TAZ
Hippo signaling "off": nuclear
TAZ
YAP
YAP
TEAD
CYR61 ↑
CTGF ↑
MCP1 ↑
Cell matrix remodeling
Inflammatory response
D
BMP signaling & Hypoxia
Chemoattraction?
Enhancement sprouting?
VEGF
VEGFR1
No signal
HIF1α
Hypoxia
pSMAD1/5/8
HIF1α
VEGFR1 ↑
VEGFR2 ↓
VEGF ↓
HIF1α ↑
ETS1
BMP4 ↑
VEGF ↑
ETS1 ↑
ILK
HIF1α
BMP2 ↑
ALK3 ↑
BMPR2 ↑