Chapter 6

General discussion

6.1 The regulation of the BMP signaling pathway

6.2 Developing FOP therapies by targeting ALK2

6.2.1 FOP disease models

6.2.2 Strategies and challenges in the development of FOP therapies based on the BMP signaling pathway

6.3 Restoration of BMPR2 signaling in PAH

6.3.1 BMPR2 in PAH

6.3.2 Strategies and challenges to restore BMPR2 expression

6.4 Soluble endoglin modifies signaling by BMP9

6.5 Concluding remarks

Bone morphogenetic proteins (BMPs) play vital roles in development and maintenance of adult tissue homeostasis. Due to the extensive biological activities they are involved in, disruptions in BMP signaling will lead to marked defects or severe pathologies in the body. In this thesis, I focused on the disturbed BMP signaling in fibrodysplasia ossificans progressiva (FOP) and pulmonary arterial hypertension (PAH). The aim of this thesis is to reveal how BMP signaling contributes to the pathology of these diseases, which might contribute to the development of effective therapeutic strategies for these diseases with unmet clinical need.

6.1 The regulation of the BMP signaling pathway

BMPs are multi-functional growth factors that are important for determining embryonic patterning and tissue morphogenesis (1). Dimeric BMP ligands initiate intracellular signaling by binding to BMP type I and type II serine/threonine kinase receptors on the cell surface to form a ternary complex. In the heteromeric complex, BMP type II receptor activates the type I receptors by phosphorylating specific serine and threonine residues in their juxtamenbrane glycine-serine-rich (GS) domains (2).

BMP signaling is extensively regulated at multiple levels, which we have described in **chapter 1**. The most important regulators for my research are the 12 kDa FK506 binding protein (FKBP12) and endoglin.

FKBP12 is widely expressed throughout the body and shows a conserved structure among different species. It was known to mediate the effects of the immunosuppressant drugs FK506 and rapamycin (3, 4). Further research showed that FKBP12 also acts as a natural ligand to bind to the GS domain of transforming growth factor beta (TGF-β) type I receptors and inhibits the leaky activation of TGF-β type I receptors in the absence of TGF-β ligands (5-7). Intact TGF-β type II receptor kinase activity is necessary for the release of FKBP12 from the type I receptors, since a mutation in the ATP binding site of the kinase domain of TGF-β type II receptors prevents the dissociation of FKBP12 from the type I receptors (5).

Interruption of the interaction between FKBP12 and TGF-β type I receptors is correlated with human diseases and could be used for drug targeting. The weak binding between FKBP12 and R206H mutant activin receptor-like kinase (ALK) 2 in FOP patients resulted in the activated response of heterotopic ossification progenitor cells to low concentration of BMP ligands, and finally

leads to ectopic bone formation in soft tissues with the involvement of other inflammatory factors (Figure 1) (8-10). On the other hand, FKBP12 may also be a drug target for curing PAH by activating BMP signaling, which we have discussed in **chapter 4** (Figure 1).

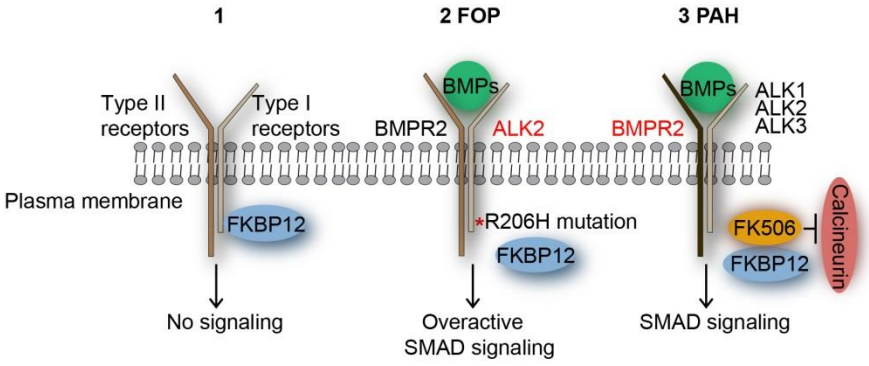

Figure 1. Schematic overview of the BMP signaling pathway. 1, FKBP12 binds to the GS domain of BMP type I receptors to prevent the signal leakage in the absence of BMPs; 2, The FOP mutation R206H in BMP type I receptor ALK2 inhibits the interaction between the ALK2 GS domain and FKBP12; 3, In PAH with dysfunctional BMPR2, FK506 can activate downstream SMAD signaling by binding to calcineurin in the presence of FKBP12, and removing FKBP12 from TGF-β/BMP type I receptor ALK1, ALK2 and ALK3. Adapted from the original figure in **Chapter 4**.

Co-receptors like endoglin that can interact with TGF-β type I and type II receptors add further regulation specificity to the BMP signaling pathway (11). Endoglin (CD105) is highly expressed in proliferating endothelial cells (ECs) (12). Various disease conditions, including hereditary hemorrhagic telangiectasia (HHT), preeclampsia and solid tumors have been associated with endoglin. In **chapter 5**, we have investigated the soluble form of endoglin and how it regulates the activity of BMP9. Finally, crosstalk with other signaling pathways could add additional layers of complexity to the BMP signaling pathway.

6.6 Developing FOP therapies by targeting ALK2

FOP is caused by a heterozygous mutation in the ALK2 gene. Most patients have the same mutation (c.617G>A; R206H) that is located in the GS domain of

ALK2 (13). Other mutations have been identified both in the GS domain and in the kinase domain of ALK2 with various phenotypes (Figure 2) (10). The clinical features of FOP include malformation of the great toes and progressive heterotopic ossification (HO) in the soft tissues. HO begins in childhood and can be triggered by traumas or occurs without warning (14). Researchers have made great progress on illustrating the molecular mechanisms of FOP since the identification the FOP mutant gene in 2006. However, the underlying mechanisms involved in the HO in FOP are still not clearly clarified, and to date there is no cure or even treatment for HO in FOP.

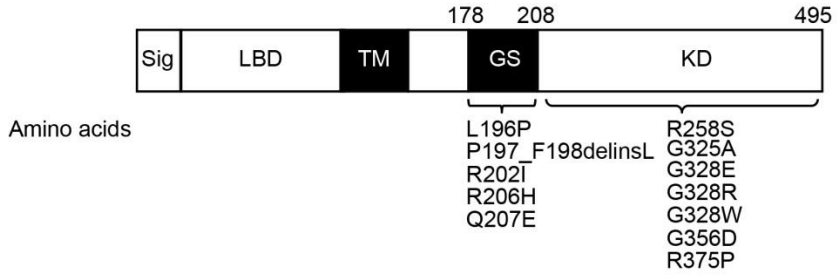

Figure 2: Schematic overview of the ALK2 structural protein domains and the FOP mutations in the GS and kinase domains. Signal peptide (Sig), Ligand binding domain (LBD), Transmembrane domain (TM), GS domain (GS), and kinase domain (KD).

6.2.1 FOP disease models

It is not easy to obtain tissues from FOP patients, because physical and surgical injuries can induce new traumas and trigger HO. Previous research in FOP animal models have helped us to understand the role of BMP signaling in the pathology of FOP. For instance, Yu *et al.* showed that blocking mutant *ALK2* by an ALK2 kinase small molecule inhibitor might be a useful treatment, as revealed from experiments in a FOP mouse model expressing constitutively active ALK2 (15). Chakkalakal *et al.* showed the first direct evidence that the ALK2 R206H mutation is the cause of FOP using a chimeric mouse knock-in model (16). The work on animal models might also provide novel research and treatment targets for FOP. Recently, a FOP conditional knock-in mouse model was introduced by Hatsell *et al.*(17). This mouse model recreated FOP disease phenotypes by exhibiting HO progressively in various locations of the body.

Importantly, in this FOP mouse model the novel finding that activin A signals through mutant R206H ALK2 to induce FOP was verified. Blocking activin A activity with antibodies prevented the HO phenotype in this FOP mouse model, which indicates that activin A is a potential therapeutic target in FOP (17).

Validation in human samples of disease phenotypes and molecular mechanisms found in animal models will facilitate us to better understand the pathology of human diseases. Previously, researchers obtained FOP patients' samples in the form of lymphoblastoid cell lines or connective tissue of the discarded milk teeth of FOP children (13, 18). Due to the rarity feature of this disease and limited accessibility of patient's tissues, we have focused on modeling FOP disease using human induced pluripotent stem cells (hiPSCs). This can provide an alternative human research model that complements the *in vivo* studies of FOP mouse models. hiPSCs can provide a large amount of cells for research, and, more importantly, hiPSCs have the potential to differentiate into various cell linages that can mimic the disease phenotypes in the pathological cells. There are several publications on the establishment of hiPSCs from FOP patients and their subsequent differentiation into chondrocytes and osteoblasts (19, 20). Our research on this topic is described in **chapter 2**.

Our FOP hiPSC disease model needs to be improved on many aspects before applying it in high-throughput drug development. One crucial issue of the application of hiPSCs is to choose proper controls. Previous publications have shown that there are variations in different hiPSC clones and these variations (resulting from (epi) genetic changes) may arise during reprogramming and culturing (21-25). We found that the small-molecule ALK2 kinase inhibitor LDN-212854 could rescue the excessive osteoblast differentiation of FOP hiPSC-pericytes (**chapter 2)**. However, we also observed clone variations between FOP or wild-type hiPSCs derived from different donors. The further application of hiPSCs may need to use multiple control hiPSC lines from different donors or gene-edited hiPSCs from the same genetic background (19).

6.2.2 Strategies and challenges in the development of FOP therapies based on the BMP signaling pathway

The identification of *ALK2* mutation in FOP patients provides a specific druggable target for this disease. The first approach to inhibit HO progression in FOP was to block the activity of the mutant ALK2 receptors (14). Based on this principle, the small-molecule inhibitor of BMP type I receptor LDN-193189 was used to inhibit ALK2 mediated SMAD1/5 phosphorylation and to reduce ectopic ossification in the FOP mouse model expressing constitutively active $ALK2^{Q207D}$ and concomitant inflammation (15). Thereafter other small-molecule BMP type I receptor kinase inhibitors have been discovered, including LDN-212854, DMH1 and K02288 (26-28).

Targeting the overactive ALK2 by anti-sense oligonucleotides (AONs) or siRNAs that suppress (mutant) ALK2 expression is the other possible strategy for the treatment and prevention of HO in FOP patients (29-31). Previous researches showed that the allele specific siRNA technique can be used to target the disease-causing ALK2 (30, 31). The siRNAs were applied in the patient' cells to restore BMP activity and osteogenic differentiation (30, 31). In **chapter 3**, we used the AON-mediated exon skipping technique to target mouse wild-type *Alk2* to prevent the increased BMP signaling in FOP. AON-mediated exon skipping has been reported to reframe the mutated dystrophin mRNA and restore protein synthesis of dystrophin protein in skeletal muscle of Duchenne muscular dystrophy patients (32, 33). Systemic delivery of AONs is less challenging than of siRNAs. However, the application of the AON technique in FOP is still in the preliminary stage. We have attempted to specifically target the ALK2 R206H allele with human ALK2 AONs, but so far this has not been successful. The other problem, targeting HO progenitor cells by ALK2 AON, also needs to be solved.

Recently work indicated that TGF-β type II receptors need to cooperate with the mutated ALK2 in FOP. BMP type II receptor (BMPR2) and activin receptor type IIb (ActR2B) are involved in the activation of BMP signaling by mutant ALK2 (R206H, G325A) (34, 35). Thus, BMPR2 could be another novel therapeutic target for FOP drug development, for instance development of AONs and siRNAs targeting BMPR2.

As already indicated above, a critical issue for developing effective therapies for FOP is to identify the target cells for the treatment. However, the obtained results on the identification of the HO progenitor cells are still inconclusive. Earlier linage tracing experiments in mouse models suggested that Tie2-expressing cells contribute to HO lesions (36). Research conducted by

Medici *et al.* showed that TIE2 and the endothelial marker von Willebrand factor (vWF) are co-expressed in FOP patient lesions, and a HO mouse model supported the endothelial original of HO in FOP (37). However, Wosczyna *et al.* reported that $Tie2^+$ multipotent mesenchymal cells are a predominant source of progenitors, but not the $Tie2^+$ ECs (38). As Tie2 is not a specific cell linage marker and at least expressed in ECs, hematopoietic stem cells and pericyte precursors, it is possible that other cell lineages may also contribute to HO in FOP. Other cells like circulating osteogenic precursors, skeletal myoblasts and vascular smooth muscle cells (SMCs) were also found in FOP lesions, and may contribute to HO in FOP as well (36, 39, 40).

Besides the activating mutations in the ALK2 gene, HO induction also correlates with soft tissue injury and resultant inflammation in tissue microenvironments (41). Anti-inflammatory glucocorticoid drugs have been applied for the management of early flare-ups in limited cases (42). In addition, anti-inflammatory and anti-angiogenic drugs like cyclo-oxygenase-2 (cox-2) inhibitors and non-steroidal anti-inflammatory drugs (NSAIDs) have been applied in clinical treatments (43). As already described above, a recent publication by Hatsell *et al.* indicates the involvement of activin A in HO in a FOP conditional knock-in mouse model (17). Activin A regulates both the innate and adaptive immune responses in response to injury and inflammation (44). Activin A might thus link the activated SMAD signaling and inflammatory flare-ups in FOP lesions, and bring a new drug target to cure FOP (17). It should be noted that the experimental work on FOP described in chapter 2 and 3 was performed before the publication of the paper by Hatsell *et al.*(17). By performing chondrogenic and osteogenic differentiation of FOP hiPSCs or ALK2 AON transfected cells treated with Activin A in the presence or absence of activin inhibitors, we might not only validate the role of Activin A in HO but also provide a different platform for developing efficient treatments for FOP.

6.3 Restoration of BMPR2 signaling in PAH

6.3.1 BMPR2 in PAH

PAH is a chronic and progressive disease characterized by high pulmonary artery pressure, which will result in failure of the right heart if left untreated. Heterozygous mutations in the *BMPR2* gene are detected in most of the patients with heritable PAH (~70%) and some patients with idiopathic PAH (~20%) (45,

46). The majority of BMPR2 mutations (~70%) result in premature termination of the transcript through the process of nonsense-mediated decay (NMD^+). The remaining mutations (NMD^-) cause disease due to the dominant negative effects (45). Mutations in the *ALK1* or *endoglin* genes are also associated with the pathology of PAH (47, 48). Besides, reports have described mutations in other BMP signaling components, like *SMAD1*, *SMAD5* and *SMAD8* in PAH patients (49, 50). Conditional heterozygous or homozygous deleted BMPR2 gene in mouse pulmonary ECs results in PAH (51), as well as mice expressing dominant-negative BMPR2 in vascular SMCs after birth (52).

New DNA sequencing techniques such as RNA sequencing and whole-exome sequencing have helped to identify more PAH related genes. *Caveolin-1* and *potassium channel subfamily K member 3* (*KCNK3*) were identified as the two mutated genes in PAH patients without BMPR2 mutation (53, 54), and *TopBP1* was identified in idiopathic PAH without BMPR2 mutation (55). However, these mutations are extremely rare compared to BMPR2 mutations (56).

Although there is a strong correlation between mutations in BMPR2 and PAH, the low penetrance of BMPR2 mutations suggests that other genetic and environmental factors combine with the disrupted BMP signaling to contribute to the development of PAH. For instance, the incidence of PAH is elevated in women, and that alterations in estrogen metabolism are associated with the increased penetrance in female patients (57). Suppression of BMP signaling by estrogen promoted the proliferation of female pulmonary artery smooth muscle cells (PASMCs) and predisposed women to PAH (58).

Loss function of BMPR2 in ECs increases pulmonary EC apoptosis and promotes endothelial permeability (59, 60). Hamid *et al.* illustrated that unaffected mutation carriers have higher levels of wild-type BMPR2 transcripts than familial PAH patients, indicating that the expression level of wild-type BMPR2 might be important in disease pathogenesis of familial PAH patients, especially patients with nonsense mediated decay mutations in BMPR2 (61). Furthermore, PAH patients without BMPR2 mutations showed lower expression levels of BMPR2, for instance PAH patients associated with human immunodeficiency virus (HIV) infection (62). By adenoviral transfer of BMPR2 into the pulmonary vascular endothelium, Reynolds *et al.* showed that upregulating BMPR2 expression in two established PAH rat models can rescue PAH disease phenotypes (63). Thus, these examples demonstrate the critical

role of BMPR2 signaling in PAH and BMPR2 might act as a potential regulator linking different PAH pathologies.

6.3.2 Strategies and challenges to restore BMPR2 expression

Current treatments of PAH mainly focus on reversal of pulmonary vasoconstriction or decrease of EC and SMC proliferation, through targeting of the prostacyclin, endothelin, or nitric oxide pathways (64). However, these treatments can relieve disease symptoms and slow down the progression of PAH, but cannot prevent the disease. Understanding of the molecular mechanism of PAH pathology has enabled us to consider several novel treatments by restoring BMPR2 levels.

Strategy 1:

One strategy is to increase BMPR2 expression on the mRNA or protein levels. As mentioned above, the majority of BMPR2 mutations (around 70%) result in the premature termination of translation of the BMPR2 transcripts (45), the approach to promote the read-through of premature stop codons in BMPR2 could be beneficial for these patients. Drake *et al.* showed that ataluren normalizes full-length BMP2 protein expression level by permitting ribosomal read-through of premature stop codons (65). In addition, ataluren also corrected BMP-regulated miRNA processing and restored the hyperproliferative phenotype of pulmonary artery endothelial cells (PAECs) and PASMCs (65).

NMD^- mutations may lead to the restoration of BMPR2 proteins in the endoplasmic reticulum and Golgi and fail to reach the cell surface. Chemical chaperones can help with the correct folding of BMPR2 protein and enhances the trafficking of BMPR2 to the cell surface (66). Thirdly, Durrington *et al.* demonstrated that BMPR2 is degraded through lysosomes and that the endogenous mammalian E3 ligase Itch may be involved in this process (67). The antimalarial drug chloroquine has been reported to promote cell surface expression of BMPR2 by blocking lysosomal degradation (68, 69).

A recent publication indicated that BMP9 restores BMPR2 signaling in part by upregulating BMPR2 expression. Long *et al.* reported that BMP9 could prevent EC apoptosis and enhance monolayer integrity *in vitro*. Furthermore, BMP9 reversed the development of PAH in heterozygous knock-in mice expressing a mutant human BMPR2 gene and two other experimental PAH models (60).

Strategy 2:

Another option is using reagents that activate BMPR2 downstream signaling by activating either BMP type I receptors or SMAD signaling. An example of this using the compound FK506 has been described in **chapter 4**. By performing high throughput screening of US food and drug administration (FDA) approved drugs and bioactive compounds in BMP reporter cell lines, we identified FK506 (tacrolimus) as the best BMP activator. FK506 activates BMP signaling via a dual mechanism. Firstly, it acts as a calcineurin inhibitor. Calcineurin is a calcium-dependent serine-threonine phosphatase which activates nuclear factor of activated T cells (NFAT) dependent transcription by dephosphorylating NFATs (70). Bonnet *et al.* demonstrated that NFAT activation is associated with human and experimental PAH, and inhibition of NFAT with calcineurin/NFAT inhibitor Cyclosporine A reverses monocrotaline induced PAH in rats (71). Besides, calcineurin was shown to antagonize the intensity of BMP signaling by directly dephosphorylating receptor-regulated SMADs (R-SMAD) during neural differentiation of human and mouse embryonic stem cells (ESCs) (72). In **chapter 4**, we showed that low dose of FK506 mildly inhibits NFAT activity and its downstream target Interleukin (IL)-2. Thus, inhibition of calcineurin in PAH patients could help to relief inflammatory responses and probably activates BMP downstream signaling by counteracting R-Smad dephosphorylation. The other mechanism by which FK506 can activate BMP signaling is by interacting with FKBP12 to prevent the binding of FKBP12 to the BMP type I receptors ALK1, ALK2 and ALK3, resulting in enhanced phosphorylation of these type I receptors and activation of downstream signaling (Figure 1).

A clinical trial on the effects of low-dose FK506 reported therapeutic benefit for three cases of end-stage PAH (73). FK506 is a potent immunosuppressive drug being widely used in solid organ transplantations (74). It has been found that organ transplanted patients treated with FK506 are at high risk of renal injury, which might have happened due to the wide expression of calcineurin (75, 76). Thus, even though FK506 has shown significant clinical benefits, long-term use of this agent when treating PAH needs to be carefully monitored for the toxicity issue.

Besides FK506, we have tested compounds developed by Arcarios BV (Netherlands) for their effects on the BMP signaling pathway. For this drug screening we used C2C12 cells (a mouse myoblast cell line) stably transfected

with a reporter construct containing a BMP-responsive element linked to the luciferase gene (BRE-luc). By adding 135 ng/ml BMP9, the concentration resulting in 20% of maximal response (EC-20) and distinguishing activators requiring the exogenous ligand (Figure 3A), we have identified two agents (compound 6 and compound 13) that could potentiate BMP9-induced luciferase activity in C2C12 cells (Figure 3B). In addition to BMP9, we have also screened Arcarios compounds with other BMP ligands (BMP2, BMP6 and BMP7), but none of these compounds activated luciferase activity with these other ligands (data not shown). The two co-activators could sustain BMP9-induced phospho-SMAD1/5 (pSMAD1/5) activity for about ten hours (Figure 3C). In a three days differentiation assay, they also could potentiate the BMP9 induced alkaline phosphatase activity (ALP) activity (Figure 3D). The above results indicate that compound 6 and 13 selectively activate BMP9 signaling but not the signaling of other BMPs we have tested.

BMP9 specifically signals through ALK1 signaling in ECs and ALK2 in myoblasts (77, 78), and it can selectively enhance endothelial BMPR2 signaling in established PAH animal models (60). Further *in vitro* and *in vivo* experiments with those co-activators are currently being conducted in our laboratory. As we performed the drug screening in a murine myoblast cell line, further validation experiments in PAH target cells (ECs and SMCs) are needed. We also need to verify the effects of these compounds in PAH patient cells, and finally to test them in PAH animal models.

In summary, none of above treatment strategies is currently approved for patients; some of them are tested in clinical trials. Activating BMP signaling as therapeutic approach for PAH is challenging due to the complexity of BMP signaling. Thus, deeper understanding of the molecular mechanisms and regulation of the BMP signaling pathway, and also other signal pathways involved in PAH, are urgently needed and would be helpful for the development of PAH therapies.

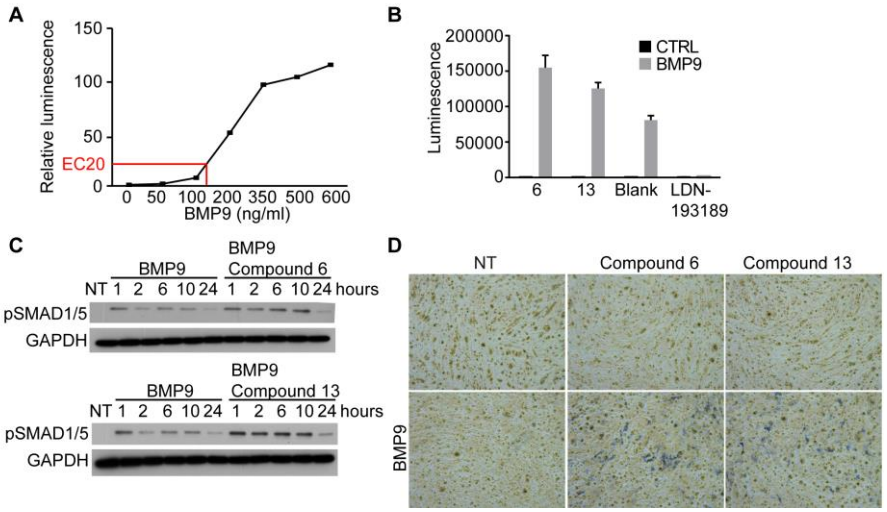

Figure 3: Compound 6 and compound 13 are coactivators of BMP signaling. A, BRE-luciferase activity assay testing different concentrations of BMP9 in C2C12 cells. 135 ng/ml BMP9 (EC-20) was applied in later experiments. B, BRE-luciferase activity testing compound 6 (10 μM), compound 13 (10 μM) with or without BMP9 (135 ng/ml) in C2C12 cells. C, pSMAD1/5 analysis following the treatment with only BMP9 (135 ng/ml), BMP9 with compound 6 (10 μM), or compound 13 (10 μM) atdifferent time points. D, ALP staining of C2C12 cells cultured with BMP9 (135 ng/ml) and/or compound 6 or 13 (10 μM) on day 3 of differentiation with 2% FBS.

6.4 Soluble endoglin modifies signaling by BMP 9

BMP9 is a TGF-β ligand mainly produced by the liver which can circulate in its active form (79). The activity of BMPs is modulated by co-receptors and soluble antagonists like noggin and other regulators that have been discussed in **chapter 1.** Crossveinless 2 (CV2 or BMPER) is the only known regulator of BMP9; its functions as an activator or an inhibitor of BMP signaling rely in part on the concentration of CV2 (80, 81). In **chapter 5,** we have described that the role of soluble endoglin (sEng) in regulating BMP9 signaling.

Endoglin is homodimeric transmembrane glycoprotein that functions as a co-receptor for TGF-β signaling (82). It is essential for cardiovascular development and mutations in *endoglin* are associated with the vascular disease HHT characterized by telangiectasias and arteriovenous malformations (83, 84). sEng sheds from the membrane bound endoglin upon proteolytic cleavage by matrix metalloproteinase (MMP) 14 at position 586 in carcinoma tissues (85).

sEng is associated with preeclampsia, a pregnancy associated vascular disorder characterized by hypertension and proteinuria (86). The expression level of sEng correlates with disease severity and dramatically falls after delivery (86). Rat preeclampsia models with increasing expression of sEng recapitulate the elevated mean arterial pressure, vascular damage in the placenta as well as other preeclampsia symptoms (86, 87). Besides preeclampsia, the level of sEng in plasma might be a marker for diagnosis of the vascular disorder PAH. Malhotra *et al.* found in PAH patients elevated sEng and soluble vascular endothelial growth factor receptor (VEGFR) 1, another anti-angiogenic marker (88).

BMP9 binds to ALK1 and endoglin in ECs and activates downstream signaling by phosphorylating SMAD1/5 (77). Recently researches showed that sEng directly can bind to BMP9 and sequester its activity (11, 89). However, we have shown in **chapter 5** that sEng may have dual effects on BMP9 at lower concentrations. Membrane bound endoglin can both promote and inhibit SMAD1/5 and SMAD2/3 downstream TGF-β signaling, which relies on the endoglin expression levels and the receptors (e.g. ALK1 and ALK5) involved in certain cellular contexts (90-92). sEng might work as a ligand trap depending on the dosage of sEng and TGF-β family receptors as well.

Endoglin is a key mediator of BMP9 induced angiogenic signaling (93, 94), but the molecular mechanism is still not complete clear. The role of BMP9 in angiogenesis largely relies on the dosage and the ECs types targeted. Scharpfenecker *et al.* found that BMP9 inhibits basic fibroblast growth factor (bFGF) induced ECs proliferation and migration and blocks vascular endothelial growth factor (VEGF) induced angiogenesis *in vitro* (78). However, in another report Suzuki *et al.* showed that BMP9 promotes angiogenesis in matrigel plug assays *in vivo* and also enhances tumor angiogenesis in a pancreatic carcinoma xenograft model (95). sEng specifically binds to BMP9 to inhibit VEGF-induced vessel formation *in vivo* (89), which indicates that sEng scavenges BMP9 or BMP10 ligands to disturb the balance for normal angiogenesis.

6.5 Concluding remarks

The studies in this thesis focused on the role of BMP signaling in disease contexts and identification of novel strategies for treatment of FOP and PAH based on the understanding of disease pathology. Despite the misregulation of BMP signaling in FOP (overactive) and PAH (insufficient), local inflammation

also plays a critical role in disease progressions. In **chapter 2** and **3**, a FOP hiPSC disease model and a mouse Alk2 AON were introduced. Although we found that targeting *ALK2* gene is sufficient to block osteoblast differentiation *in vitro* in the studies in **chapter 2** and **3**, experiments to test the combination effects of ALK2 and inflammation inhibitors in our disease model might be beneficial for the development of novel treatment strategies. By screening FDA proved drug libraries, we have shown in **chapter 4** that the chemical compound FK506 can activate BMP signaling via a dual mechanism of action, as a calcineurin inhibitor and BMP activator. FK506 has been reported to be beneficial for end-stage PAH patients (73). The research on PAH indicated that the combination of BMP signaling modulators with other modulators, especially of local inflammation, would be helpful for the development of efficient treatments for PAH and probably FOP. In **chapter 5**, we have demonstrated that sEng regulates BMP9 activity. Revealing the molecular mechanism of the interactions of sEng and BMP9 with other TGF-β/SMAD signaling components could be relevant for the development of diagnostic tools and treatment of human diseases, especially PAH and preeclampsia.

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