BMP signaling in vascular and heterotopic bone diseases

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Summary

Bone morphogenetic proteins (BMPs) are multifunctional regulators in embryonic development and tissue homeostasis. Disruptions in BMP signaling lead to various diseases, such as skeletal diseases, vascular diseases and cancer. Studies in this thesis mainly focused on the role of BMP signaling in disease contexts, and the identification of possible novel treatments for fibrodysplasia ossificans progressiva (FOP) and pulmonary arterial hypertension (PAH) based on the understanding of the disease pathology.

FOP is a rare disease which severely affects the health condition and life span of the patients. The majority of FOP research was conducted in murine cell lines or mouse models and some were conducted in human material obtained from blood or milk teeth. In order to mimic the disease phenotypes in human cells, especially in heterotopic ossification progenitor cells, we have established human induced pluripotent stem cell (hiPSC) lines from FOP patients samples. Our FOP iPSC disease model recreated disease phenotypes by showing enhanced mineralization in FOP pericytes and impaired endothelial cell (EC) differentiation ability, which might be due to activated SMAD signaling. Furthermore, FOP iPSC derived pericytes could be applied for drug development for FOP (**chapter 2**). The research on FOP iPSCs indicated that this model might be a useful tool for high-throughput screening and verification of new disease targets.

The discovery in 2006 that an activating mutation in the BMP type I receptor activin receptor-like kinase (ALK) 2 is associated with FOP provided a druggable target for the development of FOP treatment. One option is to block the aberrant and enhanced ALK2 signaling with chemical compounds targeting the ALK2 kinase activity. Another possible strategy is to knockdown mutant ALK2 expression by anti-sense oligonucleotides (AON) or siRNAs. Our study in **chapter 3** showed that an ALK2 AON can specifically target exon 8 of wild-type mouse *Alk2 in vitro* and decrease ALK2 expression in various ECs. Furthermore, the ALK2 AON could downregulate BMP/SMAD signaling and block osteoblast differentiation in ECs.

In contrast to FOP, BMP signaling is reduced in PAH. By screening an FDA approved drug library, we identified in **chapter 4** the chemical compound FK506 as an activator of BMP signaling. By binding the 12-kDa

FK506-binding protein FKBP12, FK506 was found to activate BMP signaling via a dual mechanism, as a calcineurin inhibitor and a derepressor of the BMP type I receptors. In addition, low-dose FK506 could rescue EC dysfunction and improve EC targets of BMP signaling, such as apelin expression, in several experimental animal models. Thus, FK506 is a valuable drug in treating PAH as well as other vascular diseases with defective BMP signaling.

In **chapter 5**, we have shown that soluble endoglin (sEng) can regulate BMP9 activity. sEng together with BMP9 can bind to ALK1 (and possibly transforming growth factor β (TGF-β) receptor type 2) and participate in signaling itself at the cell membrane. sEng and BMP9 are two important TGF-β signaling pathway components for the maintenance of vascular homeostasis, and are also involved in inflammatory responses in healthy and pathological conditions. Research to reveal the molecular mechanism of the interactions of sEng and BMP9 with the different membrane receptors would be beneficial for developing novel diagnosis markers and therapeutic strategies for vascular disorders, especially PAH and preeclampsia.

In summary, this thesis describes how disrupted BMP signaling is implicated in the pathology of FOP and PAH. In addition to BMP signaling, other environmental factors like local inflammatory stimuli are involved in disease progression, and relevant for the development of effective therapies. Finally, alternations in BMP signaling are involved in various other human diseases in addition to the diseases discussed in this thesis. Thus, research on developing effective treatments for FOP and PAH might have potential therapeutic value for other BMP signaling related disorders and help to understand the role of BMP signaling in different cellular contexts.