Summary

New blood vessels arise from pre-existing vessels when the supply of oxygen and nutrients and removal of waste products for the formation of new tissues is limited by diffusion (also termed as angiogenesis). Upon activation and under the guidance of vascular growth factors, endothelial cells (ECs) sprout from mother vessels and branch into nascent vessels. Subsequently pericytes/smooth muscle cells are recruited to the newly formed sprout for vessel stabilization. The primary vascular plexus is remodeled into a highly hierarchical vascular tree. Whereas angiogenesis is beneficial during wound healing, pregnancy, etc. and a prerequisite for development, abnormal angiogenesis is implicated in numerous vascular disorders, such as tumor angiogenesis, atherosclerosis, hypertension and other cardiovascular diseases.

The transforming growth factor (TGF)-β signalling pathway plays a major role in angiogenesis. It has been shown that TGF-β signalling affects both endothelial and mural cell function in genetic studies and *in vitro* cell culture models. Aberration of the TGF-β signalling cascade leads to abnormal remodeling and maturation of the primitive vascular plexus and decreased vessel wall integrity in adults. Targeted deletion of TGF-β signalling receptors in mice, such as ALK1, ALK5, TβRII or endoglin, results in embryonic lethality due to impaired vascular development. In humans, mutations in ALK1, ALK5, TβRII or endoglin are associated with human vascular diseases such as HHT and pulmonary hypertension (PAH). Vascular endothelial growth factor (VEGF) is a multifunctional molecule that is involved in vascular growth and remodeling. Similarly, in genetic mouse models abnormalities in or mis-regulation of the VEGF signalling pathway results also in embryonic death or severe vascular dysfunction. Perturbation in VEGF signalling also contributes to the pathology of tumor angiogenesis and cardiovascular diseases in humans.

This thesis is focused on the characterization of the crosstalk between the TGF-β and VEGF signalling pathways, on EC function, the effect of bone morphogenetic protein (BMP)9 on EC function and the role of endoglin in VEGF-induced angiogenesis. The results of these studies may give us insights into the impacts/effects of these two angiogenic signalling cascades on EC function. This can be beneficial for the understanding of the etiology of certain vascular diseases and the development of new treatment modalities in the future.

The key findings in this thesis are:

- Inhibition of the TGF-β type I receptor by a pharmaceutical kinase inhibitor enhances VEGF-induced angiogenesis both *in vitro* and *in vivo*. The crosstalk between TGF-β and VEGF leads to the induced expression of the pro-angiogenic integrin α5. *[Chapter 2]*
- BMP-9 is a potent stimulator of the ALK1 signalling pathway in endothelial cells and it inhibits VEGF-stimulated angiogenesis at high dosing. *[Chapter 3]*

- BMP-9 directly binds to endoglin and it potently induces endoglin expression at the transcriptional level. *[Chapter 3]*
- Endoglin is required for VEGF-induced angiogenesis in endothelial cells. VEGF-induced EC sprouting is decreased in ECs with endoglin-specific knockdown, but reduced endoglin expression does not affect major VEGF-induced signalling cascades. *[Chapter 4]*
- Absence of endoglin impairs the remodeling of vascular structures embryoid bodies, but doesn't affect endothelial cell differentiation in embryoid bodies. *[Chapter 4]*
- Shedding of endoglin is mediated in part by MMP14. *[Chapter 5]*
- The soluble form of endoglin exerts inhibitory effects on VEGF-induced angiogenesis. *[Chapter5]*

Our studies demonstrate that a crosstalk at the receptor level between VEGF signalling and TGF-β signalling affects EC function and angiogenesis *in vitro* and *in vivo*. In other words, these two signalling pathways work, together with other pathways, in a coordinated manner to maintain the balance in EC function or the optimal angiogenic output via the regulation of gene expression. Once the balance between TGF-β and VEGF signalling is disturbed, downstream gene expression profiles change followed by cell morphology, as well as cell functions such as migration and proliferation. These changes affect EC function and vessel formation. Consequently the development of the vasculature will be modified accordingly. Therefore, the studies described in this thesis further confirm the crucial role of TGF-β in EC function and provide some novel molecular mechanisms. On endothelial cells, TGF-β transduces its effect by binding to two distinct TGF-β type I receptors, ALK1 and ALK5. The two receptors result in distinctive impacts on endothelial cell behavior due to the local environment. Activation of ALK1 by BMP9 leads to inhibition of VEGF-induced angiogenesis, but the inhibition of ALK5 by pharmacological compounds results in enhancement of vascular network stimulated by VEGF.

Endoglin, an auxiliary TGF-β receptor, has been well studied and is considered as an important angiogenic molecule. It is shown in this thesis that endoglin is required for efficient VEGF-induced angiogenesis. The role of endoglin in VEGF signalling further supports the notion for interplay between the TGF-β and VEGF pathways in controlling EC function. Moreover, we show that soluble endoglin (sEng) and an endoglin neutralizing antibody (Tracon105) function as a suppressor/inhibitor of VEGF-induced angiogenesis (chapter 4). sEng negatively influences the angiogenic potential of EC and elevated level of sEng was found to be associated with EC dysfunction in pregnant women with hypertension and pre-eclampsia. Interestingly, aberrant concentrations of soluble fms-like tyrosine kinase 1(sFlt-1), also known as the soluble form of the VEGF receptor, contributes the onset of pre-eclampsia as well. This indicates again the interplay and non-redundancy of these two pathways in maintenance of EC homeostasis. Detection of circulating sEng and sFlt-1 hold promise for the early diagnostic potential for patients with pre-eclampsia.