Chapter 7

General Discussion

The TGF-β family signalling pathways are highly fine-tuned cellular signalling networks and their biological effects dependent on the local microenvironment and context. Signalling cascades of TGF-β family members have been shown to be important in vascular development and angiogenesis. Mutations in TGF-β family receptors or Smads have been linked to vascular disorders such as HHT, PAH and Marfan Syndrome, as described in the introduction of this thesis. Mice deficient in specific TGF-β family signalling components, i.e. ligands, receptors or Smad transcription factors, frequently develop embryonic lethality due to vascular defects. The exact mechanism by which signalling of TGF-β family members, and perturbations therein, affects vascular function remains largely unknown. Therefore, this discussion will focus on our current perspectives of mechanisms underlying the crosstalk between TGF-β and VEGF, the perspective of BMP9 and endoglin in endothelial cell biology and the emerging role of TGF-β family signalling in angiogenic sprout remodeling.

The interplay between TGF-β and VEGF signalling pathways in endothelial cell function

TGF-β signalling in endothelial cells

In endothelial cells (ECs), after the binding to type II receptor (TβRII), TGF-β signals via two opposing type I receptors (TβRI) and thereby activate distinct Smad signalling pathways: (i) the ALK5/Smad2/3 signalling pathway leading to inhibition of EC migration and proliferation, and (ii) the ALK1/Smad1/5 cascade that resulting in the induction EC migration (1). In addition, endoglin, a co-receptor for TGF-β ligands, promotes ALK1- but inhibits ALK5-induced signalling (2). Although the TGF-β/ALK1 pathway appears to directly antagonize TGF-β/ALK5 signalling, the presence of ALK5 is a requirement for efficient TGF-β/ALK1 signalling (3). Interestingly, when ECs are plated on fibronectin the requirement of ALK5 for TGF-β/ALK1 signalling is bypassed (4). To intervene with the pro-angiogenic effects of the TGF-β superfamily, tools have been generated to specifically inhibit the function of ALK1 and endoglin. Soluble chimeric forms of ALK1 (ALK1-Fc) or endoglin (endoglin-Fc) fused to an antibody Fc domain can attenuate the ALK1 signalling pathway possibly by scavenging pro-angiogenic ligands of the TGF-β superfamily. As a result, the fine-balance between TGF-β family and VEGF signalling is disturbed resulting in impaired VEGF-induced EC sprouting *in vitro* and *in vivo* (5 - 6).

TGF- β regulates VEGF expression

Besides the direct effects that TGF-β has on EC behavior, TGF-β also regulates EC function indirectly by regulating the expression of angiogenic factors in non-endothelial cell types, such as smooth muscle cells, macrophages and tumor cells. Notably, in macrophages TGF-β induces

vascular endothelial growth factor (VEGF) production in a Smad-dependent manner to mediate angiogenic responses (8). In addition, TGF-β induces the expression of VEGF in tumor cells and tumor associated-stromal fibroblasts via the ALK5/TβRII complex (9, 10). SB-431542, an ALK4/5/7 kinase inhibitor (11), has been shown to exert an inhibitory effect on VEGF secretion in human cancer cell lines (12 - 14). Moreover, TGF-β synergizes with hypoxia, a potent stimulator of angiogenesis, in stimulation of VEGF promoter activity in a Smad3-dependent manner (9, 15). In contrast, the activation of the ALK1/BMPRII complex upon BMP9 binding has been shown to suppress VEGF expression both in cells *in vitro* and in mouse aorta, lungs, liver, and intestine (16), indicating distinct regulatory effects of ALK1 and ALK5 on VEGF expression by different TGF-β family members.

Thus the regulation of VEGF production is mediated by different TGF-β superfamily members via distinct signalling transduction routes in diverse cell types. Therefore, the effect of TGF-β family members on local angiogenesis is mediated in part through modulation of VEGF signalling (17 - 19). This cross-talk between VEGF and TGF-β signalling orchestrates in an intricately regulated manner the vascular balance, and interfering with either one will result in vascular malformations

VEGF and TGF-β intracellular crosstalk

VEGF and TGF-β family members play prominent roles in angiogenesis, and the interaction between the two signalling pathways modulates EC behavior. While VEGF controls endothelial cell functions in angiogenesis such as migration, proliferation, sprouting and permeability, TGF-β family members are prerequisite for vascular network modeling via the regulation of EC migration, death and survival (20). *In vitro* TGF-β/ALK5 signalling can induce apoptotic effects and inhibit EC proliferation, migration and sprouting (Chapter 2, 1, 3). Interestingly, treatment with a sub-optimal dose of the ALK5 kinase inhibitors SB-43152 or LY-364947 synergistically enhanced EC capillary formation and EC sprouting in response to VEGF, suggesting that crosstalk between TGF-β and VEGF signalling regulates endothelial cell behavior (Chapter 2, 21). This synergistic effect in EC sprouting is also observed when using TGF-β neutralizing antibodies in combination with VEGF (Chapter 2, 21). Transcriptional analysis revealed that inhibition of ALK5 signalling in combination with VEGF stimulation promotes the expression of anti-apoptotic factors and angiogenesis-associated integrins and down-regulates pro-apoptotic genes (Chapter 2). In the similar research line, Watabe *et al.* reported that the inhibition of TGF-β improves stem cell derived EC survival and endothelial sheet formation (22). Furthermore, TGF-β was shown to be able to convert VEGF activity from being anti-apoptotic to pro-apoptotic in EC by shifting the activation of p38 MAP kinase from the pro-survival p38β to the pro-apoptotic p38α MAP kinase (23, 24,). Remarkably the activation of p38α by TGF-β is transient and the ECs become refractory to TGF-β induced apoptosis due to reduced levels of p38α and increased levels of p38β MAP kinase (24), showing that the crosstalk between TGF-β and VEGF signalling dynamically regulates EC biology.

Since modulation of a signal transduction pathway can occur at different levels, e.g. at the level of the receptor or downstream modulators, we made attempts to identify at what level the crosstalk between VEGF and TGF-β occurred. Unfortunately, we did not observe any significant changes in either Smad signalling, p38 or ERK MAP kinase activity after treatment with ALK5 kinase inhibitor or co-stimulation with TGF-β and VEGF (unpublished data). VEGR2 triggers different and diverse signalling pathways to regulate EC function. Yet we cannot exclude the possibility of involvement of other downstream signalling events such as endothelial cell permeability-related nitric oxide synthase (eNOS/NOS3) activation, cell migration related focal adhesion kinase (FAK) activity or PI3 Kinase activity. In addition, our studies (Chapter 2) have only addressed the role of TGF-β in EC angiogenic property both *in vitro* and *in vivo*. A more extensive investigation on the effect of the other TGF-β super family members such as BMPs and their receptors on EC function will improve the current understanding of the TGF-β/VEGF crosstalk.

In summary, TGF-β and VEGF signalling intimately intertwine and crosstalk with each other to regulate EC function. Inhibition of TGF-β signalling potentiated the VEGF-induced angiogenesis (22, Chapter 2). However the fine-tune of this crosstalk in EC is also dependent on various factors: ligand gradient, time windows and local vascular bed condition. The interpretation of TGF-β/VEGF crosstalk can not only be based on any particular specific biological setting, but requires the local EC status and its interaction with neighboring other cell types in the local microenvironment.

Crosstalk between VEGF and TGF-β at the receptor level

Although our experiments did not provide evidence for direct interaction of the downstream intracellular VEGF and TGF-β signalling cascades, other studies offered alternative clues pointing to possible crosstalk at the receptor levels. Glinka and co-workers showed that in cancer cells the co-receptors of VEGF, neuropillin-1 and 2 have affinity for ALK5 and TβRII, and over-expressing of neuropillin-1 and 2 increased the levels of phosphorylated Smad2/3 (25) indicating neuropillins potentiates ALK5/Smad2/3 pathway. Furthermore, neuropillin-1 and -2 capture and activate latent TGF-β to be accessible by changing the conformation of the bound form of latent TGF-β. Given that neuropillin-1 and -2 are required for efficient VEGF signalling and enhance ALK5/Smad2/3 pathway (22, 26), it is possible that neuropillins may be a modulator for the interplay between TGF-β and VEGF signalling in endothelial function.

VE-cadherin is another interesting potential mediator for TGF-β and VEGF interplay. Clustered VE-cadherin improved the sensitivity of Smad activation upon TGF-β stimulation by enhancement of TβRI/TβRII assembly; disruption of VE-cadherin clustering results in reduction of TGF-β-induced inhibition of EC growth and migration (27). In addition, TGF-β induces assembly of the adherens junction complex by separating VEGF receptor 2 (VEGFR2) from VE-cadherin but increasing β-catenin association with both VEGFR2 and VE-cadherin (28). These studies suggest

that the interplay between TGF-β and VEGF pathways may be regulated by the formation of complexes containing TGF-β receptors and VEGF receptors with VE-cadherin as the core. It is plausible that the affinity of VE-cadherin for VEGFR2 and TGF-β receptor determines the TGF-β and VEGF crosstalk.

BMP9: both inhibit and stimulate angiogenesis

Out of more than 30 TGF-β superfamily ligands, there are over 20 BMP subfamily members. BMPs signal through both BMP receptors and activin type II receptors to relay their signals to the downstream cascades via different BMP type I receptors such as ALK1,-2,-3, and -6, while they are also regulated by intrinsic antagonists (e.g. noggin) (29). BMPs exert broad functions as a result of great complexity between combinations of ligands, type I and II receptors. BMP9 is a secreted circulating protein (30) that is highly expressed in the damaged liver (31) and binds to the extracellular domain of ALK1 and endoglin (30, 32). BMP9 signals via ALK1 to modulate endothelial cell function (Chapter 3; 33). Mutations in ALK1 are associated with the vascular disease HHT2. Mutations found in the extracellular domain of ALK1 interfere with the binding of BMP9, whilst HHT2 mutations in the intracellular domain are shown to reduce ALK1 activation upon BMP9 stimulation (34, 35), suggesting that there might be a role of BMP9 in the vascular disease HHT2.

BMP9 has an important role in angiogenesis (33, 36, 37). However, its effect on EC function and the mechanism of action remain unclear. Chapter 3 demonstrates an inhibitory effect of BMP9 on VEGF-induced angiogenesis. This result is supported by observations by David *et al.*, who reported that BMP9 functions as a circulating vascular quiescence factor (38). A recent *in vivo* study has confirmed that activation of ALK1 by BMP9 inhibits retina angiogenesis and blockade of the BMP9/ALK1 signalling by ALK1-Fc induced hypervascularization of retina (39). Interestingly, BMP9 has also been shown to stimulate EC proliferation and to be a pro-angiogenic factor in Matrigel plug vascularization and tumor angiogenesis in a xenograft model (40, 41). In addition, BMP9 has been shown to stimulate endothelial tube formation *in vitro* (43). Moreover, anti-hALK1 and anti-BMP9 antibodies can inhibit endothelial cell sprouting (37). These different results can in part be ascribed to differences in experimental setup: a low dose of BMP9 was found to have a pro-angiogenic effect, while a high concentration exerts an inhibitory effect on EC function (41; Chapter 3). However other factors can also contribute to the different results obtained. Differential receptor usage and/or different levels of activation of intracellular effectors of BMP9 may underlie its complex effects in different types of ECs.

The role of endoglin in endothelial cell function

Endoglin is expressed at the cell surface as a disulfide-linked homodimer, especially in active ECs (42, 44 45). It exists as long-form (L-endoglin) and short-form (S-endoglin) based on the different length of the cytoplasmic domain (46, Chapter 7). Apart from the membrane-bound forms, endoglin can be shedded by Matrix Metalloproteinase-14 (MMP14) existing as a soluble form (sol-Eng) (Chapter 3), which will be discussed later in detail.

In Chapter 4 we demonstrate that the lack of endoglin decreases the potential of VEGF signalling and VEGF-induced sprouting, resulting in defective endothelial cell sprouting. Mutations in endoglin are associated with the human vascular disease HHT1 (47). Endoglin heterozygous mice exhibit vascular lesions due to capillary malformation, resembling HHT1 syndromes (48 - 50). EC-specific deletion of endoglin in adult mice caused severe cerebrovascular dysplasia with the injection of adeno associated virus carrying VEGF; while one copy loss of endoglin only develops minimal irregular vascular formation (51). Our *in vitro* observations together with *in vivo* findings in the literature underscore the importance of endoglin in VEGF-induced vascular physiology. Yet the requirement of endoglin for efficient VEGF responses is likely not only restricted to ECs since endoglin is also expressed by fibroblasts, smooth muscle cells, and macrophages. Therefore, it is worthwhile to investigate the role of endoglin in VEGF-induced angiogenesis also by addressing other modulators of EC sprouting and the angiogenic microenvironment in order to achieve more insights.

Different forms of endoglin through differential splicing and membrane shedding

It remains very difficult to clarify the role of endoglin in EC function. Up to now, most of the studies about endoglin have focused on the characterization of the functional role of L-endoglin, which is usually referred to endoglin. A few studies have tried to elucidate the role of S-endoglin in EC function. Surprisingly S-endoglin has binding preference for ALK5 rather than ALK1, which provides a possible explanation for the different effects of L-endoglin on EC function (52, 53). Endoglin has been reported to impose opposing effects on EC. While endoglin was shown to be pro-angiogenic and required for EC function (2, Chapter 4), another study demonstrates that endoglin inhibits EC migration (54). The discrepancies among studies are not only due to differences in the experimental setup, such as the source of ECs model system, cell density, or even the expression ratio of S-endoglin/L-endoglin; but also the engagement of endoglin in distinct TGF-β/BMP receptor complexes in response to diverse TGF-β superfamily ligands. Endoglin is a pivotal modulater for ALK1 and ALK5 signalling in ECs. Yet further studies are required to elucidate the precise role of different forms of endoglin in EC function regulation upon TGF-β treatment in different biological environments.

Endoglin with its interactors other than TGF-β receptors in EC function

As addressed previously endoglin is required for VEGF-induced sprouting, however the exact mechanism remains unknown. Apart from its function as co-receptor for TGF-β/BMP receptor complexes, endoglin interacts with VE-cadherin (27). Our preliminary data suggested that endoglin-deficiency reduced the endogenous interaction between VE-cadherin and VEGFRII in ECs (unpublished data). Moreover VE-cadherin modulates the sensitivity of VEGFR2 signalling to cell density (55). These results suggest the possibility of a potential association between endoglin and VEGFR signalling activity via its interaction with VE-cadherin (Fig.1A). It would be interesting to investigate the effect of endoglin deficiency on VE-cadherin signalling as VE-cadherin fine tunes VEGFR2 and TGF-β signalling (Fig.1B).

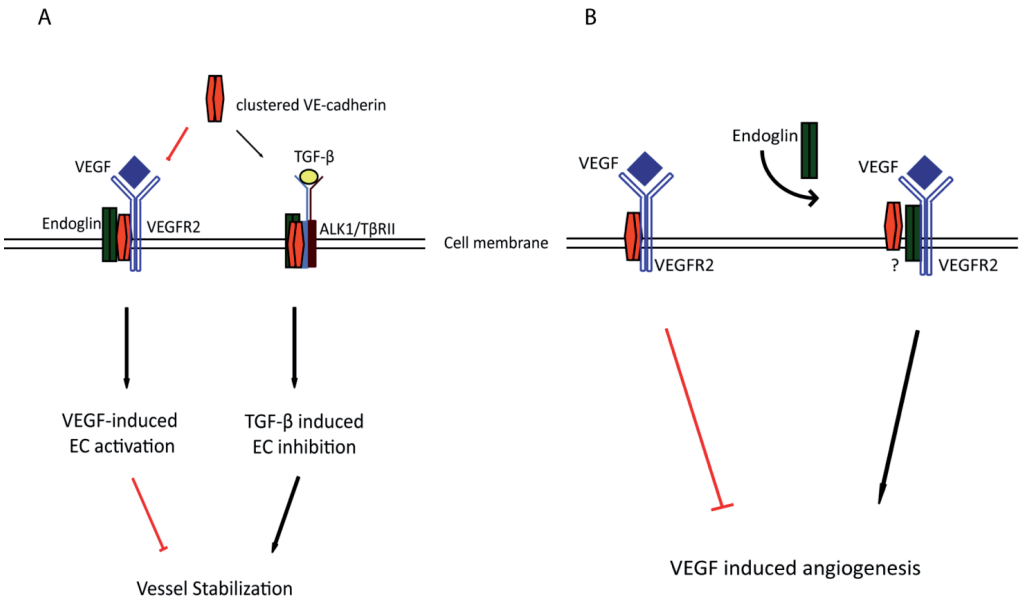

Fig 1 *Schematic possible roles of VE-cadherin and endoglin in controlling the angiogenic balance by regulating VEGF and TGF-β signalling pathways. (A) Clustered VE-cadherins may potentiaate TGF-β signalling and exert inhibitory effect on VEGFR2 signalling in ECs at high cell density, resulting in vessel stabilization. (B) Endoglin is indispensable for VEGF-induced angiogenesis. However, it is not well understood how endoglin deficiency affects VEGF-induced angiogenesis. It is possible that the endoglin may release the inhibitory effect of VE-cadheirn on VEGF signalling by altering the receptor complex composition.*

To further investigate the mechanism of the impact of endoglin on VEGFR2 signalling, we examined the effect of endoglin deficiency on VEGFR2. As tyrosine 1175 of VEGFR2 is an essential phosphorylation site for VEGFR2 activation (56, 57), the phosphorylation level of this site was examined in ECs with endoglin deficiency. Surprisingly, the tyrosine 1175 phosphorylation level was

strongly reduced in endoglin knockdown cells at 5 min of VEGF stimulation (Fig. 2). Subsequently we studied the phosphorylation level of downstream signalling components of VEGFR2 such as ERK and p38 MAP kinases. Unfortunately no changes in ERK and p38 phosphorylation were observed in endoglin deficient ECs upon VEGF stimulation (data not shown). It is possible that the ERK and p38 MAP kinases were not affected in this particular experimental setting due to the high amount of VEGF stimulation (25ng/ml), which may saturate the sensitivity of VEGF-induced MAP Kinase activity. Furthermore, a previous study showed that VEGFR2 preferentially signals to PI3 Kinase in confluent endothelial cells but promotes MAP Kinase activation in the sparse cells or VE-cadherin deficient ECs (55) (Fig. 1A). Thus VEGFR2 signalling may behave differently dependent on the local cell condition. In order to further characterize the influence of endoglin on VEGF signalling future studies should include the other downstream cascades of VEGFR2 such as FAK, AKT, eNOS, Src and PLCγ. Finally, it is not yet known whether lack of endoglin will influence the effect of VE-cadherin on VEGFR2 signalling.

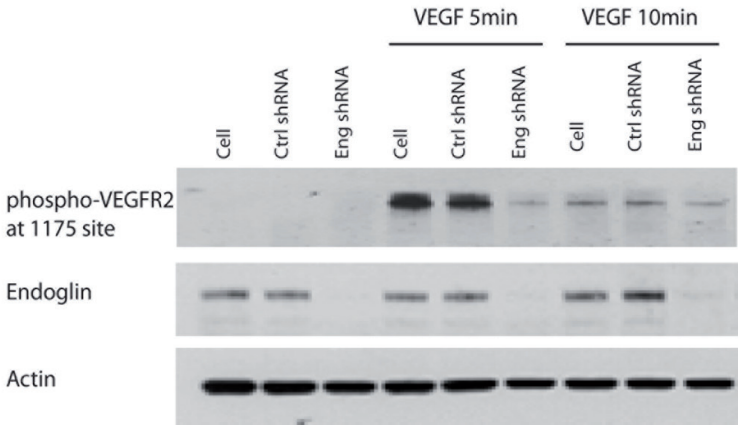

Fig. 2 *Phosphorylation level of VEGFR2 is reduced upon VEGF stimulation in endoglin knockdown ECs. HUVEC cells were stimulated with VEGF (25ng/ml) for 5min and 10 min. Non-infected cells (cell), control shRNA infected cells (Ctrl shRNA) and shRNA-mediated endoglin depleted cells (Eng shRNA) were used. Afterwards cells were lysed and samples subjected to SDS-PAGE and subsequent western blotting. Membranes were either probed with an antibody that specifically recognizes phosphorylated VEGFR2 at the site of 1175 and with an antibody directed against endoglin. An anti-β-actin antibody was used to confirm equal loading.*

The interaction between endoglin and VE-cadherin also provides a hint that endoglin may be involved in cell junctions/contacts. Our preliminary data of electron microscopic analysis showed that endoglin was localized at the cell-cell contacts (data not shown). Tight alignment of endoglin at the cell-cell contact in addition to its interaction with VE-cadherin could further enhance TGF-β signalling in EC stabilization (Fig. 3A). Furthermore, endoglin has been shown to be situated at the cell membrane and to control focal adhesion via its interaction with zyxin (58), and mediate the

activation of PI3 kinase and AKT at the cell membranes, leading to the stabilization of EC sprouting (59). These studies demonstrate the possible function of endoglin in EC junction, which can be important for vessel integrity during angiogenesis.

It is reported that endoglin not only interacts with integrin α5β1 to modulate the crosstalk between TGF-β/ALK1 signalling and fibronection/α5β1signalling, but also co-internalize with α5β1 to propagate integrin α5β1 signalling (4). The fibronection/α5β1signalling suppresses TGF-β induced EC migration in endoglin-depending manner, which could also be in part the explanation of the opposing role of endoglin in EC function in different experiment settings. Of note, the co-localization of endoglin and α5β1 in endocytosis is required for developmental angiogenesis in zebrafish (4), take together with the evidence that VEGF promotes α5β1 internalization to induce EC migration, It is plausible that endoglin could regulate VEGF-induced angiogenesis via its co-endocytotic partner integrin α5β1 leading to enhanced ALK1 signalling (Fig. 3B).

In summary, all these results indicate that endoglin does not function merely as a co-receptor for TGF-β signalling in controlling EC function. Endoglin appears to be involved in EC cell junctions and endocytosis. Current understanding of this possible function is limited. Screening for novel endoglin interactors will provide more insight in endoglin biology. Novel interactors and/or regulators may reveal a new function of endoglin in angiogenesis, in TGF-β/BMP signalling and beyond.

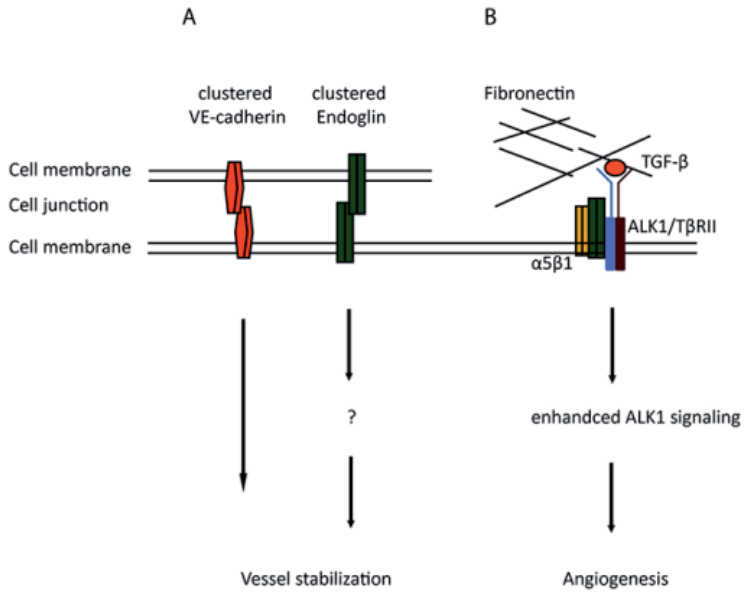

Fig. 3. *Possible role of endoglin in EC functions. (A) Endoglin is situated at the cell-cell contacts. Similar to clustered VE-cadherin, it is possible that the clustered endoglin contribuates to the vessel stabilization. (B) Fibronectin/α5β1 promotes ALK1signalling in a endoglin-dependet fashion, thereby promoting angiogenesis (Tian et al, 2012).*

The emerging therapeutic value of soluble endoglin and its pathological role in pre-eclampsia

Soluble endoglin (sol-Eng) is a circulating factor in serum (60). Elevated levels are observed in patients with pre-eclampsia (60, 61), cancer patients with metastasis (62, 63) and diabetic patients (64). Sol-Eng can be generated via matrix metalloproteinase (MMP)-14 mediated shedding of membrane bound endoglin on endothelial cells in colorectal cancer. The MMP14-mediated cleavage occurs at a site close to the transmembrane domain of endoglin (Chapter 5). Interestingly, it has been shown recently that MMP-14 can induce the release of soluble endoglin in pre-eclamptic placenta (65). The cleaved sol-Eng contains the extracellular domain, retaining the ability of binding to TGF-β and BMP-9 (32, 61). The reported sizes of sol-Eng appear to be variable in different studies. The sol-Eng from pre-eclampsia patients is around 65kDa; but the one cleaved by MMP14 is around 80kDa in tumor cells. This suggests that the endoglin may harbor multiple potential cleavage sites and that the generation of sol-Eng in tumors and pre-eclampsia may occur via different mechanisms. In chondrocytes an increase in the ALK1/ALK5 ratio leads to MMP13 production (66), but it is not known yet whether MMP13 harbors cleavage potential for endoglin. Yet it remains to be examined whether the ALK1/ALK5 ratio regulates MM14 production resulting in modulation of sol-Eng production.

Circulating sol-Eng may have systemic effects on angiogenesis. Sol-Eng contributes to pre-clampsia in concert with soluble VEGFR1 (solFlt1) (61). Elevated sol-Eng levels in the brain causes brain arteriovenous malformation via modulation of MMP activity but without changing the expression of membrane bound forms of endoglin (67). Yet further studies are required to establish a comprehensive understanding of the role of sol-Eng *in vivo*. High levels of circulating sol-Eng might lead to EC dysfunction due to its anti-angiogenic effect, which provides a potential therapeutic window for the endoglin neutralizing antibody to restore EC function. On the other hand, the anti-angiogenic effect of sol-Eng may be interesting for cancer treatment in the future, as an adjuvant therapy to selectively ablate blood vessels and thereby limiting tumor growth.

Future Perspective: TGF-β signalling shapes the sprout tip

A vessel sprout consists of tip and stalk cells. Tip cells are localized at the leading edge of vessel sprouts. The gradient of VEGF guides the direction of tip cells (68). With enriched filopodia-like cell protrusion at their migrating front tip cells are sensitive to local directional cues and thereby orientate the direction of sprouts accordingly (68, 69), while adjacent stalk cells contribute to the extension of the nascent sprouts (Fig. 4). However, the tip and stalk cell phenotypes are subject to a highly dynamic process of phenotype selection along vascular sprouts. As discussed before, in addition to their direct effects on EC behavior, a role for TGF-β family members in other events of vascular modeling is emerging, such as tip-stalk cell phenotype specification.

The TGF-β downstream components Id-1 and its upstream phosphorylated Smad1/5 (p-Smad1/5) exhibit distinct localization patterns in EC sprouts. At embryonic day 9.5 phosphorylated Smad1/5 is ubiquitously expressed in tip and stalk cells, whereas Id-1 is only distributed in the stalk cells (70). In the absence of Smad1/5 signalling, the stalk cells acquire plasticity, becoming tip-cell-like cells (70). This suggests that Smad1/5 signalling is important for stalk/tip cell selection and that Smad1/5 and Id-1 may exert divergent roles in the modulation of tip and stalk cell behavior. Moreover, p-Smad1/5 interacts with the Notch intracellular domain (NICD) to potentiate the expression of the Notch signalling downstream targets Hey1 (Herp2) and JAG1 (71, 72), while Id-1 form heterodimers with Hes-1 for Hes-1 stabilization in ECs (70). It thus appears that the interaction between Smad1/5 and Notch signalling regulates tip and stalk cell morphogenesis. Any impairment of the interplay may affect downstream target gene expression, and thereby result in loss of tip and stalk cell specification. Along with this notion, Larrviée *et al.* reported that blockade of ALK1 in tip cells displayed loss of stalk cell phenotype (39). Hence, TGF-β superfamily signalling cooperates with Notch signalling to specify the tip and stalk cell selection in early vascular sprouting events.

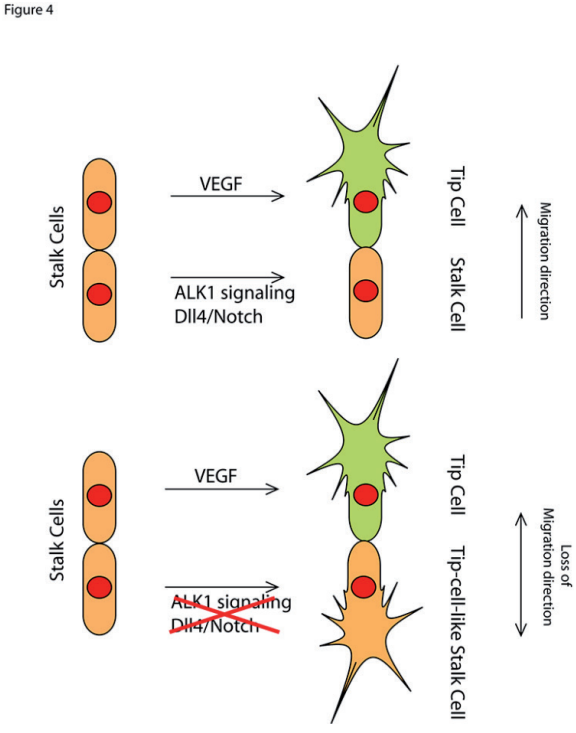

Fig. 4 *Scheme of stalk/tip cell phenotype selection. Stalk cell (orange) and tip cell (green) are under dynamic selection by several signalling pathways. The VEGF pathway drives the transformation of stalk cells into tip cells. ALK1-Smad1/5 signalling integrated with Notch signalling to maintain the adjacent stalk cell phenotype. Impaired ALK1 or Notch signalling turns the adjacent stalk cells into tip-like cells with hypervascularization, resulting in the loss of directed EC migration.*

Chapter 4 demonstrated the requirement of endoglin for VEGF-induced EC sprouting both *in vitro* and *ex vivo. In vivo* endoglin deficiency mice displayed abnormal vascular structure development upon VEGF stimulation. In addition, endoglin is reported to promote EC proliferation and migration (2, 54). These studies open the possibility that endoglin may hold an important function in tip cell development as it is a critical determinant in controlling endothelial behavior.

The crucial role of TGF-β superfamily signalling in angiogenesis has been underlined by numerous studies both *in vivo* and *in vitro.* The exact molecular mechanisms of TGF-β superfamily signalling in vascular development remain complex and require further elucidation. However, the increasing knowledge of TGF-β superfamily signalling and its crosstalk with other signalling pathways rationalize its physiological and pathological contributions to vascular remodeling. Intervention of TGF-β holds great potential as therapeutic agents in anti-angiogenesis therapy of cancer.

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