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Review

TGF-β receptor function in the endothelium

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Abstract

Genetic studies in mice and humans have revealed the pivotal role of transforming growth factor-β (TGF-β) signaling during angiogenesis. Mice deficient for various TGF-β signaling components present an embryonic lethality due to vascular defects. In patients, mutations in the TGF-β type I receptor ALK1 or in the accessory TGF-β receptor endoglin are linked to an autosomal dominant disorder of vascular dysplasia termed Hereditary Haemorrhagic Telangiectasia (HHT). It has puzzled researchers for years to explain the effects of TGF-β being a stimulator and an inhibitor of angiogenesis in vitro and in vivo. Recently, a model has been proposed in which TGF-β by binding to the TGF-β type II receptor can activate two distinct type I receptors in endothelial cells (ECs), i.e., the EC-restricted ALK1 and the broadly expressed ALK-5, which have opposite effects on ECs behavior. ALK1 via Smad1/5 transcription factors stimulates EC proliferation and migration, whereas ALK5 via Smad2/3 inhibits EC proliferation and migration. Here, the new findings are presented concerning the molecular mechanisms that take place in ECs to precisely regulate and even switch between TGF-β-induced biological responses. In particular, the role of the accessory TGF-β receptor endoglin in the regulation of EC behavior is addressed and new insights are discussed concerning the possible mechanisms that are implicated in the development of HHT.

Keywords: Angiogenesis; Hereditary Haemorrhagic Telangiectasia; Smad; TGF-β

1. Introduction

Transforming growth factor-β (TGF-β) is a multifunctional cytokine involved in the regulation of proliferation, differentiation, migration, and survival of many different cell types [1]. The actions of TGF-β are highly dependent on the cellular context. Three isoforms are present in mammals, i.e., TGF-β1, TGF-β2, TGF-β3, which show partly overlapping as well as distinct functions. TGF-β is secreted in a latent form, which first needs to be activated by proteases or thrombospondin before it can bind to its specific type I and type II serine/threonine kinase receptors. In TGF-β signaling, one TGF-β type II receptor (TβR-II) and two distinct TGF-β type I receptors, i.e., the endothelium restricted activin receptor-like kinase (ALK)1 and the broadly expressed ALK5, have been implicated. After ligand binding and activation of type I receptors, signals are transduced from the membrane to the nucleus via intracellular effectors, termed Smads [2,3]. Whereas ALK1 activation induces the phosphorylation of Smad1, Smad5, and Smad8, ALK5 promotes Smad2 and Smad3 phosphorylation [4].

Gain and loss of function studies in mice have revealed that the TGF-β signaling pathways have an important role both during embryogenesis and in maintenance of homeostasis during adult life [5]. TGF-β has been shown to act as an inhibitor or a stimulator of angiogenesis in vitro and in vivo, depending on experimental conditions [6,7]. Perturbed TGF-β signaling has been implicated in various human diseases [8,9]. During tumor development, TGF-β can exert opposite effects. During the early stages, it acts as an inhibitor of proliferation. However, when tumor cells have selectively escaped from the antimitotic response of TGF-β and often secrete large amounts of TGF-β, it promotes cell

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invasion, metastasis, and indirectly creates a favorable tumor microenvironment by promoting neovascularization [10,11]. Mutations in the TGF-β type I receptor ALK1 or the accessory receptor endoglin that are both highly expressed in endothelial cells (ECs), have been linked to human vascular disorder, termed Hereditary Haemorrhagic Telangiectasia (HHT) [12,13]. Here, we review the function of TGF-β during angiogenesis by controlling the function of ECs and important new insights into TGF-β receptor signaling pathways in ECs.

2. TGF-β signal transduction

2.1. Serine/threonine kinase receptors

TGF-β signals through a heteromeric complex of type I and type II transmembrane serine/threonine kinase receptors [2,3]. The overall structures of type I and type II receptors are similar. They are composed of small cysteine-rich extracellular parts, single transmembrane regions, and intracellular parts that contain serine/threonine kinase domains (Fig. 1A). TGF-β ligands have a high affinity for type II receptor and upon binding to this receptor, a specific type I receptor is recruited. Once this heteromeric complex of two type I and two type II receptors is formed, a conserved 30 amino acid domain, the GS domain of the type I receptor is phosphorylated by the type II receptor [14]. Phosphorylation of serine and threonine residues in GS-domain of type I receptors by TβRII results in a conformational change in the type I receptor. Subsequently, phosphorylation of signaling molecules named Smads takes place that propagate the signal to the nucleus (Fig. 2) [15]. Studies on mutated type I receptors indicate that at least two type I receptors are necessary for signaling [16,17]. Although the exact stoichiometry in the heteromeric signaling complex is not known, it is likely to be minimally a heterotetramer with two type I and two type II receptors [18].

2.2. Smad intracellular effectors

Smad proteins are nuclear effectors for TGF-β receptors [2,3]. There are three distinct types of Smads: Receptor-regulated (R-), Common mediator (Co)-Smads and Inhibitory Smads (I-). R-Smads, i.e., Smad1, Smad2, Smad3,

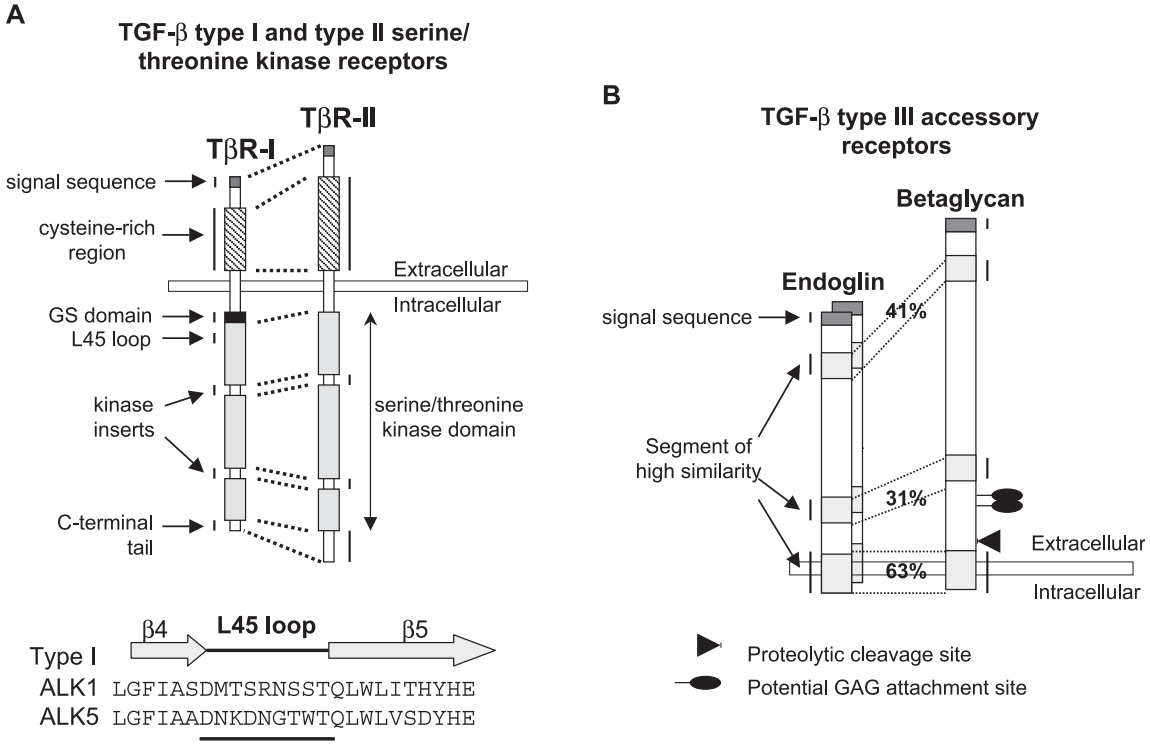

Fig. 1. Schematic representation of TGF-β receptors. (A) TGF-β type I (TβR-I) and TGF-β type II (TβR-II) are single transmembrane protein serine/threonine kinases with two kinase inserts. The extracellular domains are rich in cysteine residues. The carboxyl terminal tail is shorter in the TβR-I compared to the TβR-II. The glycine–serine rich (GS) domain, which regulates the receptor activation, and the L45 loop (an exposed nine-amino acid sequence between kinase subdomains IV and V), are only found in TβR-I. A comparison of amino acid sequences in L45 loop region between activin receptor-like kinase (ALK)1 and ALK5 is shown below. The two β strands (β4 and β5) that flank the L45 loop are shown as arrows. (B) Endoglin and betaglycan (TGF-β type III receptors or TβR-III) are single transmembrane TGF-β accessory receptors that lack an enzymatic motif in their short intracellular domains. The percentages of identical amino acids in specific regions of the human endoglin and betaglycan are shown. Their cytoplasmic tails contain many serine and threonine residues and a putative PDZ domain at the last 3 Carboxy terminal residues. Proteolytic clevage site and potential glycosaminoglycan (GAG) side chains that are rich in heparin sulfate and chondroitin sulfate are indicated. (This figure has been modified with permission from Miyazono et al. [98] and Elsevier.)

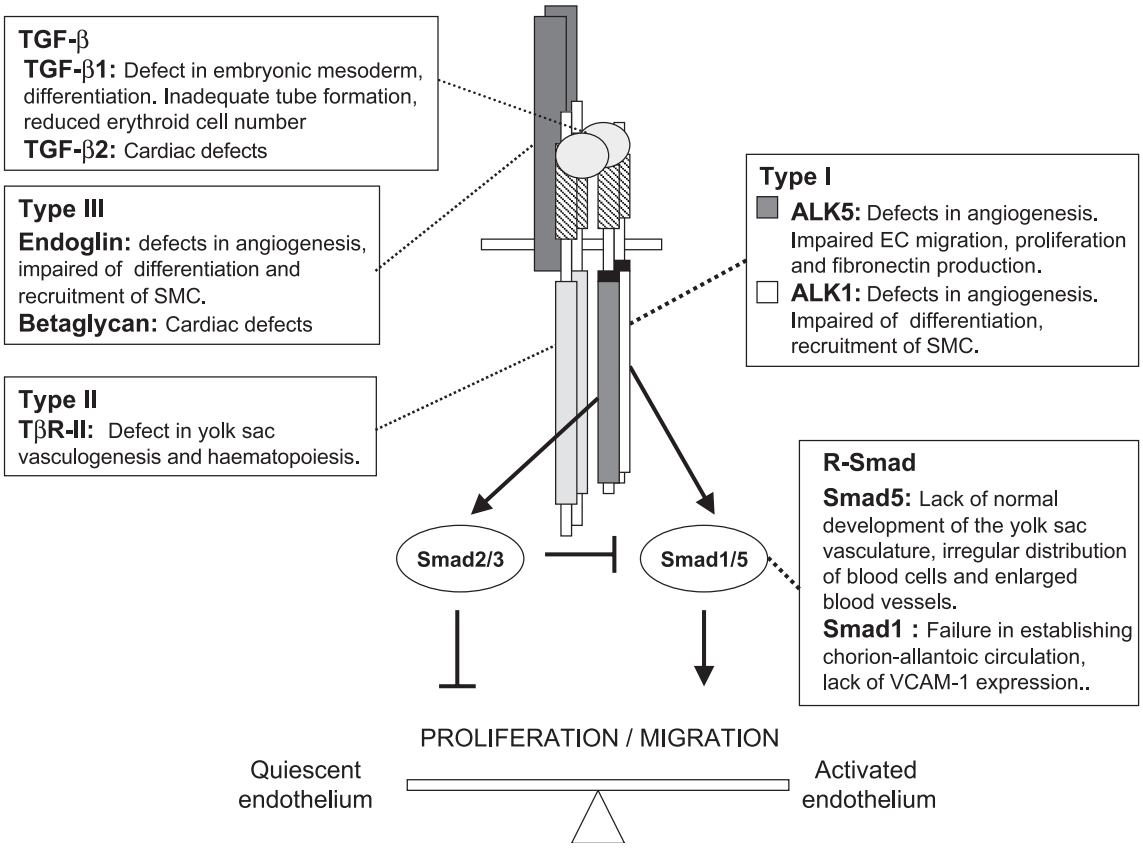

Fig. 2. Regulation of EC behavior by TGF-β signaling and corresponding vascular defects observed in mice deficient in TGF-β components. TGF-β switches EC behavior via two distinct TGF-β type I receptor (TβR-I)/Smad pathways. Upon TGF-β-induced heteromeric complex formation, activin receptor-like kinase (ALK)5 and ALK1 are phosphorylated and activated by TβR-II kinase. Signaling of TGF-β through ALK5 and subsequent Smad2/3 phosphorylation leads to inhibition of EC proliferation and migration. Signaling of TGF-β through ALK1 via phosphorylation of Smad1/5 induces EC proliferation and migration. Moreover, ALK1 signaling indirectly inhibits ALK5-induced Smad-dependent transcriptional responses. Vascular defects of mice deficient in TGF-β signaling components are listed. Abbreviations: VCAM-1, vascular cell adhesion molecule-1;SMC, smooth muscle cell.

Smad5, and Smad8, are phosphorylated by the type I receptor at their extreme C-terminal serine residues [19]. The L3 loop of R-Smads interacts with L45 loop in type I receptors, a region which determines signaling specificity among different type I receptors (Fig. 1A) [2,3]. Activated R-Smads subsequently interact with Smad4 (Co-Smad). This complex translocates to the nucleus where it participates in transcriptional regulation of target genes [20,21]. R-Smads and Smad4 share two conserved domains, termed Mad homology (MH) MH1 and MH2 domain. Both the MH1 and the MH2 domains can interact with select sequence-specific transcription factors. With the exception of Smad2, the MH1 domains of Smads can bind to DNA, whereas the MH2 domains mediate Smad oligomerization and Smad-receptor interaction and it is involved in the transcriptional activation. The I-Smads, i.e., Smad6 and Smad7 prevent the activation of R-Smads by different mechanisms. They compete with R-Smads for receptor interaction [22,23], recruit ubiquitin ligases to the activated receptor, thus promoting its proteosomal degradation [24,25] or recruit phosphatases thereby dephosphorylating the activated type I receptor [26]. I-Smads have MH2 domains but their N-terminal regions only share little sequence similarity with R-Smads and Smad4. The expression of I-Smads is quickly induced upon TGF-β stimulation and upon shear stress of the endothelium [27].

3. TGF-β and maintenance of vascular integrity

During embryogenesis, the cardiovascular system is the first organ to develop and to become functional, allowing growth and maintenance of organ integrity. Two processes are responsible for the formation of blood vessels: (i) vasculogenesis which defines the primary in situ differentiation of endothelial precursors from mesoderm, and their organization into a primary capillary plexus and (ii) angiogenesis which defines the formation of new vessels by a process of sprouting from preexisting vessels [6]. In addition to its role during development, angiogenesis is required for the maintenance of functional and structural integrity of the organism in postnatal life. In this case, angiogenesis is tightly regulated and is limited by the metabolic demands of the tissues concerned.

Angiogenesis can be viewed as two separate, but balanced phases. An activation phase that includes:

increased vascular permeability, basement membrane degradation, EC proliferation and migration. A resolution phase that includes inhibition of EC proliferation and migration, basement membrane reconstitution and stabilization of the vessel by recruitment and differentiation of mesenchymal cells into pericytes and smooth muscle cells (SMC) [28]. The recruitment of mesenchymal cells into the new vessel is mediated by factors, such as platelet-derived growth factor-BB (PDGF-BB) or TGF-β. Upon contact of mesenchymal cells with ECs, latent TGF-β is activated and then induces the differentiation of mesenchymal cells into pericytes and smooth muscle cells. [29]. Pericytes contribute in part to EC survival and stability through expression of vascular endothelial growth factor (VEGF). ECs/mesenchymal cells coculture studies have shown that increased VEGF production by pericytes was found to be critically dependent on heterotypic cell contact mediated production of active TGF-β [30].

Gene knockouts of TGF-β, its receptors and downstream signaling proteins have demonstrated the essential role of TGF-β signaling in (cardio) vascular development (Fig. 2). Mice deficient in TGF-β1 die in utero due to vascular defects [31]. TGF-β2-null embryos display, among other developmental defects, severe cardiac malformations [32,33]. Mice lacking TβR-II or ALK5 die at around E10.5 due to defects in vascular development of the yolk sac [34,35]. ECs isolated from ALK5-deficient mice exhibit impaired fibronectin production and migration in culture that may contribute to the abnormal vessel formation in the yolk sac in vivo [35]. ALK1 knockout mice die at E10.5–11.5. Although, EC differentiation and vasculogenesis appear normal, ALK1 mutant mice develop defects in angiogenesis and vascular smooth muscle cell development. ALK1 knockout embryos fail to form branching capillary network, and the blood vessels are dilated [36]. Smad1-deficient mice demonstrate a failure in establishing chorion–allontoic circulation [37,38], whereas Smad5-deficient embryos have defects in yolk sac vasculature with enlarged blood vessels [39,40]. Mice deficient in the accessory receptor, termed endoglin, exhibit embryonic lethality at E10.5–11.5 with cardiovascular and angiogenesis defects associated with abnormal vascular smooth muscle cell development [41–43]. Mice deficient in the more broadly expressed TGF-β type III receptor, termed betaglycan, exhibit lethal proliferative defects in heart and apoptosis in liver [44].

4. Balancing the activation state of the endothelium via ALK1 and ALK5

One of the aspects that puzzled researchers for years is that TGF-β exerts bifunctional effects on EC proliferation; it can both stimulate and inhibit proliferation of ECs. Low doses of TGF-β stimulate EC proliferation and migration, while high doses of TGF-β inhibit these responses. Other regulatory effects of TGF-β on ECs such as induction of protease activity and extracellular matrix remodeling are highly dependent on the source of ECs and culture conditions used. Recent results have reported that TGF-β regulates the activation state of the endothelium via a fine balance between ALK5 and ALK1 signaling [4]. Whereas the TGF-β/ALK5 pathway leads to inhibition of EC migration and proliferation, the TGF-β/ALK1 pathway induces EC migration and proliferation. Transcriptional profiling using microarrays with constitutively active forms of ALK1 (caALK1) or ALK5 (caALK5) has demonstrated remarkable differences between the target genes regulated by ALK1 or ALK5 in ECs [45]. ALK1 specifically stimulates the expression of Id-1, an inhibitor of basic helix-loop-helix (bHLH) proteins that promotes EC proliferation and migration. ALK5 specifically induces expression of fibronectin expression, an extracellular matrix protein, and the plasminogen activator inhibitor type 1 (PAI-1). PAI-1 is a negative regulator of EC migration in vitro [46] and angiogenesis in vivo [47]. Treatment of EC-derived from embryonic stem cells with SB-431542, a synthetic ALK5 kinase inhibitor, facilitates proliferation and sheet formation [48]. Furthermore, TGF-β may be involved in vascular permeability as expression of Claudin-5, an EC-specific component of tight junctions, was greatly upregulated by SB-431542 [48].

Other studies have reported that ALK1 stimulates the resolution phase of angiogenesis [49,50]. The discrepancies between the ascribed roles of ALK1 in different studies are not clearly understood. It may be caused by differences in the cell lines and culture conditions used or by possible secondary adaptative processes that may take place in the embryo during development in order to counteract the lack of ALK1 expression [7].

The existence of two type I receptor pathways activated by one ligand, side-by-side in one cell type, raised the question of how their activation is controlled and why these two cascades coincide. Goumans et al. [51] observed that ALK5-deficient ECs are not only defective in TGF-β/ALK5, but also in their TGF-β/ALK1 responses. ALK5 was found to be important for recruitment of ALK1 into a TGF-β receptor complex, and, moreover, the kinase activity of ALK5 is essential for efficient ALK1 activation. Another level of crosstalk between ALK1 and ALK5 was also identified. ALK1 not only induced responses opposite to those of ALK5, but it was found to directly antagonize ALK5/Smad2/3 signaling. Ectopic expression of Smad5 or Smad3 was found to potentiate or attenuate, respectively, the ALK1 inhibitory effect on ALK5-induced transcriptional responses. This implies that the antagonism is exerted at the Smad level. The requirement for ALK5 in TGF-β/ALK1 signaling, and the opposing actions of ALK1 and ALK5 provide ECs with an intricate mechanism for precisely regulating, and even switching between TGF-β-induced biological responses (Fig. 2).

Other signaling pathways, distinct from Smad pathways, have also been identified that regulate EC behavior in response to TGF-β. In fact, TGF-β can activate MAP kinases and small GTP-ases [52,53]. However, the effects observed are highly dependent on cellular context and whether this occurs in vivo remains to be investigated. In one study, TGF-β through the activation of p38 has been shown to induce the phosphorylation of myosin light chains and increase EC monolayer permeability [54]. In addition to the direct TGF-β-induced Smad independent signaling, there is an important role for Smad-independent pathways that are induced by Smad-mediated changes in gene expression. In capillary ECs, TGF-β was shown to induce autocrine secretion of TGF-α, a survival factor that activates PI3K and ERK/MAP kinases [55].

5. Accessory TGF-β receptors in endothelial cells

TGF-β type III receptors, betaglycan and endoglin, are structurally related proteins that have a more indirect role in TGF-β signal transduction. Both proteins are transmembrane receptors with short intracellular domains that lack any enzymatic motif but contain many serine and threonine residues. (Fig. 1B). An important function of betaglycan is to facilitate the binding of TGF-β to TβR-II. This is particularly important for TGF-β2, which has only low intrinsic affinity for TβR-II. Large vessels that do not express betaglycan respond much more potently to TGF-β1 and TGF-β3 than TGF-β2, suggesting that betaglycan expression correlates with TGF-β2 responsiveness [56–58]. Recently, TβR-II has been reported to phosphorylate betaglycan. This modification of the phosphorylation status of the cytoplasmic tail of betaglycan was shown to facilitate a complex formation between betaglycan and β-arrestin, which in turn could induce the endocytosis of betaglycan and TβR-II receptors [59]. Endoglin may also interact with β-arrestin as the intracellular domains of betaglycan and endoglin are highly similar (Figs. 1B and 3B).

Endoglin is a component of the receptor complex of TGF-β [60,61]. In contrast to betaglycan, it binds only ligands when it is associated with TβR-II. As TβR-II binds TGF-β1 and β3 with higher affinity, endoglin interacts much more efficiently with TGF-β1 and TGF-β3 than TGF-β2 [61,62]. Both extracellular and intracellular domains of endoglin interact with TβR-II and ALK5. The cytoplasmic domain of endoglin that is rich in serine and threonine residues is phosphorylated by ALK5 or TβR-II [63]. Ectopic expression of endoglin has been shown to inhibit TGF-β-induced growth inhibition in monocytes and myoblasts [62,64] and more recently TGF-β-induced extracellular matrix synthesis in myoblasts [65]. Moreover, in a multistage model for mouse skin carcinogenesis, reduction of endoglin expression in mice had an effect similar to transgenic mice overexpressing

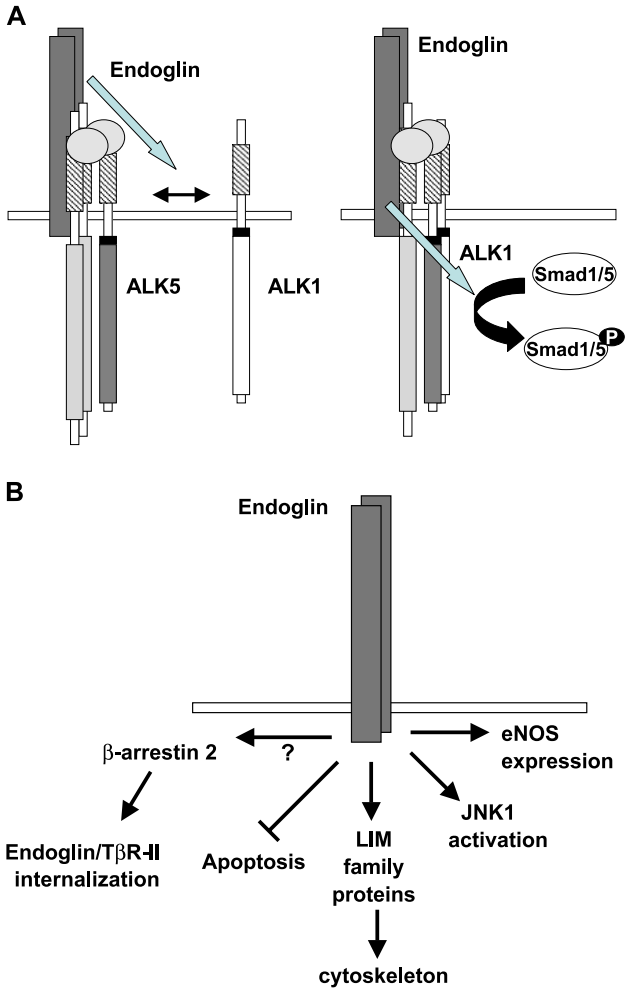

Fig. 3. Models illustrating the role of endoglin in TGF-β/activin receptor-like kinase (ALK)1 signaling and in regulation of EC behavior independent of TGF-β. (A) Two models (that are not mutually exclusive) by which endoglin may promote TGF-β/ALK1 signaling are shown: (left panel) by recruiting ALK1 into ALK5/TβR-II complex and (right panel) by stimulating ALK1 kinase activity or Smad recruitment into the complex. (B) Endoglin may regulate EC behavior independent of TGF-β by (i) regulating cytoskeleton organization, (ii) protecting ECs from hypoxia-induced apoptosis, (iii) stimulating JNK1 phosphorylation, and (iv) regulating endothelial nitric oxide synthase (eNOS) expression. As found for betaglycan, endoglin may also be phosphorylated by TβR-II on serine residues in the intracellular domain, which subsequently allows for the recruitment of β-arrestin 2 and mediate the degradation of the TβR-II/endoglin complex.

TGF-β, suggesting that endoglin in tumor cells may attenuate TGF-β signaling [66]. Treatment of ECs with antisense oligonucleotides or with neutralizing antibody for endoglin was found to potentiate the inhibitory effect of TGF-β on EC migration, or EC growth [67] [68]. These TGF-β responses are mediated via ALK5 [4] and thus suggest that endoglin is a negative regulator of TGF-β/ALK5 signaling. Consistent with this notion, ectopic expression of endoglin was recently shown to inhibit Smad3 transcriptional activity [69].

Endoglin is predominantly expressed in vascular ECs [70]. Other sites of endoglin expression include syncytiotrophoblasts of full-term placenta, stromal cells and hema-

topoietic stem cells (HSCs) [71,72]. Endoglin is highly expressed in activated ECs, its expression being potently induced by hypoxia [73–75]. TGF-β1 itself is also a promoter of endoglin expression, whereas TNF-α suppresses endoglin expression in vascular ECs [76]. Elevated levels of endoglin have been detected in cancer patients and are positively correlated with tumor metastasis. In addition to its function as a marker for tumor angiogenesis, endoglin antibodies coupled with toxins or radioactivity have successfully been used to target ECs in antiangiogenic therapy [71,75].

Mutation in the accessory TGF-β receptor endoglin and ALK1 can give rise to HHT (see discussion further below), suggesting that they act in a common pathway. Consistent with this notion, TGF-β/ALK1 signaling was recently shown to require endoglin. Moreover, the level of endoglin expression determines growth capacity of EC. In the absence of endoglin, ECs do not grow and ALK1 signaling is abrogated whereas ALK5 signaling is stimulated. Endoglin may thus function as a modulator of the balance between TGF-β/ALK1 and TGF-β/ALK5 signaling pathways. These recent results favor a model, in which endoglin stimulates TGF-β/ALK1 signaling and indirectly inhibits TGF-β/ALK5 signaling, thus promoting the activation phase of angiogenesis [77]. How endoglin promotes TGF-β/ALK1 signaling remains unclear; endoglin may be involved in heteromeric complex formation between ALK1 and ALK5 and/or recruitment of Smad1/5 to ALK1 and/or recruitment of ALK1 to particular subcellular components (Fig. 3A). An alternative model has been proposed by Guerrero-Esteo et al. [63], in which endoglin ectopically expressed altered the phosphorylation status of TβR-II and ALK5, increased Smad2 phosphorylation and promoted Smad2-driven reporter activity. Further studies are awaited to clarify the differences.

6. HHT are linked to mutations in endoglin and ALK1

HHT (also termed Osler–Weber–Rendu disease) is an autosomal dominant disorder of vascular dysplasia that affects many organs. Characteristic symptoms include skin and mucosal telangiectases, pulmonary, cerebral and hepatic arteriovenous malformations, and hemorrhage associated with these vascular lesions. The characteristic lesions in this disorder are telangiectases that consist of focal dilatations of postcapillary venules and arterial venous malformations (AVMs). Two variants of HHT, HHT1 and HHT2, have been described that are linked to endoglin and ALK1, respectively. Mutations in endoglin include deletions, insertions, and missense mutations and splice site changes. A majority of the identified mutations represent null alleles. Thus, the predominant mechanism underlying the HHT1 phenotype seems to be a model of haplo-insufficiency, leading to the reduced levels of endoglin protein at the cell surface of vascular ECs [78–80]. Mutations in ALK-1 have been found and include small deletions, insertions, and nonsense mutations leading to truncated proteins as well as missense mutations. Expression analysis suggested that some of the mutations were null alleles because of apparent instability of the mutant transcript [81]. This supports again a haploinsufficiency model for HHT2 as proposed for HHT1 [81,82]. HHT1 has a higher prevalence of pulmonary AVMs than HHT2 families who generally have a milder phenotype and later onset. Clinical manifestations of HHT are highly heterogeneous within and between families [83]. The upregulation of endoglin during the monocyte–macrophage transition is impaired and age dependent in both HHT types. Thus, endoglin levels may have predictive value with respect the clinical severity of HHT [84]. Mice heterozygous for endoglin or ALK1 demonstrate at the adult stage characteristics of HHT. In line with HHT patients, the phenotype obtained in mice also depends on the genetic background [85,86]. Therefore, genetic and epigenetic factors have been postulated to account for this diversity.

Interestingly, patients with characteristics of HHT without mutation in endoglin or ALK1 have been reported, suggesting that other genes might be linked to HHT [87–89]. Recently, Gallione et al. [90] reported that mutation in MADH4 (Smad4) can cause a syndrome consisting of both Juvenile Polyposis and HHT phenotypes. This observation suggests that HHT could be linked to a reduction of both TGF-β/ALK1 and TGF-β/ALK5 signaling. Consistent with this, vascular defects associated with a reduction of ALK5 expression have been reported in VEGF-induced microvessel of endoglin heterozygous mice [91]. Moreover, ECs from endoglin heterozygous mouse embryos show a reduction of ALK5 expression [77]. Taken together based on these results, we propose the following model: in ECs with reduced endoglin level, ALK5 signaling is increased. In order to compensate this “overactivity of ALK5 signaling” leading to inhibition of EC growth, ECs adapt by downregulating ALK5 expression. In patients, HHT may thus not only be caused by a reduction in ALK1/endoglin signaling but may result from a more general defect in TGF-β signaling in vivo.

Recent reports have suggested that endoglin can mediate certain effects independently of TGF-β signaling (Fig. 3B). Using endoglin knockdown approach, Li et al. [92] have demonstrated that endoglin can act as antiapoptotic factor in ECs under hypoxic stress. This effect is mediated in the absence of TGF-β. Certain endoglin antibodies have antiangiogenic properties in vitro and in vivo, while not affecting the binding of TGF-β to endoglin [68]. Endoglin has been shown to regulate cell migration in a TGF-β-independent manner. The cytoplasmic tail of endoglin, but not of betaglycan, was shown to bind LIM domain-containing proteins and associated adapter proteins. This was shown to affect formation of focal adhesion and to

regulate actin cytoskeletal reorganization [93,94]. Ectopic expression of endoglin can induce JNK1 phosphorylation [69], which may negative regulate Smad3 activation. Endoglin was shown to regulate nitric oxide (NO)-dependent vasodilation. Moreover, a downregulation of endothelial nitric oxide synthase (eNOS) expression was detected in endoglin heterozygous mice, whereas modulation of endoglin expression in cultured ECs regulates eNOS expression [95]. Further studies are needed to examine the relevance of these findings for HHT and other vascular diseases.

7. Conclusions

Whereas genetic studies in mice and human have demonstrated that TGF-β is a key player in the development and physiology of the vascular system, the molecular mechanisms and target cells by which the pleiotropic TGF-β elicits its pro- and antiangiogenic properties were still unclear. However, recent studies have provided important new insights that reconcile previously published observations. TGF-β can activate in ECs two distinct type I signaling pathways, i.e., ALK1 and ALK5 with opposite effects on EC behavior. Both genetic and biochemical data indicate a need for endoglin in TGF-β/ALK1 signaling. These results provide a framework for further studies to investigate underlying mechanisms by which subverted TGF-β signaling leads to vascular disorders like HHT or contributes to tumor neovascularization.

The vascular defects observed in HHT are likely caused by a loss of EC function, which indirectly affect proper EC-smooth muscle cell/pericyte interactions and result in poor vascular integrity. Consistent with this notion, analysis of yolk sacs from endoglin-deficient mice revealed defective paracrine TGF-β signaling from ECs to adjacent mesothelial cells [96]. Furthermore, downregulation of the endoglin/ALK1 pathway may indirectly potentiate TGF-β/ALK5 signaling. The latter pathway has been shown to induce expression of smooth muscle markers in ECs, such as SM22α [45]. Whether the endoglin/ALK1 pathway serves to maintain EC integrity by preventing their transdifferentiation into smooth muscle cells or pericytes is an interesting area for future research.

While there is ample evidence that endoglin is expressed in activated human ECs in, e.g., tumor vessels, little information is available on ALK1 expression in human tissues. Interestingly, using a mutant mouse line for ALK1 (ALK1lacZ) in which exons of ALK1 were replaced with beta-galactosidase gene, ALK1 expression was found to be greatly diminished in adult arteries, but induced in newly forming arterial vessels during wound healing and tumor angiogenesis [97]. The generation of highly specific ALK1 antibodies is eagerly awaited to investigate the ALK1 distribution in various tumor tissues and correlate ALK1 expression with that of endoglin and phosphorylated Smad1/5.

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