Chapter 1

TGF-β signalling in vascular biology and dysfunction

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Abstract

Transforming growth factor (TGF)-β family members are multifunctional cytokines that elicit their effects on cells, including endothelial and mural cells, via specific type I and type II serine/threonine kinase receptors and intracellular Smad transcription factors. Knock-out mouse models for TGF-β family signalling pathway components have revealed their critical importance in proper yolk sac angiogenesis. Genetic studies in humans have linked mutations in these signalling components to specific cardiovascular syndromes such as hereditary hemorrhagic telangiectasia, primary pulmonary hypertension and Marfan syndrome. In this review, we present recent advances in our understanding of the role of TGF-β receptor signalling in vascular biology and disease, and discuss how this may be applied for therapy.

Keywords**:** angiogenesis, BMP, Marfan syndrome, Smad, pre-eclampsia, pulmonary hypertension, TGF-β

Introduction

Transforming growth factor (TGF)-β is the prototypic member of a large family of evolutionarily conserved pleiotropic cytokines. Thirty three members are present in mammals, including three TGF-β isoforms, activins, and bone morphogenetic proteins (BMPs) [1-3]. TGF-β family members have critical and specific roles during embryogenesis and in maintaining the homeostasis of adult tissues. Perturbations in their signalling pathways have been linked to a diverse set of developmental disorders and diseases, including cancer, fibrosis, auto-immune and cardiovascular diseases.

All TGF-β family ligands are generated as dimeric precursor proteins and subsequently cleaved by proteases and secreted [4]. Members of the TGF-β family elicit their cellular effects by binding to a complex of type II and type I serine/threonine kinase transmembrane receptors (Figure 1). Five type II receptors and seven type I receptors, also termed activin receptor-like kinases (ALKs) are present in mammals [1-3]. Within the ligand-induced heteromeric receptor complex, the constitutively active type II receptor phosphorylates the type I receptor on specific serine and threonine residues in the intracellular juxtamembrane region. TGF-β signals in most cells via TGF-β type II receptor (TβRII) and ALK5, activins via activin receptor type IIA (ActRIIA) and IIB and ALK4, and BMPs via BMP type II receptor (BMPRII), ActRIIs and ALK1, 2, 3 and 6. In endothelial cells (ECs) TGF-β can, in addition to ALK5, also signal via ALK1 [5, 6]. Upon type I receptor activation, receptor-regulated Smads (R-Smads) are recruited to, and phosphorylated by the type I receptor at the two serine residues in their extreme carboxyl termini. ALK4 and 5 (and 7) induce R-Smad2 and 3 phosphorylation, while ALK1, 2, 3 and 6 mediate phosphorylation of R-Smad1, 5 and 8. Activated R-Smads form complexes with the common mediator Smad4, and translocate into the nucleus, where they can regulate, together with other partner proteins, the transcription of specific target genes. Inhibitory (I)-Smads, i.e. Smad6 and -7, can inhibit the activation of R-Smads by competing with R-Smads for type I receptor interaction and by recruiting specific ubiquitin ligases or phosphatases to the activated receptor complex thereby targeting it for proteosomal degradation or de-phosphorylation, respectively [1-3].

Access of ligands to the signalling type I and type II receptors is regulated by soluble ligand binding proteins and by accessory type III receptors. Examples of the latter class that have been most intensively studied are endoglin and betaglycan [4, 7]. Both receptors are structurally related trans-membrane proteins with long extracellular and short intracellular domains and without an enzymatic motif. Compared with the type I and type II receptors, betaglycan is present in higher abundance and binds TGF-β1 and 3 with lower affinity [8]. When TGF-β encounters a target cell expressing betaglycan, it is likely to interact first with betaglycan, which then presents it to TβRII. Betaglycan facilitates TGF-β signalling, in particular for TGF-β2 that binds with very weak affinity to TβRII alone [9, 10]. Endoglin (CD105) is a homodimer that interacts with TGF-β1 and TGF-β3, but only when it is associated with TβRII [11, 12]. Both the endoglin extracellular and intracellular domains interact with TβRII and ALK5 [7]. Endoglin is able to bind directly to BMPs [13-15].

In contrast to factors such as vascular endothelial growth factors (VEGFs) and angiopoietins that have prominent effects on EC behavior [16], TGF-β was initially discovered through its effects on fibroblasts [17] and subsequently shown to affect among other cell types, epithelial-, immune-, stem-, endothelial- and mural cells. This together with its highly cellular context dependent properties, frequently having opposite effects depending on the cellular differentiation state or the presence of other specific extracellular cues, has left the elucidation of the complex role of TGF-β family members in the cardiovascular system somewhat under-investigated. However, phenotypic and molecular characterizations of knock-out mice for TGF-β signalling components have demonstrated their critical role in angiogenesis, and importantly several cardiovascular syndromes were directly linked to mutations in their genes [4]. TGF-β family members have now gained a prominent spot among other key cytokines that control vascular function. Our understanding of their complex role in cardiovascular biology and interplay with VEGF and other angiogenesis regulators is proceeding at a rapid pace. In this review we focus on recent insights into the function of TGF-β family members in the cardiovascular system and discuss how dys-regulation of their signalling pathways contributes to vascular pathologies.

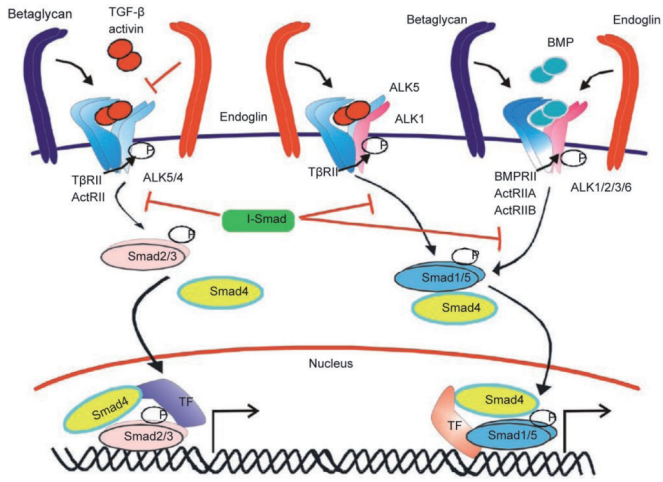

Fig.1 *Signal transduction by TGF-β family members is mediated via specific heteromeric complexes of type I and type II serine/threonine kinase receptors. In most cells TGF-β interacts with TβRII and ALK5, but in endothelial cells it can also signal via ALK1. BMPs signal via BMPRII, ActRIIA and ActRIIB, and type I receptors ALK1, 2, 3 and 6. Co-receptors betaglycan and endoglin can facilitate TβRII/ALK5 and TβRII/ALK1 signalling. Soluble versions of the co-receptors betaglycan and endo- glin have been shown to sequester TGF-β and BMP9, respectively. Intracellular signalling can be divided into two main Smad signalling pathways: ALK5 induces phosphorylation of Smad2 and Smad3, and ALK1, 2, 3 and 6 mediate phosphorylation of Smad1, 5 and 8. Activated R-Smads form heteromeric complexes with common mediator Smad4, which accumulate in the nucleus, where they can act as transcription factor complexes and regulate the expression of specific target genes*

Vascular morphogenesis

Neovascularization, the formation of new functional microvascular networks, is a tightly controlled process regulated by several converging signalling pathways that are tightly coordinated in time and space. The formation of new blood vessels can occur via two processes, vasculogenesis and angiogenesis, both of which result in the formation of endothelial-lined tubes [16]. During vasculogenesis new vessels arise de novo from a mass of proliferating cells, classically forming an inner core of hematopoietic precursor cells and an outer layer of ECs termed a blood island, followed by their subsequent migration, fusion and organization into a primary capillary plexus [18]. Angiogenesis refers to the formation of new capillary networks by sprouting from pre-existing vessels [18, 19]. Neovascularization is involved in growth and development, wound healing and several pathological situations such as tumor growth and metastasis, and cardiovascular disorders [20]. While angiogenesis occurs during embryogenesis and in adult life, vasculogenesis was initially thought to occur only in the embryo, but some studies have suggested that circulating endothelial progenitor cells may also contribute to vessel formation in the adult [21]. The formation of new capillaries involves EC activation, migration, alignment, proliferation, tube formation, branching and maturation of intercellular junctions and the surrounding basement membrane. All new blood vessels begin as simple EC-lined capillaries, but during vessel maturation, some vessels remain as capillaries covered by pericytes, and others develop into large vessels with support from a layer of smooth muscle cells (SMCs), forming a strong vessel wall.

Angiogenesis is a carefully balanced process, under the control of and fine tuned by a multitude of factors, including stimulators like VEGF and inhibitors such as thrombospondin [20]. Considering the context-dependent effects of TGF-β in other cell systems, it is not surprising that its effect on blood vessel formation is biphasic [4]. It can act as both a promoting and an inhibitory factor of angiogenesis for which the underlying mechanisms are starting to be uncovered.

Insights from knock-out mice

In vivo studies show that loss of TGF-β signalling components leads to abnormal formation of the primitive vascular plexus and decreased vessel wall integrity caused by irregular capillary vessel formation or impaired differentiation and recruitment of vascular SMCs. Targeted deletions of ALK1, ALK5, TβRII and endoglin in mice result in remarkably similar phenotypes (Figure 2). Embryos lacking any one of these components die during mid-gestation due to impaired vascular development, exhibiting hyper-dilated, leaky vessels [22], highlighting the importance of TGF-β signalling in the vascular sys- tem. The primary target cell for TGF-β is the EC since mice deficient in endothelial TβRII or ALK5 expression showed vascular defects in the yolk sac and embryonic lethality at embryonic day (E)10.5, which pheno-copied mice lacking receptors in all cells [23]. TGF-β

not only affects the ECs, but is also important for proper differentiation and function of SMCs and pericytes. Mice lacking TβRII specifically in vascular SMCs also showed vascular defects in the yolk sac but at later stages of development, allowing the embryo to survive to E12.5 [23].

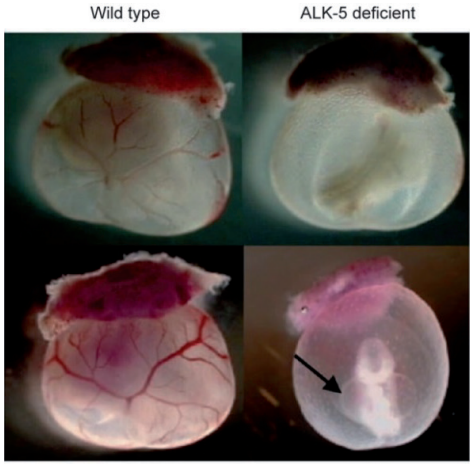

Fig.2 *TβRI/ALK5-deficient embryos exhibit severe defects in vascular development. Gross morphology of whole-mount yolk sacs in mutant embryos is compared with wild type littermates. Arrow indicates the pericardial effusion in the mutant embryo probably caused by a circulation defect [116].*

At the intracellular level Smad1-, Smad2- or Smad4- deficient mice demonstrate pre-angiogenesis lethality in embryos [22]. In Smad1-deficient embryos this is accompanied with defects in chorion-allantoic circulation [24, 25]. Smad5 knock-out and endothelial specific Smad4 knock-out have phenotypes reminiscent of TGF-β receptor knock-out mice [26, 27]. Smad3-lacking mice are viable but die of impaired immunity and colon cancer [22].

The I-Smads, Smad6 and 7 were initially identified as EC shear stress response genes [28] and are highly expressed in specific regions of the heart [29, 30]. Both I-Smads are strongly induced by TGF-β family members and participate in a negative feedback loop [1-3]. Studies of mice deficient in Smad6 or Smad7 demonstrated their critical role in development and heart homeostasis [30, 31]. Mice lacking Smad6 are born, but develop multiple heart abnormalities, including septation defects, ossification of the aorta, and have high blood pressure. While some of the Smad7-deficient mice die shortly after birth because of heart complications and abnormal heart rate, the majority die in utero due to cardiovascular defects, including outflow tract malformation and abnormality in ventricular septum and non-compaction.

Role of TGF-β signalling in ECs

TGF-β has been proposed to regulate the activation state of ECs by differentially activating two type I receptors, ALK5 and ALK1 [5, 6]. ALK5 is widely expressed in almost all tissues, but the expression of ALK1 is restricted to ECs and during embryogenesis at sites of angiogenesis [32].

TGF-β/ALK5 signalling induces Smad2/3 phosphorylation and blocks angiogenesis by inhibiting EC proliferation, tube formation and migration [6, 33]. ALK5 induces the expression of fibronectin and plasminogen activator inhibitor type 1 (PAI-1), a negative regulator of EC migration [34]. Interestingly, in mouse embryonic stem cell-derived ECs, the ALK5 kinase inhibitor SB-431542 enhances EC growth and integrity via up-regulation of the tight junction component Claudin-5, suggesting a role for ALK5 signalling in regulating vascular permeability [35]. ALK5 indeed has been reported to increase TGF-β-induced EC permeability and actin cytoskeleton remodeling [36]. By enhancing TβRII/ALK5 assembly clustered VE-cadherin promotes persistent and elevated TGF-β-induced Smad2/3 activation, indicating a positive role for VE-cadherin in TGF-β/ALK5-induced vessel stabilization [37]. Taken together, TGF-β/ALK5 signalling plays an important role in keeping the endothelium quiescent.

In contrast to TGF-β/ALK5, TGF-β/ALK1 signalling induces Smad1/5 activation and has been shown to stimulate EC migration, proliferation and tube formation [33]. Caveolin1 was shown to associate with ALK1 and to promote TGF-β/ALK1-induced responses [38]. An important intracellular effector of ALK1 is Id1; its upregulation was shown to be required for TGF-β/ALK1-induced EC migration and tube formation [6]. However, an inhibitory effect of ALK1 signalling on EC proliferation, migration and spouting has also been reported [39, 40]. BMP9, identified as a ligand for the ALK1 and BMPR-II complex in ECs, was shown to inhibit EC migration and VEGF-induced angiogenesis [13, 14]. These observations suggest that the effect of ALK1 signalling on angiogenesis is dependent on the context and specific ligand by which it is activated.

ALK1 and ALK5 signalling not only elicit opposite responses, but also physically interact with each other in ECs. ALK5-deficient ECs are not only defective in TGF-β/ALK5 signalling but also exhibit impaired TGF-β/ ALK1 responses; ALK5 was found to be necessary for recruitment of ALK1 into a TGF-β receptor complex, and the kinase activity of ALK5 is essential for maximal ALK1 activation [33]. Furthermore, ALK1 can directly antagonize ALK5/Smad2/3 signalling at the level of Smads [5, 6]. The cross-talk between ALK1 and ALK5 signalling provides ECs with a TGF-β-dependent switch to fine tune EC function.

BMPs also play an important role in EC function. BMP6 promotes EC migration and tube formation via Smad1/5/8 phosphorylation [41]. BMP4 was shown to induce proliferation and migration of ECs via stimulation of VEGF-A and Tie-2 signalling [42]. *In vivo* BMPs were also found

to induce tumor angiogenesis [43]. BMP endothelial cell precursor-derived regulator (BMPER) interacts with BMP4, and regulates BMP4-mediated angiogenesis [44]. Interestingly, inhibition of BMPRII using specific siRNA in human pulmonary arterial ECs was found to induce EC apoptosis via an increase in activated caspase-3 [45]. Upon exposure to hypoxia, BMPRII, phosphorylated Smad1/5/8 and Id1 expression were strongly reduced in these ECs, which may be of relevance to the pathogenesis of hypoxia-induced pulmonary hypertension [46]. BMP9, which interacts with ALK1, is reported to be a circulating vascular quiescence factor [15].

Interestingly, there are ECs that express betaglycan and those that express endoglin. ECs expressing betaglycan respond to all three isoforms of TGF-β, whereas ECs that express endoglin (and not betaglycan) respond with high potency to TGF-β1 and -β3, but not -β2 [47]. In ECs that co-express betaglycan and endoglin, both proteins were shown to be part of a common TGF-β receptor complex [48]. The co-receptor endoglin is predominantly expressed in highly proliferating vascular ECs, but is also reported to be detected on hematopoietic cells, syncytiotrophoblasts of term placenta, stromal cells and mesenchymal cells. Ectopic expression of endoglin inhibits TGF-β-induced growth inhibition in ECs, monocytes and myoblasts [12, 49] and extracellular matrix deposition [50]. Endoglin regulates the fine-tuning between the ALK1 and ALK5 signalling pathways. It is required for TGF-β/ALK1 signalling and indirectly inhibits TGF-β/ ALK5 signalling [49, 51]. Suppression of endoglin gene expression using siRNA resulted in impaired TGF-β/ ALK1 signalling responses [49]. ECs derived from *Eng–/–* embryos were unable to proliferate in culture, displayed reduced migration, VEGF secretion and eNOS expression [49, 52]. Interestingly, up-regulation of endoglin protects ECs from the apoptotic action of TGF-β1 [53]. Endoglin was recently shown to interact via its C- terminal PDZ binding motif with the scaffolding protein GIPC, which promotes endoglin cell surface retention [54]. Elevated endoglin expression levels are used as a marker of tumor angiogenesis and endoglin antibodies coupled with toxin or radioactivity have been successfully used to target ECs in anti-angiogenic therapy [55, 56]. Two endoglin splice forms have been reported, termed long (L) and short (S)-endoglin with pro- and anti-angiogenic activity, respectively [57]. While the L-form is most abundantly expressed and promotes TGF-β/ALK1 signalling, S-endoglin expression was recently shown to be specifically high in senescent ECs and it preferentially promotes TGF-β/ALK5 signalling [58,59]. Transgenic mice overexpressing S-endoglin in ECs showed hypertension. Taken together, whereas L-endoglin contributes to the activation phase of angiogenesis and contributes to pathological neovascularization, S-endoglin is induced during EC senescence and may lead to age-associated pathologies such as hypertension [59].

Role of TGF-β signalling in vascular SMCs

Vascular SMCs express multiple type I and type II receptors for TGF-β family members [60]. TGF-β is a potent stimulator of vascular SMC differentiation by activating the genetic program that

includes a large set of SMC differentiation marker genes [61]. Myocardin, an important coactivator of serum response factor, was shown to potently enhance TGF-β/Smad3-mediated activation of SM22α actin transcription [62]. The zinc finger E-box binding transcription factor DeltaEF1 was also shown to have an important effector role in this respect by forming a complex with serum response factor and Smad3 [63]. TGF-β-induced growth inhibition of vascular SMCs was found to be ALK5-mediated via both Smad3-dependent and p38 MAP kinase-dependent signalling pathways [64]. TGF-β was also shown to inhibit SMC migration in a non-Smad3-dependent pathway via up-regulation of cysteine rich protein 2 expression [65, 66]. Interestingly, Smad3-deficient vascular SMCs demonstrated reduced growth inhibition by TGF-β, but did not show any attenuated TGF-β-mediated migratory response [64, 67].

Endoglin is also important for the formation of vascular SMCs. Ectopic endoglin expression in neural crest stem cells causes pericardial hemorrhage associated with altered vascular SMC investment in the walls of major vessels, suggesting a direct role of endoglin in myogenic differentiation [68].

Vascular SMC differentiation and function are also influenced by BMPs. The effect of BMPs depends on the source of SMCs studied as well as the local environment. While BMP2 stimulates vascular SMC migration, it prevents platelet-derived growth factor induced vascular SMC proliferation via induction of the PPARγ/apoE axis [69, 70]. BMP7 also inhibits the growth of vascular SMCs and helps maintain the vascular SMC phenotype in culture [71]. In pulmonary SMCs both BMP7 and BMP4 induce apoptosis via a caspase 8- and 9-dependent mechanism [72]. Interestingly, BMP4 induces micoRNA- 21, leading to the repression of PDCD4, an inhibitor of smooth muscle cell gene expression; SMC differentiation is thereby stimulated [73].

Interaction between ECs and vascular SMCs

Tight regulation and close coordination between ECs and vascular SMCs are needed to form a mature vascular network. Vascular SMCs form abundant gap junctions with ECs and upon receiving signals from SMCs, ECs line up and recruit more SMCs [74].

TGF-β is an important regulator of the EC-SMC interaction. ECs produce latent TGF-β that upon EC-SMC interaction can be activated and induce SMC differentiation and function [75]. Targeted deletions of several TGF-β signalling components have revealed the importance of TGF-β signalling in vSMC-EC contact. ALK1- and Endoglin-deficient mice display defective remodeling of the primary capillary plexus of the yolk sac and aberrant development of SMCs [4, 5, 22, 76]. Mice lacking TβRII, Smad5, Smad1 and TGF-β1 show defects in vasculature structure or blood vessel organization [22] indicative of a defect in EC lining and impaired SMC development.

TGF-β can also stimulate SMCs to produce VEGF partly in a p38 MAP kinase [77], and a reactive oxygen species generation-dependent manner [78]. The produced VEGF can influence both the ECs as well as SMCs, by inducing their growth and differentiation. Interestingly, VEGF was found to inhibit TGF-β release by ECs [79].

Besides influencing their growth, TGF-β and BMP are also potent SMC differentiation factors and crucial regulators to switch SMCs from an undifferentiated phenotype to a contractile phenotype [61]. Furthermore, TGF-β can also induce trans-differentiation of ECs (Endo-MT) into smooth muscle-like cells. TGF-β induces the expression of α-smooth muscle actin and smooth muscle myosin in ECs in a Snail-dependent manner [80] and blocking TGF-β signalling with neutralizing antibodies abrogates the induction of smooth muscle markers in ECs [75].

Vascular disorders and TGF-β signalling

In recent years multiple cardiovascular disorders have been linked to alterations in TGF-β/BMP signalling pathways, several of which will be discussed below. Increased understanding of the molecular mechanisms has been achieved by studying mouse models that mimic these human diseases. Importantly, these mouse models also allow for testing of therapeutic strategies that aim to normalize the perturbed signalling balance.

Hereditary hemorrhagic telangiectasia (HHT)

Hereditary hemorrhagic telangiectasia (HHT), or Osler-Rendu-Weber syndrome, is an autosomal dominant vascular dysplasia, characterized by the development of mucocutaneous telangiectasias and arteriovenous malformations in the brain, lungs, liver, and gastrointestinal tract (Figure 3). There is variability in the organs affected, even between individuals within families [81]. Three genes are causally related to HHT, i.e. endoglin mutated in HHT type 1 (HHT-1) [82]; ALK1 mutated in HHT-2 [83] and mutation in Smad4 causing a syndrome consist- ing of both juvenile polyposis and an HHT phenotype [84]. *In vivo*, EC-specific deletion of ALK1 caused vascular malformations mimicking all pathologic features of HHT-2 vascular lesions [85], while endoglin heterozygous mice have dilated and fragile blood vessels which resemble clinical manifestations of HHT-1 patients [76, 86].

While circulating levels of TGF-β were reduced in HHT-1 patients compared to control, this was not found in HHT-2 patients, suggesting that reduced endoglin levels on HHT-1 ECs might lead to reduced TGF-β1 secretion [7]. Since TGF-β is important for SMC differentiation, and recruitment of SMCs to new vessels as well as their growth requires TGF-β, this reduced TGF-β secretion might explain the formation of fragile and leaky vessels in HHT-1 patients. Multiple clinical trials in HHT patients are ongoing with anti-angiogenesis agents, including bevacizumab, a neutralizing antibody

against VEGF, thalidomide and interferon α-2b to inhibit bleedings and other vascular malformations associated with HHT (http://www.hht.org/).

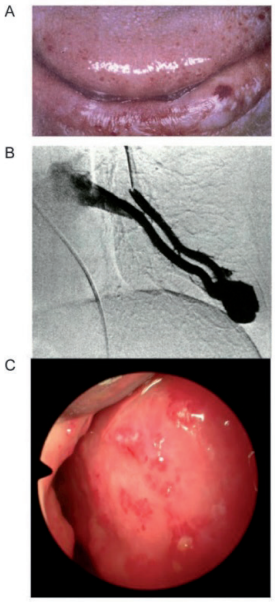

Fig. 3 *Clinical symptoms of hereditary hemorrhagic telangi- ectasia (HHT) include (A) bleedings in tongue and lower lip, (B) arteriovenous malformations (pulmonary angiogram is shown), and (C) nasal telangiectases (courtesy of Dr U Geisthoff). Panels A and B were reproduced from [117] with permission.*

Marfan syndrome and Loeys-Dietz syndrome (MFS and LDS)

Marfan syndrome (MFS) is a genetic connective tissue disorder caused by mutations in the fibrillin gene [87]. Typical MFS can affect the skeletal system, ocular system, cardiovascular system, pulmonary system as well as other organs, with defects in aorta and heart valves giving the most severe complications. Fibrillin contains several motifs homologous to latent TGF-β binding protein (LTBP), and has been shown to interact with LTBP and control TGF-β bioavailability [4]. TGF-β is bound and kept inactive by the LTBP-fibrillin complex. In fibrillin-1-deficent mice, a model of MFS, fibrillin-1 deficiency was found to diminish the sequestration of latent TGF-β to the extracellular matrix, thereby leading to increased TGF-β activation [88]. Marfan syndrome type 2 is caused by mutation in the TβRII gene locus. Three prominent features of Marfan's syndrome in humans, i.e. dilatation of the aortic root, air-space widening and skeletal myopathy, can be prevented and even reversed in the mouse model by treatment with losartan, which blocks the angiotensin II type 1 receptor and antagonizes TGF-β signalling [89, 90].

The Loeys-Dietz syndrome is a recently described autosomal dominant aortic-aneurysm syndrome presenting with cardiovascular and skeletal manifestations consistent with those seen in MFS, along with other features not present in MFS. Typical Loeys-Dietz syndrome is characterized by a mutation in either TβRI or TβRII [91]. The molecular mechanism of Loeys-Dietz syndrome is complex and poorly understood; although mutations in TβR are inactivating its function, they lead paradoxically to overactive TGF-β signalling, as measured by accumulation of phosphorylated

Smad2 in the nucleus and expression levels of connective-tissue growth factor [92]. Thus TGF-β antagonists may also alleviate manifestations of Loeys-Dietz syndrome. In a recent clinical study with 18 MFS patients, losartan decreased the rate of progressive aortic-root dilation [93] (http://www.marfan.org/ nmf/index.jsp).

Pre-eclampsia

Pre-eclampsia, which involves a raise in blood pressure, is a major cause of maternal, fetal, and neonatal mortality. The clinical manifestations of pre-eclampsia reflect widespread EC dysfunction, resulting in vasoconstriction, endo-organ ischemia and increased vascular permeability [94]. Soluble endoglin (solEng), a placenta- derived 65 kD soluble form of endoglin and soluble fms-like tyrosine kinase 1 (sFlt1, an inhibitor of VEGF) are both considered to be clinical indicators for pre-eclampsia [95]. Just before the onset of pre-eclampsia circulating levels of solEng are found to be markedly el- evated and it cooperates with sFlt in the pathogenesis of pre-eclampsia in rats [96]. SolEng was found to inhibit TGF-β-mediated activation of eNOS, thereby affecting vascular tone. SolEng disrupts EC function, inhibiting EC tube formation and enhancing capillary permeability. SolEng is formed by proteolytic cleavage, but the protease involved remains to be identified. Upon identification of this protease involved in endoglin shedding, it will be interesting to explore whether its targeting could be beneficial for treatment of pre-eclampsia. Circulating solEng levels may be a useful diagnostic marker in pre-eclampsia [4].

Pulmonary arterial hypertension

Pulmonary arterial hypertension (PAH) is a disease caused by constriction of small pulmonary arteries leading to an increase in blood pressure (Figure 4). Aberrant proliferation and dysfunction of ECs and SMCs contribute to vascular remodeling [97, 98]. Heterozygous germ line mutations in the BMP receptor II gene (BMPRII) have been found in more than 80% of familial PAH patients. In addition, about 20% of patients with idiopathic PAH were reported to have mutations in BMPRII [99, 100]. A recent study has implicated mutations of ALK1 in children with PAH [101]. Over-expression of a mutant form of BMPRII in SMCs of transgenic mice causes an increase in pulmonary arterial pressure and pulmonary arterial muscularization, resembling some manifestations of PAH patients [102]. Mutations in BMPRII also can deregulate Id gene expression [103]. BMPRII signalling was found to be essential for BMP-mediated regulation of vascular SMC growth and differentiation [104]. Clinical trials with prostanoids, endothelin antagonists and phosphodiesterase inhibitors have shown promising results in the treatment of PAH. It will be interesting to examine the interplay between the targets of these therapeutics and deregulated BMPRII signalling in PAH [105].

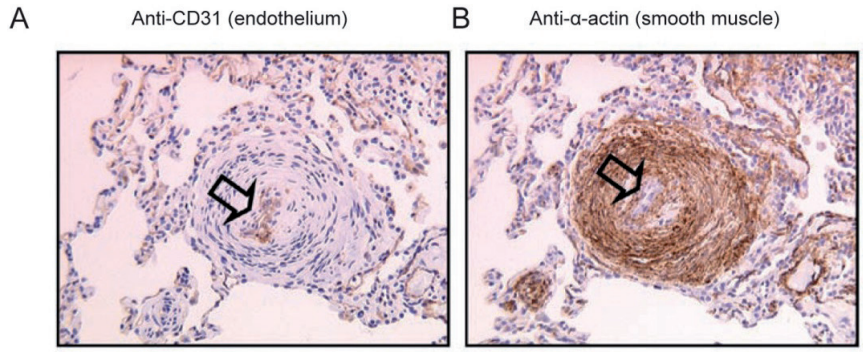

Fig.4 *Photomicrographs of lung sections from patients with primary pulmonary hypertension (PPH). The occlusion of small pulmonary arteries that is typical of PPH is shown. Stainings of lung sections from PPH patients are shown using anti- bodies against (A) CD31 (also known as platelet endothelial cell adhesion molecule-1), an endothelium-specific marker (arrow is pointing to single layer of ECs); and (B) SMC α-actin (arrow is pointing to concentric layers of SMCs). Figure was repro- duced from [97], with permission from Dr N Morrell and Lippincott, Williams and Wilkins.*

Cardiac remodeling and hypertrophy

Cardiac remodeling describes the alteration in size, shape and function of the left ventricle in response to changes in hemodynamic loading conditions, neurohormonal activation, or induction of local mediators that alter the structural characteristics of the myocardium. TGF-β is a crucial regulator of cardiac remodeling through its direct and potent actions in mediating cardiomyocyte growth, fibroblast activation and extracellular matrix deposition [106].

TGF-β levels are markedly induced in the hypertrophic myocardium. Angiotensin II via the angiotensin type 1 receptor promotes cardiomyocyte growth and stimulates fibroblast proliferation and expression of extracellular matrix components [107, 108]. Angiotensin, a potent vasoconstrictor, stimulates TGF-β1 mRNA and protein expression and may through its induction of thrombospondin lead to TGF-β activation [109], indicating that TGF-β1 acts downstream of angiotensin signalling [107]. In addition, angiotensin has been shown to directly lead to Smad3 activation via a mechanism that is not well understood [110]. The renin-angiotensin system (RAS) plays an important role in cardiac remodeling and clinical trials have documented the beneficial effects of angiotensin II inhibition in patients with myocardial infarction and heart failure [111]. Indeed, losartan has been approved for the reduction of cardiovascular events in patients with hypertension and left ventricular hypertrophy [112]. Furthermore it has been reported that TGF-β stimulation alters the program of differentiation-related gene expression in isolated cardiac myocytes, promoting the synthesis of fetal contractile proteins, characteristic of pressure-overload hypertrophy [113]. Over-expression of TGF-β in transgenic mice results in cardiac hypertrophy which is characterized by both interstitial fibrosis and hypertrophic growth of cardiac

myocytes [114]. The local production of TGF-β in hypertrophic myocardium and the link between the RAS system and TGF-β strongly implicate the role of TGF-β in hypertrophic response. All these results demonstrate the importance of TGF-β in mediating hypertrophic cardiac remodeling, however, limited knowledge is available on the signalling pathways responsible for TGF-β action in hypertrophy.

Concluding remarks

The pivotal importance of TGF-β family members in angiogenesis is underscored by the observations that nearly all knock-out mice for specific TGF-β family signalling components die during midgestation due to a yolk sac angiogenesis defect and that mutations in the genes encoding TGF-β pathway components are linked to an increasing number of human pathologies with vascular dysfunction. In concordance with these findings *in vitro* studies with different TGF-β family members have revealed potent effects on the function of ECs and vascular SMCs, affecting e.g., proliferation, differentiation, migration and extracellular matrix production. However, results obtained by different laboratories are sometimes in apparent conflict, but this can largely be attributed to context-dependent functions of TGF-β family members. Ligand concentration, cell density, cell type, media and matrix coating may determine specific cellular responses to TGF-β family members. TGF-β also plays an important role in the interplay between EdCs and vascular SMCs. Recent data indicate that TGF-β is capable of mediating EndoMT, a transdifferentiation of ECs into SMC- like cells [115].

With misregulation of TGF-β signalling at the heart of cardiovascular disorders, the cognate signalling components represent interesting targets for therapy. The first large-scale clinical trials with losartan to treat MFS are underway and results are eagerly awaited. Results from one vascular disorder may open opportunities for development of therapies in other vascular pathologies. Whereas high levels of circulating solEng may lead to pre-eclampsia by causing an EC dysfunction in multiple organs, solEng may be explored for anti-angiogenic activity in curbing tumor angiogenesis.

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