[Frontiers in Bioscience 14, 4848-4861, June 1, 2009]

Transforming growth factor-beta signaling and tumor angiogenesis

Evangelia Pardali, Peter ten Dijke

Department of Molecular Cell Biology, Leiden University Medical Center, Leiden, The Netherlands

TABLE OF CONTENTS

1. ABSTRACT

Transforming growth factor-beta (TGF-beta) family members are secreted multifunctional cytokines that play pivotal roles in development and disease. The prototypic member of this family, TGF-beta, plays a dual role in carcinogenesis, acting as a tumor suppressor in early stages and as tumor promoter in late stages of tumor progression. Numerous studies support the notion that pathological angiogenesis is one of the hallmarks of cancer. Tumor angiogenesis is regulated by a network of growth factors, including members of the TGF-beta family. TGF-beta acts in a context-dependent manner and can either stimulate or inhibit tumor angiogenesis. In this review, we discuss our current understanding on how TGF-beta family members affect endothelial and smooth muscle cell function and how perturbed TGF-beta signaling may contribute to tumor angiogenesis and tumor progression.

2. INTRODUCTION

Survival and maintenance of mammalian tissues is dependent upon adequate supply of oxygen and nutrients and the removal of toxins by blood vessels. The cardiovascular system circulates blood around the body and it is the first functional organ system to develop in mammals. Its maintenance is critical for tissue survival and proper function (1).

During embryonic development, blood vessels develop *de novo* from endothelial precursors, called angioblasts, which differentiate into a primitive capillary network. This process is referred to as vasculogenesis (2-4). The primitive capillary network is then remodeled by a process termed angiogenesis; new blood vessels sprout and branch from the pre-existing capillaries in order to develop the vascular system (5). The latter is a complex series of

events that includes: i) an initiation/activation phase, characterized by destabilization of the vessel and increased permeability, degradation of the extra cellular matrix (ECM) by proteolytic enzymes, endothelial cell (EC) proliferation and migration and ii) a resolution phase, during which endothelial cells stop proliferating and pericytes and vascular smooth muscle cells (VSMCs) are recruited to ensure stabilization, remodeling and maturation of the newly formed vessels (6). Progenitor ECs and mesenchymal cells may participate in this process by incorporating into the neovessels or by secreting factors that will regulate angiogenesis (7). The vasculature in the adult is mainly quiescent, but angiogenesis does take place in adult life to maintain physiological homeostasis and tissue integrity after wound healing, inflammation, ischemia and during the female menstrual cycle (8).

Angiogenesis is tightly regulated by a balance between pro- and anti-angiogenic signals. An alteration of this equilibrium can promote deregulated vessel growth and may result in different pathologies, including arthritis, diabetes, psoriasis and juvenile hemangiomas (5,8). Importantly, angiogenesis is needed for tumors to grow beyond a few cubic millimeters (9). In addition, the new intra-tumoral blood vessels provide a way for tumor cells to enter the circulation and to metastasize to distant organs (10).

Genetic studies in man and mice have provided evidence for the important role of TGF-beta family members and their signaling components in vasculogenesis and (tumor) angiogenesis. TGF-beta family members and their receptors are expressed by both endothelial cells and mural cells (such as pericytes and VSMCs), and depending on ligand concentration and cellular context they can elicit different, even opposite effects (11). Cancer cells often secrete high amounts of TGF-beta that can act directly on vascular cells and regulate their activity. In addition, TGF-beta can induce an influx of inflammatory cells that secrete inflammatory cytokines thereby indirectly regulating angiogenesis (12). Interestingly TGF-beta can also have direct effects on tumor cells. At the early stages of tumor progression, it acts as a tumor suppressor (growth inhibition and pro-apoptosis) and at late stages acts as a tumor promoter. Moreover, TGF-beta by inducing epithelial to mesenchymal differentiation (EMT) and invasion (13) of epithelial cancer cells and by suppressing anti-tumor immune responses (14) further promotes tumor progression. Clearly, TGF-beta has a multifaceted role in tumor progression.

3. TGF-BETA SIGNALING PATHWAYS

Members of the TGF-beta family, which includes TGF-betas, bone morphogenetic proteins (BMPs) and activins, are secreted pleiotropic dimeric proteins (15). They are involved in a number of patho-physiological events, such as embryonic development and angiogenesis, immune surveillance, wound healing, malignant transformation and tumor angiogenesis. TGF-beta isoforms (TGF-beta 1, -2 and –3) are produced as inactive latent forms that need to be activated before binding can occur to the TGF-beta signaling receptors that are located within the plasma membrane (11). Proteases, integrins and components of the extracellular matrix play an important role in this activation process (11). BMPs appear to be secreted in an active form; their availability is regulated by many soluble extracellular antagonists such as noggin, chordin and DAN (16).

TGF-beta family members signal through heteromeric complexes of type I (also known as activin receptor-like kinase, ALK) and type II transmembrane serine/threonine kinase receptors (17,18). Several type I and II receptors have been identified that have different affinities for individual members of the TGF-beta family. TGF-betas signal via a type II receptor (TbRII) and a type I receptor (TbRI, also known as ALK5) (19). In most cell types ALK5 is the predominant type I receptor mediating responses to TGF-beta but there is evidence that suggests that ALK1 and ALK2 can also form complexes with TbRII and transduce TGF-beta signals (20). BMP ligands bind to BMP and activin type II receptors (BMPRII and ActRII or ActRIIB) in combination with the type I receptors ALK1, -2, -3 or -6. In addition to these signaling receptors, co-receptors have been identified that modulate signaling by presenting or sequestering ligand to the type I and type II receptors (17). Betaglycan and Endoglin are transmembrane co-receptors with short intracellular domains that lack enzymatic motifs. Betaglycan binds all three TGF-beta isoforms and facilitates their binding, in particular to TGF-beta2, to the signaling receptors (21). Endoglin expression enhances BMP and TGF-beta /ALK1 signaling, while it has inhibitory effects on TGF-beta /ALK5 signaling (22,23). Soluble Betaglycan and Endoglin have both been shown to interfere with the TGF-beta signaling pathway (24,25).

Upon ligand binding to the heteromeric receptor complex, the type II receptor, which is constitutively active, phosphorylates the type I receptor (26). Once activated, the type I receptor propagates the signal into the cell by phosphorylating the receptor-regulated (R-)Smads (i.e. Smad1, Smad2, Smad3, Smad5 and Smad8) at two carboxy terminal serine residues. Whereas TGF-beta and activin predominantly induce Smad2 and Smad3 phosphorylation, BMPs stimulate Smad1, Smad5 or Smad8 phosphorylation (27,28). Phosphorylated R-Smads form an heteromeric complexe with Smad4 (Co-Smad) (29), and translocate to the nucleus, where in cooperation with cell-type-specific transcriptional activators, co-activators and co-repressors, they regulate the transcription of specific target genes (30) (Figure 1). Inhibitory (I)-Smads, Smad6 and Smad7 interfere with TGF-beta signaling by competing with R-Smads for receptor interaction (31). Moreover, I-Smads may recruit phosphatases and ubiquitin ligases to the activated receptors thereby inactivating the receptor by promoting their dephosphorylation and degradation, respectively (32,33). In addition to the Smad signaling TGF-beta and BMP signaling can result in activation or inhibition of the TAK1, Erk, Jnk, MAPK, PI3K and other non-Smad signaling pathways (30) (Figure 1). Consequently, TGF-beta and BMP effects are the result of an extensive interplay between Smad and non-Smad signaling pathways.

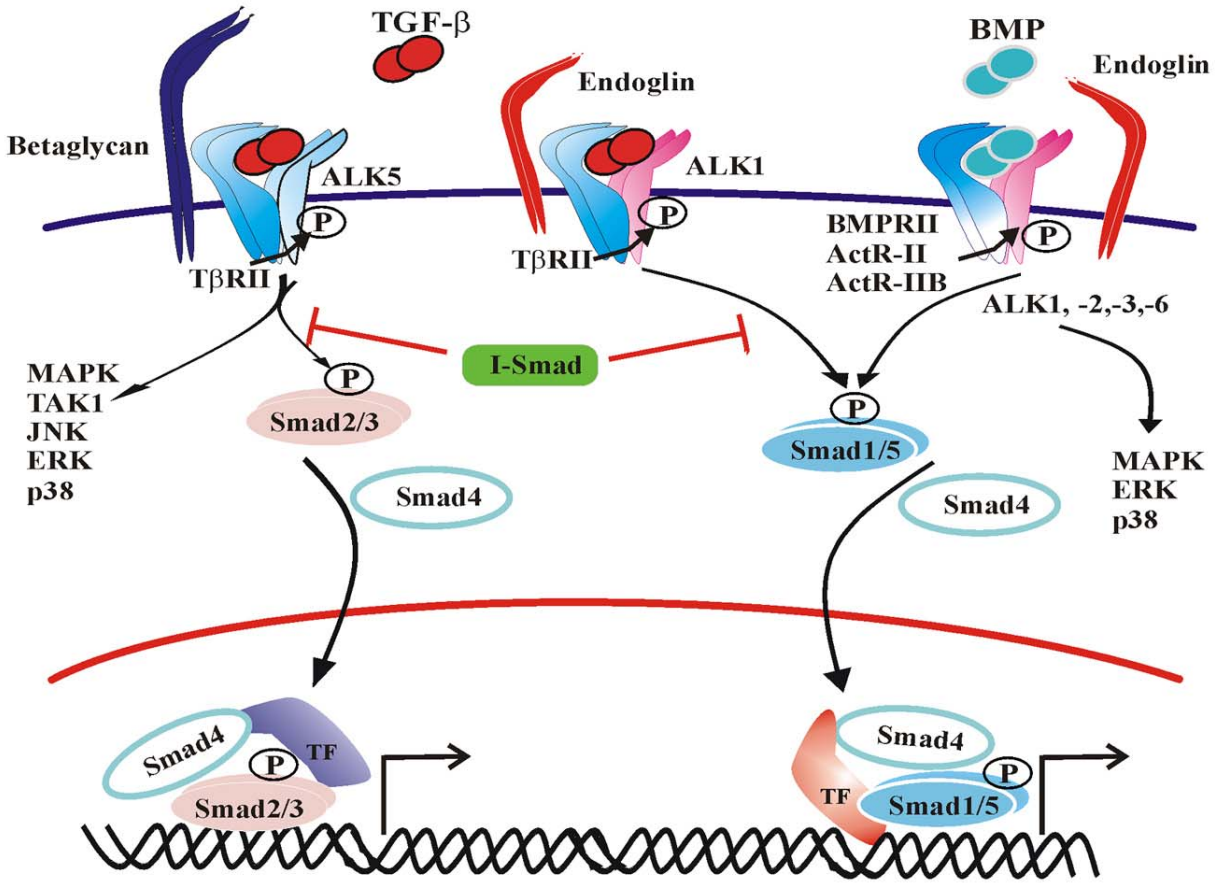

Figure 1. Overview of the TGF-beta and BMP signaling pathways. TGF-beta and BMP homodimers induce heteromeric complex formation between specific type II and type I (ALK) receptors (i.e. ALK1, 2, 3, 5 and 6). The type II receptors then transphosphorylates the type I receptors on specific serine and threonine residues in the juxtamembrane domain, leading to type I receptor activation. Subsequently, the type I receptor propagates the signal into the cell by phosphorylating receptor regulated (R)-Smads, which can form heteromeric complexes with Smad4. Upon nuclear accumulation, they can contact promoter/enhancer DNA elements in target genes directly and indirectly by interacting with other transcription factors (TF) and regulate gene transcriptional responses in collaboration with transcriptional activators or repressors. In addition to the Smad-dependent pathways, non-Smad pathways can be activated by TGF-beta.

4. TGF-BETA AND VASCULAR DEVELOPMENT

TGF-beta exerts diverse functional effects on ECs. At low concentrations, TGF-beta promotes EC proliferation and migration (34-38), while these responses are inhibited at high concentrations (39-41). Recent studies have demonstrated that, in mouse ECs, TGF-beta signals through two distinct type receptor pathways, i.e. the ALK5 and the ALK1-dependent pathway, resulting in activation of Smad2/3 and Smad1/5/8, respectively (42,43). Transcriptional profiling studies in ECs have shown that ALK1 regulates expression of genes involved in EC proliferation and migration, while ALK5 regulates genes involved in cell-cell interactions, cell adhesion and extracellular matrix remodeling (42,44,45). Moreover, TGF-beta/ALK1/Smad1 signaling was found to antagonize ALK5/Smad2/3 signaling by attenuating Smad transcriptional responses (43). Interestingly, ALK5 deficient mouse EC show defects both in TGF-beta/ALK5 responses and TGF-beta/ALK1 responses suggesting that ALK5 is essential for ALK1 recruitment into the TGF-beta receptor complex and its activation (42). In contrast to the above described studies, it was shown that ALK1 overexpression could inhibit proliferation and migration of a human microvascular EC line (46). Those results are consistent with the phenotype observed in ALK1 deficient mice which have fragile blood vessels (47). In the same study, it was shown that ALK1 knockout embryos exhibited increased mRNA levels for genes involved in the activation phase of angiogenesis, suggesting that ALK1 promotes the resolution phase of angiogenesis (47). Those discrepancies may be due to the different cell types used in the studies or to the adaptive processes that take place in the ALK1 deficient embryos. Interestingly, a new member of the TGF-beta superfamily BMP9 was show shown to bind with high affinity to ALK1 on ECs (48,49). BMP9, as well as BMP10, display inhibitory effects on EC proliferation and migration (22,49). This could suggest that

ALK1 is involved in both the activation and in the resolution phase of angiogenesis. In this case the type of ligand (TGF-beta or BMP9/10) and the receptor complexes that are activated, results in activation of different Smad and non-Smad transcriptional activations that will determine the fate of the ECs.

Endoglin is predominantly expressed in activated highly proliferating ECs. Both TGF-betas and BMPs, and also hypoxia have been shown to potently induce Endoglin expression (49,50). Ectopic expression of Endoglin was shown to inhibit TGF-beta/ALK5-induced anti-proliferative response in several cell systems (51,52). Abrogation of Endoglin expression in ECs results in inhibition of proliferation and migration (23). It was also shown that on ECs, Endoglin potentiates the TGF-beta/ALK1 pathway and in this way it inhibits indirectly the TGF-beta/ALK5 signaling. Interestingly, it was shown that mouse embryonic ECs from Endoglin heterozygotes mice had not only impaired ALK1, but also impaired ALK5 signaling as a result of downregulated ALK5 expression levels (53). Consistent with this finding, blood outgrowth endothelial cells (BOECs) from hereditary hemorrhagic telangiectasia (HHT) patients with Endoglin mutations (see below) were found to have impaired TGF-beta/ALK1 and TGF-beta/ALK5 signaling due to downregulation of ALK5 expression (54). Endoglin was also shown to play important role in BMP9/ALK1 induced transcriptional responses on ECs (22). Taken together those results suggest that Endoglin may regulate EC function by balancing TGF-beta and BMP responses.

Pericytes and VSMCs play important roles in blood vessel formation and stability. Several studies have highlighted the critical importance of pericytes and VSMCs in vascular homeostasis, vascular disorders and tumor angiogenesis. Furthermore it is evident that intercellular communication between ECs and pericytes and VSMCs also play an important role in vascular stability. Collectively, this suggests that pericytes and VSMCs may be potential therapeutic targets (55,56). Several factors including TGF-betas and BMPs have been shown to regulate mural cell migration, proliferation and differentiation. Also genetic studies in mice have demonstrated the importance of the TGF-beta signaling for *de novo* formation of VSMCs (see below). TGF-beta potentiates vascular smooth muscle differentiation by increasing the expression of alpha-smooth muscle actin and smooth muscle myosin (57,58). Moreover, TGF-beta promotes proliferation of VSMCs at low concentrations, whereas, at high concentrations inhibits VSMCs proliferation and migration (59-61). TGF-beta effects on VSMCs are mediated by the ALK5 pathway and they involve Smad2/3 as well as activation of p38 mitogen activated protein kinase (MAPK), Erk and JNK signaling cascades (58,62).

5. BMP AND VASCULAR DEVELOPMENT

Several studies have investigated the role of the BMP signaling pathway on EC function. BMP2, 4, and 6 have been shown to stimulate EC proliferation, migration and capillary tube formation *in vitro* (63-66). In line with those results, it was shown that BMP treatment of bone explants could stimulate angiogenesis (67). This effect could be inhibited by soluble BMP receptor 1A and the BMP2, 4 and 7 inhibitor, noggin. Moreover, addition of a vascular endothelial growth factor-A (VEGF-A) neutralising antibody, inhibited both basal and BMP stimulated angiogenesis, suggesting that (endogenous) BMPs may induce angiogenesis through VEGF (67). BMP2 and 4 pro-angiogenic effects are mediated not only by the Smad1/5/8 pathway, but also by activation of the ERK pathway (64,68). Interestingly, BMP4 was shown to induce expression and phosphorylation of VEGFR2 and Tie2 proteins in mouse stem cell derived endothelial cells and human microvascular endothelial cells (69). Those results suggest that BMP4 may exert its pro-angiogenic effects by activating also the VEGF/VEGFR2 and Angiopoietin-1/Tie2 signaling cascades (69). The direct pro-angiogenic effects of BMP6 have been shown to be mediated by the upregulation of transcription factor inhibitor of differentiation 1 (Id1) expression in ECs (66). Independent studies have identified, in addition to Id1, cycloeoxygenase 2 (Cox2) as an essential downstream component mediating BMP6-dependent EC activation (70). BMP9 and BMP10 were shown to bind to ALK1 and potently inhibit EC migration and growth (22,49). Surprisingly, Drm/gremlin, a BMP2, BMP4 and BMP7 antagonist (71,72), was shown also to potentiate angiogenesis by inducing proliferation, migration and sprouting of ECs *in vivo* and *in vitro* (73). BMP4 did not influence the pro-angiogenic effects of Drm/gremlin, suggesting that Drm/gremlin may interact directly with the ECs and in this way promote angiogenesis. Indeed it has been demonstrated that Drm/gremlin can bind directly to a cell-membrane protein complex on different ECs types and to induce phosphorylation and activation of focal adhesion kinase (FAK) and mitogen activated protein kinase ERK. Interestingly, addition of recombinant BMP4 could not prevent binding of Drm/gremlin to the ECs (73). Further studies are awaited to unravel the role of Drm/gremlin in angiogenesis.

6. ROLE OF TGF-BETA SIGNALING IN MAINTENANCE OF VASCULAR INTEGRITY

TGF-beta and BMPs as well as many of their signaling components, are expressed in the embryonic and adult vasculature. Genetic studies in mice and human have shown that disruption of TGF-beta and BMP signaling pathways results in defects in vasculogenesis, and angiogenesis (74). TGF-beta1 deficient mice have fragile vessels and die due to defective yolk sac vasculogenesis (75,76). TbRII deficient mice are highly reminiscent of TGF-beta null mice (77); they die at midgestation and exhibit severe deficiencies in yolk sac hematopoiesis and vasculogenesis with defects in angiogenesis. Mice deficient in TbRI expression die also at midgestation due to severe defects in vascular development of yolk sac and placenta (78). A recent report suggested that ALK5 is not expressed in ECs but only on VSMCs (79). However, analysis of mice with ECs or smooth muscle cell specific deletion of either TbRII or TbRI has clearly shown that both receptors

play important roles in ECs function as well as in VSMCs (80,81).

The defective vascular phenotype described in TGF-b1 and TbRII knockout mice is highly reminiscent of the vascular lesions described in patients with hereditary hemorrhagic telangiectasia (HHT), an autosomal dominant vascular disorder. There are two major types of the disease, HHT1 and HHT2 that are linked to mutations in the Endoglin and ALK1 genes, respectively (82,83). Both Endoglin and ALK1 are expressed in the vascular endothelium. Endoglin deficient mice are embryonic lethal due to defective heart and vascular development probably due to impaired differentiation and recruitment of VSMCs (84-86). ALK1 knockout mice also die during embryogenesis and exhibit defective vascular remodeling and dilated vessels and defective differentiation and recruitment of VSMCs (47,87). Moreover, the vascular defects seen in Endoglin deficient mice and the endothelium-specific knockouts of TbRII and TbRI are to a certain extent due to reduced levels of TGF-beta expression and defective TGF-beta/ALK5 signaling in adjacent mesenchymal cells (80). As a result, mesenchymal cell differentiation into VSMCs and their association with the endothelial tubes are impaired. Those results suggested that TGF-beta signaling in EC promotes TGF-beta expression, synthesis, and release by these cells which in turn, induces differentiation of VSMCs from surrounding mesenchymal cells, but also sustains TGF-beta expression in ECs through an autoregulatory loop. Smad4 mutations have also been implicated in HHT, since a subset of patients with juvenile polyposis has been shown to develop vascular malformations and frequent nosebleeds (88). Smad5 deficient mice display defects in vasculogenesis with enlarged blood vessels and decreased numbers of VSMCs (89,90). Another vascular disorder that has been associated with BMP signaling components is primary pulmonary hypertension (PPH), which was linked to mutations in BMPRII (91). This disease is characterized by uncontrolled remodeling of the pulmonary arteries due to increased proliferation of smooth muscle cells and increased pulmonary EC apoptosis (92,93). Interestingly, certain HHT2 patients develop PPH-like syndromes, suggesting that ALK1 mutations may also be involved in PPH (94,95).

7. TUMOR ANGIOGENESIS

Several studies revealed that tumors cannot grow more than 1-2mm^3 if the supply of oxygen and nutrients is limited. Many tumors as well as metastatic lesions may be dormant for years. Several mechanisms can explain tumor dormancy such as cancer cell cycle arrest, balancing cancer cell proliferation by apoptosis, an active immune system or limitations in blood supply. Tumor angiogenesis is essential to escape this period of dormancy. This process, also known as the angiogenic switch, is regulated by a variety of pro- and anti-angiogenic factors (10). It is now evident that cancer is not a single cell disease and that tumor progression depends on the signals from the tumor cells as well as the surrounding stroma. Several factors have been shown to promote tumor angiogenesis and tumor growth, such as VEGF, basic fibroblast growth factor (bFGF), platelet derived growth factor (PDGF), placental growth factor (PlGF), as well as TGF-betas and BMPs.

7.1. TGF-beta and tumor angiogenesis

TGF-beta expression is upregulated in many cancers, such as breast and pancreatic cancer, and it correlates with poor prognosis (96,97). Moreover, over-expression of TGF-beta1 in Chinese hamster ovary cells and human prostate cancer cells significantly stimulates tumor growth and angiogenesis upon injection into mice (98-100). Prostate carcinoma cells overexpressing TGF-beta showed enhanced tumor angiogenesis in tumor xenografts, while local administration of neutralizing antibodies to TGF-beta1 strongly reduced tumor angiogenesis (101). Moreover, intraperitoneal injection of TGF-beta antibodies reduced angiogenesis and tumorigenicity of a renal carcinoma cell line in nude/athymic mice (102).

Inhibition of the TGF-beta signaling was shown in several *in vivo* models to inhibit tumor growth and tumor angiogenesis. Ectopic expression of soluble TbRIII (sRIII) significantly inhibited the growth of tumors formed by human colon carcinoma HCT116 and breast carcinoma MDA-MB-435 cells in nude mice, in part due to the inhibition of angiogenesis. It also reduced the metastatic potential of the MDA-MB-435 cells. Treatment of human EC with recombinant sRIII significantly inhibited their ability to form a capillary structures on matrigel (103-105). Moreover, administration of sRIII into MDA-MB-231 xenograft-bearing athymic nude mice significantly inhibited the tumor growth by inhibiting angiogenesis (106). Growth of three-way differential reactive stroma tumors was inhibited by administration of TGF-beta1 latency-associated peptide (LAP) or TGF-beta1 neutralizing antibody due to inhibition of angiogenesis (107). SB-431542, a TbRI/ALK5 kinase inhibitor, was shown to inhibit the tumor-promoting effects of TGF-beta, including TGF-beta-induced EMT, cell motility, migration and invasion, and VEGF secretion in human cancer cell lines (108-110).

The mechanism by which TGF-beta induces tumor angiogenesis is not fully understood, but presumably is the product of direct and indirect effects. TGF-beta can activate EC proliferation and migration, it can induce capillary formation of EC when they are cultured on a collagen matrix and promote angiogenesis *in vivo* in the chicken chorioallantoic membrane assay (38). TGF-beta can induce expression of VEGF in various cells in the tumor microenviroment such as tumor cells, stromal fibroblasts and cells of the immune system (macrophages) (111) (Figure 2). VEGF expression in response to TGF-beta is enhanced by hypoxia (50). TGF-beta was shown to potentiate tumor angiogenesis by inducing VEGF expression in a Ras-transformed mouse mammary carcinoma model (112). In addition, TGF-beta can recruit inflammatory cells that produce angiogenic signals (113). Moreover, TGF-beta can induce the expression of various extracellular matrix components such as matrix metalloproteinases (MMPs), collagen, urokinase plasminogen activator and integrins (114-116) (Figure 2).

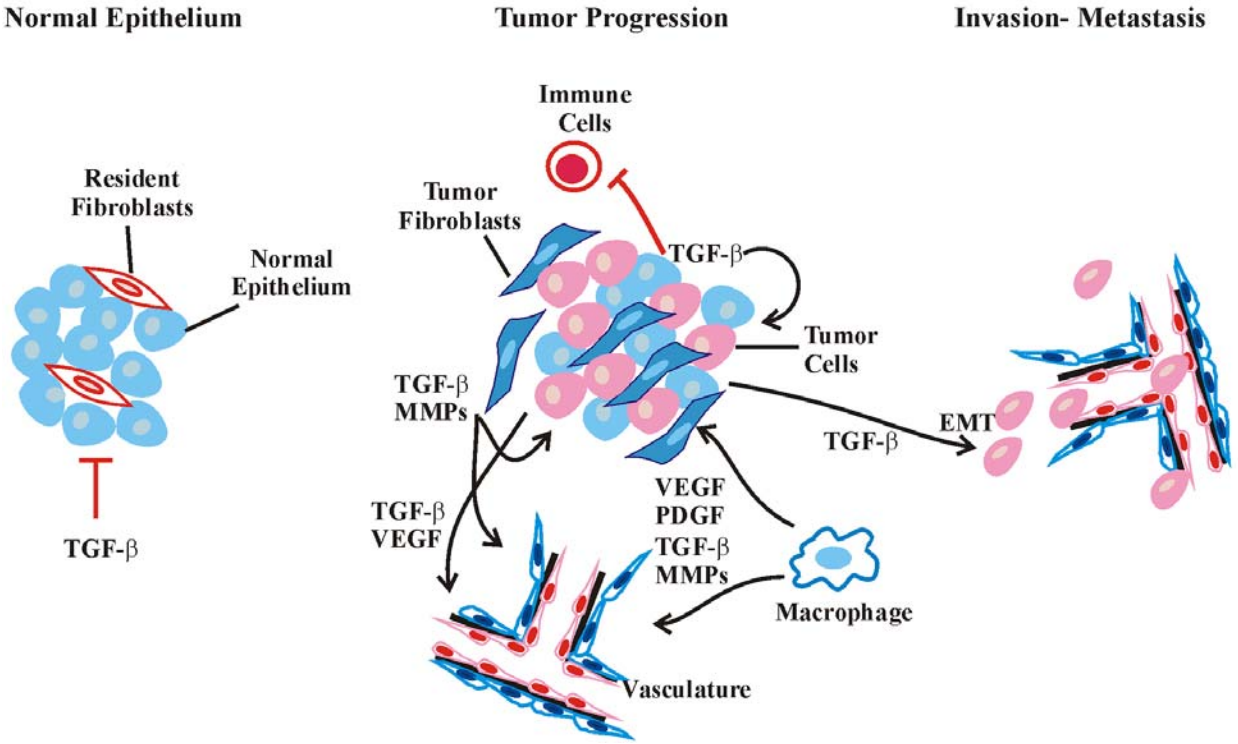

Figure 2. Working model on the role of TGF-beta in tumor angiogenesis.Normal epithelial cells are sensitive to TGF-beta - mediated growth inhibition, however oncogenic initiation results in an increase in TGF-beta expression within the tumor micro-environment and subsequent loss of TGF-beta inhibitory growth control. The tumor-promoting effects of TGF-beta are mediated by regulation of tumor growth, angiogenesis and immune system. TGF-beta can induce angiogenesis directly by acting on the vasculature and indirectly by inducing expression of pro-angiogenic factors (VEGF, PDGF) and extracellular matrix components (MMPs). Finally, TGF-beta can induce epithelial to mesenchymal transition (EMT) of the tumour cells and production of MMPs, which contribute to extracellular matrix degradation, resulting in migration, invasion and tumor metastasis.

Extracellular matrix components have been found to play important role in both the initiation and resolution phase of angiogenesis as well as in the regulation of tumor angiogenesis (117). In addition degradation of ECM components by MMPs may release or activate certain angiogenic growth factors and in this way promote angiogenesis.

In contrast to the above described studies in other models TGF-beta was shown to inhibit tumor angiogenesis. Blocking endogenous TGF-beta signaling by over-expression of sTbRII in a mouse hepatoma cell line MH129F, resulted in enhanced tumor formation and increased angiogenesis *in vivo*. MH129F/TRIIs tumors showed increased expression of VEGF, KDR/Flk-1 receptor (VEGFR2) and CD31 staining. Those results suggest that endogenous TGF-beta can act as a negative regulator of the VEGF/Flk-1-mediated angiogenesis pathway in hepatoma progression (118). In another study it was demonstrated that expression of the type-1 repeats of thrombospondin (TSP)-1 in the human squamous cell carcinoma cell line A431 inhibits tumor growth and angiogenesis through activation of endogenous TGF-beta (119). In addition, studies in the primary gallbladder wall tumor microenvironment revealed that elevated TGF-beta1 levels inhibited tumor growth and angiogenesis (120). High levels of TGF-beta1 (i.e. activation of ALK5 pathway) may inhibit the processes of angiogenesis by maintaining the microvasculature in a quiescent state. It was also suggested that TGF-beta1 may suppress angiogenesis by down-regulating cell adhesion molecules and in this way inhibiting rolling and extravasation of pro-angiogenic leukocytes (120).

7.3. Role of Smads in tumor angiogenesis

Several studies tried to elucidate the role of R-Smads in tumor angiogenesis. In NRK52E proximal tubular cells it was shown that TGF-beta/Smad2 pathway induces production of the antiangiogenic factors TSP-1 and soluble Flt-1 (VEGFR1), while TGF-beta/Smad3 pathway induces VEGF expression (121). Consistent with those results, conditioned media from Smad2 knockout cells, but not Smad3 knockout cells, stimulated endothelial proliferation. However, in another study it was shown that introduction of Smad3 into human gastric cancer cells, which did not express Smad3, restored TGF-beta responsiveness and decreased their tumorogenicity and tumor angiogenesis (122). This was due to the inhibitory effects of Smad3 on VEGF and CD31 expression. Smad4 was also shown to affect expression of pro- and anti-angiogenic factors.

Ectopic expression of Smad4 in pancreatic carcinoma cells suppressed tumor growth *in vivo* by suppressing tumor angiogenesis (123). Smad4 restoration resulted in decreased expression levels of VEGF and increased the expression of TSP-1 and reduced vascular density (123). Thus, Smad intracellular signaling can influence angiogenesis by altering the balance between pro-(VEGF) and anti-(TSP-1, sFlt-1) angiogenic factors in a cell dependent manner.

7.3. Role of Endoglin in tumor angiogenesis

Endoglin is predominantly expressed on activated ECs. Several studies provided evidence for the role of Endoglin in tumor angiogenesis and tumor progression. Endoglin expression is upregulated on the ECs of tumors compared to the ECs from normal adjusted tissues and Endoglin upregulation correlates with poor prognosis (124-127). Moreover, tumor vascularization and tumor growth are decreased in Endoglin heterozygotes mice (128). Those defects were associated with lower levels of endothelial nitric oxide synthase (eNOS) in the tumors of Endoglin heterozygotes compared with those of Endoglin wild type mice (128). Systemic administration of Endoglin antibodies conjugated with immunotoxins and immuno-radioisotopes can target ECs and selectively inhibit tumor angiogenesis and carcinoma development without affecting the vasculature in the normal tissues (129-132). The above studies suggest that Endoglin may be a powerful diagnostic and prognostic marker and a vascular target in therapeutic anti-angiogenic approaches in cancer.

7.4. BMP and tumor angiogenesis

BMPs have also been reported to enhance tumor angiogenesis. Recombinant BMP2 could enhance angiogenesis in Matrigel plugs containing A549 cells in nude mice (133). The BMP2, 4 and 7 antagonist noggin abrogated BMP2 induced angiogenic response (64). In a murine breast cancer xenograft model, human MCF-7 cells expressing BMP2 developed vascularized tumors while the parental MCF-7 cells failed to form tumors, suggesting that BMP2 can promote tumor angiogenesis *in vivo* (65). Tumors derived from melanoma cells with impaired BMP4 expression, displayed reduced tumor growth or large necrotic areas owing to lack of angiogenesis (134,135). BMPs have also been shown to induce VEGF expression. Moreover, it has also been suggested that BMP2 might promote tumor angiogenesis by recruiting endothelial progenitor cells and by triggering mesenchymal stem cells of the tumor stroma to secrete angiogenic growth factors such as VEGF and PlGF (136). Finally, Drm/gremlin was shown to be expressed by the stroma of human tumor xenografts in nude mice and in ECs of human lung tumors vasculature when compared with non-neoplastic lung (73).

8. FUTURE PERSPECTIVES

The realization that tumor growth does not depend only on uncontrolled tumor cell proliferation but also on angiogenesis has opened a new road in cancer therapy. Inhibition of tumor angiogenesis and selective destruction of tumor blood vessels offers a unique strategy for cancer treatment including preventing recurrence (137). Despite the fact that the recent insights into the molecular basis of angiogenesis have resulted in development of new treatment strategies to inhibit angiogenesis, many questions remain unanswered. The better understanding of the non-neoplasticand tumor angiogenesis will reveal specific markers as targets for the development or improvement of new, effective angiogenesis inhibitors.

TGF-beta signaling plays a central role in tumor development, metastasis and in tumor angiogenesis. Several therapeutic strategies targeting TGF-beta signaling, such as the delivery of small-molecule inhibitors of TGF-beta receptors, soluble-receptors (soluble Fc-TbRII and sTbRIII) and antisense-compound inhibitors, have been shown to prevent the growth and metastasis of certain cancers (138). However, there may be adverse effects caused by inhibition of TGF-beta signaling, including induction of cancers. In a recent study using low doses of TbbRI kinase inhibitor in experimental intractable solid tumors, resulted in decreased VSMCs coverage of the tumor endothelium and promoted the accumulation of anticancer drugs in the tumour tissue (139). Endoglin has a unique tissue distribution since is highly expressed on actively proliferating ECs and in tumor associated angiogenic vasculature. Although the exact molecular mechanism by which Endoglin acts remains unclear, emerging evidence identifies Endoglin as a suitable vascular target for anti-angiogenic therapy in solid tumors and other angiogenic diseases (130,132). Nevertheless, additional studies are required to define the most appropriate antibody-based therapeutic approaches for Endoglin and their application in clinical trials.

Considering the dynamic regulatory roles of TGF-beta in tumor progression and angiogenesis, anti-TGF-beta-based therapeutic strategies must be carefully considered before administration. A better understanding of the regulation of angiogenesis by TGF-beta family members is of great importance for the rational development of new therapeutic strategies targeting the TGF-beta signaling pathway.

9. ACKNOWLEDGEMENTS

We thank Dr D. Baker for careful reading of the manuscript and for his valuable comments. Our studies are supported by the FP6 EC Integrated Project Angiotargeting 504743 and STREP Tumor-Host-Genomics, the Dutch Cancer Society RUL 2005-3371 and the Ludwig Institute for Cancer Research.

10. REFERENCES

1. Carmeliet, P.: Angiogenesis in life, disease and medicine. *Nature* 438, 932-936 (2005)

2. Jain, R.: Molecular regulation of vessel maturation. *Nat Med* 9, 685-693 (2003)

3. Risau, W.: Embryonic angiogenesis factors. *Pharmacol Ther* 51, 371-376 (1991)

4. Risau, W., and I. Flamme: Vasculogenesis. *Annu Rev Cell Dev Biol* 11, 73-91 (1995)

5. Carmeliet, P., and R. Jain: Angiogenesis in cancer and other diseases. *Nature* 407, 249-257 (2000)

6. Folkman, J., and P.A. D'Amore: Blood vessel formation: what is its molecular basis? *Cell* 87, 1153-1155 (1996)

7. Ribatti, D.: The discovery of endothelial progenitor cells. An historical review. *Leuk Res* 31, 439-444 (2007)

8. Carmeliet, P.: Angiogenesis in health and disease. *Nat Med* 9, 653-660 (2003)

9. Bergers, G., and L. Benjamin: Tumorigenesis and the angiogenic switch. *Nat Rev Cancer* 3, 401-410 (2003)

10. Hanahan, D., and J. Folkman: Patterns and emerging mechanisms of the angiogenic switch during tumorigenesis. *Cell* 86, 353-364 (1996)

11. ten Dijke, P., and H. Arthur: Extracellular control of TGFbeta signalling in vascular development and disease. *Nat Rev Mol Cell Biol* 8, 857-869 (2007)

12. Shah, A.H., and C. Lee: TGF-beta-based immunotherapy for cancer: breaching the tumor firewall. *Prostate* 45, 167-172 (2000)

13. Sporn, M.B., A.B. Roberts, L.M. Wakefield, A.B. Glick, and D. Danielpour: Transforming growth factor-beta and suppression of carcinogenesis. *Princess Takamatsu Symp* 20, 259-266 (1989)

14. Li, M.O., Y.Y. Wan, S. Sanjabi, A.K. Robertson, and R.A. Flavell: Transforming growth factor-beta regulation of immune responses. *Annu Rev Immunol* 24, 99-146 (2006)

15. Massague, J.: TGF-beta signal transduction. *Annu Rev Biochem* 67, 753-791 (1998)

16. Reddi, H.A.: Interplay between bone morphogenetic proteins and cognate binding proteins in bone and cartilage development: noggin, chordin and DAN. *Arthritis Res* 3, 1-5 (2001)

17. ten Dijke, P., K. Miyazono, and C.H. Heldin: Signaling via hetero-oligomeric complexes of type I and type II serine/threonine kinase receptors. *Curr Opin Cell Biol* 8, 139-145 (1996)

18. ten Dijke, P., K. Miyazono, and C.H. Heldin: Signaling inputs converge on nuclear effectors in TGF-beta signaling. *Trends Biochem Sci* 25, 64-70 (2000)

19. Franzen, P., P. ten Dijke, H. Ichijo, H. Yamashita, P. Schulz, C.H. Heldin, and K. Miyazono: Cloning of a TGF beta type I receptor that forms a heteromeric complex with the TGF beta type II receptor. *Cell* 75, 681-692 (1993)

20. Ebner, R., R.H. Chen, S. Lawler, T. Zioncheck, and R. Derynck: Determination of type I receptor specificity by the type II receptors for TGF-beta or activin. *Science* 262, 900-902 (1993)

21. Wang, X.F., H. Lin, E. Ng-Eaton, J. Downward, H.F. Lodish, and R.A. Weinberg: Expression cloning and characterization of the TGF-beta type III receptor. *Cell* 67, 797-805 (1991)

22. David, L., C. Mallet, S. Mazerbourg, J.J. Feige, and S. Bailly: Identification of BMP9 and BMP10 as functional activators of the orphan activin receptor-like kinase 1 (ALK1) in endothelial cells. *Blood* 109, 1953-1961 (2007)

23. Lebrin, F., M.J. Goumans, L. Jonker, R.L. Carvalho, G. Valdimarsdottir, M. Thorikay, C. Mummery, H.M. Arthur, and P. ten Dijke: Endoglin promotes endothelial cell proliferation and TGF-beta/ALK1 signal transduction. *EMBO J* 23, 4018-4028 (2004)

24. Venkatesha, S., M. Toporsian, C. Lam, J. Hanai, T. Mammoto, Y.M. Kim, Y. Bdolah, K.H. Lim, H.T. Yuan, T.A. Libermann, I.E. Stillman, D. Roberts, P.A. D'Amore, F.H. Epstein, F.W. Sellke, R. Romero, V.P. Sukhatme, M. Letarte, and S.A. Karumanchi: Soluble endoglin contributes to the pathogenesis of preeclampsia. *Nat Med* 12, 642-649 (2006)

25. Vilchis-Landeros, M.M., J.L. Montiel, V. Mendoza, G. Mendoza-Hernandez, and F. Lopez-Casillas: Recombinant soluble betaglycan is a potent and isoform-selective transforming growth factor-beta neutralizing agent. *Biochem J* 355, 215-222 (2001)

26. Wrana, J.L., L. Attisano, R. Wieser, F. Ventura, and J. Massague: Mechanism of activation of the TGF-beta receptor. *Nature* 370, 341-347 (1994)

27. Abdollah, S., M. Macias-Silva, T. Tsukazaki, H. Hayashi, L. Attisano, and J.L. Wrana: TbetaRI phosphorylation of Smad2 on Ser465 and Ser467 is required for Smad2-Smad4 complex formation and signaling. *J Biol Chem* 272, 27678-27685 (1997)

28. Nakao, A., T. Imamura, S. Souchelnytskyi, M. Kawabata, A. Ishisaki, E. Oeda, K. Tamaki, J. Hanai, C.H. Heldin, K. Miyazono, and P. ten Dijke: TGF-beta receptor-mediated signalling through Smad2, Smad3 and Smad4. *EMBO J* 16, 5353-5362 (1997)

29. Lagna, G., A. Hata, A. Hemmati-Brivanlou, and J. Massague: Partnership between DPC4 and SMAD proteins in TGF-beta signalling pathways. *Nature* 383, 832-836 (1996)

30. Feng, X.H., and R. Derynck: Specificity and versatility in tgf-beta signaling through Smads. *Annu Rev Cell Dev Biol* 21, 659-693 (2005)

31. Itoh, S., and P. ten Dijke: Negative regulation of TGF-beta receptor/Smad signal transduction. *Curr Opin Cell Biol* 19, 176-184 (2007)

32. Ebisawa, T., M. Fukuchi, G. Murakami, T. Chiba, K. Tanaka, T. Imamura, and K. Miyazono: Smurf1 interacts with transforming growth factor-beta type I receptor through Smad7 and induces receptor degradation. *J Biol Chem* 276, 12477-12480 (2001)

33. Valdimarsdottir, G., M.J. Goumans, F. Itoh, S. Itoh, C.H. Heldin, and P. ten Dijke: Smad7 and protein phosphatase 1alpha are critical determinants in the duration of TGF-beta/ALK1 signaling in endothelial cells. *BMC Cell Biol* 7, 16 (2006)

34. Fajardo, L.F., S.D. Prionas, H.H. Kwan, J. Kowalski, and A.C. Allison: Transforming growth factor beta1 induces angiogenesis *in vivo* with a threshold pattern. *Lab Invest* 74, 600-608 (1996)

35. Koh, G.Y., S.J. Kim, M.G. Klug, K. Park, M.H. Soonpaa, and L.J. Field: Targeted expression of transforming growth factor-beta 1 in intracardiac grafts promotes vascular endothelial cell DNA synthesis. *J Clin Invest* 95, 114-121 (1995)

36. Roberts, A.B., M.B. Sporn, R.K. Assoian, J.M. Smith, N.S. Roche, L.M. Wakefield, U.I. Heine, L.A. Liotra, V. Falanga, J.H. Kehrl, and A. Fauci: Transforming growth factor type beta: rapid induction of fibrosis and angiogenesis *in vivo* and stimulation of collagen formation *in vitro*. *Proc Natl Acad Sci U S A* 83, 4167-4171 (1986)

37. Roberts, A.B., and M.B. Sporn: Regulation of endothelial cell growth, architecture, and matrix synthesis by TGF-beta. *Am Rev Respir Dis* 140, 1126-1128 (1989)

38. Yang, E.Y., and H.L. Moses: Transforming growth factor beta 1-induced changes in cell migration, proliferation, and angiogenesis in the chicken chorioallantoic membrane. *J Cell Biol* 111, 731-741 (1990)

39. Baird, A., and T. Durkin: Inhibition of endothelial cell proliferation by type beta-transforming growth factor: interactions with acidic and basic fibroblast growth factors. *Biochem Biophys Res Commun* 138, 476-482 (1986)

40. Bensaid, M., F. Malecaze, F. Bayard, and J.P. Tauber: Opposing effects of basic fibroblast growth factor and transforming growth factor-beta on the proliferation of cultured bovine retinal capillary endothelial (BREC) cells. *Exp Eye Res* 48, 791-799 (1989)

41. D'Amore, P., and S.R. Smith: Growth factor effects on cells of the vascular wall: a survey. *Growth Factors* 8, 61-75 (1993)

42. Goumans, M.J., G. Valdimarsdottir, S. Itoh, A. Rosendahl, P. Sideras, and P. ten Dijke: Balancing the activation state of the endothelium via two distinct TGF-beta type I receptors. *EMBO J* 21, 1743-1753 (2002)

43. Goumans, M.J., G. Valdimarsdottir, S. Itoh, F. Lebrin, J. Larsson, C. Mummery, S. Karlsson, and P. ten Dijke: Activin receptor-like kinase (ALK)1 is an antagonistic mediator of lateral TGFbeta/ALK5 signaling. *Mol Cell* 12, 817-828 (2003)

44. Lux, A., F. Salway, H.K. Dressman, G. Kröner-Lux, M. Hafner, P.J. Day, D.A. Marchuk, and J. Garland: ALK1 signalling analysis identifies angiogenesis related genes and reveals disparity between TGF-beta and constitutively active receptor induced gene expression. *BMC Cardiovasc Disord* 6, 13 (2006)

45. Ota, T., M. Fujii, T. Sugizaki, M. Ishii, K. Miyazawa, H. Aburatani, and K. Miyazono: Targets of transcriptional regulation by two distinct type I receptors for transforming growth factor-beta in human umbilical vein endothelial cells. *J Cell Physiol* 193, 299-318 (2002)

46. Lamouille, S., C. Mallet, J.J. Feige, and S. Bailly: Activin receptor-like kinase 1 is implicated in the maturation phase of angiogenesis. *Blood* 100, 4495-4501 (2002)

47. Oh, S.P., T. Seki, K.A. Goss, T. Imamura, Y. Yi, P.K. Donahoe, L. Li, K. Miyazono, P. ten Dijke, S. Kim, and E. Li: Activin receptor-like kinase 1 modulates transforming growth factor-beta 1 signaling in the regulation of angiogenesis. *Proc Natl Acad Sci U S A* 97, 2626-2631 (2000)

48. Brown, M.A., Q. Zhao, K.A. Baker, C. Naik, C. Chen, L. Pukac, M. Singh, T. Tsareva, Y. Parice, A. Mahoney, V. Roschke, I. Sanyal, and S. Choe: Crystal structure of BMP-9 and functional interactions with pro-region and receptors. *J Biol Chem* 280, 25111-25118 (2005)

49. Scharpfenecker, M., M. van Dinther, Z. Liu, R. van Bezooijen, Q. Zhao, L. Pukac, C.W. Lowik, and P. ten Dijke: BMP-9 signals via ALK1 and inhibits bFGF-induced endothelial cell proliferation and VEGF-stimulated angiogenesis. *J Cell Sci* 120, 964-972 (2007)

50. Sanchez-Elsner, T., L.M. Botella, B. Velasco, A. Corbi, L. Attisano, and C. Bernabeu: Synergistic cooperation between hypoxia and transforming growth factor-beta pathways on human vascular endothelial growth factor gene expression. *J Biol Chem* 276, 38527-38535 (2001)

51. Letamendia, A., P. Lastres, N. Almendro, U. Raab, H.J. Buhring, S. Kumar, and C. Bernabeu: Endoglin, a component of the TGF-beta receptor system, is a differentiation marker of human choriocarcinoma cells. Int J Cancer 76, 541-546 (1998)

52. Lastres, P., A. Letamendia, H. Zhang, C. Rius, N. Almendro, U. Raab, L.A. Lopez, C. Langa, A. Fabra, M. Letarte, and C. Bernabeu: Endoglin modulates cellular responses to TGF-beta 1. J Cell Biol 133, 1109-1121 (1996)

53. Lebrin, F., M.J. Goumans, L. Jonker, R. Carvalho, G. Valdimarsdottir, M. Thorikay, C. Mummery, H. Arthur, and P. ten Dijke: Endoglin promotes endothelial cell

proliferation and TGF-beta/ALK1 signal transduction. EMBO J 23, 4018-4028 (2004)

54. Fernandez, L., F. Sanz-Rodriguez, R. Zarrabeitia, A. Perez-Molino, R.P. Hebbel, J. Nguyen, C. Bernabeu, and L.M. Botella: Blood outgrowth endothelial cells from Hereditary Haemorrhagic Telangiectasia patients reveal abnormalities compatible with vascular lesions. *Cardiovasc Res* 68, 235-248 (2005)

55. Armulik, A., A. Abramsson, and C. Betsholtz: Endothelial/pericyte interactions. *Circ Res* 97, 512-523 (2005)

56. von Tell, T.D., A. Armulik, and C. Betsholtz: Pericytes and vascular stability. *Exp Cell Res* 312, 623-629 (2006)

57. Hautmann, M.B., C.S. Madsen, and G.K. Owens: A transforming growth factor beta (TGFbeta) control element drives TGFbeta-induced stimulation of smooth muscle alpha-actin gene expression in concert with two CArG elements. *J Biol Chem* 272, 10948-10956 (1997)

58. Seay, U., D. Sedding, S. Krick, M. Hecker, W. Seeger, and O. Eickelberg: Transforming growth factor-beta-dependent growth inhibition in primary vascular smooth muscle cells is p38-dependent. *J Pharmacol Exp Ther* 315, 1005-1012 (2005)

59. Ding, R., D.C. Darland, M.S. Parmacek, and P.A. D'Amore: Endothelial-mesenchymal interactions *in vitro* reveal molecular mechanisms of smooth muscle/pericyte differentiation. *Stem Cells Dev* 13, 509-520 (2004)

60. Goumans, M.J., D. Ward-van Oostwaard, F. Wianny, P. Savatier, A. Zwijsen, and C. Mummery: Mouse embryonic stem cells with aberrant transforming growth factor beta signalling exhibit impaired differentiation *in vitro* and *in vivo*. *Differentiation* 63, 101-113 (1998)

61. Hirschi, K.K., S.A. Rohovsky, and P.A. D'Amore: PDGF, TGF-beta, and heterotypic cell-cell interactions mediate endothelial cell-induced recruitment of 10T1/2 cells and their differentiation to a smooth muscle fate. *J Cell Biol* 141, 805-814 (1998)

62. Chen, S., and R.J. Lechleider: Transforming growth factor-beta-induced differentiation of smooth muscle from a neural crest stem cell line. *Circ Res* 94, 1195-1202 (2004)

63. Boyd, N.L., S.K. Dhara, R. Rekaya, E.A.Godbey, K. Hasneen, R.R. Rao, F.D. West, B.A. Gerwe, and S.L. Stice: BMP4 promotes formation of primitive vascular networks in human embryonic stem cell-derived embryoid bodies. *Exp Biol Med (Maywood)* 232, 833-843 (2007)

64. Langenfeld, E.M., and J. Langenfeld: Bone morphogenetic protein-2 stimulates angiogenesis in developing tumors. *Mol Cancer Res* 2, 141-149 (2004)

65. Raida, M., J.H. Clement, R.D. Leek, K. Ameri, R. Bicknell, D. Niederwieser, and A.L. Harris: Bone morphogenetic protein 2 (BMP-2) and induction of tumor angiogenesis. *J Cancer Res Clin Oncol* 131, 741-750 (2005)

66. Valdimarsdottir, G., M.J. Goumans, A. Rosendahl, M. Brugman, S. Itoh, F. Lebrin, P. Sideras, and P. ten Dijke: Stimulation of Id1 expression by bone morphogenetic protein is sufficient and necessary for bone morphogenetic protein-induced activation of endothelial cells. *Circulation* 106, 2263-2270 (2002)

67. Deckers, M., G. van der Pluijm, S. Dooijewaard, M. Kroon, V. van Hinsbergh, S. Papapoulos, and C. Lowik: Effect of angiogenic and antiangiogenic compounds on the outgrowth of capillary structures from fetal mouse bone explants. *Lab Invest* 81, 5-15 (2001)

68. Zhou, Q., J. Heinke, A. Vargas, S. Winnik, T. Krauss, C. Bode, C. Patterson, and M. Moser: ERK signaling is a central regulator for BMP-4 dependent capillary sprouting. *Cardiovasc Res* 76, 390-399 (2007)

69. Suzuki, Y., K. Montagne, A. Nishihara, T. Watabe, and K. Miyazono: BMPs promote proliferation and migration of endothelial cells via stimulation of VEGF-A/VEGFR2 and Angiopoietin-1/Tie2 signaling. *J Biochem* 143, 199-206 (2008)

70. Ren, R., P.C. Charles, C. Zhang, Y. Wu, H. Wang, and C. Patterson: Gene expression profiles identify a role for cyclooxygenase 2-dependent prostanoid generation in BMP6-induced angiogenic responses. *Blood* 109, 2847-2853 (2007)

71. Balemans, W., and W. van Hul: Extracellular regulation of BMP signaling in vertebrates: a cocktail of modulators. *Dev Biol* 250, 231-250 (2002)

72. Bardot, B., L. Lecoin, I. Fliniaux, E. Huillard, M. Marx, and J.P.H. Viallet: Drm/Gremlin, a BMP antagonist, defines the interbud region during feather development. *Int J Dev Biol* 48, 149-156 (2004)

73. Stabile, H., S. Mitola, E. Moroni, M. Belleri, S. Nicoli, D. Coltrini, F. Peri, A. Pessi, L. Orsatti, F. Talamo, V. Castronovo, D. Waltregny, F. Cotelli, D. Ribatti, and M. Presta: Bone morphogenic protein antagonist Drm/gremlin is a novel proangiogenic factor. *Blood* 109, 1834-1840 (2007)

74. Goumans, M.J., and C. Mummery: Functional analysis of the TGFbeta receptor/Smad pathway through gene ablation in mice. *Int J Dev Biol* 44, 253-265 (2000)

75. Dickson, M.C., J.S. Martin, F.M. Cousins, A.B. Kulkarni, S. Karlsson, and R.J. Akhurst: Defective haematopoiesis and vasculogenesis in transforming growth factor-beta 1 knock out mice. *Development* 121, 1845-1854 (1995)

76. Martin, J.S., M.C. Dickson, F.M. Cousins, A.B. Kulkarni, S. Karlsson, and R.J. Akhurst: Analysis of

homozygous TGF beta 1 null mouse embryos demonstrates defects in yolk sac vasculogenesis and hematopoiesis. *Ann N Y Acad Sci* 752, 300-308 (1995)

77. Oshima, M., H. Oshima, and M.M. Taketo: TGF-beta receptor type II deficiency results in defects of yolk sac hematopoiesis and vasculogenesis. *Dev Biol* 179, 297-302 (1996)

78. Larsson, J., M.J. Goumans, L.J. Sjostrand, M.A. van Rooijen, D. Ward, P. Leveen, X. Xu, P. ten Dijke, C.L. Mummery, and S. Karlsson: Abnormal angiogenesis but intact hematopoietic potential in TGF-beta type I receptor-deficient mice. *EMBO J* 20, 1663-1673 (2001)

79. Seki, T., K.H. Hong, and S.P. Oh: Nonoverlapping expression patterns of ALK1 and ALK5 reveal distinct roles of each receptor in vascular development. *Lab Invest* 86, 116-129 (2006)

80. Carvalho, R.L., L. Jonker, M.J. Goumans, J. Larsson, P. Bouwman, S. Karlsson, P. ten Dijke, H.M. Arthur, and C.L. Mummery: Defective paracrine signalling by TGFbeta in yolk sac vasculature of endoglin mutant mice: a paradigm for hereditary haemorrhagic telangiectasia. *Development* 131, 6237-6247 (2004)

81. Carvalho, R.L., F. Itoh, M.J. Goumans, F. Lebrin, M. Kato, S. Takahashi, M. Ema, S. Itoh, M.A. van Rooijen, P. Bertolino, P. ten Dijke, and C.L. Mummery: Compensatory signalling induced in the yolk sac vasculature by deletion of TGF receptors in mice. *J Cell Sci* 120, 4269-4277 (2007)

82. McAllister, K.A., K.M. Grogg, D.W. Johnson, C.J. Gallione, M.A.Baldwin, C.E.Jackson, E.A. Helmbold, D.S. Markel, W.C. McKinnon, J. Murrell, M.K. McCormick, M. Pericak-Vance, P. Heutink, B. Oostra, T. Haitjema, C. Westerman, M. Porteous, A. Guttmacher, M. Letarte, and D. Marchuk: Endoglin, a TGF-beta binding protein of endothelial cells, is the gene for hereditary haemorrhagic telangiectasia type 1. *Nat Genet* 8, 345-351 (1994)

83. McDonald, M.T., K.A. Papenberg, S. Ghosh, A.A. Glatfelter, B.B. Biesecker, E.A. Helmbold, D.S. Markel, A. Zolotor, W.C. McKinnon, J.L. Vanderstoep, C. Jackson, M. Iannuzzi, F. Collins, M. Boehnke, M. Porteous, A. Guttmacher, and D.A. Marchuk: A disease locus for hereditary haemorrhagic telangiectasia maps to chromosome 9q33-34. *Nat Genet* 6, 197-204 (1994)

84. Arthur, H.M., J. Ure, A.J. Smith, G. Renforth, D.I. Wilson, E. Torsney, R. Charlton, D.V. Parums, T. Jowett, D.A. Marchuk, J. Burn, and A.G. Diamond: Endoglin, an ancillary TGFbeta receptor, is required for extraembryonic angiogenesis and plays a key role in heart development. *Dev Biol* 217, 42-53 (2000)

85. Li, D.Y., L.K. Sorensen, B.S. Brooke, L.D. Urness, E.C. Davis, D.G. Taylor, B.B. Boak, and D.D Wendel: Defective angiogenesis in mice lacking endoglin. *Science* 284, 1534-1537 (1999)

86. Sorensen, L.K., B.S. Brooke, D.Y. Li, and L.D. Urness: Loss of distinct arterial and venous boundaries in mice lacking endoglin, a vascular-specific TGFbeta coreceptor. *Dev Biol* 261, 235-250 (2003)

87. Urness, L.D., L.K. Sorensen, and D.Y. Li: Arteriovenous malformations in mice lacking activin receptor-like kinase-1. *Nat Genet* 26, 328-331 (2000)

88. Gallione, C.J., G.M. Repetto, E. Legius, A.K. Rustgi, S.L. Schelley, S. Tejpar, G. Mitchell, E. Drouin, C.J. Westermann, and D.A. Marchuk: A combined syndrome of juvenile polyposis and hereditary haemorrhagic telangiectasia associated with mutations in MADH4 (SMAD4). *Lancet* 363, 852-859 (2004)

89. Chang, H., D. Huylebroeck, K. Verschueren, Q. Guo, M.M. Matzuk, and A. Zwijsen: Smad5 knockout mice die at mid-gestation due to multiple embryonic and extraembryonic defects. *Development* 126, 1631-1642 (1999)

90. Yang, X., L.H. Castilla, X. Xu, C. Li, J. Gotay, M. Weinstein, P.P. Liu, and C.X. Deng: Angiogenesis defects and mesenchymal apoptosis in mice lacking SMAD5. *Development* 126, 1571-1580 (1999)

91. Thomson, J., R. Machado, M. Pauciulo, N. Morgan, M. Yacoub, P. Corris, K. McNeil, J. Loyd, W. Nichols, and R. Trembath: Familial and sporadic primary pulmonary hypertension is caused by BMPR2 gene mutations resulting in haploinsufficiency of the bone morphogenetic protein tuype II receptor. *J Heart Lung Transplant* 20, 149 (2001)

92. Lane, K.B., R.D. Machado, M.W. Pauciulo, J.R. Thomson, J.A. Phillips, J.E. Loyd, W.C. Nichols, and R.C. Trembath: Heterozygous germline mutations in BMPR2, encoding a TGF-beta receptor, cause familial primary pulmonary hypertension. The International PPH Consortium. *Nat Genet* 26, 81-84 (2000)

93. Teichert-Kuliszewska, K., M.J. Kutryk, M.A. Kuliszewski, G. Karoubi, D.W. Courtman, L. Zucco, J. Granton, and D.J. Stewart: Bone morphogenetic protein receptor-2 signaling promotes pulmonary arterial endothelial cell survival: implications for loss-of-function mutations in the pathogenesis of pulmonary hypertension. *Circ Res* 98, 209-217 (2006)

94. Trembath, R.C., J.R. Thomson, R.D. Machado, N.V. Morgan, C. Atkinson, I. Winship, G. Simonneau, N. Galie, J.E. Loyd, M. Humbert, W.C. Nichols, J. Berg, A. Manes, J. McGaughran, M. Pauciulo, L. Wheeler, and N.W. Morrell: Clinical and Molecular Genetic Features of Pulmonary Hypertension in Patients with Hereditary Hemorrhagic Telangiectasia. *N Engl J Med* 345, 325-334 (2001)

95. Harrison, R.E., J.A. Flanagan, M. Sankelo, S.A. Abdalla, J. Rowell, R.D. Machado, C.G. Gregory Elliott, I.M. Robbins, H. Olschewski, V. McLaughlin, E. Gruenig, F. Kermeen, M. Halme, A. Raisanen-Sokolowski, T.

Laitinen, N.W. Morrell, and R.C. Trembath: Molecular and functional analysis identifies ALK-1 as the predominant cause of pulmonary hypertension related to hereditary haemorrhagic telangiectasia. *J Med Genet* 40, 865-871 (2003)

96. Friess, H., Y. Yamanaka, M. Buchler, M. Ebert, H.G. Beger, L.I. Gold, and M. Korc: Enhanced expression of transforming growth factor beta isoforms in pancreatic cancer correlates with decreased survival. *Gastroenterology* 105, 1846-1856 (1993)

97. Gorsch, S.M., V.A. Memoli, T.A. Stukel, L.I. Gold, and B.A. Arrick: Immunohistochemical staining for transforming growth factor beta 1 associates with disease progression in human breast cancer. *Cancer Res* 52, 6949-6952 (1992)

98. De Jong, K.P., R. Stellema, A. Karrenbeld, J. Koudstaal, A.S. Gouw, W.J. Sluiter, P.M. Peeters, M.J. Slooff, and E.G. de Vries: Clinical relevance of transforming growth factor alpha, epidermal growth factor receptor, p53, and Ki67 in colorectal liver metastases and corresponding primary tumors. *Hepatology* 28, 971-979 (1998)

99. Stearns, M.E., F.U. Garcia, K. Fudge, J. Rhim, and M. Wang: Role of interleukin 10 and transforming growth factor beta1 in the angiogenesis and metastasis of human prostate primary tumor lines from orthotopic implants in severe combined immunodeficiency mice. *Clin Cancer Res* 5, 711-720 (1999)

100. Ueki, N., M. Nakazato, T. Ohkawa, T. Ikeda, Y. Amuro, T. Hada, and K. Higashino: Excessive production of transforming growth-factor beta 1 can play an important role in the development of tumorigenesis by its action for angiogenesis: validity of neutralizing antibodies to block tumor growth. *Biochim Biophys Acta* 1137, 189-196 (1992)

101. Lokeshwar, B.L., and N.L. Block: Isolation of a prostate carcinoma cell proliferation-inhibiting factor from human seminal plasma and its similarity to transforming growth factor beta. *Cancer Res* 52, 5821-5825 (1992)

102. Ananth, S., B. Knebelmann, W. Gruning, M. Dhanabal, G. Walz, I.E. Stillman, V.P. Sukhatme: Transforming growth factor beta1 is a target for the von Hippel-Lindau tumor suppressor and a critical growth factor for clear cell renal carcinoma. *Cancer Res* 59, 2210-2216 (1999)

103. Bandyopadhyay, A., Y. Zhu, M.L. Cibull, L. Bao, C. Chen, and L. Sun: A soluble transforming growth factor beta type III receptor suppresses tumorigenicity and metastasis of human breast cancer MDA-MB-231 cells. *Cancer Res* 59, 5041-5046 (1999)

104. Bandyopadhyay, A., Y. Zhu, S.N. Malik, J. Kreisberg, M.G. Brattain, E.A. Sprague, J. Luo, F. Lopez-Casillas, and L.Z. Sun: Extracellular domain of TGFbeta type III receptor inhibits angiogenesis and tumor growth in human cancer cells. *Oncogene* 21, 3541-3551 (2002)

105. Lei, X., A. Bandyopadhyay, T. Le, and L. Sun: Autocrine TGFbeta supports growth and survival of human breast cancer MDA-MB-231 cells. *Oncogene* 21, 7514-7523 (2002)

106. Bandyopadhyay, A., F. Lopez-Casillas, S.N. Malik, J.L. Montiel, V. Mendoza, J. Yang, and L.Z. Sun: Antitumor activity of a recombinant soluble betaglycan in human breast cancer xenograft. *Cancer Res* 62, 4690-4695 (2002)

107. Tuxhorn, J.A., S.J. McAlhany, F. Yang, T.D. Dang, and D.R. Rowley: Inhibition of transforming growth factor-beta activity decreases angiogenesis in a human prostate cancer-reactive stroma xenograft model. *Cancer Res* 62, 6021-6025 (2002)

108. Halder, S.K., R.D. Beauchamp, and P.K. Datta: A specific inhibitor of TGF-beta receptor kinase, SB-431542, as a potent antitumor agent for human cancers. *Neoplasia* 7, 509-521 (2005)

109. Hjelmeland, M.D., A.B. Hjelmeland, S. Sathornsumetee, E.D. Reese, M.H. Herbstreith, N.J. Laping, H.S. Friedman, D.D. Bigner, X.F. Wang, and J.N. Rich: SB-431542, a small molecule transforming growth factor-beta-receptor antagonist, inhibits human glioma cell line proliferation and motility. *Mol Cancer Ther* 3, 737-745 (2004)

110. Matsuyama, S., M. Iwadate, M. Kondo, M. Saitoh, A. Hanyu, K. Shimizu, H. Aburatani, H.K. Mishima, T. Imamura, K. Miyazono, and K. Miyazawa: SB-431542 and Gleevec inhibit transforming growth factor-beta-induced proliferation of human osteosarcoma cells. *Cancer Res* 63, 7791-7798 (2003)

111. Teraoka, H., T. Sawada, T. Nishihara, M. Yashiro, M. Ohira, T. Ishikawa, H. Nishino, and K. Hirakawa: Enhanced VEGF production and decreased immunogenicity induced by TGF-beta 1 promote liver metastasis of pancreatic cancer. *Br J Cancer* 85, 612-617 (2001)

112. Breier, G., S. Blum, J. Peli, M. Groot, C. Wild, W. Risau, and E. Reichmann: Transforming growth factor-beta and Ras regulate the VEGF/VEGF-receptor system during tumor angiogenesis. *Int J Cancer* 97, 142-148 (2002)

113. Pertovaara, L., A. Kaipainen, T. Mustonen, A. Orpana, N. Ferrara, O. Saksela, and K. Alitalo: Vascular endothelial growth factor is induced in response to transforming growth factor-beta in fibroblastic and epithelial cells. *J Biol Chem* 269, 6271-6274 (1994)

114. Duivenvoorden, W.C., H.W. Hirte, and G. Singh: Transforming growth factor beta1 acts as an inducer of matrix metalloproteinase expression and activity in human

bone-metastasizing cancer cells. *Clin Exp Metastasis* 17, 27-34 (1999)

115. Edwards, D.R., G. Murphy, J.J. Reynolds, S.E. Whitham, A.J. Docherty, P. Angel, and J.K. Heath: Transforming growth factor beta modulates the expression of collagenase and metalloproteinase inhibitor. *EMBO J* 6, 1899-1904 (1987)

116. Sehgal, I., and T.C. Thompson: Novel regulation of type IV collagenase (matrix metalloproteinase-9 and -2) activities by transforming growth factor-beta1 in human prostate cancer cell lines. *Mol Biol Cell* 10, 407-416 (1999)

117. Kalluri, R.: Basement membranes: structure, assembly and role in tumour angiogenesis. *Nat Rev Cancer* 3, 422-433 (2003)

118. Kim, K.Y., S.Y. Jeong, J. Won, P.D. Ryu, and M.J. Nam: Induction of angiogenesis by expression of soluble type II transforming growth factor-beta receptor in mouse hepatoma. *J Biol Chem* 276, 38781-38786 (2001)

119. Yee, K.O., M. Streit, T. Hawighorst, M. Detmar, and J. Lawler: Expression of the type-1 repeats of thrombospondin-1 inhibits tumor growth through activation of transforming growth factor-beta. *Am J Pathol* 165, 541-552 (2004)

120. Gohongi, T., D. Fukumura, Y. Boucher, C.O. Yun, G.A. Soff, C. Compton, T. Todoroki, and R.K. Jain: Tumor-host interactions in the gallbladder suppress distal angiogenesis and tumor growth: involvement of transforming growth factor beta1. *Nat Med* 5, 1203-1208 (1999)

121. Nakagawa, T., J.H. Li, G. Garcia, W. Mu, E. Piek, E.P. Böttinger, Y. Chen, H.J. Zhu, D.H. Kang, G.F. Schreiner, H.Y. Lan, and R.J. Johnson: TGF-beta induces proangiogenic and antiangiogenic factors via parallel but distinct Smad pathways. *Kidney Int* 66, 605-613 (2004)

122. Han, S.U., H.T. Kim, D.H. Seong, Y.S. Kim, Y.S. Park, Y.J. Bang, H.K. Yang, and S.J. Kim: Loss of the Smad3 expression increases susceptibility to tumorigenicity in human gastric cancer. *Oncogene* 23, 1333-1341 (2004)

123. Schwarte-Waldhoff, I., O.V. Volpert, N.P. Bouck, B. Sipos, S.A. Hahn, S. Klein-Scory, Jutta Lüttges, Günter Klöppel, Ulrich Graeven, Christina Eilert-Micus, Annette Hintelmann, Wolff Schmiegel: Smad4/DPC4-mediated tumor suppression through suppression of angiogenesis. *Proc Natl Acad Sci U S A* 97, 9624-9629 (2000)

124. Burrows, F.J., E.J. Derbyshire, P.L. Tazzari, P. Amlot, A.F. Gazdar, S.W. King, M. Letarte, E.S. Vitetta, and P.E. Thorpe: Up-regulation of endoglin on vascular endothelial cells in human solid tumors: implications for diagnosis and therapy. *Clin Cancer Res* 1, 1623-1634 (1995)

125. Calabro, L., E. Fonsatti, G. Bellomo, A. Alonci, F. Colizzi, L. Sigalotti, M. Altomonte, C. Musolino, and M. Maio: Differential levels of soluble endoglin (CD105) in myeloid malignancies. *J Cell Physiol* 194, 171-175 (2003)

126. Kumar, S., A. Ghellal, C. Li, G. Byrne, N. Haboubi, J.M. Wang, and N. Bundred: Breast carcinoma: vascular density determined using CD105 antibody correlates with tumor prognosis. *Cancer Res* 59, 856-861 (1999)

127. Takahashi, N., A. Haba, F. Matsuno, and B. Seon: Antiangiogenic therapy of established tumors in human skin/severe combined immunodeficiency mouse chimeras by anti-endoglin (CD105) monoclonal antibodies, and synergy between anti-endoglin antibody and cyclophosphamide. *Cancer Res* 61, 7846-7854 (2001)

128. Duwel, A., N. Eleno, M. Jerkic, M. Arevalo, J.P. Bolanos, C. Bernabeu, and J.M. Lopez-Novoa: Reduced tumor growth and angiogenesis in endoglin-haploinsufficient mice. *Tumour Biol* 28, 1-8 (2007)

129. Bredow, S., M. Lewin, B. Hofmann, E. Marecos, and R. Weissleder: Imaging of tumour neovasculature by targeting the TGF-beta binding receptor endoglin. *Eur J Cancer* 36, 675-681 (2000)

130. Costello, B., C. Li, S. Duff, D. Butterworth, A. Khan, M. Perkins, S. Owens, A.F. Al-Mowallad, S. O'Dwyer, and S. Kumar: Perfusion of 99Tcm-labeled CD105 Mab into kidneys from patients with renal carcinoma suggests that CD105 is a promising vascular target. *Int J Cancer* 109, 436-441 (2004)

131. Matsuno,F., Y. Haruta, M. Kondo, H. Tsai, M. Barcos, and B.K. Seon: Induction of lasting complete regression of preformed distinct solid tumors by targeting the tumor vasculature using two new anti-endoglin monoclonal antibodies. *Clin Cancer Res* 5, 371-382 (1999)

132. Seon, B.K., F. Matsuno, Y. Haruta, M. Kondo, and M. Barcos: Long-lasting complete inhibition of human solid tumors in SCID mice by targeting endothelial cells of tumor vasculature with antihuman endoglin immunotoxin. *Clin Cancer Res* 3, 1031-1044 (1997)

133. Langenfeld, E.M., S.E. Calvano, F. Abou-Nukta, S.F. Lowry, P. Amenta, and J. Langenfeld: The mature bone morphogenetic protein-2 is aberrantly expressed in non-small cell lung carcinomas and stimulates tumor growth of A549 cells. *Carcinogenesis* 24, 1445-1454 (2003)

134. Rothhammer, T., I. Poser, F. Soncin, F. Bataille, M. Moser, and A.K. Bosserhoff: Bone morphogenic proteins are overexpressed in malignant melanoma and promote cell invasion and migration. *Cancer Res* 65, 448-456 (2005)

135. Rothhammer, T., F. Bataille, T. Spruss, G. Eissner, and A.K. Bosserhoff: Functional implication of BMP4 expression on angiogenesis in malignant melanoma. *Oncogene* 26, 4158-4170 (2007)

136. Raida, M., A.C. Heymann, C. Gunther, and D. Niederwieser: Role of bone morphogenetic protein 2 in the

crosstalk between endothelial progenitor cells and mesenchymal stem cells. *Int J Mol Med* 18, 735-739 (2006)

137. Folkman, J.: Tumor angiogenesis. *Adv Cancer Res* 19, 331-358 (1974)

138. Yingling, J., K.L. Blanchard, and J.S. Sawyer: Development of TGF-beta signalling inhibitors for cancer therapy. *Nat Rev Drug Discov* 3, 1011-1022 (2004)

139. Kano, M.R., Y. Bae, C. Iwata, Y. Morishita, M. Yashiro, M. Oka, T. Fujii, A. Komuro, K. Kiyono, M. Kaminishi, K. Hirakawa, Y. Ouchi, N. Nishiyama, K. Kataoka, and K. Miyazono: Improvement of cancer-targeting therapy, using nanocarriers for intractable solid tumors by inhibition of TGF-beta signaling. *Proc Natl Acad Sci U S A* 104, 3460-3465 (2007)

Abbreviations: TGF: transforming growth factor; TbR: TGF-beta receptor; ALK: activin receptor-like kinase; BMP: bone morphogenetic proteins; BMPRII: type II BMP receptor; ActRII: type II Activin receptor; sRIII: soluble type III receptor; HHT: hereditary hemorrhagic telangiectasia; PPH: primary pulmonary hypertension; VEGF: vascular endothelial growth factor; VEGFR: VEGF receptor; bFGF: basicfibroblast growth factor; PDGF: platelet derived growth factor; PlGF: placental growth factor; EC(s): endothelial cell(s); VSMCs: vascular smooth muscle cells; BOECs: blood outgrowth endothelial cells; MAPK: mitogen activated protein kinase; FAK: focal adhesion kinase; TAK1: TGF-beta activated kinase 1; LAP: latency-associated peptide; TSP-1: thrombospondin-1; eNOS: endothelial nitric oxide synthase; ECM: extracellular matrix; EMT: epithelial to mesenchymal transition; MMP: matrix metalloproteinase.

Key Words: TGF- beta, BMP, Smad, Tumor, Angiogenesis, Endoglin, ALK, HHT, Review

Send correspondence to: E. Pardali, Department of Molecular Cell Biology, Building 2, Room R-02-024, Leiden University Medical Center, Postzone S-1-P, Postbus 9600, 2300 RC Leiden, The Netherlands, Tel: 31-71-526 9273, Fax: 31-71- 5268270, E-mail: e.pardali@lumc.nl