Available online at www.sciencedirect.com
ScienceDirect
Biochimica et Biophysica Acta 1775 (2007) 21 – 62
Biochimica et Biophysica Acta
BBA
www.elsevier.com/locate/bbacan

Review

Actions of TGF-β as tumor suppressor and pro-metastatic factor in human cancer

Katerina Pardali [1], Aristidis Moustakas*

Ludwig Institute for Cancer Research, Box 595 Biomedical Center, Uppsala University, SE-751 24 Uppsala, Sweden

Received 15 April 2006; received in revised form 24 June 2006; accepted 26 June 2006
Available online 8 July 2006

Abstract

Transforming growth factor-β (TGF-β) is a secreted polypeptide that signals via receptor serine/threonine kinases and intracellular Smad effectors. TGF-β inhibits proliferation and induces apoptosis in various cell types, and accumulation of loss-of-function mutations in the TGF-β receptor or Smad genes classify the pathway as a tumor suppressor in humans. In addition, various oncogenic pathways directly inactivate the TGF-β receptor-Smad pathway, thus favoring tumor growth. On the other hand, all human tumors overproduce TGF-β whose autocrine and paracrine actions promote tumor cell invasiveness and metastasis. Accordingly, TGF-β induces epithelial–mesenchymal transition, a differentiation switch that is required for transitory invasiveness of carcinoma cells. Tumor-derived TGF-β acting on stromal fibroblasts remodels the tumor matrix and induces expression of mitogenic signals towards the carcinoma cells, and upon acting on endothelial cells and pericytes, TGF-β regulates angiogenesis. Finally, TGF-β suppresses proliferation and differentiation of lymphocytes including cytolytic T cells, natural killer cells and macrophages, thus preventing immune surveillance of the developing tumor. Current clinical approaches aim at establishing novel cancer drugs whose mechanisms target the TGF-β pathway. In conclusion, TGF-β signaling is intimately implicated in tumor development and contributes to all cardinal features of tumor cell biology.

Keywords: Cancer; Epithelial–mesenchymal transition; Metastasis; Smad; TGF-β; Tumor suppressor

Contents

Abbreviations: ALK, activin receptor-like kinase; APC, adenomatous polyposis coli; ARTS, apoptotic response to TGF-β signals; BMP, bone morphogenetic protein; CDK, cyclin-dependent kinase; CKI, cyclin-dependent kinase inhibitor; CTL, cytotoxic T lymphocyte; DAPK, death-associated protein kinase; EMT, epithelial–mesenchymal transition; ERK, extracellular signal-regulated protein kinase; HGF/SF, hepatocyte growth factor/scatter factor; Id, inhibitor of differentiation; JNK, Jun N-terminal kinase; JPS, juvenile polyposis syndrome; LEF/TCF, lymphoid enhancer factor/T-cell factor; MAPK, mitogen activated protein kinase; MET, mesenchymal–epithelial transition; MH, Mad homology; MKK, MAPK kinase kinase; MMTV, mouse mammary tumor virus; NF-κB, nuclear factor κB; PDGF, platelet derived growth factor; PI3K, phosphoinositide 3′ kinase; PKA/B/C, protein kinase A/B/C; PML, promyelocytic leukemia; PTEN, phosphatase and tensin homologue; PTHrP, parathyroid hormone-related peptide; RNAi, RNA interference; SHIP, SH2-containing 5′-inositol phosphatase; Ski, Sloan-Kettering Institute retrovirus oncogene; SnoN, Ski-related novel gene; MDM2/X, murine double minute 2 or X gene; TERT, telomerase reverse transcriptase; TGF, transforming growth factor; TIEG1, TGF-β-inducible early response gene-1; TβRI, TGF-β receptor type I; TβRII, TGF-β receptor type II; Treg, regulatory $CD4^+CD25^+$ T cell; VEGF, vascular endothelial growth factor

* Corresponding author. Tel.: +46 18 160414; fax: +46 18 160420.
E-mail address: aris.moustakas@licr.uu.se (A. Moustakas).
[1] Current address: Molecular Medicine, Dept. of Genetics and Pathology, Rudbeck Laboratory, Uppsala University, SE-751 85 Uppsala, Sweden.

0304-419X/$ - see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.bbcan.2006.06.004

1. How does TGF-β work?

1.1. TGF-β, a secreted cytokine with opposing modes of action

Transforming growth factor-β (TGF-β) was discovered as a secreted polypeptide factor from chemically or virally transformed fibroblasts that could elicit transformation of normal fibroblasts in classical in vitro assays [1,2]. Soon after its discovery, TGF-β was found to also act as an inhibitor of cell proliferation, thus establishing a dual role of TGF-β in cell growth control, which is cell type-dependent [3,4]. Similar to the dual role TGF-β plays in regulating cell proliferation, this growth factor was rapidly established to affect a large variety of cellular processes during embryonic development and adult tissue homeostasis, and the action of TGF-β in one target cell type or developmental stage was frequently reported to be the opposite in a neighboring cell type or a subsequent developmental stage [5]. This intriguing feature of TGF-β biology is well established even in the field of cancer research. TGF-β is known to act as a tumor suppressor in early stages of tumorigenesis, but it can also promote advanced tumor cell invasiveness and metastasis [6,7].

1.2. Relevance of TGF-β to the six cardinal features of cancer biology

The complex roles TGF-β plays during cancer progression make this polypeptide factor and its signaling pathway become involved in essentially all six cardinal changes of normal cell physiology that characterize cancer cells [8]. Accordingly, TGF-β indirectly promotes self-sufficiency to growth signals, as it is over-produced by cancer cells and induces production of several mitogenic factors such as hepatocyte growth factor/scatter factor (HGF/SF) or platelet derived growth factor (PDGF) in the tumor stroma [9]. The TGF-β pathway is targeted by loss-of-function mutations in various human cancers, thus relieving tumor cell growth from a major cytostatic agent and an inducer of apoptosis of diverse cell types [10]. TGF-β also protects the genome from harmful recombination and suppresses telomerase activity, offering

another reason why tumor cells inactivate its signaling pathway [11]. Finally, via an array of cellular mechanisms, TGF-β signals to the tumor stromal environment, by targeting fibroblasts, myofibroblasts, the vasculature and surveilling immune cells, thus promoting tumor cell survival, invasiveness, angiogenesis and metastasis in advanced cancer stages (reviewed in [12]). The elucidation of mechanisms by which TGF-β regulates the function of the diverse set of cells that participate in tumor development is progressing rapidly [13–15], thus offering novel territories for pharmaceutical intervention.

1.3. The signaling pathway: Smads and alternative signaling inputs

1.3.1. TGF-β and its receptors

TGF-β is the prototype of 34-some extracellular ligands that form the TGF-β superfamily of morphogenetic factors, additionally including bone morphogenetic proteins (BMPs), growth differentiation factors (GDFs), müllerian inhibiting substance (MIS), activins and others [16,17]. All these cytokines regulate cell proliferation, apoptosis, differentiation and migration and each ligand presents unique features of action, while they all share a common machinery to transmit intracellular signals. In this review we primarily discuss TGF-β, which is represented by three isoforms, TGF-β1, -β2 and -β3, and wherever possible, we refer to other members of the superfamily.

TGF-β is secreted as an inactive latent disulfide-linked homodimeric polypeptide that is bound to other extracellular proteins such as latent TGF-β binding proteins (LTBPs) that tether the ligand in the extracellular matrix (reviewed in [18,19]). The mature, bioactive ligand that consists of the processed C-terminal homodimeric polypeptide is produced upon proteolytic cleavage of the latent complex. Recently, it has been postulated that interactions between the latent TGF-β with thrombospondin or integrin receptors might possibly exert direct physical forces on the latent complex in order to release mature TGF-β [18].

The mature ligand binds directly to the protein core of a transmembrane proteoglycan receptor, β-glycan or TGF-β type III receptor (reviewed in [16,20]). β-glycan is not expressed in all cell types, thus revealing that the function of this co-receptor is not absolutely necessary for TGF-β signaling, and at least in endothelial cells, β-glycan function seems to be replaced by a related transmembrane glycoprotein named endoglin (reviewed in [21]). These co-receptors form complexes with the signaling receptor serine/threonine kinases and mediate ligand presentation to them (Fig. 1). Two related receptors transmit biological signals, the TGF-β type II receptor (TβRII) and TGF-β type I receptor (TβRI), the latter also named activin receptor-like kinase 5 (ALK5) (reviewed in [16,20]). TβRII and TβRI form hetero-tetrameric complexes of two identical TβRII/TβRI receptor heterodimers, bound to dimeric TGF-β. In the receptor complex, upon TGF-β binding, each receptor subunit reorients along the transmembrane domain axis in a manner that the intracellular kinase domains of TβRII face the juxtamembrane segments of the corresponding TβRI within each pair of heteromeric receptors [22–24]. The end result is trans-phosphorylation of specific serine and threonine residues in the juxtamembrane segment of TβRI by the TβRII kinase (Fig. 1). Receptor trans-phosphorylation results in a conformational change of TβRI so that its dormant catalytic center can be activated (reviewed in [20]). This model of TGF-β receptor signaling seems to apply to most other cases of receptor serine-threonine kinases that transmit signals by various other ligands of the large TGF-β superfamily [16]. However, in endothelial and possibly in other cell types, a more complex scenario takes place (reviewed in [21]). Accordingly, endothelial cells express the accessory receptor endoglin, TβRII, TβRI and a fourth receptor, the type I receptor named ALK1. Thus, depending on signaling inputs from other pathways that define the proliferation and differentiation state of endothelial cells, this cell type presents dual signaling pathways downstream of TGF-β [25]. TβRII activates TβRI, which then elicits endothelial cell cycle arrest by activating the TGF-β branch of Smad signaling (i.e. Smad2 and Smad3, see below). Alternatively, endoglin promotes signaling of TβRII to TβRI and then TβRI switches on ALK1 activity within the hetero-tetrameric complex, while ALK1 switches off TβRI, leading to induction of endothelial proliferation [26,27]. ALK1, in contrast to TβRI, activates the so-called BMP branch of Smad signaling (i.e., Smad1, Smad5 and Smad8) [16]. Recent evidence points to a possible new ligand for the ALK1 receptor, namely the bone morphogenetic protein 9 (BMP-9) [28]. This will possibly demand re-evaluation of the above signaling model of endothelial cells, as for example it is formally possible that TβRI activates rapid BMP-9 signaling via ALK1, thus making the activation of ALK1 by TGF-β indirect. Future research in the mechanisms of TGF-β receptor assembly and regulation is amply warranted to examine such signaling scenarios in the context of not only normal but also malignant cells.

1.3.2. Smad signaling

The activated TβRI phosphorylates the intracellular effector proteins Smads, which constitute a small, evolutionarily conserved family of signal transducers (Fig. 1) (reviewed in [17]). Smads are modular proteins with conserved N-terminal Mad-homology 1 (MH1), intermediate linker and C-terminal MH2 domains. The MH1 domain participates in nuclear localization, DNA-binding and protein–protein interactions. The linker domain accepts regulatory phosphorylations by other signaling kinases (e.g. mitogen activated protein kinases (MAPKs) or cyclin-dependent kinases (CDKs)), and recruits ubiquitin ligases that regulate Smad and TGF-β receptor half-life. The MH2 domain is a major protein–protein interaction domain, including phospho-serine-binding activity. The catalytically active TβRI phosphorylates the C-terminal serine residues of a subclass of Smads named R-Smads (receptor-activated Smads), which are Smad2 and Smad3, two distinct proteins that play non-redundant functions in eliciting the biological effects of TGF-β. Receptor-phosphorylated R-Smads exhibit high affinity for Smad4, also termed Co-Smad, which is not phosphorylated by receptors but rapidly oligomerizes with

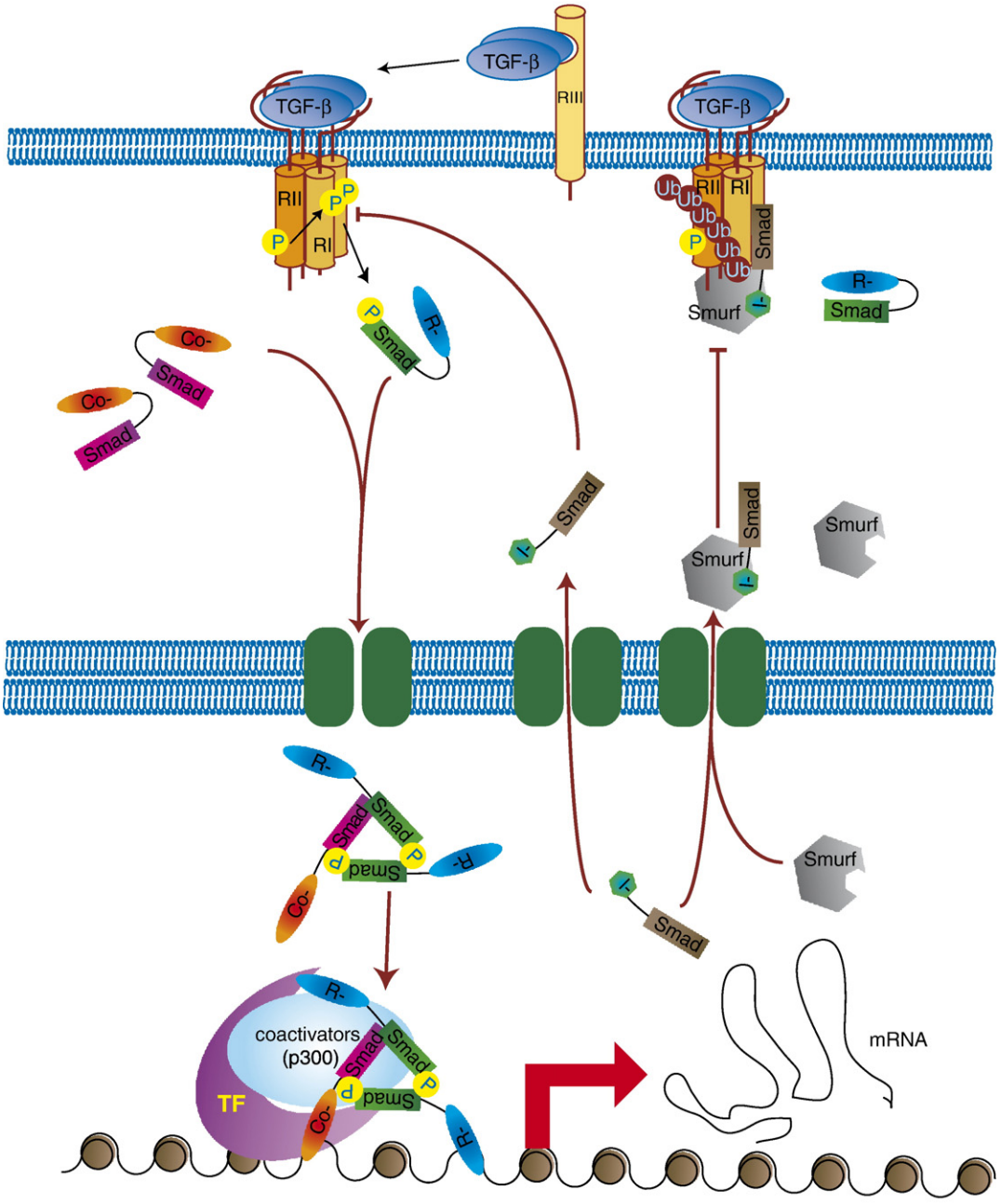


Fig. 1. TGF-β signaling by Smads. TGF-β bound to β-glycan on the cell surface is presented to the signaling TβRII/TβRI heterotetrameric receptor complex. TβRII trans-phosphorylates TβRI, which then phosphorylates R-Smads that move to the nucleus and form complexes with the Co-Smad. The heterotrimeric Smad complex binds to DNA and interacts with transcriptional co-factors (TF) and co-activators to regulate transcription. I-Smads and Smurfs reside in the nucleus and are exported in response to TGF-β signaling, to form complexes with each other and with the receptor, leading to receptor ubiquitylation (Ub) and endocytosis followed by lysosomal degradation (not shown). The I-Smad also inhibits R-Smad phosphorylation by the receptor complex.

phosphorylated Smad2 and Smad3, thus forming functional trimeric protein complexes (Fig. 1). All monomeric Smad proteins constantly shuttle in and out of the nucleus, but formation of the receptor-activated R-Smad/Co-Smad complexes favors their nuclear accumulation [29]. In the nucleus, the active R-Smad/Co-Smad complexes bind directly to DNA (Smad-binding elements) and associate with a plethora of transcription factors, co-activators or co-repressors, thus leading to transcriptional induction or repression of a diverse array of genes (roughly 500 in mammalian cells) (reviewed in [16,17]).

Within this basic signal transduction pathway, a negative regulatory feedback loop is intricately built that involves primarily three types of proteins: inhibitory Smads (I-Smads), ubiquitin ligases of the Smurf family and phosphatases (reviewed in [17]). TGF-β/Smad signaling rapidly induces I-Smad (e.g. Smad7) and Smurf (e.g. Smurf1 and Smurf2) gene expression. Smad7 is then recruited to the TGF-β receptor complex and competitively inhibits the phosphorylation of R-Smads by TβRI (Fig. 1). Smad7 also recruits phosphatases that dephosphorylate and inactivate the receptor complex. Finally,

Smad7 directly binds and activates the catalytic activity of Smurf ubiquitin ligases, leading to ubiquitylation of TβRI (Fig. 1), and thus promoting endocytosis and final lysosomal degradation of the receptor–ligand complex (reviewed in [30]). Finally, nuclear phosphatases such as PPM1A dephosphorylate the C-terminal tails of R-Smads and lead to disassembly of the transcriptionally active R-Smad/Co-Smad, initiating a molecular cascade for termination of the transcriptional Smad signal [31,32]. Despite the importance of these negative regulators of TGF-β/Smad signaling, the exact sequence of molecular events and the full details of the regulatory processes present significant gaps that await future elucidation.

1.3.3. Alternative signaling inputs

In addition to activation of the Smad pathway, TGF-β mobilizes the activity of several other intracellular pathways, including MAPKs, phosphoinositide 3′ kinase (PI3K), small GTPases of the Ras superfamily and more (reviewed in [33]). Such alternative signal transducers often regulate the Smad pathway itself and mediate signal transduction by various other growth or morphogenetic factors. In conclusion, TGF-β transmits biological signals to normal or cancer cells via the central Smad pathway and via alternative signaling proteins that regulate the quantitative output of the pathway, and offer nodal points for crosstalk with other signal transduction pathways that govern the complex life of cells. Understanding the critical steps of the TGF-β pathway that become perturbed during tumorigenesis essentially amounts to explaining complex but defined signaling networks, whose activity cannot be properly regulated at critical stages of adult tissue homeostasis.

2. TGF-β as tumor suppressor

2.1. Human cancer mutations

Genetic studies have identified a plethora of mutations in the genes encoding for the two receptors and the three Smads that mediate TGF-β signaling (reviewed in [10]). All reported tumor mutations in the TGF-β pathway target the receptors and Smads, however, misregulation of the ligand is abundant in human cancer (see below).

2.1.1. Receptor mutations

The TβRII gene contains characteristic short nucleotide repeats (poly-adenine tract) in its kinase-coding portion, that makes it a hot-spot in human cancers that exhibit DNA replication errors, also known as microsatellite instability. This phenomenon leads to the generation of mutant receptor transcripts (e.g., frameshifts after single nucleotide deletion) and loss of expression of the functional receptor [34–36]. This type of mutation occurs late during colon tumorigenesis, at the stage of adenoma to carcinoma transition [37], and is also abundant in gastric, pancreatic, biliary tract, lung and brain (glioma) tumors (reviewed in [10]). The activin type II receptor (AcvRII) undergoes similar replication error-dependent mutations of a poly-adenine tract in gastrointestinal tumors and in one case of a non-microsatellite unstable pancreatic cancer [38].

In head-and-neck tumors, G–C transversions either inactivate the TβRII kinase activity, thus blocking the ligand-dependent activation step of TβRI, or constitutively activate this kinase, also leading to aberrant signaling [39,40]. In T-cell lymphomas, a different point mutation in the TβRII kinase domain dominantly inhibits the function of the wild type receptor encoded by the second, non-mutated chromosomal copy [39,40]. Advanced, recurrent breast cancers, but not primary breast tumors, accumulate point mutations in the kinase domain of TβRII [41]. Comparable mutations in the kinase domain or the poly-adenine tract of TβRII have been described in ovarian and lung cancers, and, interestingly, in a significant proportion of microsatellite stable colon cancers [42–44]. A common feature of all studied genetic lesions of TβRII is that such tumors express very low or even undetectable levels of this receptor and thus, even in cases where no specific mutational defect can be mapped on TβRII, loss of its expression becomes a major mechanism by which tumor cells acquire resistance to the TGF-β tumor suppressive effects (see discussion on epigenetic control).

The next component in the signal transduction cascade, *TβRI/ALK5*, can also be mutated in human tumors, as for example pancreatic and biliary cancers suffer from homozygous deletions of the gene [45]. Frameshift or missense mutations in TβRI have been described in metastatic head-and-neck tumors and in ovarian cancers [44,46]. On the other hand, metastatic breast and head-and-neck cancers accumulate a point mutation in the conserved TβRI kinase domain, leading to suboptimal TGF-β signaling during lymph node metastasis [46,47]. However, the prevalence of such a TβRI-dependent mechanism during breast, lung and colon cancer metastasis has been challenged [48]. In addition to TβRI, the activin type I receptor (*AcvRI/ALK4*) gene can be point mutated and inactivated in pancreatic cancers [49]. Thus, at least in the pancreas, both type II and type I receptors for activin can be mutationally inactivated. The only receptor of the BMP branch of this superfamily of cytokines that has been genetically linked to human cancer is the type I receptor BMPRIA/ALK3. ALK3 accumulates germline nonsense mutations that are transmitted to children that inherit juvenile polyposis (JPS), Cowden and Bannayan–Riley–Ruvalcaba familial syndromes [50,51]. These patients develop gastrointestinal hamartomas, benign colonic epithelial polyps that predispose to gastrointestinal cancer. Despite the lack of mutational data, the second BMP-specific type I receptor, BMPRIB/ALK6 mediates growth suppression of prostate cancer cells by BMP-7 [52]. When a constitutively active form of this receptor is introduced into such tumor cells, upon xenografting to nude mice, results in significant reduction of tumor size. These experiments demonstrate a potential tumor suppressor role for BMP/ALK6 signaling in prostate cancer and possibly relate to the findings that link BMP family members to the process of prostate tumor progression.

2.1.2. Smad mutations

The *Smad2* gene maps on chromosome 18q21, in a genetic locus that exhibits frequent loss of heterozygosity, and which is closely linked to two other tumor suppressors, Smad4 and DCC, but also to the *Smad7* gene [53]. A number of inactivating missense or nonsense point mutations have been mapped primarily in the MH2 domain and less frequently in the MH1 domain of Smad2 in colorectal, ovarian, cervical, lung and liver cancers [44,53–55]. The mechanism of inhibition of TGF-β signaling by cancer mutations in Smad2 varies: some create truncated MH2 domains that cannot be phosphorylated by the receptor, others mutate the critical L3 loop of the MH2 domain that allows docking of Smad2 to TβRI, yet others inhibit the process of Smad2 dissociation from the receptor upon phosphorylation [53,55,56]. A single Smad2 MH2 domain point mutant, first identified in colorectal cancers, inhibits Smad2 phosphorylation but additionally inactivates the transcriptional activity of both Smad2 and Smad3 by yet unknown nuclear mechanisms [57]. Alternatively, many MH1 and MH2 domain point mutants result in protein instability, thus creating rapidly degrading Smad2 variants that cannot transmit proper signals [58,59]. In all cases, loss of Smad2 function appears sufficient in inhibiting the physiological TGF-β signaling pathway.

In contrast to Smad2, the second R-Smad of the TGF-β pathway, Smad3 has not been found to be mutated in colon, breast, pancreatic, lung, ovarian, parathyroid or hematological cancers, neither in JPS [60–65]. All these studies conclude that Smad3 may suffer loss of heterozygosity or it can exhibit characteristic DNA polymorphisms, which are silent at the protein level. In one report on gastric carcinoma cells, Smad3 expression was severely downregulated, and reconstitution of wild type Smad3 could rescue the growth suppressive response of these cells to TGF-β in vitro [66]. In pediatric T-cell acute lymphoblastic leukemia (ALL), Smad3 protein expression is also absent, while no genetic mutations can be detected in the *Smad3* gene and its mRNA expression is normal [67]. However, at this moment, no specific mutational or epigenetic mechanism exists that explains how *Smad3* gene expression can be silenced and thus contribute to resistance to TGF-β signaling by the tumor cells. This is curious as mechanistic experiments in vitro and in vivo suggest Smad3 as a major mediator of anti-proliferative effects of TGF-β (see below). If the above conclusion stands true, it is possible that TGF-β-mediated growth suppression is not a primary mechanism that explains the tumor suppressor action of TGF-β.

The most frequently mutated Smad gene in human cancer is *Smad4*, which was originally discovered as a novel tumor suppressor gene of chromosome 18q21.1 that is deleted in pancreatic cancers (DPC4) with relatively high frequency (48%) [68]. In addition to pancreatic tumors, Smad4 deletions and intragenic mutations have since been mapped in hepatocellular, breast, bladder, biliary tract and ovarian carcinomas, intestinal, colorectal and lung cancers, and tumors of prostate and cervical origin, albeit with lower frequencies compared to tumors of pancreatic origin [10,54,69–72]. The molecular mechanisms by which *Smad4* mutations, other than complete deletions of the gene, alter the flow of TGF-β signaling have been studied quite extensively. The majority of MH2 missense mutations and short C-terminal truncations of Smad4 lead to defective homo-oligomerization or hetero-oligomerization with phosphorylated R-Smads, as analyzed at the atomic level by crystallography and molecular modeling [73–75]. Furthermore, as discussed for Smad2, MH1 and MH2 domain missense mutations and C-terminal truncations lead to Smad4 protein instability and proteasomal degradation that is catalyzed by the ubiquitin ligase SCF^{Skp2} [59,76–78].

Based on its tumor suppressor role, *Smad4* can be used as a marker gene for prediction of colon cancer progression, thus offering the possibility to define better chemotherapeutic protocols [79]. Smad4 accumulates primarily intragenic mutations in epithelial cells of colorectal cancer patients, and in JPS, such germline mutations often lead to C-terminal truncation of the Smad4 protein [80–82]. Interestingly, JPS patients with mutations in Smad4 exhibit a more expanded and massive form of gastric polyposis compared to patients with mutations in the *BMPR1A* gene discussed above or in other unidentified genes, suggesting a more aggressive phenotype resulting from loss-of-function of Smad4 [82]. Furthermore, a rare set of patients that exhibit the combined syndromes of JPS (previously linked to Smad4 or BMPRIA) and of hereditary hemorrhagic telangiectasia (HHT), a vascular disorder with causal links to the genes of the co-receptor *endoglin* or the type I receptor *ALK1*, were found to carry only mutations in Smad4 [83]. This finding suggested the need for more sophisticated medical screening of JPS patients as they might also develop defects in their vasculature. Studies of colorectal cancers from various stages of malignancy, including metastases to liver, have demonstrated that the Smad4 mutations are selected at late stages of carcinogenesis and their frequency increases as tumors progress towards metastasis, whereas early stage adenomas almost never accumulate mutations in Smad4, again arguing that the TGF-β pathway is not a classic initiator of tumorigenesis [84]. Similarly, loss of Smad4 expression during pancreatic tumorigenesis is clear at advanced tumor stages of in situ carcinomas but not at earlier stages [85]. Despite that, a careful genetic, molecular and cytogenetic analysis of *Smad4* mutagenesis and expression in colorectal cancers has suggested a model whereby this gene is selectively inactivated after the onset of the microsatellite instability pathway but clearly prior to the onset of chromosomal instability and aneuploidy, suggesting a more intermediate stage in colorectal tumor progression as the sensitive step to loss of Smad4 function [86].

R-Smads of the BMP signaling pathways, Smad1, Smad5 and Smad8, have also been proposed to mediate signals by TGF-β, at least in breast cancer and normal endothelial cells [25,87]. Accordingly, these three Smad genes have been screened for loss or gain-of-function mutations in various human cancers. Several such screens have not identified any mutations in the *Smad1* gene, while its expression is severely repressed in cervical carcinomas and inversely, Smad1 is

overexpressed in follicular lymphoma [63,88,89]. Similarly, the *Smad5* gene was not found to be mutated in several gastric and a large set of human tumor cell lines, but its expression was found elevated in hepatocellular carcinomas [90,91]. Due to its chromosomal location on 5q31.1, a locus that is frequently deleted in myelodysplastic syndromes and acute myeloid leukemia (AML), the *Smad5* gene has been proposed as a candidate tumor suppressor. However, mutational analysis in leukemic patients could only reveal hemizygosity but no loss-of-function mutations, while a spliced variant of Smad5 that lacks its normal C-terminal sequence was found constitutively expressed in leukemic but also hematopoietic stem cells [92–94]. This truncated Smad5 fails to signal properly or form functional complexes with Smad4. Smad8 shows similar behavior as the only known cases so far indicate decreased or complete loss of its expression in various cancers, including breast, prostate and colorectal cancers [95,96]. Thus, no evidence for clear tumor suppressor function of the BMP-specific R-Smads has emerged to this date.

The inhibitory Smad6 and Smad7 attenuate normal TGF-β or BMP signaling, and accordingly the prediction has been that these proteins might be overexpressed in human tumors that acquire resistance to TGF-β-mediated growth inhibitory responses. This prediction has been proven in some cancer cases, while in many others, no obvious alterations in *I-Smad* gene structure or expression have been found. Thus, failure to identify I-Smad mutations or aberrations in expression has been reported in hepatocellular, ovarian or certain colorectal carcinomas [97–99]. On the other hand, Smad7 overexpression has been found in malignant follicular thyroid carcinomas, endometrial and another cohort of colorectal cancers, and in gastric tumors or in the gastric mucosa of *Helicobacter pylori*-positive patients [100–104]. Both Smad6 and Smad7 seem to be overexpressed in pancreatic cancer and in squamous cell carcinomas of the esophagous [105–107]. In the cases of esophageal, gastric and colorectal carcinomas, I-Smad overexpression was suggested to have prognostic significance as the degree of I-Smad expression correlated with poor survival rate of the patients and more aggressive and invasive tumor behavior [100,102,107]. Such tumor studies have revealed that Smad7, in addition to acting as a general inhibitor of TGF-β receptor-Smad signaling, it could promote tumor growth by inactivating the retinoblastoma protein, which leads to E2F-mediated transcriptional derepression in colon cancer cells, while Smad7 could also induce expression of the *thioredoxin* gene, leading to accelerated growth and resistance to apoptosis of pancreatic carcinoma cells [108,109]. Despite these observations, the role of inhibitory Smads and especially of Smad7 remains rather obscure and is possibly complex, as we discuss again later in the metastasis sections.

In conclusion, the genetic evidence from human tumors, so far supports a clear role of the two TGF-β receptors, of Smad4 and less frequently of Smad2 as tumor suppressors implicated in the progression of several types of human cancers. The rest of the signaling components may be selectively misregulated during tumorigenesis, but their action as tumor suppressors cannot be supported by current genetic evidence.

2.2. Epigenetic control of receptor-Smad expression

Many human tumors often downregulate or overexpress specific components of the TGF-β signaling pathway, which results in loss of the tumor suppressor action of the pathway. The accessory receptor β-glycan is silenced in various tumors such as breast cancer, and this leads to relative but not absolute insensitivity to the growth inhibitory action of TGF-β [110]. Thus, restoration of high levels of β-glycan in breast cancer cells leads to restoration of an autocrine TGF-β pathway that partially suppresses tumor cell growth. In a similar manner, renal cell carcinomas lose expression of β-glycan at earlier stages of carcinogenesis, and when they become metastatic, they also lose TβRII expression [111]. Isolation of renal carcinoma cell lines that represent the various stages of tumorigenesis and reconstitution of the expression of β-glycan, TβRII or both together indicated that only the combined presence of β-glycan and TβRII could lead to significant suppression of the transformed cell phenotype.

The best mechanistic examples of epigenetic control of TGF-β pathway components come from studies on the loss of expression of the two signaling receptors, TβRII and TβRI. Squamous cell carcinomas harbor point mutations in the *TβRII* promoter region, leading to significant inhibition of its transcription [112]. In pancreatic carcinoma cells, defective TβRII expression correlates with decreased activity of the transcription factor Sp1 that binds to and regulates TβRII transcription [113]. In this case, the DNA methyl transferase inhibitor 5′-aza-2′-cytidine enhances Sp1-mediated transcription and restores TβRII expression and growth suppression by TGF-β. This study emphasizes the role of DNA methylation in the control of TβRII expression, a fact that is corroborated by additional studies in lung carcinomas, which also exhibit TβRII silencing due to methylation of the CpG islands of this promoter and also due to enhanced histone deacetylation at the same regulatory regions of the *TβRII* gene [114,115]. Similar to TβRII, hypermethylation of CpG islands in the *TβRI* promoter results in suppression of its expression in gastric carcinomas, thus revealing a common mechanistic theme in repression of TGF-β signaling in human tumors [116].

The evidence for misregulation of Smad gene expression has been summarized above. Recent studies that are based on large scale tissue microarray technology have confirmed the generality of the importance of Smad level regulation in human tumors such as breast and head-and-neck carcinomas [117,118]. However, in contrast to TGF-β receptors, no specific mechanism for misregulation of Smad gene expression has yet been presented. This is largely because analysis of the regulatory sequences of the various Smad genes has not yet been performed in a systematic manner. Despite that, it is worth mentioning that for example Smad3, for which the genetic evidence for a clear tumor suppressor role is absent, is essentially not expressed by choriocarcinomas, thus contributing to their

relative insensitivity to TGF-β [119]. The clearest case of epigenetic regulation of a Smad gene though comes from *Smad8*, the BMP-specific R-Smad, whose expression is lost in a significant proportion of breast and colon cancers due to DNA hypermethylation of *Smad8* regulatory sequences [95]. This example highlights that much more remains to be discovered about the role of Smad signaling during tumorigenesis, as Smad8 is the least studied member of this family. The specific contribution of epigenetic control of this pathway clearly deserves further attention and promises improvement of our understanding of the tumor suppressor role of TGF-β.

2.3. Mouse models of tumorigenesis that demonstrate tumor suppressor activity

Although genetic and molecular studies in human cancer patients are most important for the establishment of a new tumor suppressor gene, complementary evidence from mouse models makes the findings more convincing and further contributes to the understanding of molecular mechanisms. Several mouse models have been created over the past 10 years in the TGF-β signaling pathway, and many of these models address the question of the tumor suppressor action of this pathway.

2.3.1. TGF-β models

Transgenic mice with targeted expression of TGF-β1 in their mammary gland due to a mouse mammary tumor virus (MMTV) enhancer-promoter that drives expression of the transgene, exhibit hypo-proliferation and poor development of their mammary ducts, and at the same time fail to develop breast tumors [120]. Interestingly, when these mice are crossed with another mouse model that expresses TGF-α under the control of MMTV in the breast, and which develops mammary hyperplasia and carcinomas, the bigenic mouse shows suppression of mammary carcinoma development. Further challenging of the mice with chemical carcinogens such as 7,12-dimethylbenz[α] anthracene, which induces potently mammary carcinoma formation in normal mice, proves that the TGF-α/TGF-β1 mice develop significant resistance to carcinogenesis induced by this chemical, thus enforcing the notion that ectopic expression of TGF-β1 in the breast acts as a tumor suppressor and protects mice from mammary tumor development. The inverse experiment is also illustrative as heterozygote knockout mice for TGF-β1 express only 10–30% of the normal levels of TGF-β1 in various tissues, and are essentially normal. However, such mice exhibit mild cellular hyperproliferation in their lungs and livers, and when challenged with chemical carcinogens develop more frequent and bigger tumors compared to wild type mice [121]. These experiments proved that TGF-β1 protects against tumorigenesis and a single copy of its gene is not sufficient to confer the full protective effect. More recent studies in the above two mouse models with an emphasis on estrogen receptor α (ERα)-positive mammary epithelial cells, support the previous findings and further suggest that lowering the dose of TGF-β1 in the mouse leads to an increase in the population of ERα-positive epithelial cells that exhibit high proliferative index [122]. Conversely, when the dose of TGF-β1 was increased in the mammary gland, the ERα-positive epithelial population decreased significantly. Thus, escape of ERα-positive mammary cells from the negative control of TGF-β contributes to breast carcinogenesis.

2.3.2. TGF-β receptor models

The same as above conclusions have been derived from transgenic mouse models where TβRII signaling has been perturbed using a dominant-negative TβRII construct that pairs with the normal receptor and blocks physiological signaling in vitro in cell lines but also in vivo. Accordingly, mice expressing the dominant negative TβRII under the control of MMTV exhibit high transgene expression in the breast and lower in the lung and a few other organs [123]. Branching of mammary alveoli in these mice is significantly enhanced, and no signs of spontaneous tumorigenesis were detected. However, upon challenge with 7,12-dimethylbenz[α]anthracene, the incidence, numbers and size of developing tumors increased dramatically in the breast and to a lesser extent in the lung. These results were confirmed in an independent MMTV-dominant negative TβRII mouse model, and when these mice were crossed with the MMTV-TGF-α model, inhibition of endogenous TGF-β signaling effectively decreased the latency of mammary carcinoma development, followed by dramatic suppression of tumor cell invasion [124]. A similar conclusion is derived in a mammary epithelium-specific knockout of the *TβRII* gene, as such mice develop alveolar hyperplasia, and when crossed with an MMTV-polyoma virus middle T antigen mouse, the middle T-driven mammary tumors are more aggressive and metastasize to lung with an increased rate [125]. Models demonstrating the tumor suppressor effect of TGF-β are not limited only to the breast and lung, but additionally the dominant negative TβRII or the conditional knockout strategy for this gene have been used in a rat prostate pre-malignant cell line and in colonic epithelium respectively, and in both cases enhancement of tumorigenesis could be observed [126,127]. Thus, in vivo mouse models amply corroborate the genetic data from human cancers, supporting a role of TβRII as a tumor suppressor gene.

2.3.3. Smad models

The first knockout model for Smad3 reported significant frequency of colon cancer development with lymph node metastasis, and suggested strongly the role of Smad3 as a tumor suppressor, despite weak evidence on this direction from human studies [128]. This result has been challenged by the generation of two additional Smad3 knockout mice, which did not reveal any spontaneous tumor development in addition to the immunological or mild limb malformation phenotypes observed [129,130]. Furthermore, one of the Smad3 knockout models and its heterozygote littermate were exposed to a classic protocol for skin carcinogenesis, showing reduced formation of papillomas and no evidence for squamous cell carcinomas, which appeared to be dependent on the dose of the *Smad3* gene [131]. The latter study emphasizes a role of Smad3 in mediating the effects of TGF-β not so much in the tumor cell per se but more in the infiltrating macrophages, for which TGF-β acts as a potent chemo-attractant. The three distinct phenotypes of these

independent Smad3 knockout models have been attributed to the different genetic background of the mouse strains used. Despite that, the first Smad3 knockout mouse remains an interesting model for the study of colorectal cancer.

The *Smad2*, *Smad4* and *Smad7* genes reside on chromosome 18 in humans and on a syntenic stretch of chromosome 18 of the mouse, and in addition the adenomatous polyposis coli (APC) tumor suppressor is closely linked on the same mouse chromosome, thus allowing the generation of compound heterozygous mice for mutations in the *APC* and *Smad2* genes [132]. Such compound heterozygotes in the cis configuration give rise to intestinal polyps and invasive adenocarcinomas, which are causally related to the APC mutation only, as the result is identical to single APC mutant heterozygote animals. This result suggests that in the mouse, loss of heterozygosity in the Smad2 locus does not contribute significantly to colon cancer development. In a second study of the compound heterozygote APC/Smad2 knockout mouse model, the same general result was found, confirming that Smad2 does not contribute to the initiation process of colorectal tumorigenesis [133]. However, the latter study reported abnormally large tumors with multiple invasions that were clearly different from the smaller and less invasive tumors generated by the APC heterozygote alone.

These results with the compound APC/Smad2 mutant are drastically different from the similar compound heterozygote mouse model for APC and Smad4 [134]. In these mice, the intestinal polyps were much more aggressive compared to the APC mutant alone or the APC/Smad2 double mutant, and support the conclusion that loss of heterozygosity of the Smad4 locus contributes more essentially to the development of colorectal cancer compared to loss of the Smad2 locus. The constitutive Smad4 knockout mouse dies from early embryonic defects, however, the apparently normal heterozygote, upon aging (more than 1 year) develops polyps in the stomach and duodenum, and the tumor epithelial cells suffer from loss of heterozygosity [135]. This mouse model best resembles human JPS. Furthermore, conditional inactivation of Smad4 in the mammary gland has no apparent effects on normal mammary gland development; however, mutant mammary epithelial cells were hyperproliferative and changed their differentiation towards a squamous epithelial phenotype [136]. The net result was development of squamous cell carcinomas, in which the β-catenin levels increased dramatically, indicating that normal TGF-β/Smad4 signaling induces degradation of β-catenin and thus promotes EMT (see Section 3.3.1). We therefore conclude that mouse models support a strong tumor suppressor role for Smad4 and less for Smad2.

Similar to the human studies for expression levels of various Smad proteins, analysis of Smad expression in animal models supports the general conclusion that specific Smad expression is altered during tumorigenesis in a tissue-specific manner. For example, in a rat model for prostate cancer, Smad2, 3, 4, 6 and Smad7 protein levels were found rather elevated in tumor areas that associated with apoptosis, suggesting an overall activation of the levels and of signaling by TGF-β in the prostate cancer model [137]. In the chemical carcinogenesis model of mouse skin, Smad1, 2, 3, 4 and Smad5 protein levels were found to be significantly reduced in the carcinoma cells, while Smad7 levels increased dramatically, thus supporting the general attenuation of endogenous TGF-β signaling [138]. Interestingly, Smad7 induction in a mouse model of gastric cancer with aberrant STAT3 signaling, leads to inhibition of endogenous TGF-β signaling, which is critical for the formation and survival of gastric polyps that eventually develop to gastric adenomas [139]. This model provides a good example of crosstalk between cytokine-STAT and TGF-β signaling via the negative regulator of the latter pathway, Smad7. Consistent with a pro-tumorigenic action of Smad7, a transgenic mouse model with pancreas-specific expression of Smad7 develops pancreatic intraepithelial neoplasia (PanIN), the precursor stage to pancreatic carcinoma [140]. The premalignant lesions of the pancreas showed characteristic overproliferation of the epithelial cells and increased fibrosis around the lesions, which are strongly indicative of loss of TGF-β signaling efficacy in this tissue. The same conclusion was derived from studies of pancreatic carcinoma and colon adenocarcinoma cell lines stably transfected with Smad7, which lost their ability to be growth inhibited by TGF-β and showed enhanced anchorage-independent growth in vitro [105,141]. Upon xenografting into nude mice, the Smad7-expressing cells gave rise to tumors with increased growth compared to control carcinoma cells. On the other hand, ectopic expression of Smad7 in highly malignant and metastatic melanoma cells that exhibit relative resistance to the growth inhibitory action of TGF-β and constitutive Smad signaling, leads to inhibition of melanoma cell invasiveness and anchorage-independent colony growth in vitro, and significant reduction in tumor formation in xenografted nude mice [142]. The latter supports fully the general concept, as stated from the beginning in this review, that TGF-β actions are context and tissue-dependent.

2.4. Cellular mechanisms of tumor suppressor action

2.4.1. The cytostatic program

2.4.1.1. Cell cycle arrest at the G1 phase. TGF-β arrests the cell cycle of epithelial, endothelial and hematopoietic cells at the early G1 phase via Smad-mediated transcriptional regulation of critical regulators of the cell cycle (Fig. 2) [143]. The first direct transcriptional target of the TGF-β pathway that explained how this cytokine inhibits proliferation of epithelial cells was c-Myc, whose expression was repressed by TGF-β [144]. At the same time, it was discovered that TGF-β maintains pRb (retinoblastoma protein) in its hypophosphorylated status during arrest of the cell cycle in early G1 phase [145]. The two processes were immediately linked as oncoproteins from DNA viruses such as HPV-16 E7, adenovirus E1A and SV-40 large T antigen were all shown to abrogate TGF-β-mediated epithelial growth arrest and c-Myc repression in a manner that depended on the ability of these proteins to block the function of pRb [146]. These findings fit well with evidence from retinoblastoma tumor cells that exhibit severe loss of TGF-β receptors and lack of growth inhibitory responses to TGF-β [147]. Today, we appreciate a rather detailed

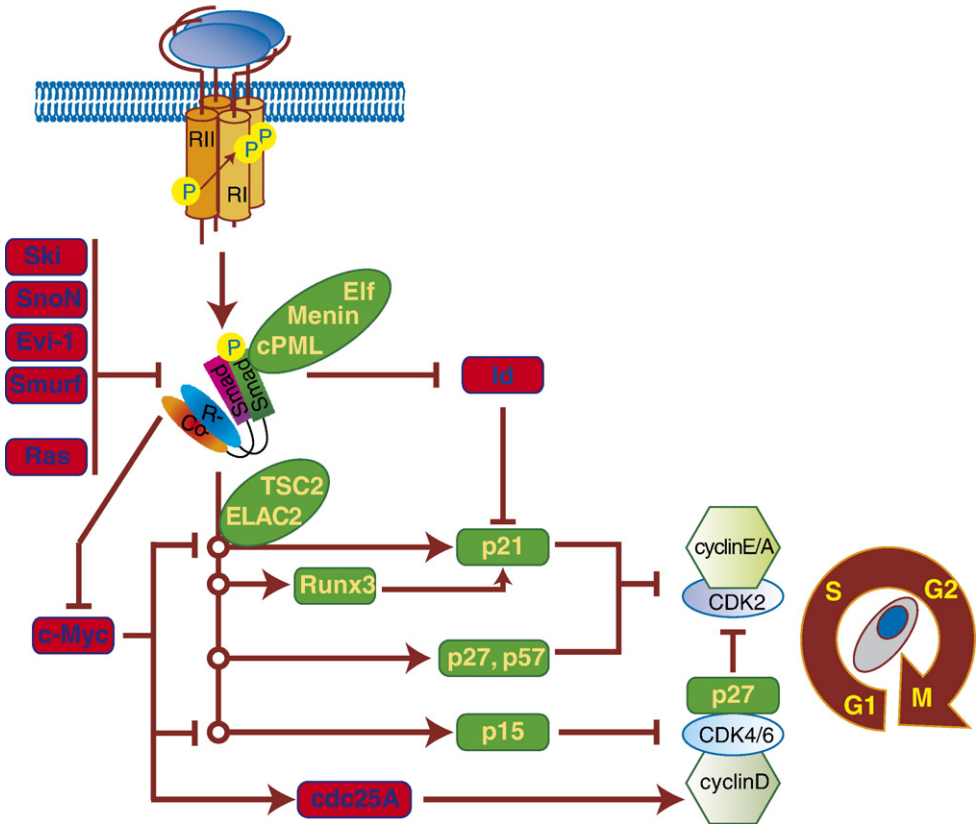


Fig. 2. The cytostatic response to TGF-β/Smad signaling. The TGF-β receptor activates Smad signaling that induces directly (circular nodes) transcription of downstream genes: *p21*, *Runx3*, *p27*, *p57* and *p15*, or represses transcription of *c-Myc* and *Id*. *Runx3* is used to propagate further *p21* response, while c-Myc represses transcriptionally *p21* and *p15* and Smad-mediated downregulation of c-Myc is required prior to *p21* and *p15* induction. c-Myc also induces expression of the phosphatase Cdc25A. Oncogenic stimuli such as Ski, SnoN, Evi-1, Smurf and Ras directly interact or post-translationally modify the Smad complex thus repressing its transcriptional activity. Tumor suppressors, Elf, menin and cPML interact with activated Smads and enhance their signaling pathway directly. Tumor suppressors, TSC2/tuberin and ELAC2 interact with activated Smads and enhance their transcriptional output towards *p21* gene regulation. The CKIs and Cdc25A directly affect the function of cyclin/CDK complexes, while p27 is displaced from the cyclin-D/CDK4/6 complexes via p15 to cyclin-E/A/CDK2 complexes, leading to arrest of the cell cycle at the early G1 phase. Oncogenic or anti-cytostatic components are shown in red ovals, while tumor suppressor or pro-cytostatic components are shown in green ovals.

mechanism of how c-Myc transcriptional repression is mediated by TGF-β. Smad3 carries from the cytoplasm to the nucleus the co-repressor protein and pRb family member p107 together with the transcription factor E2F4, and the complex is recruited together with Smad4 to a transcriptional inhibitory element on the c-Myc promoter, residing adjacent to an E2F4-binding site [148–151]. Thus, repression of a major mitogenic transcription factor is a key event in the epithelial cytostatic program of TGF-β (Fig. 2).

The TGF-β pathway directly affects components of the cell cycle machinery. Accordingly, TGF-β inhibits translation of the CDK4 mRNA [152]. The tumor suppressor p53 binds to specific sequences of the 5′ untranslated region of the CDK4 mRNA, and the mechanism by which TGF-β/Smad signaling instructs p53 to act as a translational repressor remains to be elucidated [153]. A better understood mechanism of regulation of cell cycle components by TGF-β is the transcriptional and post-translational control of cell cycle inhibitors (CKIs) of the Ink4 and Kip/Cip families (Fig. 2). The CKI $p27^{Kip1}$ is induced by TGF-β to bind to cyclinE-CDK2 complexes and inhibit them [154]. TGF-β also induces transcriptionally the expression of $p15^{Ink4B}$ [155] and of $p21^{Cip1}$ [156,157], and the scenario of CDK inhibition works as follows, at least in epithelial cells: increase of $p15^{Ink4B}$ levels leads to binding to CDK4 and CDK6, thus displacing $p27^{Kip1}$ and $p21^{Cip1}$ from these kinases, which can then associate with available CDK2 and inhibit its activity; the concomitant increase in $p21^{Cip1}$ ensures a maximal block of CDK2 activity and cell cycle arrest at the G1 to S phase transition [157]. In contrast, in hematopoietic cells, TGF-β employs the CKI $p57^{Kip2}$, whose expression is also transcriptionally induced and this CKI plays similar roles as $p21^{Cip1}$ and $p27^{Kip1}$ in arresting the cell cycle of these cells [158]. Finally, TGF-β also represses expression of the Cdc25A tyrosine phosphatase that dephosphorylates and activates G1 phase CDKs (Fig. 2) [159]. An E2F-p130 repressor complex mediates Cdc25A transcriptional repression and the involvement of Smads in this process remains unexplored. It is formally possible that regulation of Cdc25A is indirect and is mediated by the direct repression of c-Myc (Fig. 2).

2.4.1.2. Cytostatic control by Smads. Recent studies have clarified the mechanisms by which Smad signaling induces

transcription from the *p15*^{Ink4B} and *p21*^{Cip1} genes. A large complex of Smad2, Smad3 and Smad4, together with transcription factor Sp1 binds to a GC-rich proximal *p15*^{Ink4B} promoter region and activates expression of this gene [160]. Interestingly, the direct binding of c-Myc to Smad2 and Smad3 inhibits this transcriptional event on the *p15*^{Ink4B} promoter [161]. When c-Myc levels prevail in the nucleus, c-Myc pairs with the *p15*^{Ink4B} initiator element-binding protein Miz1, forming repressor complexes that block expression of this gene [162]. Conversely, upon TGF-β signaling, c-Myc levels decrease and relieve *p15*^{Ink4B} from active repression, while at the same time allowing Miz1 to cooperate with the Smad–Sp1 complex and elicit full activation of this gene [163]. A rather similar transcriptional scenario operates during induction of p21^{Cip1} by TGF-β. Smad3 and Smad4 make protein complexes with Sp1 that bind with high affinity to the proximal GC-rich promoter of this gene and elicit its transactivation [164,165]. Simultaneously, TGF-β activates protein kinase Cα (PKCα) that phosphorylates the regulatory calcium-binding protein S100C/A11, which translocates to the nucleus, binds to transcription factor Sp1 and recruits it to the GC-rich p21^{Cip1} promoter [166,167]. In addition, p53, a major transactivator of p21^{Cip1} expression, or its relatives, p63 and p73, bind to a distal enhancer element of this gene and via direct interaction with Smads, stabilize the ternary transcriptional complex and provide robust induction of its expression [168]. Similar to the action of p53, the Smad3/Smad4 complex binds directly to a distal enhancer element of the *p21*^{Cip1} gene, and this binding is stabilized by transcription factors of the FoxO family that recognize DNA elements lying adjacent to the Smad binding sites on the enhancer [169]. Thus, TGF-β signaling employs a plethora of signaling components and transcription factors, acting at both enhancer and proximal promoter sites to mediate rapid but also sustained induction of p21^{Cip1} expression. The precise role of Smad4 in the Smad complex that mediates p21^{Cip1} induction by TGF-β has been challenged. For example, in pancreatic carcinoma cells that lack the Smad4 locus, or in immortalized keratinocytes in which the normal levels of Smad4 are depleted after short interfering (si) RNA transfection, p21^{Cip1} can be transcriptionally induced by TGF-β via the action of Smad2 or Smad3 alone [170,171]. Similar to the p15^{Ink4B} mechanism of repression by elevated c-Myc levels, this proto-oncogene also binds to the *p21*^{Cip1} promoter via transcription factor Sp1 and blocks its transactivation by the incoming Smad signal [172]. Based on the combined models of c-Myc, p15^{Ink4B} and p21^{Cip1} transcriptional regulation, it is obvious that c-Myc repression is a critical prerequisite for the execution of the full cytostatic response of epithelial cells (Fig. 2).

Transcriptional induction of p21^{Cip1} is not a unique response to TGF-β, but actually all pathways of the TGF-β superfamily can potently induce expression of this CKI in mediating the cytostatic response [173]. Furthermore, TGF-β seems to additionally stabilize p21^{Cip1} protein levels post-transcriptionally via an as yet poorly understood mechanism that clearly involves the action of Smad4 [171,174]. Thus, regulation of this CKI by TGF-β appears to be a major mechanism within the cytostatic program and loss of this specific response is a common feature described in a large variety of tumor cells [175]. The critical role of the p21^{Cip1} response to TGF-β is also highlighted by the fact that K-Ras transformed colon carcinoma cells that are growth stimulated by TGF-β elicit the opposite response in terms of p21^{Cip1} regulation, whereby this CKI is downregulated at the protein level and thus permits cell growth [176].

More recently, another group of target genes of the TGF-β pathway has been implicated in the cytostatic response of epithelial cells. This is the Id family of transcriptional regulators, whose expression is repressed by TGF-β while induced by BMP family members in epithelial cells [177–179]. Transcriptional repression of Id1, Id2 and Id3 is mediated by Smad signaling (Fig. 2), and at least in the case of Id1, Smad complexes engage transcription factor ATF3 to repress this gene, while in the case of Id2, Smads utilize the c-Myc antagonists, Mad2 and Mad4 to elicit repression [177,178]. Downregulation of endogenous epithelial Id2 and Id3 by TGF-β is absolutely necessary for growth arrest to take place, and at least Id2 antagonizes the anti-proliferative effects of the CKI p21^{Cip1} in a direct manner, thus explaining why TGF-β needs to repress Id2 expression while inducing p21^{Cip1} expression [173,179].

During TGF-β-mediated cytostasis, Smads act in association with other factors some of which are tumor suppressors (e.g. p53). Another tumor suppressor co-factor is the transcription factor Runx3, whose expression is lost during gastric carcinogenesis, leading to resistance to growth inhibitory responses by TGF-β [180]. Runx3 cooperates with Smads during p21^{Cip1} transcriptional induction in stomach epithelial cells (Fig. 2), thus providing a tissue-specific mechanism of the cytostatic, p21^{Cip1}-mediated response [181]. A second tissue-specific mechanism for integrated transcriptional regulation of p21^{Cip1} during prostate epithelial cell cytostasis (Fig. 2) involves the tumor susceptibility protein ELAC2 [182]. ELAC2 acts as a scaffold protein that coordinates the Smad–Sp1 protein complex together with transcription factor FAST-1 (FoxH1) on the p21^{Cip1} enhancer-promoter, as ELAC2 interacts with all these proteins. The tumor suppressor protein tuberous sclerosis complex 2 (TSC2, tuberin) also interacts with Smad2 and Smad3 proteins and potentiates the transcriptional regulation of p21^{Cip1} and p27^{Kip1}, while repressing cyclin A expression and thus eliciting growth arrest of hematopoietic cells [183]. TSC2 is an established downstream effector of signaling by the LKB1 tumor suppressor [184]. TSC2 mediates the anti-proliferative effects of LKB1 in epithelial cells and represents an interesting nodal point of convergence between two major tumor suppressor pathways, TGF-β and LKB1. ELF (embryonic liver fodrin), a spectrin family scaffolding protein, interacts with phosphorylated Smad3 and with Smad4 and promotes their nuclear translocation, while loss of ELF in mouse knockouts results in defective TGF-β/Smad signal transduction (Fig. 2) [185]. ELF function within the TGF-β pathway seems to explain why expression of this protein is lost in various human cancers, such as colon, liver and stomach [186,187]. The promyelocytic leukemia (PML)

tumor suppressor that is mutated in acute promyelocytic leukemias (APL) localizes in the nucleus, in the so-called PML bodies but also in the cytoplasm. The cytoplasmic form of PML (cPML) directly interacts with R-Smads and promotes proper TGF-β receptor endocytosis and R-Smad phosphorylation, thus being essential for TGF-β signaling (Fig. 2) [188]. Accordingly, APL cells are resistant to TGF-β and reconstitution of wild type cPML restores TGF-β-mediated growth arrest and apoptosis in the leukemic cells.

2.4.1.3. Cytostatic control by other pathways. In addition to the above mechanisms of the cytostatic response to TGF-β that center primarily on the function of Smads, alternative pathways can be involved. The ability of Smad4-deficient epithelial cells to undergo growth arrest by TGF-β supports the action of alternative signaling mechanisms [170,171,189]. For example, Smad2 activates Erk1/2 in carcinoma cells growing in suspension cultures, while Smads bind to the regulatory subunit of the cAMP-dependent protein kinase A (PKA) and activate this enzyme independent from modulation of the cAMP levels in the cells [190,191]. These mechanisms link to the transcriptional activation of the CKI $p21^{Cip1}$ gene during inhibition of cell growth. Alternatively, the TGF-β receptor complex binds the regulatory subunit Bα of the protein phosphatase PP2A and TGF-β induces assembly of the tri-subunit (PP2A-Bα, Aβ, Cα) phosphatase and its association with p70 S6 kinase, thus dephosphorylating and inhibiting the p70 S6 kinase [192,193]. Finally, the small GTPase RhoA activates the ROCK1 kinase, which phosphorylates and inactivates the cell cycle-specific phosphatase Cdc25A in epithelial cells [194]. Thus, Cdcd25A is targeted by dual transcriptional and post-translational mechanisms by TGF-β signaling. A similar mechanism of Rho and p38 activation seems to operate during growth arrest of mammary epithelial cells in a Smad-co-dependent manner [195]. Interestingly, the Rho-based mechanism could explain why TGF-β inhibits epithelial growth while inducing fibroblast mitogenesis, at least in NIH-3T3 cells [194].

In conclusion, the status of our knowledge on the cytostatic response to TGF-β strongly implicates regulation of various genes by Smads, which together with additional effectors orchestrate a global cellular network aiming at the precise block of the cell cycle prior to the G1 restriction point.

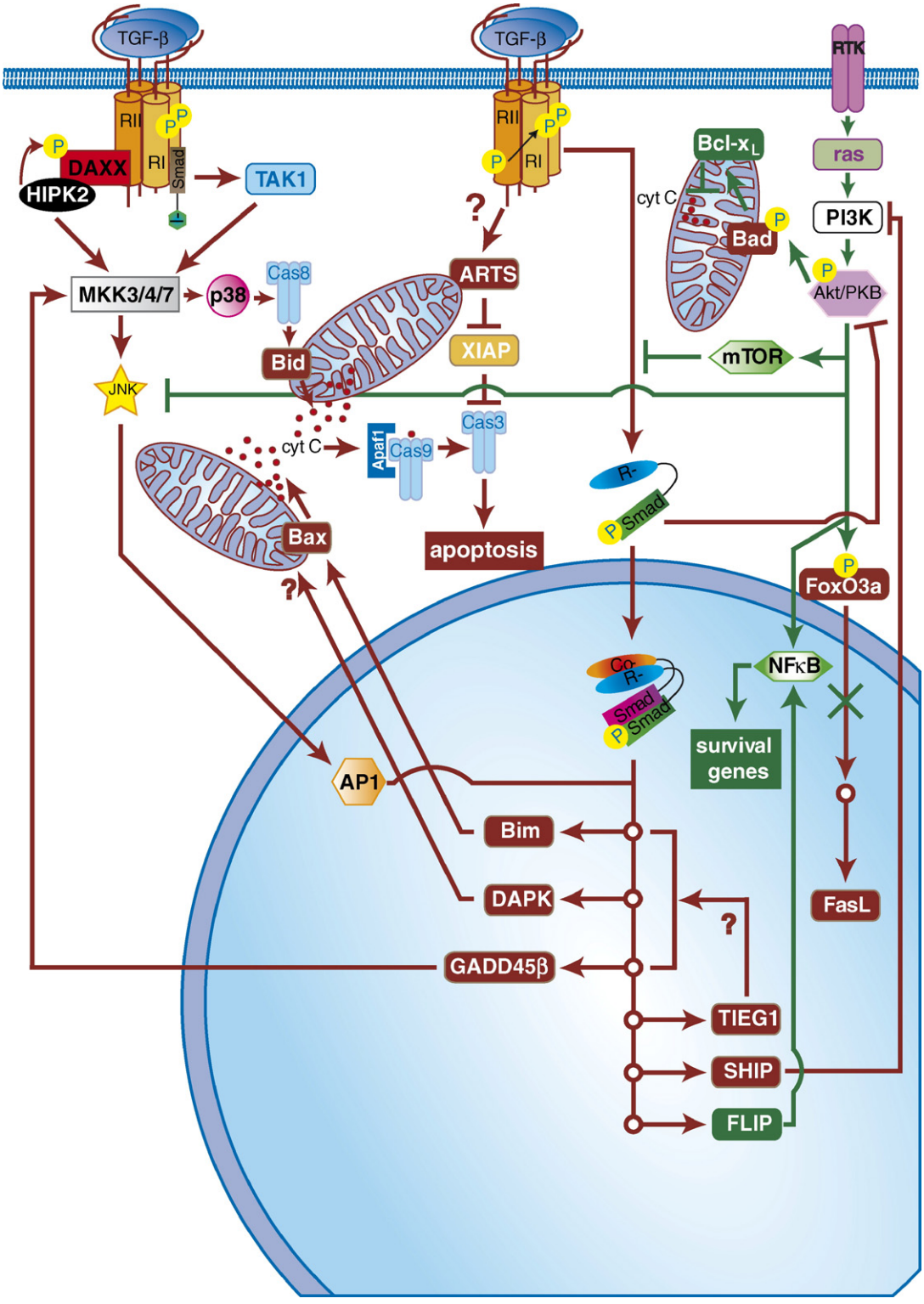
2.4.2. The apoptotic program

Inherent to the tumor suppressor action of TGF-β is its ability to induce apoptosis in a cell type-specific manner, and mutations in components of the signaling pathway are thought to contribute to resistance to pro-apoptotic responses. Interestingly, TGF-β is also known to promote cell survival under certain conditions, such as in specific neuronal populations, and even in carcinoma cells. The plethora of reports on the apoptotic responses of various cell types to TGF-β obliges us here to summarize some of the mechanisms that have been established so far.

2.4.2.1. Apoptotic control by Smads. Smad signaling regulates expression of several genes whose function directly feeds to the apoptotic machinery (Fig. 3), and in this respect the apoptotic gene program resembles the cytostatic program outlined above. Such critical gene targets of Smad signaling are the *TGF-β-inducible early response gene-1* (*TIEG1*), the signaling factor *GADD45β*, the Bcl-2 homology domain-only factor *Bim*, the *death-associated protein kinase* (*DAPK*) and the phospholipid phosphatase *SHIP*. TIEG1 is a zinc-finger transcription factor which regulates expression of other pro-apoptotic genes, although specific transcriptional mechanisms have not been yet outlined [196,197]. GADD45β is a signaling protein that interacts with and activates the mitogen-activated protein kinase kinase 4 (MKK4), which then activates the MAPK p38, leading to caspase-8 and Bad activation, thus mobilizing mitochondrial release of cytochrome C and further caspase pathways [198,199]. Transcriptional induction of the pro-apoptotic gene *Bim* also feeds to the same mitochondrial pathway as Bim activates the pro-apoptotic factor Bax that induces cytochrome C release and caspase-dependent apoptosis [200]. DAPK also acts on mitochondria by modulating the membrane action potential of these organelles, and although not clear yet, DAPK may mobilize mitochondrial cytochrome C release and caspase activation [201]. Finally, Smad-mediated

Fig. 3. The apoptotic response program. TβRII in the TGF-β receptor complex directly binds DAXX, which recruits HIPK2 and becomes phosphorylated by HIPK2, leading to activation of MKK3/4/7. The same kinases can be activated by TAK1 which is activated by Smad7 (I-Smad) bound to the receptor complex. MKKs then phosphorylate and activate JNK or p38 MAPKs. JNK activates the AP-1 transcriptional complex, leading to induction of pro-apoptotic genes (red circular nodes) in cooperation with Smads. p38 activates caspase-8 (Cas8), which activates the pro-apoptotic factor Bid, leading to cytochrome C (cyt C) release and activation of the apoptosome (cyt C/Apaf1/Caspase-9 (Cas9) complex), which activates caspase-3 (Cas3) and executes apoptosis. The TGF-β receptor complex signals by unknown mechanism (?) to mitochondrial ARTS, which inhibits XIAP, the inhibitor of caspase 3, leading to apoptosis. The activated nuclear Smad complex induces transcription of pro-apoptotic genes such as *Bim*, *DAPK*, *GADD45β*, *TIEG1*, and *SHIP*. Bim activates the pro-apoptotic Bax, which leads to cytochrome C release and caspase activation. DAPK modulates the action potential of the mitochondrial membrane and induces apoptosis via yet unknown molecular mechanisms (?). GADD45β interacts with and activates MKK4, thus activating the p38 pro-apoptotic pathway. TIEG1 is a transcription factor that regulates additional pro-apoptotic genes, but it is not clear whether these include those listed in the figure (?). SHIP inhibits PI3K. Smad3 can directly interact and inhibit the activity of Akt/PKB in addition to transactivating pro-apoptotic target genes. Activated Smads also induce expression of the pro-survival factor FLIP, which exits the nucleus and activates the transcriptional activity of NF-κB, thus inducing the expression of other anti-apoptotic factors. Growth factors signaling via receptor tyrosine kinases (RTK) activate the Ras/PI3K/Akt/PKB pathway. Akt phosphorylates the pro-apoptotic protein Bad, thus activating the anti-apoptotic protein Bcl-x_L, which blocks cytochorome C release. Akt also activates mTOR, which inhibits R-Smad phosphorylation by the TGF-β receptor complex, and directly inhibits the pro-apoptotic JNK, while activating the pro-survival NF-κB pathway. In addition to NF-κB Akt phosphorylates the pro-apoptotic transcription factor FoxO3a, leading to its cytoplasmic retention and transcriptional inactivation of its target pro-apoptotic genes such as Fas ligand (FasL). All pro-apoptotic events are shown in dark red and all pro-survival events are shown in green.

transcriptional induction of the lipid phosphatase SHIP inhibits phospholipid phosphorylation catalyzed by PI3K thus inhibiting activation of Akt/PKB, a major pro-survival kinase, which leads to induction of apoptosis of hematopoietic cells (Fig. 3) [202]. Another major mechanism that directly targets mitochondrial functions is the regulation of the mitochondrial septin family member ARTS (Fig. 3) [203]. TGF-β, via unknown signaling effectors, leads to ARTS delocalization from the mitochondrion to the nucleus. During this translocation, ARTS interacts with and inactivates XIAP, a major inhibitor of apoptosis, which results in activation of caspase 3 and apoptosis [204]. Signaling pathways, such as Ras/PI3K, which can be activated by growth factors or hormones like insulin that signal via receptor tyrosine kinases (RTK), protect cells from TGF-β



mediated apoptosis by enhancing the activity of Akt/PKB [205]. According to one model, the ratio of Smad3 protein over Akt/PKB levels defines whether an epithelial cell undergoes apoptosis in response to TGF-β, because Smad3 interacts and sequesters Akt/PKB, in a manner independent from Akt/PKB kinase activity (Fig. 3) [206,207]. According to another model, Akt/PKB signals via its downstream effector, mammalian target of Rap (mTOR), in a kinase-dependent manner, leading to inhibition of Smad3 phosphorylation by its receptor, by an as yet unresolved mechanism [208]. Alternatively, TGF-β can enhance epithelial cell survival via rapid activation of the PI3K/Akt pathway, which phosphorylates the pro-apoptotic transcription factor FoxO3a/FKHRL1 leading to its cytoplasmic sequestration and inhibiting transcriptional induction of Fas ligand (FasL) by FoxO3a [209]. Akt/PKB also phosphorylates the transcription factor NF-κB, which is involved in regulation of several pro-survival genes such as Bcl-2 family members, FLIP and XIAP (reviewed in [210]). TGF-β signaling transiently activates NF-κB via the TGF-β-activated kinase 1 (TAK1)-mediated pathway, but this is not sufficient to elicit pro-survival signals [211]. However, upon long-term exposure to TGF-β, a great variety of cell types inactivate the NF-κB pathway, via transcriptional induction of the inhibitor of NF-κB (IκBα), thus favoring a pro-apoptotic fate [212,213]. The adaptor molecule CD2-associated protein (CD2AP) can activate the PI3K/Akt pathway, thus enhancing cell survival [214]. Interestingly, the TGF-β receptor complex seems to activate the PI3K enzyme after forming protein complexes with its regulatory subunit, p85 [215]. It is formally possible that CD2AP or another protein serves as a bridge between the receptor complex and p85 of PI3K.

In addition, TGF-β, in a Smad3-dependent manner induces expression and activates the Fas receptor, without affecting its cognate Fas ligand, leading to caspase-8 activation and apoptosis of gastric carcinoma cells [216]. Smad3 also mediates the caspase-dependent cleavage of BAD in rat hepatoma cells [217], and whether activation of DAPK is an intermediate step in this mechanism remains unclear. In lymphoma cells, TGF-β induces apoptosis by activating the caspase cascade but like in the gastric carcinomas, Fas ligand is not an intermediate effector of the process [218]. On the other hand, Fas ligand-dependent induction of apoptosis has also been observed in lung epithelial cells responding to TGF-β, which leads to caspase 3 activation [219].

2.4.2.2. Apoptotic control by other pathways. A common feature in the vast array of mechanisms that lead to apoptosis in response to TGF-β is the involvement of MAPKs, such as p38 and c-Jun N-terminal kinase (JNK). TβRII directly associates with the Fas receptor adaptor protein Daxx, which mediates JNK activation in response to TGF-β and during apoptosis of lymphocytes or hepatocytes [220]. The Daxx-JNK pathway additionally involves as an intermediate regulator, the homeodomain-interacting protein kinase 2 (HIPK2), which interacts with and phosphorylates Daxx, induces the activities of mitogen-activated protein kinase kinase 4 (MKK4) and MKK7, which ultimately activate JNK and induce apoptosis (Fig. 3) [221]. TGF-β-activated JNK crosstalks directly with Smad3 and Smad4 in regulation of gene expression, as it phosphorylates Jun family members that interact with the Smads and bind to regulatory sequences of genes involved in eliciting the apoptotic response [222–224]. Whether this pathway directly crosstalks with Smad-induced expression of GADD45β in regulating pro-apoptotic MAPKs remains unexplored. In addition to Daxx, another direct link between TGF-β receptors and pro-apoptotic effectors such as p38 MAPK and JNK, is the inhibitory Smad7, leading to apoptosis of normal and tumor epithelial cells [225–229]. Smad7, like GADD45β is another immediate-early target gene of Smad signaling [230]. TAK1, MKK3 and MKK4 seem to act as upstream inducers of p38 and JNK, in response to the Smad7 signal, and whether the TGF-β receptors directly phosphorylate and activate any of these effector kinases, which are tethered to the receptor complex via Smad7 remains to be elucidated (Fig. 3). Alternatively, the mixed lineage kinase MLK3 can also be the upstream activator of p38 in response to TGF-β in apoptosing hepatoma cells [231]. Finally, nuclear Smad7 also antagonizes the pro-survival signal of transcription factor NF-κB during TGF-β-induced apoptosis of epithelial cells [232].

In conclusion, the apoptotic response of normal or tumor cells to TGF-β is complex, incorporating both pro-survival and pro-apoptotic pathways and a series of cytoplasmic and nuclear effectors, many of which are kinases. In all cases, the net decision of whether TGF-β will elicit apoptosis or might favor survival depends on alternative signaling inputs that the cell receives.

2.4.3. Suppression of immortalization and maintenance of genomic stability

In contrast to the overwhelming number of studies that analyzed how TGF-β mediates cell cycle arrest and apoptosis, the effects of this cytokine on genome integrity and the process of tumor cell immortalization are poorly analyzed. Studies of the TGF-β1 knockout mouse revealed that TGF-β plays a protective role on the genome. In TGF-β1-null keratinocytes exposure to the drug *N*-phosphonoacetyl-L-aspartate (PALA) leads to enhanced gene amplification when compared to wild type cell counterparts [233]. Conversely, ectopic expression of TGF-β1 in keratinocytes could protect the cells from genomic instability induced by PALA. These findings led to the proposal that loss-of-function mutations in the tumor suppressor TGF-β pathway would lead to higher risk for genomic instability, thus leading to aneuploidy and further cancer progression. The model has been verified in vitro using keratinocytes that are either null for TGF-β1 or express the dominant negative TβRII, and simultaneously overexpress a hyperactive *Ha-Ras* oncogene [234]. Cells lacking endogenous TGF-β or its signaling receptor undergo spontaneous transformation with higher frequency, and this phenomenon is preceded by large scale aneuploidy and accumulation of chromosomal abnormalities, phenotypes that can be suppressed upon exposure of cells to exogenous TGF-β1. This protective effect of TGF-β on genomic stability also supports the extensive evidence on activation of latent TGF-β1 by DNA damage caused by

ionizing radiation [235]. Interestingly, the well-established role of apoptosis induced by radiation in vivo has been directly linked to the activation of endogenous TGF-β1 using knockout mice for this gene [236]. Furthermore, p53 activation by DNA damage seems to also involve the intermediate activation of latent TGF-β1, which mediates p53 phosphorylation by as yet unknown mechanisms.

On the other hand, recent studies have proposed the opposite function of TGF-β as an inducer of genomic instability in cell culture systems in vitro [237]. These studied revealed a novel mechanism based on which TGF-β induced proteasomal degradation of the DNA repair factor Rad51, thus inhibiting proper DNA repair upon genotoxic stress. In a similar but distinct scenario, the TGF-β-activated Smad3 forms nuclear complexes with the tumor suppressor protein BRCA1, thus inhibiting the activity of BRCA1 towards DNA repair upon induction of DNA damage [238]. Whether the two distinct mechanisms of Rad51 degradation and Smad3/BRCA1 complex formation are linked remains to be examined. Establishing also conditions during which TGF-β induces genomic instability in vivo is quite important, as this will support strongly a protumorigenic role of this cytokine as we discuss extensively later.

Senescence is a physiological response of all cell types and represents a form of resistance to malignancy, usually associated with the onset of genomic instability. Based on the role of TGF-β as a protector of genome integrity, sparse but interesting reports support the model that TGF-β can also induce senescence of tumor cells, thus expressing a tumor suppressor role that aims at shifting tumor cells back to normalcy [239]. Inability of tumor cells to undergo senescence usually links to abnormal activation of telomerase, which is often observed as an overexpression of its catalytic subunit TERT [8]. Accordingly, ectopic expression of TERT blocks the growth inhibitory effect of TGF-β1 in mammary epithelial cells [240]. More important is the finding of an elegant genetic screen for negative regulators of TERT expression, which uncovered two novel regulators of TERT expression: Smad-interacting protein 1 (SIP1) and the tumor suppressor Menin [11]. Both proteins directly interact with Smad3, and SIP1 recruits co-repressors leading to transcriptional repression of TGF-β-regulated genes, while Menin cooperates with TGF-β signaling in mediating Smad3 binding to DNA and transcriptional regulation [241–243]. Thus, it is clear that TGF-β, together with its cooperating factors represses TERT expression and protects cells from spontaneous immortalization. The above result is corroborated by studies in rat cells where TGF-β, via Smad3, transcriptionally represses expression of TERT [244]. c-Myc downregulation is a prerequisite for TERT repression by TGF-β as c-Myc is a major transcriptional inducer of TERT expression.

In summary, the specific mechanisms by which TGF-β suppresses genomic instability and immortalization remain open to further investigation, while the conditions during which TGF-β switches into an agent that promotes genomic instability and tumorigenesis need better definition, as they are crucially important for the full understanding of how TGF-β controls the evolution of human tumors.

2.4.4. Inactivation of TGF-β signaling by oncogenes

While the various mechanisms of genetic and epigenetic inactivation of TGF-β signaling components explain the tumor suppressor action of this pathway in certain types of cancer, the large majority of tumors do not exhibit any of these alterations. Yet, most tumor cells exhibit relative resistance to TGF-β signaling, which is often expressed as resistance to growth inhibition or apoptosis. Such tumor cells may have a fully functional TGF-β signaling pathway, but specific regulatory events in the transmission of the signal may have been altered. Today, we recognize a large set of such regulatory mechanisms. Many of them have recently explained the molecular action of classical oncogenes, which suppress TGF-β signaling in order to promote oncogenesis.

2.4.4.1. Myc. As described above, the discovery that TGF-β represses c-Myc expression during epithelial growth arrest was intimately connected with the finding that viral oncogenes such as adenoviral E1A or SV-40 large T antigen block both growth inhibitory responses and c-Myc repression by TGF-β [146]. In addition to blocking c-Myc repression, the adenoviral oncoprotein E1A also affects the immediate-early transcriptional induction of $p15^{Ink4B}$ and $p21^{Cip1}$ by TGF-β [245]. Initially it was proposed that these oncoproteins sequester pRb or its relatives in mediating their inhibitory effects. However, based on the current understanding of the transcriptional mechanisms that regulate expression of these genes by TGF-β, it appears that the oncoproteins either inhibit the interaction of Smad proteins with the co-activator p300/CBP [246–249], or alternatively, based on their direct ability to bind to Smads, block their transactivation potential [250].

Not only is c-Myc expression repressed by TGF-β/Smad signaling, but additionally, aberrant expression of Myc in tumor cells leads to resistance to the growth suppressor response, since c-Myc actively represses expression of critical cell cycle regulatory genes like $p15^{Ink4B}$ and $p21^{Cip1}$ (Fig. 2) [148,163,172,251]. Myc becomes the mediator of loss of TGF-β anti-proliferative responses for another oncogenic pathway, the Wnt/β-catenin pathway [252]. Accordingly, in colon, breast and liver cancers, hyperactive β-catenin/LEF/TCF signaling leads to sustained expression of c-Myc; this secondarily makes cells unable to elicit epithelial growth arrest in response to TGF-β. Interestingly, the Wnt pathway directly interacts and crosstalks with TGF-β signaling during embryonic development, as β-catenin and LEF/TCF form complexes with Smads during regulation of common target genes [253,254]. Furthermore, the negative regulator of Wnt/β-catenin signaling, axin, associates with Smad3 and promotes TGF-β signaling [255]. In addition, axin forms complexes with Smad7 and promotes degradation of the latter by the ubiquitin ligase Arkadia [256]. These data suggest that hyperactivation of Wnt signaling due to axin disregulation, also affects TGF-β signaling in a negative manner.

2.4.4.2. Ras. A second major oncogene whose action affects normal TGF-β signaling is Ras and its downstream Raf/MAPK pathway. Oncogenic Ras superactivates Erk1/2, which directly

phosphorylate specific serine residues in the linker domain of Smad2 and Smad3 [257]. Linker phosphorylation results in cytoplasmic retention of the Smads, thus blocking their physiological nuclear function and thus providing an interesting mechanism by which the Ras pathway subverts the activity of the Smad pathway in tumor cells (Fig. 2). However, this model has been challenged by independent investigations that analyzed modulation of Smad signaling induced by mitogenic stimuli. Accordingly, physiological HGF/SF signaling can induce Smad2 phosphorylation mediated by the MAPK pathway, which possibly targets the C-terminal tail of Smad2 instead of its linker, leading to positive activation of Smad signaling [258]. Hyperactive Raf induces high Erk activity and does not perturb basic Smad functions, but surprisingly blocks the apoptotic response of kidney epithelial cells to TGF-β, which to a large extent depends on proper Smad signaling [259]. Since the phosphorylation status of Smads by the activated Erk was not analyzed in this study, it is hard to judge what the effects of Smad linker phosphorylation were in this cell system. More convincingly, two recent studies demonstrated that linker phosphorylation of Smad3 and Smad4 by the p38 MAPK and Raf/Erk pathways respectively, elicited positive TGF-β signaling responses, leading to mammary epithelial growth arrest in the former case, and enhanced Smad4-dependent transcriptional activation in the latter [195,260]. Thus, despite apparently contradicting reports, the importance of downregulation of Smad signaling activity by oncogenic Ras signaling remains an interesting possibility that deserves further attention and cell type specificity or quantitative differences in the strength of Ras signaling between the various systems may well explain the observed discrepancies.

In agreement with the above, not only Erk1/2 but also JNK was reported mediating phosphorylation of the linker region of Smad2 and Smad3, resulting in the same negative effects on TGF-β signaling, and such post-translational modifications have been firmly established in a series of colorectal adenocarcinomas [261]. In fact, the more invasive or metastatic the tumor, the higher the degree of Smad linker phosphorylation was found. These mechanisms explain why in colon cancer cells with loss of Smad4 expression, plain reconstitution of Smad4 is not sufficient to restore growth arrest responses and proper regulation of *c-Myc* and $p21^{Cip1}$ gene responses [262]. This is due to the concomitant presence of the *Ki-Ras* oncogene in such cells that attenuates endogenous Smad signaling. Ectopic expression of a mutant Smad3 that cannot be phosphorylated by Erk1/2 in its linker together with wild type Smad4 can rescue the cytostatic response in such tumor cells, emphasizing the synergistic inactivation of TGF-β signaling by both Smad4 genetic and epigenetic control and by Ras-mediated suppression of the pathway. Interestingly, the same model explains why TGF-β sometimes acts as a potent mitogenic factor of carcinoma cells. In prostate carcinoma cells with hyperactive Ha-Ras, endogenous TGF-β signaling appears normal, yet not only TGF-β fails to induce cytostasis, but it even enhances cell proliferation [263]. Pharmacological inhibition of MEK1, anti-sense oligonucleotides targeting Ha-Ras or dominant negative Raf mutants, all block aberrant Ras signaling and concomitantly restore TGF-β-mediated cytostasis and transcriptional induction of $p15^{Ink4B}$ and $p21^{Cip1}$. Similarly, in colon carcinoma cells that overexpress Ki-Ras, TGF-β induces proliferation and enhances their tumorigenic aggressiveness [264]. The mechanism behind this phenotype seems to be aberrant post-translational processing of the β-glycan receptor, but whether such an effect on the receptor modulates the ability of Ki-Ras to attenuate endogenous Smad signaling has not been determined. An equally important and even more widespread action of Ras signaling is to induce robust TGF-β secretion from carcinoma cells, while at the same time modulating Smad signaling. Based on this model, Ras-mediated tumorigenesis and metastasis depends critically on the induction of TGF-β and an autocrine response of the carcinoma cells to TGF-β signaling, a process that defines the potency of EMT and tumor cell invasiveness and metastatic rate in vivo [265,266]. Similar to Ras, other oncogenic signaling pathways could possibly inactivate normal Smad function by post-translational modifications. A good such example is activation of PKC by tumor promoting agents such as phorbol esters, leading to direct phosphorylation of serines in the MH1 domain of Smad3, thus inactivating the DNA-binding activity of Smad3, which results in suppression of the cytostatic and apoptotic response of epithelial cells to TGF-β [267]. It is therefore clear that Ras signaling can convert TGF-β from a tumor suppressor to a tumor promoter and for this reason the crosstalk between Ras and TGF-β will be addressed again later.

2.4.4.3. Ski and SnoN. TGF-β signaling misregulation has recently explained the molecular action of the oncogene *Ski* and of its related protein SnoN. Ski is a well-recognized viral oncogene with presumed nuclear functions, however, its oncogenic activity was rationalized upon discovering that Ski directly interacts with Smad2, Smad3 and Smad4 [268–271]. Ski confers resistance to the TGF-β cytostatic response and when bound to the nuclear Smad complex on target genes, recruits co-repressors of the N-CoR family and histone deacetylases, thus blocking the positive transcriptional activity of Smads (Fig. 2). Additionally, Ski was shown to stabilize the DNA-binding activity of transcriptionally inactive Smad complexes and was also found capable of inhibiting the phosphorylation of Smad2 and Smad3 by the TGF-β receptor complex [272,273]. A similar mechanism was described for the close relative of Ski, the SnoN protein (Fig. 2), which additionally was found to be proteasomally degraded by TGF-β signaling, in order for Smad proteins to be able to act transcriptionally at physiological levels [274,275]. Furthermore, sustained TGF-β signaling induces SnoN mRNA expression, thus restoring the levels of SnoN in the cell. According to this model, SnoN acts as a repressor of endogenous TGF-β-responsive genes, which must be removed for proper TGF-β signaling to occur, and eventually needs to be restored to bring the cell to its default state. This elegant model proposed that tumor cells with abnormally high Ski or SnoN levels would exhibit defective TGF-β signaling and resistance to the cytostatic or apoptotic responses. Interestingly, in order for Ski and SnoN to act as potent repressors of Smad

transcriptional activity, they must bind to both Smad3 and Smad4 [276–278]. Accordingly, mutant Ski or SnoN proteins that fail to associate with Smads are also defective oncogenes in vitro.

The in vivo relevance of the interaction between Smads and Ski or SnoN has been analyzed in mouse models and in human tumors. In mice where only one copy of the *Ski* or *SnoN* gene has been knocked out, spontaneous tumorigenesis was observed, which was enhanced further upon exposure to chemical carcinogens [279,280]. These results defined *Ski* and *SnoN* as tumor suppressor genes instead of oncogenes, and mouse embryonic fibroblasts derived from these heterozygous mice exhibited hyperproliferation in vitro and potent oncogenic transformation upon ectopic expression of Ki-Ras. This dual role of Ski and SnoN in tumorigenesis mimics the role of TGF-β in human cancer. In human tumors, the oncogenic potential of Ski and SnoN is compatible with the model of inactivation of TGF-β responses. Cutaneous melanomas express high Ski levels and as these tumors progress towards metastasis, the Ski subcellular distribution shifts from nuclear to cytoplasmic [281]. Such cytoplasmic Ski is equally potent in binding and suppressing Smad signaling in melanomas. SnoN seems to exhibit the inverse behavior as normal cells express primarily cytoplasmic SnoN, while SnoN is predominantly nuclear in tumor cells [282]. The cytoplasmic form of SnoN cannot be easily degraded by TGF-β signaling, as this degradative pathway primarily operates in the nucleus via the action of ubiquitin ligases such as Smurf2 and the anaphase promoting complex [283–285]. Thus, in normal cells, cytoplasmic SnoN is more stable and can repress endogenous Smad signaling even more potently than nuclear, oncogenic SnoN, which presents an unresolved problem, since it raises the question of how can TGF-β signaling bypass the obstacle of cytoplasmic SnoN during normal tissue homeostasis. Despite these mechanistic difficulties in understanding the precise, yet complex mode of action of Ski and SnoN, additional tumor studies support high expression of these proteins in human cancers. SnoN has been proposed to have prognostic value in breast carcinomas, and Ski overexpression correlates with the more advanced stages of squamous cell carcinomas of the esophagous [286,287]. In esophageal cancers, SnoN disregulation is also important, as such carcinoma cells exhibit resistance to TGF-β-mediated degradation of SnoN, thus maintaining inhibitory Smad–SnoN complexes on DNA, such as the promoter of c-Myc, thus, stably repressing the transcriptional activity of physiological Smad signaling [288]. Finally, regulation of SnoN expression by TGF-β has been recently proposed to explain why this cytokine acts as a mitogen and transforming factor in AKR-2B and NRK fibroblasts, the cellular system that led to the discovery of TGF-β [289]. According to this model, these fibroblast cell lines express sustained levels of SnoN in response to TGF-β and this correlates with their ability to become transformed. In summary, Ski and SnoN have provided a plethora of new mechanistic insight about the functional inactivation of physiological Smad signaling during tumorigenesis, and these two tumor suppressors/oncogenes promise to offer even more revelations to the mode of action of TGF-β during cancer progression.

2.4.4.4. Other oncogenes and tumor suppressors. We will close this section by enumerating a small number of additional proteins that promote or inactivate Smad signaling and thus act as tumor suppressors or oncogenes (Fig. 2). The ubiquitin ligase Smurf2 that induces Smad, SnoN and TGF-β receptor down-regulation and inhibits overall TGF-β signaling, has been found overexpressed in squamous cell carcinomas of the esophagous, and the degree of expression of this protein correlated with poor prognosis in such cancer patients [290]. The oncoprotein Evi-1 interacts with Smad3 and inhibits its transcriptional function, thus leading to resistance to the cytostatic effect of TGF-β [291]. Menin is a nuclear tumor suppressor protein that normally interacts with nuclear Smad complexes and cooperates in their transcriptional function [242]. Endocrine tumors carry inactivating Menin mutations that truncate this protein such that it antagonizes TGF-β signaling instead of promoting this pathway. The ubiquitin ligase of p53 and oncogene *MDM2* was found to confer resistance to the growth inhibitory response of epithelial cells to TGF-β and thus explain TGF-β resistance in breast cancer [292]. It was then shown that MDM2 and its relative MDMX repress the transcriptional activity of Smads, by enforcing cytoplasmic retention of Smad4 and by sequestering the coactivator p300 from nuclear Smad complexes, in cells that overexpress these proteins [293,294]. However, the physiological relevance of MDM2 acting as an inhibitor of TGF-β signaling and thus enforcing resistance to the cytostatic response of epithelial cells to TGF-β has been strongly challenged, as MDM2 overexpression was proposed to lead to genomic instability that enhances the mutational load of tumor cells, thus acquiring additional defects that impinge on the functionality of the TGF-β pathway [295]. Despite that, the established link between Smad signaling and p53 and the role of MDM2 in regulating p53 function may necessitate further examination of the Smad-MDM2 connection during tumorigenesis.

In summary, a set of well-established mechanisms seem to mediate the tumor suppressor action of TGF-β. The rapid pace of research in this field promises even wider but also deeper understanding of such mechanisms. In addition, diverse cellular pathways that drive mitogenesis and tumor formation target the TGF-β pathway. This area of research is particularly open to new discoveries and promises to explain fully how tumor cells manipulate TGF-β signaling in order to convert it from a tumor suppressor to a tumor promoter.

3. TGF-β as tumor promoter or pro-metastatic factor

3.1. TGF-β is commonly overproduced by cancer cells

One of the long-standing enigmas of TGF-β biology with relation to cancer has been why do human tumors universally oversecrete bioactive TGF-β, when this cytokine acts as a growth inhibitor? Since the early days of TGF-β cDNA cloning, it was found that human tumors and tumor cell lines express higher levels of TGF-β1 mRNA compared to normal tissues [296]. Ectopic expression of latent or mature TGF-β1 in transformed tumor 293 cells resulted in enhanced metabolic rate

and synthesis of cell adhesion markers. When introduced into nude mice such engineered tumor cells exhibited increased rate of tumor formation compared to their parental counterparts [297]. Under in vivo conditions, plasma levels of bioactive TGF-β measured prior to bone marrow transplantation and after chemotherapy correlate well with the chance of such patients to develop fibrotic disorders in their lungs and livers, which complicates the effectiveness of breast cancer therapy [298]. This finding correlates with the abundant TGF-β1 levels detected immunohistochemically at the edges of breast tumor spread and in the corresponding lymph node metastases [299,300]. In addition to breast cancer, hepatocellular and lung carcinomas also overproduce TGF-β1 in vivo and the higher the level of this cytokine the higher the degree of neovascularization observed in these tumors and the higher their chance for metastasis [301,302]. Prostatic tumors also stain positively for bioactive TGF-β1 and the level of the cytokine correlates with loss of TβRII expression, the degree of malignancy, tumor invasiveness and metastatic potential [303,304]. Furthermore, the plasma levels of TGF-β1 in prostate cancer patients can serve as a good predictor of further progression of the disease after surgical removal of the gland [305]. The higher the TGF-β1 level in plasma the higher is the chance for the patient to progress further into disease post-operatively. The same positive correlation has been made for colorectal cancer progression and plasma levels of TGF-β1 seem to reflect the degree of *TGF-β1* gene overexpression in the carcinoma cells [306]. Interestingly, upon surgical resection of such colorectal tumors, the plasma levels of TGF-β1 reduce significantly, confirming the model that a major source of the cytokine is the carcinoma cell itself [307]. Like in other cancers, colorectal tumor metastasis to the liver can be securely predicted by measuring the plasma levels of TGF-β1 in these patients [308].

While the TGF-β1 isoform is the primary ligand associated with tumor progression in humans, increased levels of TGF-β2 have also been measured together with TGF-β1 in the plasma of patients with metastatic melanoma, whereas TGF-β3 levels together with TGF-β1 constitute a better prognostic marker for the progression of breast cancer and its metastasis to lymph nodes [309,310]. Furthermore, increased TGF-β2 secretion is strongly associated with the progression of glioblastoma, a finding that led to direct therapeutic approaches as we discuss later [311]. Interestingly, one case where a mechanism of *TGF-β1* gene upregulation has been established in vivo in humans, is renal carcinoma with mutations in the tumor suppressor *von Hippel-Lindau* (*VHL*) gene [312]. In such renal carcinomas, the plasma and urine levels of TGF-β1 are elevated abnormally and this is due to the loss of the negative control exerted by VHL on TGF-β mRNA stability.

TGF-β is not the only member of its superfamily that is overproduced by tumor cells. High levels of BMPs can easily be measured in aggressive bone tumors, while squamous cell carcinoma of the esophagous secrete high BMP-6 amounts, which correlate best with the more malignant grade of these tumors [313,314]. BMP-6 secretion by prostate carcinoma cells is critical for invasiveness of the tumor cells during metastasis to bone and additionally for enhanced osteoblastic activity in the bone microenvironment that supports metastatic growth [315]. In addition to BMP-6, adenocarcinomas of the prostate secrete high BMP-7 and this factor protects such tumor cells from apoptosis, while promoting cell invasiveness and tumor metastasis [316]. Lung carcinomas produce aberrant amounts of BMP-2, which promotes growth of the tumor in vivo by activating the Smad1/5 signaling pathway and its downstream gene target *Id1* [317]. An equivalent positive effect on tumor cell proliferation and dedifferentiation has been demonstrated in advanced breast carcinomas, which are estrogen receptor-positive and overexpress the BMP receptor BMPRIB/ALK6 [318]. Thus, future studies may implicate even more members of the TGF-β superfamily in the process of tumor progression, and possibly, combinatorial detection of multiple such ligands in human tumors may offer improved methods for prognosis of the disease at least in advanced stages.

3.2. Mouse models of the pro-tumorigenic activity of TGF-β

3.2.1. TGF-β models

Xenografts of breast adenocarcinoma cells into syngeneic rats lead to lung metastases that are strongly accelerated by pre-treatment of the cells with TGFβ1, an effect that is fully inhibited by neutralizing antibodies against TGF-β1 [319]. TGF-β1 enhances extravasation of these metastatic carcinoma cells based on their ability to degrade the lung basement membrane and despite its ability to inhibit the growth of the tumor cells in vitro. In a sarcoma model, stable overexpression of TGF-β1 results in profound growth inhibitory effects in vitro (tumor suppressor action), but when tested in xenograft assays in vivo, the TGF-β1-expressing tumor cells established tumors much faster and the final tumor size was bigger, an effect that was blocked by anti-TGF-β neutralizing antibodies administered to the mice [320].

These findings have been consolidated by construction of transgenic mouse models that oversecrete TGF-β1 in the skin or mammary gland [321–324]. When TGF-β1 is conditionally overexpressed in keratinocytes and the mice are exposed to long-term chemical carcinogenesis, a dual action on tumor outcome was observed: TGF-β1 suppressed tumor growth reducing the number of primary tumors, but at the same time TGF-β1 enhanced the invasiveness and metastatic potential of these tumors [321]. Under the influence of the transgenic TGF-β1, the benign skin tumor cells underwent EMT, thus forming invasive spindle carcinoma cells in vivo, which expressed high levels of TGF-β3. In this mouse skin model, TGF-β acted directly on the carcinoma cells that secreted the cytokine, leading to EMT, which was sufficient for the acceleration of malignancy in vivo [322].

In a comparable conditional mouse skin model, expression of the *TGF-β1* transgene was switched on at specific stages of carcinogenesis after exposure to the same chemical protocol [323]. When TGF-β1 was induced early during tumor formation, it suppressed tumorigenesis, while induction at later, papilloma stages, accelerated metastasis, thus demonstrating that TGF-β is capable of directly inducing the necessary

steps towards metastasis. When this mouse model was combined with keratinocyte-targeted expression of a dominant negative TβRII, TGF-β1 could not suppress benign tumor growth anymore [325]. Unexpectedly, the dominant negative receptor could not block metastasis of advanced tumor stages, and this was proposed to indicate that TGF-β promotes metastasis by a mechanism that does not depend strongly on TβRII and that involves activation of intracellular MAPK and Rho GTPase activities. This is one of the few reports where the dominant negative TβRII fails to block all measured TGF-β responses during tumor progression. Based on the current understanding of TGF-β signaling whereby all physiological effects of this cytokine are mediated by its primary ligand-binding receptor, TβRII, it is hard to reconcile this result, as it suggests that TGF-β mediates MAPK and Rho GTPase signaling via a different receptor system. One possible mechanism that can explain such results is the ability of the TGF-β receptor complex to form complexes with many other cell surface signaling proteins. An example with relevance to this discussion is the hyaluronan receptor CD44, which primarily interacts with TβRI and less with TβRII [326]. Thus, in metastatic breast carcinoma cells, hyaluronan binds to CD44 and induces signaling by TβRI towards Smad phosphorylation and activation of canonical TGF-β responses. However, TβRI in the complex with CD44 phosphorylates the latter receptor and induces stronger interaction between CD44 and the actin cytoskeleton, which promotes carcinoma cell migration. Whether such a scenario operates in the transgenic mice discussed above remains an open possibility. In addition, it must be kept in mind that recent findings on the small mucin-like protein, podoplanin, indicate that this protein elicits formation of filopodia and induces carcinoma cell migration, without affecting the overall differentiation of epithelial cells or inducing EMT [327]. Podoplanin seems to strictly be involved in the regulation of Rho GTPase activity and actin cytoskeleton reorganization at the invasive front of tumor cells. Thus, tumor cell invasiveness in the absence of EMT becomes recently molecularly established.

In a breast model, the *TGF-β1* transgene was inducibly expressed by doxycycline in the mammary epithelium, into which a polyoma virus middle T antigen was also expressed concomitantly [324]. While induction of TGF-β1 did not affect primary breast tumor formation, it strongly increased the number of lung metastases of these tumors. The expression of TGF-β1 and the activation of endogenous Smad signaling was strictly confound to the carcinoma cells, thus favoring an autocrine action of TGF-β in promoting metastasis.

3.2.2. TGF-β receptor models

In a second series of mouse models, the function of endogenous TGF-β signaling was investigated by expressing the dominant negative TβRII receptor in the carcinoma cells. A prominent tumor model of breast cancer metastasis that critically depends on EMT is the EpH4 mouse mammary epithelial cell line that stably expresses Ha-Ras, leading to oversecretion of TGF-β and followed by loss of growth suppression responses, induction of EMT, enhanced cell invasiveness and high rate metastasis [265]. Expression of the dominant negative TβRII receptor in this cell model resulted in inhibition of autocrine TGF-β signaling, inhibition of EMT and delayed tumor formation with significantly fewer metastases [328]. Neutralizing antibodies against TGF-β or soluble TβRII that sequesters TGF-β, both had the same effect as the dominant negative receptor.

In a second model of human breast carcinoma cells, MDA-MB-231, that are resistant to growth inhibitory responses and metastasize to bone, the dominant negative TβRII inhibits endogenous TGF-β signaling in this tumor cell and it significantly inhibits metastasis in xenograft experiments [329]. Conversely, ectopic expression of the constitutively active TβRI/ALK5 enhanced metastatic potential by inducing the secretion of parathyroid hormone-related peptide (PTHrP), which promotes osteoclast activation during osteolytic metastasis. In a third model of 4T1 metastatic breast carcinoma cells, the dominant negative TβRII again blocks distant metastases, emphasizing the role of endogenous TGF-β signaling for this phenotype [330].

In a fourth model of a series of human mammary epithelial MCF-10A cell lines that express Ras and exhibit various degrees of malignancy upon xenografting to nude mice, the dominant negative TβRII did not affect tumor formation by the oncogenically transformed cells [331]. This experiment also demonstrated that TGF-β is not sufficient to initiate tumorigenesis. However, in the presence of the *Ras* oncogene, which plays the initiator role, TGF-β enforces a more malignant phenotype, so that in the high grade tumor cell line, dominant negative TβRII could effectively block metastasis rate despite its inability to reduce primary tumor growth. All these models are based on the engineering of tumor cell lines and xenograft experiments in nude mice.

Transgenic mice with breast-specific (MMTV-driven) expression of the constitutively active TβRI/ALK5, when crossed to mice expressing activated Neu receptor mutants that cause breast cancer, show that the active TβRI inhibits primary mammary cancer formation but it accelerates the rate of metastasis to lung [332]. This result has been reproduced independently with the same combination of transgenic mouse crosses [333]. In the inverse experiment, dominant negative TβRII accelerates the rate of primary tumor formation and inhibits the rate of lung metastasis. This is a clear model of the dual role of TGF-β in tumor progression that is strictly based on genetic manipulation of the TGF-β receptors. An essentially similar result was obtained when the MMTV-Neu mouse was crossed to an MMTV-TGF-β1 transgenic that expressed bioactive TGF-β1 in the mammary gland, confirming that the in vivo action of TGF-β1 or its two signaling receptors is virtually redundant [334].

3.2.3. Smad models

The role of Smad signaling in the pro-metastatic action of TGF-β has also been addressed with multiple models of xenograft experiments in nude mice and with transgenic mice. Using the series of MCF-10A/Ras cells lines described above and ectopic expression of a mutant TβRI/ALK5 that fails to

bind and phosphorylate the Smads, while retaining an active kinase, it was shown that the pre-malignant cell model was pushed to form larger and more malignant tumors, indicating that Smad signaling is required for the tumor suppressor action of TGF-β [335]. Similarly, the aggressive cell model failed to metastasize to lung as efficiently in the presence of the mutant receptor, demonstrating that Smad activation by receptor is also required for the pro-metastatic action of TGF-β. The same result was obtained when the experiment was repeated using the same set of mammary cell lines after ectopic expression of dominant negative Smad2 and Smad3, implicating these two R-Smads in both tumor suppressor and pro-metastatic actions of TGF-β [336]. Similar to breast carcinoma cells, dominant negative Smads could effectively block metastasis of the skin spindle cell carcinoma model discussed previously [328]. Furthermore, mammary epithelial specific knockout of Smad4 revealed the role of Smad4 during mammary EMT and squamous cell carcinoma formation [136]. The same result was obtained using the metastatic human MDA-MB-231 cell model and RNA interference (RNAi) against Smad4, which led to significant reduction of bone metastasis in mice [337,338]. Finally, the inhibitory Smad7, upon adenoviral delivery to metastatic breast carcinoma cells led to significant decrease of metastasis in xenografted mice [339]. The evidence so far supports primarily a canonical TGF-β receptor-Smad pathway eliciting pro-metastatic effects of TGF-β in mouse tumor models, and important gene targets of the pathway that mediate the pro-metastatic activity can be PTHrP or other secreted proteins (e.g. interleukin-11, connective tissue growth factor (CTGF)) as identified by recent large scale transcriptomic analyses [340].

3.3. Mechanisms of the pro-tumorigenic action

Currently, the pro-tumorigenic role of TGF-β can be explained by a complex set of cellular mechanisms that affect the carcinoma cell itself and its associated extracellular matrix, cells from the surrounding tumor stroma, including cancer-associated fibroblasts and immune cells, and finally cells of the vascular system.

3.3.1. Epithelial–mesenchymal transition

As already mentioned, EMT is a critical differentiation switch that allows epithelial cells to migrate, invade their local tissue environment and even intravasate to the vasculature during metastasis (Fig. 4). EMT causes destruction of fully

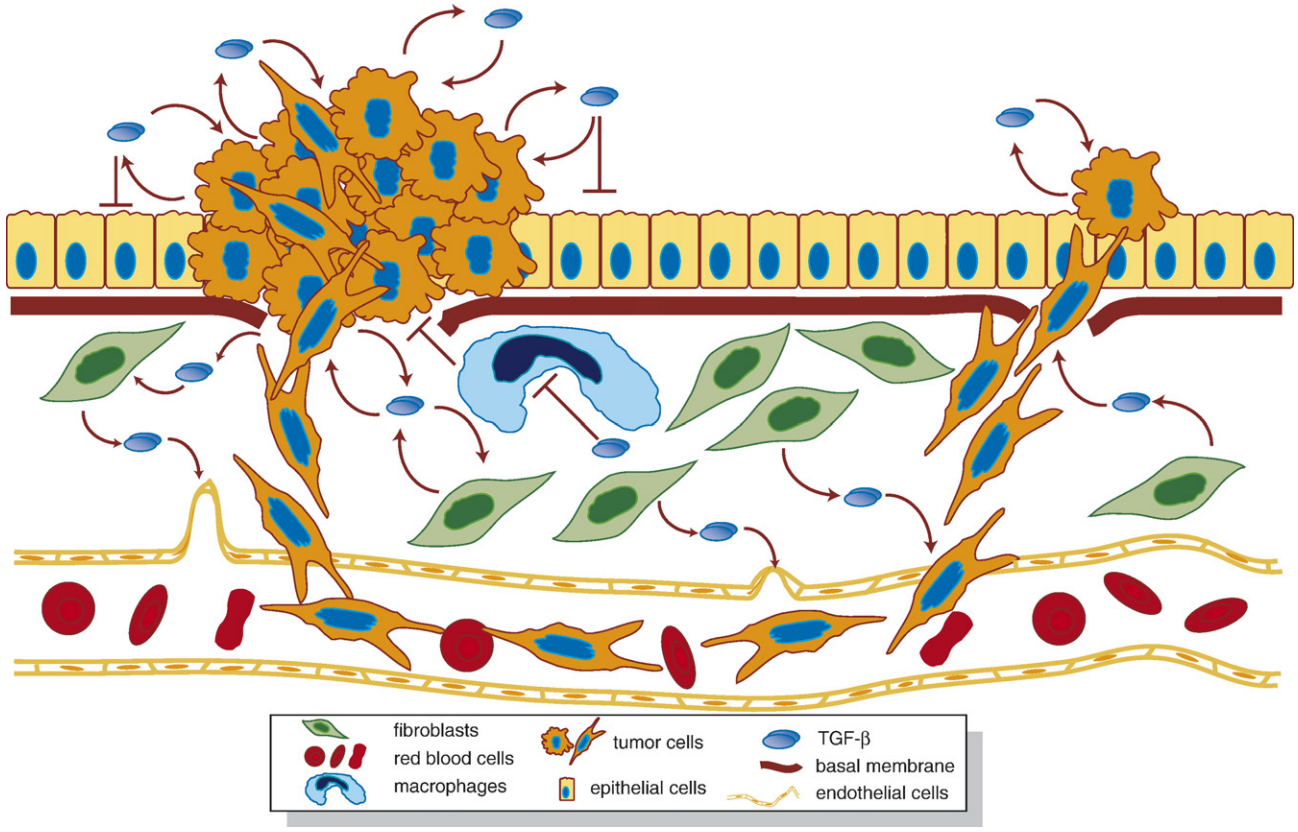


Fig. 4. TGF-β actions during carcinoma progression. A normal epithelium is disrupted by the growth of a primary tumor creating a mass of cells with lost polarity that over-proliferate and some of which switch differentiation (EMT) and become migratory and invasive, thus degrading the basement membrane. Invasive tumor cells transverse the tumor stroma that is rich in tumor-associated fibroblasts and immune cells (e.g., macrophages or natural killer cells), and eventually intravasate into neighboring blood microvessels. Metastatic tumor cells circulate in the bloodstream and extravasate at distant sites, transverse the surrounding connective tissue and establish new sites of metastasis on a new epithelial layer. TGF-β is shown being produced by primary tumor cells and acting on the same carcinoma cells or on fibroblasts; TGF-β produced by fibroblasts and acting on tumor cells, endothelial cells or inhibiting macrophage/natural killer cell function. TGF-β is also shown to act on normal epithelial cells as an inhibitor (tumor suppressor).

polarized epithelia and creation of mesenchymal, migratory cells, processes that require the disorganization of cell–cell adhesion and cell–matrix adhesion, and degradation of the extracellular matrix associated with the epithelial layer. EMT therefore represents a basic morphogenetic process that is controlled by many signaling pathways and which is utilized in many instances during normal development, but also during disease pathogenesis, including carcinoma invasiveness and tissue fibrosis [341–344].

3.3.1.1. In vitro and in vivo models of EMT. In vitro culture systems gave the first evidence that TGF-β1 could induce EMT, and today, many studies have described this widespread mammalian epithelial response in both primary and immortalized normal epithelial cells and in tumor and fibrotic epithelial cells of mammary, lung, kidney, colon, pancreatic, thyroid, lens, skin and liver origin [179,322,325,328,345–359]. EMT in response to TGF-β1 is characterized phenotypically by down-regulation of epithelial markers such as E-cadherin, specific keratins and ZO-1, and upregulation of mesenchymal markers such as fibronectin, Fsp1, α-smooth muscle actin and vimentin. During EMT, the actin cytoskeleton becomes reorganized from a cortical arrangement that supports the polarized plasma membrane to a stress-fiber network connected to focal adhesions. In addition to TGF-β1, TGF-β2 and TGF-β3 can induce EMT in vitro and in vivo, while signaling pathways that activate Smad2 and Smad3, *i.e.* TGF-β/activin/nodal/myostatin and other ligands, should in principle be able to elicit EMT, because ectopic expression of their corresponding type I receptors can induce EMT in vitro [358,360]. On the other hand, ligands that activate Smad1, Smad5 and Smad8 signaling pathways, *i.e.* BMPs/GDFs/MIS fail to elicit robust EMT [358]. In addition, BMPs can antagonize the TGF-βs in normal mammary and lens epithelial cells in vitro [179,361]. In other words, higher level of BMP inhibits TGF-β from eliciting EMT. Alternatively, BMPs induce mesenchymal–epithelial transition (MET) of fibrotic kidney cells or of mesenchymal cells generated via an EMT process [362]. In vivo, BMP-7 induces MET of adult renal fibroblasts and acts therapeutically by helping the regeneration and healing of the injured kidney (reviewed in [342]). Whether other BMP/GDF members are capable of inducing MET in other organs, and in particular during tumor progression, remains to be investigated and is of clinical importance.

As already discussed above in the mouse models, spindle cell carcinomas developed by exposure of mice to chemical carcinogens, mouse mammary carcinomas created by Ras or Raf oncogenic transformation, and both colon carcinomas and hepatocarcinomas, led to the understanding that oncogenes such as *Ras* or *Raf* induce EMT and tumor cell invasiveness in vitro and in vivo in a TGF-β-dependent manner [259,266,322,328,350]. Indeed, when the TGF-β pathway is inactivated, Ras alone cannot promote tumor cell invasiveness and metastasis. Conversely, upon activation of the TGF-β pathway, carcinoma cells that exhibit more overt and irreversible EMT lead to more aggressive and metastatic tumors [328]. Overall, it is accepted that mouse and human carcinomas oversecrete TGF-β, are sensitized to TGF-β signaling, which leads to loss of growth inhibitory or apoptotic responses to TGF-β, yet allows the development of EMT.

3.3.1.2. Control of EMT by Smads. A series of in vitro and in vivo mouse model studies using dominant negative Smad mutants, tissue specific Smad knockouts or Smad RNAi and the mutant TβRI/ALK5 that cannot bind and activate R-Smads have established the role of Smad signaling in TGF-β-induced EMT [136,179,335,336,338,353,354,356,358,360,363,364]. Surprisingly, partial depletion of Smad4 using RNAi in human HaCaT keratinocytes and colon carcinoma cells could not block TGF-β-induced EMT despite very low Smad4 levels [171]. This suggests that low levels of endogenous Smad signaling are sufficient for EMT to occur. In addition, the nuclear transcriptional regulators YY1 and c-Ski, which bind directly to Smad complexes and repress either Smad DNA-binding (YY1) or Smad transcriptional activity (c-Ski), block TGF-β-driven EMT in vitro, thus reinforcing the role of transcriptional Smad signaling in establishment of EMT [365,366].

Smad signaling mediates EMT presumably by regulation of critical gene targets that may act as effectors of EMT, or that may be required for the maintenance and full deployment of EMT. Transcriptomic screens of EMT under in vitro and in vivo conditions and screens for genes contributing to carcinoma invasiveness and metastasis are very useful as for example they have implicated TGF-β signaling as a major regulator of tumor cell invasiveness [340,349,358,367–369]. Due to the availability of such large-scale gene expression data, the number of genes that become functionally linked to EMT grows steadily. We will discuss below some specific TGF-β gene targets that provide a deeper understanding of the mechanism of EMT. Such work also aims at identification of markers of the EMT process that could serve as screening tools in the laboratory and the clinic in order to identify unequivocally EMT in various pathophysiological processes [343].

3.3.1.3. Control of EMT by other pathways. TGF-β not only activates Smad signaling but also affects the activity of various non-Smad signaling effectors, a number of which have an impact on EMT [33]. In carcinoma cells expressing an activated *Ras* oncogene, Erk1/2 and PI3K activities contribute to the establishment of EMT [266,350]. Erk, PI3K, Rho GTPase and p38 MAPK signaling are also important in immortalized and malignant human keratinocytes, human pancreatic carcinoma cells, pig thyrocytes, and normal mammary epithelial cells [348,349,351,370–373]. In addition, NF-κB signaling cooperates with Smads and contributes to EMT in vitro and metastasis in vivo in Ras-transformed breast carcinomas [374]. A possible mechanism of activation of NF-κB by TGF-β involves TAK1 that directly phosphorylates the IκB kinase 2 (IKK-2), which phosphorylates and induces degradation of IκBα, thus releasing active NF-κB [375]. Finally, integrin signaling cooperates with TGF-β receptors in eliciting EMT [376,377]. Integrin β1 function is required for p38 MAPK activation in mammary cells and integrin β1 inhibition

effectively blocks EMT [376]. During progression of human colon carcinoma cells EMT takes place and integrin β6 expression is induced, making the cells more invasive and migratory, which leads to activation of autocrine TGF-β, thus enhancing further EMT in a circular fashion [377]. In this case, integrin β6 functions upstream of TGF-β mediating its activation, instead of transmitting TGF-β signals towards EMT.

The only direct mechanism of non-Smad signaling that contributes to EMT links TGF-β receptor signaling to the polarity complex that regulates the Rho GTPase pathway [378]. In polarized epithelial cells, TβRI localizes to tight junctions by interacting with the integral membrane protein occludin, where it also interacts with the polarity protein Par6 [378]. Upon TGF-β signaling, TβRII is recruited to tight junctions and phosphorylates the type I receptor together with the type I receptor-tethered Par6. Phosphorylated Par6 can subsequently recruit the ubiquitin ligase Smurf1, which ubiquitylates and degrades RhoA [378]. This results in local disassembly of the actin cytoskeleton and tight junction dissolution, one of the hallmarks of EMT. Direct phosphorylation of Par6 by the TβRII kinase opens the possibility that this receptor has additional substrates, which may link TGF-β to non-Smad effectors. Another interesting example of EMT where at least Smad4-mediated responses do not seem to be important is the de-differentiation of Smad4-null pancreatic carcinoma cells induced by TGF-β [379]. In these cell models, TGF-β induces tyrosine phosphorylation of α- and β-catenin, which disrupts the cortical E-cadherin/catenin complexes with actin, leading to dissociation of adherens junctions. Although not yet mechanistically clear, tyrosine phosphorylation of catenins depended on Ras and PI3K activities (possibly suggesting a role for Src family kinases) and modulation of the activity of the phosphatase and tensin homologue (PTEN) that associates with the E-cadherin/catenin complexes. However, PTEN has been reported to directly interact with Smad3 and down-modulate the transcriptional signal of Smad3 [380]. Accordingly, loss-of-function of PTEN contributes to the TGF-β/Smad3-dependent enhancement of carcinoma cell motility and tumor invasiveness, emphasizing the established tumor suppressor role of PTEN as an inhibitor of the pro-tumorigenic action of TGF-β. Thus, although plasma membrane proximal signaling events downstream of TGF-β receptors may offer territory for discovery of novel signal transduction mechanisms that govern EMT and tumor cell invasiveness, the involvement of Smad protein function in such mechanisms necessitates careful consideration.

3.3.1.4. Transcriptional control of EMT. Regarding the transcriptional targets of the TGF-β pathway that are mediated in the process of EMT, *Id* (*Id2*, *Id3*) genes play critical roles [179,381]. TGF-β-specific Smads transcriptionally repress *Id* genes, whereas BMP-specific Smads induce robust levels of Ids in epithelial cells, and sustained repression of Id2 and Id3 is critical for TGF-β to downregulate E-cadherin and ZO-1 and eventually establish EMT [179]. Id2 repression releases the E12/E47 basic helix–loop–helix (bHLH) factors so they can bind to the *E-cadherin* promoter and repress the gene [381]. In contrast, the high levels of Ids induced by BMP signaling enforce global inhibition of various bHLH proteins, and failure to induce EMT. We therefore propose that regulation of *Id* gene expression explains the aforementioned antagonism between TGF-β and BMP, whereby BMP dominantly antagonizes TGF-β-induced EMT and promotes MET [179,342,361,362]. Our model proposes that during EMT of early stage carcinomas, Id levels will be reduced. However, upon metastasis and homing into a new site of tumor growth, Id levels may gradually increase in order to support more robust proliferation and survival of the transitory carcinoma cells so that a new metastatic tumor is established. In fact, Id2 levels are abnormally high in bone or lung metastatic sites of certain tumors (reviewed in [382]). However, direct proof of Id reduction in carcinomas that prepare for metastasis in vivo is an open challenge for the future.

In addition to Ids, transcriptional repressors of the *E-cadherin* gene, such as members of the Snail family of zinc finger proteins (Snail, Slug), two-handed zinc finger/homeodomain proteins (ZEB1, ZEB2), basic helix–loop–helix (bHLH) proteins (E12/E47, Twist) and high mobility group box-containing proteins (LEF-1), are involved in the EMT response to TGF-β [383]. These repressors recognize E-box DNA sequences located near the transcriptional initiation site of the *E-cadherin* gene, and recruit transcriptional co-repressors and histone deacetylases. Such regulation is critical as E-cadherin is a central component of adherens junctions, whose levels must get decreased in order for EMT to proceed. TGF-β induces interaction between Smads and ZEB1 or ZEB2, thus forming repressor complexes on the E-box region of the *E-cadherin* gene but also on other gene targets [383–385]. On the other hand, TGF-β induces expression of (a) Slug during EMT in normal heart vulval development in the chicken, (b) Snail via Smad3 or via activation of the Erk and PI3K pathways in renal epithelia cells, (c) LEF-1 via Smad signaling during normal palate development or in mammary epithelial cells that are transformed by a synthetic *Fos-estrogen receptor* oncogene [354,369,386–390]. An interesting open question is whether all these transcriptional mechanisms of *E-cadherin* repression act in concert downstream of TGF-β or whether they represent tissue-specific scenarios. We have recently addressed this problem by identifying a new key regulator of the EMT process downstream of TGF-β/Smad signaling, the high mobility group nuclear factor HMGA2, which is known to be expressed only in mesenchymal cell types [391]. Upon transcriptional induction of HMGA2 by the TGF-β pathway, the EMT process starts and this factor induces expression of Snail, Slug and Twist. Depletion of mammary epithelial cells from the inducible levels of HMGA2 prevents them from undergoing EMT and from expressing mesenchymal protein markers. This example illustrates that TGF-β is capable of eliciting a global network of transcriptional changes that involves many, if not all, of the above regulators of E-cadherin.

Despite the understanding of how E-cadherin expression is regulated, little is known about transcriptional regulation of other genes that are required for establishment of the mesenchymal phenotype. Transcriptomic analyses of the

TGF-β response of immortalized keratinocytes showed that TGF-β, via the Smad pathway, induces expression of the ligand of Notch signaling, Jagged1 [349]. Thus, TGF-β primes Notch signaling and then TGF-β cooperates with active Notch to regulate expression of the *Hey1* transcriptional repressor during the establishment of EMT [392]. Similar to the Notch example, TGF-β induces expression of PDGF ligands and receptors, thus activating autocrine PDGF signaling that promotes EMT and in vivo metastasis of mammary epithelial cells [393]. The same mechanism has been described in hepatocellular carcinomas, where TGF-β induces autocrine PDGF signaling, which promotes tumor growth in vivo [394]. These recent reports open the exciting possibility that TGF-β induces mitogenic signaling that acts towards tumor stromal cells, as discussed below, and towards the carcinoma cell itself, thus creating a more complicated scenario of signaling crosstalk during tumorigenesis. Finally, TGF-β induces expression of the adaptor protein disabled-2 (Dab2) that participates in clathrin-mediated endocytosis [395]. During EMT, Dab2 protects mammary cells from apoptosis and permits the differentiation switch to the mesenchymal phenotype. The above findings suggest that several additional gene targets of the TGF-β/Smad pathway must link to the EMT response and promise the identification of the most immediate and direct effectors of the EMT process downstream of TGF-β.

In conclusion, the EMT response to TGF-β involves a complex signaling network, including non-Smad and Smad signals that lead to the dissolution of epithelial cell adhesion, while changes in gene expression dictate the differentiation switch and the generation of the mesenchymal cell.

3.3.2. Tumor cell invasiveness

An immediate corollary to EMT is the process of cancer cell invasiveness. This process requires both a motile cellular phenotype and the ability of tumor cells to degrade and remodel their extracellular milieu. Traditionally, these cellular functions were ascribed to fibroblasts or highly tumorigenic carcinoma cells. The current understanding of EMT provides a unified framework that proposes that migratory and matrix remodeling (fibrotic) properties may not necessarily depend on the action of different cell types in the tumor environment, but rather characterize different stages of differentiation of the original carcinoma cell. We will therefore emphasize some of the features of the invasive properties of tumor cells from this perspective.

TGF-β secreted from gastric carcinoma cells best correlates with the invasive and metastatic properties of the tumors than with systemic actions away from the location of the tumor [396]. Such observations led to the concept that the action of TGF-β is more important locally as a modulator of tumor microenvironment. Such tumor invasive properties can be induced by aberrant Smad signaling as demonstrated by the analysis of cancer mutant Smad2 forms that promote tumor cell invasiveness and synergize with TGF-β stimulation to increase the malignant feature of the tumor [397]. According to these findings, the tumor suppressor action of Smad2 is also linked not just to the loss of anti-proliferative or apoptotic responses, but also to pro-invasive cell behavior. In a similar scenario, pancreatic carcinoma cells with loss-of-function mutations in Smad4, respond to autocrine TGF-β by enhanced phosphorylation of Smad2 and Smad3, an event that supports carcinoma cell invasiveness [398]. Pharmacologic treatment of such carcinomas with a specific inhibitor of the TβRI/ALK5 kinase blocked in vitro motility and invasiveness.

The cellular mechanisms that explain the motile and invasive phenotype in response to TGF-β are diverse and include both Smad-dependent gene regulation and activation of alternative signaling effectors in the carcinoma cell. Accordingly, transcriptomic screens showed that TGF-β, via Smad3, induces expression of the guanine exchange factor NET1, which activates Rho GTPases, thus supporting actin reorganization and cell motility [399]. In addition, TGF-β, induces expression of several *tropomyosin* genes in a Smad- and p38 MAPK-dependent manner [400]. High tropomyosin levels contribute to cytoskeletal contractility and metastatic carcinoma cell motility. Induction of the homeobox transcription factor CUTL1 by TGF-β leads to activation of many genes that regulate cell motility, tumor cell invasiveness and extracellular matrix deposition [401]. In addition, CUTL1 serves as a poor prognosis marker for metastatic breast carcinoma. TGF-β also induces expression of α3β1-integrin in hepatocellular carcinoma cells and motility and invasiveness of these cells depends critically on the level of this integrin receptor [402]. The motility of metastatic breast carcinoma cells that respond to autocrine TGF-β1 in vitro depends on the activity of both TβRII and TβRI kinases and additionally seems not to require Smad activation but rather the activity of the PI3K/Akt pathway [403]. The motility of epithelial cells seems to depend on the phase of the cell cycle that the TGF-β-responding cells reside [404]. Apparently, epithelial cells that have just completed their cytokinesis are significantly more motile compared to cells that prepare for DNA synthesis prior to cell division. This interesting in vitro finding might have diverse implications for the in vivo behavior of invasive, migratory tumor cells, a possibility worth exploring with the new non-invasive imaging technologies. Finally, the interplay of Smad signaling with alternative effector proteins, such as the p38 MAPK regulates the production of PTHrP from metastatic breast carcinoma cells [405]. As described under the metastatic mouse models, PTHrP is required for the productive establishment of metastatic foci to bone, based on its ability to mobilize osteoblasts at the invasive front of the tumor. These specific examples illustrate a recurrent theme of signaling effectors of TGF-β as regulators of carcinoma invasiveness. Understanding therefore the conditions under which the signaling pathway makes specific choices for regulation of cell proliferation, EMT or motility most probably involves a deeper analysis of the integrated crosstalk of TGF-β signaling with other pathways that become activated during cancer progression.

3.3.3. Actions in the tumor stroma

The previous discussion on the role of EMT and cell motility during carcinoma progression clearly emphasizes the role of the tumor microenvironment and its three-dimensional architecture

[406]. This microenvironment, in addition to the extracellular matrix components provides the functions and control of tumor growth and behavior by the embedded cellular components, which include cancer-associated fibroblasts or myofibroblasts, immune cells and microvessels. TGF-β is known to be produced by and act upon all these cell types (Fig. 4), and in this section, we will focus on the fibroblast–myofibroblast compartment [9]. Interestingly, the developmental origin of the tumor-associated fibroblasts is still debated, and an exciting possibility is that they are derived from the actual epithelial layer or the carcinoma cells based on the process of EMT. Such a possibility is already well documented in the literature and one characteristic example is human breast cancer cells that undergo EMT and derive fibroblastoid or myofibroblast-like cells, which retain weak residual features of their epithelial origin, such as specific keratins, and whose function is to communicate with the carcinoma cells and affect their proliferation [407]. These tumor-surrounding cells provide a rich source of growth and angiogenic factors in addition to chemokines, and a primary action of TGF-β acting on such fibroblasts is the release of new cytokines [408]. A prominent mitogenic factor produced by cancer-associated fibroblasts is PDGF, whose expression was first shown to be induced at the mRNA level by TGF-β in leukemic cells [409]. In human fibroblasts, TGF-β regulates expression of many PDGF ligand isoforms and of some of its receptor chains, with the net effect of promoting PDGF binding to its receptors and inducing mitogenic signaling via PDGF [410]. A second major mitogen that signals via receptor tyrosine kinases is basic fibroblast growth factor (bFGF), whose mRNA and protein levels are also induced by TGF-β, for example in human renal fibroblasts [411]. The positive effect of TGF-β on renal fibroblast proliferation can therefore be attributed to the effects of bFGF that acts downstream of the original TGF-β signal. Another important factor whose mRNA and protein expression is induced by TGF-β acting on fibroblasts is CTGF, which acts in an autocrine or paracrine manner to induce mitogenesis of fibroblasts themselves or other neighboring cells [412]. Recent screens of gene signatures that correlate with breast carcinoma metastasis to bone, have repeatedly identified the *CTGF* gene as a major target of TGF-β at the site of bone metastasis [340]. In addition to secreted growth factors, as mentioned before, TGF-β induces expression of integrin receptors that receive signals from matrix components in the extracellular milieu. For example, human lung fibroblasts respond to TGF-β by inducing expression of $\alpha_v\beta_3$-integrin, which forms complexes with TβRII, and thus promotes rapid fibroblast mitogenesis that depends on integrin receptor activation by matrix ligands [413].

Another prominent cell type of the tumor stroma is the so-called "activated" myofibroblast, whose differentiation status is characterized by the expression of α-smooth muscle actin. According to some models, such myofibroblasts create an invasive front that paves the way to the carcinoma cells that initiate metastasis. For example, colon cancer cells, under in vitro culture conditions drive the invasiveness of neighboring myofibroblasts into various extracellular matrices, and TGF-β, which is secreted by the carcinoma cells drives this process by acting on the myofibroblasts [414]. In such invasive myofibroblasts, TGF-β1 stimulates the JNK pathway and expression of N-cadherin at the tips of filopodia, while migratory and invasive behavior is effectively blocked by an antibody or siRNA against N-cadherin or by low molecular weight inhibitors of JNK. In addition, the stroma of squamous cell carcinomas is rich in such myofibroblasts, which reside at the invasive front of the tumor and are derived via an EMT process from the carcinoma cells based on the action of the carcinoma-secreted TGF-β1 [415]. Upon formation of these myofibroblasts, TGF-β1 continuously acts on them and induces expression of HGF/SF, which promotes even further carcinoma proliferation and invasion. This study demonstrates beautifully the significant contribution of tumor stromal elements on the invasive character of human tumors and implicates strongly TGF-β on every step of this process. The importance of this model has been verified elegantly by tissue-specific knockout of TβRII in fibroblasts, in a tumor mouse model [416]. The result is dramatically revealing as the loss of TGF-β signaling in the fibroblasts led to the formation of prostate neoplasms and of invasive squamous cell carcinoma in the forestomach of these mice. These types of tumors exhibited abnormally high numbers of stromal cells and the fibroblasts that could not mediate TGF-β signals secreted high amounts of HGF/SF that acted in a paracrine manner on the adjacent epithelial cells, thus leading to stimulation of over-proliferation. The same TβRII knockout experiment done specifically in mammary gland fibroblasts showed that normal mammary ductal development was inhibited significantly, as epithelial cells decreased in number while the knockout fibroblasts increased in numbers in the tissue [417]. Furthermore, the TβRII knockout mammary fibroblasts when used in xenograft experiments together with mammary carcinoma cells, they promoted significantly high tumor growth and invasion, which correlates with over-secretion of HGF/SF, macrophage-stimulating protein (MSP) and other mitogenic factors by the knockout fibroblasts. These recent mouse models open a new way of analyzing the epithelial/carcinoma–mesenchymal interactions and emphasize the complexity of the actions TGF-β has within the evolving tumor microenvironment.

Future studies concentrating on cancer-associated fibroblasts and their roles in regulating not only carcinoma cell behavior but also the function of stromal immune cells and blood microvessels promise much towards the more effective design of anti-tumor therapies.

3.3.4. Regulation of tumor angiogenesis

Another functional consequence of intense growth factor activity in the tumor microenvironment is the induction of neo-angiogenesis and microvessel formation from pre-existing normal blood vessels of the diseased tissue that sprout and provide nutritional, hormonal and immunological support to the growing tumor [418]. Mouse studies using knockouts for the TGF-β1, TβRII, TβRI/ALK5, and the endothelial-specific TGF-β co-receptor endoglin show clear defects in vasculo- or angiogenesis and essentially the mice suffer or die due to such defects of their vasculature [419–422]. A similar function has been ascribed to the type I receptor ALK1 upon knockout of its

gene in the mouse [423]. Furthermore, genetic mutations in the *ALK1* and *endoglin* genes are the causative agents for the development of hereditary hemorrhagic telangiectasia, establishing the role of the TGF-β pathway in human angiogenic syndromes [424,425]. Thus, current efforts of understanding the signaling mechanisms and the associated biology of TGF-β in endothelial cells and pericytes that form the contractile vascular wall are of primary clinical importance [426].

In vitro studies with normal endothelial and carcinoma cells established that TGF-β induces expression of selected members of the vascular endothelial growth factor (VEGF) at the mRNA level [427,428]. Hypoxic conditions in conjunction with TGF-β induce robust levels of VEGF mRNA based on the activation of hypoxia-inducible factor 1 (HIF1) and Smad proteins, which interact and promote transcription of the *VEGF* gene [429]. Thus, TGF-β can induce endothelial cell proliferation and differentiation based on its effects on VEGF expression. On the other hand, TGF-β can directly induce growth arrest and apoptosis of endothelial cells at least in vitro, by inducing cytostatic and apoptotic responses similar to those discussed above for epithelial cells. The opposing effects of TGF-β on endothelial proliferation and differentiation are currently explained by the elegant model of signaling by the type I receptors TβRI/ALK5 and ALK1 [25,26], as described in the introduction.

3.3.4.1. TGF-β promotes tumor angiogenesis. TGF-β secreted from tumor cells can play either a positive or a negative role on angiogenesis, thus acting either as a promoter of tumor growth indirectly presumably via induction of VEGF or as a tumor suppressor by inhibiting VEGF function [430]. There is ample evidence supporting both models. Chinese hamster ovary cells stably transfected with TGF-β1 and injected subcutaneously into nude mice, result in faster tumor growth compared to control cells and this correlates with massive angiogenesis inside the tumor mass, a phenotype that can be effectively blocked by anti-TGF-β neutralizing antibodies [431]. In human prostate cancer studies, overexpression of TGF-β1 best correlates with enhanced angiogenesis around the tumor, which leads to an increased frequency of metastasis of prostate carcinoma cells [304]. Treatment of tumors with TGF-β1 latency-associated peptide or neutralizing antibody against TGF-β1, both representing mechanisms of sequestration of TGF-β from access to its receptors, resulted in a decrease of blood vessel density, which then led to a significant reduction in tumor size [432]. A transgenic mouse model of head-and-neck cancer where ectopic TGF-β1 expression is targeted to head-and-neck epithelial cells, exhibited severe inflammation and increased angiogenesis of the epithelium, thus promoting its hyper-proliferation [433]. In this model, TGF-β that is misexpressed in the epithelium creates a favorable microenvironment for tumor growth. Tumor cells utilize several mechanisms by which they achieve their pro-angiogenic effects based on the action of TGF-β. For example, secreted latent TGF-β gets activated by the proteolytic cleavage of metalloproteinase-9 (MMP-9) in order for TGF-β to signal its pro-angiogenic effects [434]. For MMP-9 to activate TGF-β it must be properly localized at the cell surface, which is achieved by the interaction of MMP-9 with the transmembrane receptor CD44.

3.3.4.2. TGF-β inhibits tumor angiogenesis. On the other hand, gallbladder tumors secrete TGF-β, which inhibits angiogenesis and results in reduced tumor growth, consistent with a tumor suppressor function of TGF-β [435]. Ectopic expression, in hepatoma cells, of a mutant TβRII that represents only the extracellular, soluble domain of this receptor, which binds TGF-β with high affinity, results in inhibition of autocrine TGF-β activity and subsequent secretion of VEGF [436]. Thus, blocking autocrine TGF-β induces expression of VEGF, which results in significant angiogenic effects within the tumors that these hepatoma cells develop upon implantation into mice. A similar scenario develops in the transgenic mouse model of skin carcinogenesis with epidermal-specific expression of dominant negative TβRII. Upon treatment with chemical carcinogens, the increased number of tumors that developed with a shorter latency exhibited a dramatic degree of neovascularization, which correlated with their increased rate of metastasis [437]. Thus, inhibiting endogenous TGF-β signaling does not simply promote tumor growth but it has a profound effect on neo-angiogenesis of the tumor that leads to tumor metastasis. The same anti-angiogenic effect of TGF-β signaling was proposed when Smad4-negative pancreatic carcinoma cells were transfected with wild type Smad4 [438]. Reconstitution of Smad4 led to a clear tumor suppressor effect measured by assays of mouse tumor growth. However, these carcinoma cells did not become sensitive to TGF-β signaling, but Smad4 expression suppressed VEGF transcription and induced the anti-angiogenic protein thrombospondin-1. This resulted in development of small tumors with relatively low numbers of microvessels. This experiment proposes a function of Smad4 that may be independent of TGF-β signaling. However, Smad4 might well act downstream of several other cytokines of the TGF-β superfamily that has not been examined yet.

Neuroblastomas that are characterized by oncogenic N-Myc amplification, downregulate activin-A via direct repression of its gene by N-Myc, thus suppressing the anti-proliferative and anti-angiogenic action of this TGF-β family member [439]. Activin-A produced from neuronal cells inhibits proliferation of normal endothelial cells and blocks in vivo angiogenesis. Ectopic expression of activin-A in neuroblastomas with N-Myc amplifications suppresses their proliferation via Smad-mediated induction of cell cycle inhibitors, as well as tumor growth in vivo and angiogenesis formed by such tumors via repression of VEGF transcription [440,441]. Furthermore, screening various human neuroblastomas identified activin-A expression in differentiated, early stage tumors but loss of its expression in undifferentiated, more aggressive tumors. Thus, activin-A signaling resembles the anti-angiogenic action of TGF-β. In contrast, BMP-2 stimulates angiogenesis by lung carcinoma cells, which promotes their tumor growth [442]. BMP-2 has a similar pro-angiogenic role on breast carcinoma cell-derived tumors as it also promotes endothelial tube formation in three-dimensional in vitro cultures [443]. In both cases, BMP-2

induces expression of Id1, the transcriptional regulator that has strong biological effects as a pro-angiogenic factor.

We conclude that similar to the overall dual effect of TGF-β on tumor progression, TGF-β exhibits dual roles in regulating tumor angiogenesis, acting either as inhibitor or as enhancer of neo-vascularization. This dual property is reflected by the known actions of other family members, suggesting that the net outcome of tumor angiogenesis in vivo may depend on the concerted action of more than one TGF-β family cytokines.

3.3.5. Regulation of immunologic action

A final and very important function of TGF-β in the tumor stroma is the modulation of immune cells. TGF-β acts as an inhibitor of B or T lymphocyte proliferation and differentiation, resulting in the most potent natural immunosuppressor in the human body (reviewed in [15]). In addition, TGF-β deactivates scavenging macrophages and thus protects the developing tumor from proper immune surveillance (Fig. 4) [444]. Such a potent role of tumor-secreted TGF-β in helping escape from proper immunological control has been described in natural cases of human cancer such as pancreatic carcinomas or brain glioblastomas, where the secretion of TGF-β1 or TGF-β2 respectively has a clear immunosuppressive effect [445,446]. Direct experimental proof of this action of TGF-β comes from experimental tumors, whereby stable transfection of TGF-β1 into a highly immunogenic tumor, resulted in loss of activation of primary cytolytic T-lymphocytes (CTL), despite the continuous and robust surface expression of tumor-specific antigens and class I major histocompatibility complexes [447]. This behavior of the transfected tumor cells allowed progressive growth and expansion of the tumor. Possibly the most convincing evidence of this mechanism of TGF-β action stems from transgenic mouse models where the dominant negative TβRII is targeted for expression in T-lymphocytes [448]. The engineered T cells develop a proper and enhanced immune response against experimental tumors implanted into the mouse, proving that the T-lymphocyte is a potent cell type that provides effective therapy against cancer when its endogenous TGF-β pathway is inactivated. The same conclusion is derived from colon carcinoma studies in transgenic mice that either overexpress TGF-β1 or the dominant negative TβRII receptor in T-lymphocytes that infiltrate the tumor stroma, since these T-cells drastically inhibit carcinoma cell growth in vivo [449]. In this colon cancer study, TGF-β acting on T cells induced secretion of interleukin 6 (IL-6) that elicited STAT-mediated signaling in the carcinoma cells that is detrimental for proper immune surveillance. Inhibiting TGF-β responses in the T-cell breaks this signaling circuit and provides therapeutic benefits for the host organism. The immune-suppressive effects of TGF-β are closely linked to the function of a special class of lymphocytes, the regulatory $CD4^+CD25^+$ T cells (Treg). Treg suppress the anti-tumor activity of $CD8^+$ T cells by inhibiting their cytotoxic activity and TGF-β signaling is important for this regulatory step as dominant negative TβRII specifically targeted in $CD8^+$ cells results in resistance of these cells to the action of Treg [450]. $CD8^+$ cell resistance to TGF-β signaling then elicits strong anti-tumor activities. Interestingly, Treg themselves are under the control of TGF-β signaling as Treg-specific expression of the dominant-negative TβRII blocks the suppressive activity of Treg towards other $CD8^+$ T cells [451]. Finally, the molecular mechanism and the gene network that mediates the suppressive activity of TGF-β against tumor-fighting CTLs has been recently deciphered using transcriptomic analysis coupled to functional validation of the findings in a mouse thymoma tumor model [452]. Accordingly, TGF-β signaling inhibits expression of perforin, granzyme A and B, Fas ligand and interferon-γ, which act together in order to elicit cytotoxicity mediated by CTLs. At least in the case of interferon-γ and granzyme B, direct transcriptional repression of their genes by Smad protein complexes has been demonstrated and when endogenous TGF-β is neutralized either in the thymoma tumor cells or systemically by engineering expression of a secreted, soluble form of TβRII by the same tumor cells, then the CTL cytotoxic program is restored and mediates its anti-tumor action.

Thus, as outlined above, the activities of TGF-β that target the function of T lymphocytes provide a strong case for the tumor promoting action of this cytokine. Furthermore, T lymphocytes represent excellent targets for anti-tumor therapy in the clinic as exemplified by the various transgenic mouse models utilized so far that lead to tumor eradication upon inhibition of TGF-β signaling.

4. Can TGF-β offer novel therapeutic means against human cancer?

Significant research activity currently focuses on the development of therapeutic approaches against cancer that are based on manipulation of the TGF-β pathway [453]. Here we outline the general approaches taken by pharmaceutical industry and academic research.

4.1. Low molecular weight inhibitors: regulation at the receptor level

Several drugs developed for the treatment of cancer have been analyzed so far and found to have direct or indirect links to the TGF-β signaling pathway. Since this represents a very large body of studies, we will here outline some characteristic examples. Farnesyltransferases are enzymes that post-translationally modify the small GTPases of the Ras family, thus regulating their proper localization on cellular membranes. As we outlined in the signaling part of this review a number of small GTPases seem to mediate signals by TGF-β. Specific inhibitors for farnesyltransferases have been developed as agents that block the oncogenic activity of some of the members of the Ras family [454]. The action of a specific inhibitor against farnesyltransferases, FTI-277, was tested in a pancreatic carcinoma cell line that responds to TGF-β [455]. Interestingly, FTI-277 led to enhanced expression of TβRII mRNA, protein and cell surface ligand-binding activity, thus increasing the responsiveness of the tumor cells to TGF-β with respect to downstream transcriptional and cytostatic responses. This study established that drugs that target oncogenic proteins like Ras,

have additional beneficiary effects as they simultaneously enhance the cytostatic functions of TGF-β against tumor cell growth. However, if we consider the pro-tumorigenic actions of TGF-β, such drugs, if used systemically, they may also have detrimental effects by promoting tumor invasiveness and suppression of cytolytic T cell activities. The TβRII gene is a common target for regulation by several other drugs. (a) Catopril induces TβRII mRNA expression and leads to suppression of renal cancer growth [456]. (b) Dietary ω-3 fatty acids that are used as chemopreventive agents lead to induction of TβRII expression in a mouse model of colon cancer, thus enhancing TGF-β responsiveness of the carcinoma cells that leads to cytostasis [457]. The beneficiary effect of ω-3 fatty acids is not direct on TβRII expression, but rather is mediated by PKCβII, which acts oncogenically during colon tumorigenesis and which suppresses TGF-β signaling by repressing TβRII expression. Thus, ω-3 fatty acids derepress TβRII from the oncogenic action of PKCβII. (c) Similar to the examples that affect TβRII expression, the histone deacetylase inhibitor suberoylanilide hydroxamic acid (SAHA) induces high levels of TβRI in breast cancer cells, which leads to cytostatic responses to TGF-β in the carcinoma cells [458]. (d) In a screen for novel compounds that potentiate the cytostatic effect of TGF-β, the known anti-cancer drug rapamycin was identified as a compound that synergizes with TGF-β in eliciting cytostatic responses in epithelial cells [459]. Interestingly, rapamycin allowed TGF-β to arrest the growth of cell lines transformed by oncogenes such as *Ha-Ras*, *Myc* and *E2F1*. The molecular action of rapamycin seemed to be at the cell cycle level, by inducing the activity of cell cycle inhibitors and decreasing the activity of CDK2. (e) Finally, synthetic triterpenoids with potent cytostatic properties were shown to induce expression of TβRII and mimic the response of TGF-β [460]. These organic compounds were able to enhance Smad signaling at the phosphorylation and transcriptional level and blocked the action of I-Smads, thus providing novel means for induction of endogenous TGF-β responses in pathological conditions.

Despite these various pharmacologic agents that exhibit beneficiary side effects by affecting TGF-β signaling, current intense efforts have led to the development of the first specific low molecular weight inhibitors against the enzymatic activity of the TβRI/ALK5 and TβRII kinases [461,462]. These inhibitors are ATP-binding analogues and competitively block the catalytic pocket of the receptor kinase [463]. The current TβRI/ALK5 kinase inhibitors are relatively specific for this family of receptor kinases, excluding many other serine/threonine kinases and the BMP-specific receptor kinases, however, they inhibit in addition to TβRI/ALK5, the activin/nodal receptors ALK4 and ALK7 [464]. Despite this problem of not absolute specificity against the TGF-β receptor kinase, one of the novel TβRI/ALK5 inhibitors, SD-108, was tested against gliomas [465]. SD-108 was shown to affect all aspects of TGF-β physiology with respect to tumor growth: inhibition of autocrine or paracrine signaling by TGF-β produced by gliomas, inhibition of TGF-β-induced glioma cell migration and invasiveness, and enhancement of the cytotoxic activity of CTLs against glioma cells. Thus, the inhibitor exhibited a promising profile of activities against TGF-β signaling and since the glioma model depends primarily on the pro-tumorigenic action of TGF-β, SD-108 is beneficial against this specific tumor. In another set of studies, the TβRI/ALK5 inhibitors SB-431542, A-83-01 and LY2109761, all inhibited potently TGF-β-induced EMT in vitro, suggesting a possible use of these inhibitors against the pro-tumorigenic action of TGF-β in many types of cancers [358,462,466]. Interestingly, the latter compound, LY2019761, was demonstrated to induce expression of the Coxsackie and adenovirus receptor (CAR), whose expression is normally repressed by TGF-β [467]. This event coincides with the onset of EMT as CAR is a tight junction component that must be downregulated for EMT to take place. The clinical interest of this study lies to the fact that the new TGF-β inhibitor can be possibly used to enhance adenovirus-mediated gene delivery protocols, at least in their initial in vitro phase, prior to transplantation of the engineered cells to the patient. Whether LY2109761 could also be administered directly to the patient that is treated with adenovirus-mediated gene therapy regimes remains to be examined pre-clinically. At the current moment, some of the above-mentioned "specific" inhibitors of TβRI/ALK5 have entered phase I clinical trials against various forms of human cancer and the results of these studies are anxiously awaited.

4.2. Antibody- or affinity-based therapy

Throughout this review, we cited examples where anti-TGF-β experimental approaches have been used in mouse models. We mentioned the use of neutralizing anti-TGF-β antibodies, the soluble extracellular domain of TβRII that binds with high affinity TGF-β and neutralizes its receptor-binding activity, and the soluble extracellular domain of β-glycan, which acts in a similar manner. Some of these reagents have actually advanced to the pre-clinical level.

Using the neutralizing antibody 2G7 that has high affinity for all three mammalian isoforms of TGF-β, TGF-β1, -β2 and -β3, and the metastatic human breast cancer cell line MDA-MB-231, it was demonstrated that the antibody had a small and transient negative effect on primary tumor growth but it could suppress abdominal and lung metastases [468]. The mode of action of this antibody appeared to be primarily the dramatic increase in natural killer lymphocyte activity that apparently enhanced tumor immune surveillance. In the same tumor model, enforced expression of a soluble extracellular domain of β-glycan (sRIII) binds to TGF-β produced by the breast carcinoma cells and inhibits its activity, such that it lowered the incidence and growth of the primary tumors, but more significantly, it completely blocked their metastasis to lung [469]. In this breast cancer model system, sRIII was shown to antagonize autocrine TGF-β, by inducing apoptosis of the carcinoma cells, which exhibited enhanced PTEN levels and reduced Akt activation [470]. Along the same line of thinking, enforced expression of the extracellular, ligand-binding domain of TβRII in various human pancreatic carcinoma cells lines, followed by xenograft experiments in mice, demonstrated weak activity towards

limiting primary tumor growth and strong activity towards inhibiting metastasis [471]. This soluble TβRII antagonist also reduced rat tumors derived from implantation of gliosarcoma cells primarily because of an enhanced natural killer cell-mediated immune response against the primary tumor [454]. The same therapeutic effect has been demonstrated for a soluble extracellular domain of TβRII fused to the immunoglobulin Fc fragment (Fc:TβRII), which resulted in apoptosis of primary breast cancer and inhibition of tumor cell migration, intravasation and lung metastasis in a transgenic mouse model of MMTV-polyoma virus middle T antigen, or in xenograft mouse models with various breast cancer cell lines [472]. The same antagonist, Fc:TβRII, when expressed in the mammary gland of an MMTV-based transgenic mouse model, and the mouse was challenged either with melanoma cells or by crossing it to the MMTV-Neu oncomouse, it fully blocked metastasis [473]. In addition, this mouse model did not exhibit adverse effects from the chronic presence of the antagonist when aged animals were tested, suggesting that the inhibitor acted on the tumor-derived TGF-β without affecting the physiological action of TGF-β on all other tissues of the animal. The clinical potential of this experiment is great and in addition, this experiment pointed to the possibility of using anti-TGF-β therapy without severe adverse effects.

A monoclonal antibody against human endoglin, TEC-11, is a good marker of proliferating endothelial cells in vivo since endoglin is often overexpressed by vascular endothelial cells in the tumor stroma. When TEC-11 was coupled to the deglycosylated ricin A chain, it provided a new cytotoxic drug that targeted human endothelial cells and inhibited their proliferation only when the cells were dividing rapidly, thus promising a therapeutic advantage against tumor angiogenesis that depends on the rapid proliferation of such cells during neovascularization [474]. This principle was actually tested in mouse tumor models of breast cancer, with two additional anti-endoglin antibodies (SN6j and SN6k), which inhibited primary tumor growth and more significantly, they led to a regressed tumor size even 100 days after withdrawal from therapy [475].

Finally, very recently, the first approaches of using Smad-based therapy have surfaced [476]. In this approach, the Smad-interacting domains of FoxH1, LEF1 and CBP, all transcriptional cofactors of Smads during regulation of gene expression, have been used to create high affinity peptide aptamers that are fused to *E. coli* thioredoxin A in order to create stable and highly expressed chimeric proteins that would bind endogenous Smads and block their activity. When tested for biological activity, these anti-Smad peptide aptamers showed highly selective effects for specific gene targets without inhibiting overall TGF-β signaling. Although preliminary, this approach may eventually lead to the development of gene group-specific inhibitors that might interfere solely with the pro-tumorigenic effects of TGF-β.

In conclusion, affinity-based approaches have already shown promising effects and the soluble TβRII reagent seems to provide a rather efficacious alternative to receptor kinase inhibitors, since at least in mice, its chronic presence does not reveal obvious adverse effects. These reagents clearly act as anti-metastatic drugs with small beneficial effects against the primary tumor. Furthermore, the beginnings of anti-Smad reagents may offer future possibilities to target more effectively the pro-tumorigenic action of TGF-β, while leaving its tumor suppressor activity intact.

4.3. Anti-sense RNA approaches

Finally, we should discuss the very promising development of anti-sense oligodeoxynucleotide therapy against gliomas, which as we described above are characterized by over-secretion of TGF-β2. The oligonucleotide AP 12009 is directed against human TGF-β2 and has been administered into brain tumors by continuous infusion [477]. The lack of toxicity of this reagent was tested in rabbits and monkeys [478]. After phase I and II clinical trials, AP 12009 showed better survival time after recurrence when compared to the currently used protocol of chemotherapy against gliomas [477]. Based on this initial success, AP 12009 is now tested in a phase I/II study of pancreatic carcinoma and of malignant melanoma, in order to establish whether this antisense oligonucleotide can be used more widely as an anti-tumor reagent. Picking on the success of the antisense approach another recent effort to exploit RNAi against both TGF-β1 and TGF-β2 in human glioblastoma has been reported [479]. In human gliomas, TGF-β suppresses the differentiation of natural killer cells that attack the primary tumor, and the siRNAs cause reversion of this effect, leading to proper immunological response, which decreases significantly the glioma cell motility and invasiveness.

Thus, brain tumors are currently the major targets of anti-TGF-β therapy that is based on antisense DNA or RNAi technology. These approaches fit to the need for infusion of the drugs directly to the brain; however, the possible use of these inhibitors against other types of solid tumors is open to future trials.

5. Conclusions and perspectives

In this review we tried to provide an authoritative account of the action of TGF-β and some of its relative cytokines during tumor formation, progression and metastasis. We outlined the major signaling pathways involved and provided several examples of the action of this multifunctional cytokine in both human and experimental tumor studies. The current evidence suggests that it is possible to differentiate between the tumor suppressor and pro-tumorigenic effects of TGF-β. The latter appear to be more widespread and essentially affect many if not all solid tumors developed by humans. The tumor suppressor activity operates apparently in some distinct groups of tumors and the mechanisms that dictate the tissue specificity and the frequency by which the TGF-β pathway is inactivated by genetic mutation or epigenetic control is still not fully understood. Among the cellular mechanisms that explain the tumor promoting action of TGF-β, EMT still represents a chapter of active research that requires deeper investigation and further establishment as a major mechanism operating during human tumor progression. On the other hand, suppression of

immune surveillance, and more specifically of cytolytic T lymphocyte action against tumor cells, is a proven action of TGF-β that can be used effectively in a therapeutic approach. More efforts are needed in order to derive specific protocols that will restore such cytolytic T cell activity in cancer patients by targeting the TGF-β pathway with some of the newly developed drugs. Alternatively, if the new TGF-β inhibitors prove to have the long-feared adverse side effects due to the multifunctionality of this cytokine, the more targeted approaches towards Smad function or towards some of the regulators of the pathway that represent drugable entities is amply warranted. We are confident that the near future will prove that anti-TGF-β therapy will join the ranks of other novel therapies that target receptor tyrosine kinases or angiogenesis, in the ever-increasing efforts to conquer cancer.

Acknowledgements

The authors would like to dedicate this review article to the memory of Anita B. Roberts, an inspirational leader of the TGF-β field that transposed her passion, dedication and open-minded attitude towards science to many of us. Funding of the authors' work is provided by the X-109/2001-02 scholarship of the Alexander S. Onassis Public Benefit Foundation, Greece, and the Swedish Research Council (2005–6136) to K.P.; by the Ludwig Institute for Cancer Research, the Swedish Cancer Society (project number: 4855-B03-01XAC), the Swedish Research Council (project number K2004-32XD-14936-01A) and the Marie Curie Research Training Network (RTN) "EpiPlastCarcinoma" under the European Union FP6 program to A.M. We thank our colleagues A. Gaal, M. Kowanetz, A. Kurisaki, K. Kurisaki, P. Lönn, A. Morén, H. Niimi, S. Thuault and U. Valcourt for their contributions to the scientific work emanating from our laboratory, and Carl-Henrik Heldin for continuous encouragement and intellectual support.

References

[1] A.B. Roberts, L.C. Lamb, D.L. Newton, M.B. Sporn, J.E. De Larco, G.J. Todaro, Transforming growth factors: isolation of polypeptides from virally and chemically transformed cells by acid/ethanol extraction, Proc. Natl. Acad. Sci. U. S. A. 77 (1980) 3494–3498.
[2] H.L. Moses, E.L. Branum, J.A. Proper, R.A. Robinson, Transforming growth factor production by chemically transformed cells, Cancer Res. 41 (1981) 2842–2848.
[3] R.F. Tucker, G.D. Shipley, H.L. Moses, R.W. Holley, Growth inhibitor from BSC-1 cells closely related to platelet type β transforming growth factor, Science 226 (1984) 705–707.
[4] A.B. Roberts, M.A. Anzano, L.M. Wakefield, N.S. Roche, D.F. Stern, M.B. Sporn, Type β transforming growth factor: a bifunctional regulator of cellular growth, Proc. Natl. Acad. Sci. U. S. A. 82 (1985) 119–123.
[5] M.B. Sporn, A.B. Roberts, TGF-β: problems and prospects, Cell Regul. 1 (1990) 875–882.
[6] R.J. Akhurst, R. Derynck, TGF-β signaling in cancer—a double-edged sword, Trends Cell Biol. 11 (2001) S44–S51.
[7] A.B. Roberts, L.M. Wakefield, The two faces of transforming growth factor β in carcinogenesis, Proc. Natl. Acad. Sci. U. S. A. 100 (2003) 8621–8623.
[8] D. Hanahan, R.A. Weinberg, The hallmarks of cancer, Cell 100 (2000) 57–70.
[9] N.A. Bhowmick, H.L. Moses, Tumor–stroma interactions, Curr. Opin. Genet. Dev. 15 (2005) 97–101.
[10] L. Levy, C.S. Hill, Alterations in components of the TGF-β superfamily signaling pathways in human cancer, Cytokine Growth Factor Rev. 17 (2006) 41–58.
[11] S.Y. Lin, S.J. Elledge, Multiple tumor suppressor pathways negatively regulate telomerase, Cell 113 (2003) 881–889.
[12] R.L. Elliott, G.C. Blobe, Role of transforming growth factor Beta in human cancer, J. Clin. Oncol. 23 (2005) 2078–2093.
[13] P.M. Siegel, J. Massagué, Cytostatic and apoptotic actions of TGF-β in homeostasis and cancer, Nat. Rev., Cancer 3 (2003) 807–820.
[14] N.A. Bhowmick, E.G. Neilson, H.L. Moses, Stromal fibroblasts in cancer initiation and progression, Nature 432 (2004) 332–337.
[15] M.O. Li, Y.Y. Wan, S. Sanjabi, A.K. Robertson, R.A. Flavell, Transforming growth factor-β regulation of immune responses, Annu. Rev. Immunol. 24 (2006) 99–146.
[16] X.-H. Feng, R. Derynck, Specificity and versatility in TGF-β signaling through Smads, Annu. Rev. Cell Dev. Biol. 21 (2005) 659–693.
[17] J. Massagué, J. Seoane, D. Wotton, Smad transcription factors, Genes Dev. 19 (2005) 2783–2810.
[18] J. Keski-Oja, K. Koli, H. von Melchner, TGF-β activation by traction? Trends Cell Biol. 14 (2004) 657–659.
[19] D.B. Rifkin, Latent transforming growth factor-β (TGF-β) binding proteins: orchestrators of TGF-β availability, J. Biol. Chem. 280 (2005) 7409–7412.
[20] Y. Shi, J. Massagué, Mechanisms of TGF-β signaling from cell membrane to the nucleus, Cell 113 (2003) 685–700.
[21] F. Lebrin, M. Deckers, P. Bertolino, P. ten Dijke, TGF-β receptor function in the endothelium, Cardiovasc. Res. 65 (2005) 599–608.
[22] H.J. Zhu, A.M. Sizeland, A pivotal role for the transmembrane domain in transforming growth factor-β receptor activation, J. Biol. Chem. 274 (1999) 11773–11781.
[23] M. Huse, Y.G. Chen, J. Massagué, J. Kuriyan, Crystal structure of the cytoplasmic domain of the type I TGF β receptor in complex with FKBP12, Cell 96 (1999) 425–436.
[24] B.Y. Qin, S.S. Lam, J.J. Correia, K. Lin, Smad3 allostery links TGF-β receptor kinase activation to transcriptional control, Genes Dev. 16 (2002) 1950–1963.
[25] M.J. Goumans, G. Valdimarsdottir, S. Itoh, A. Rosendahl, P. Sideras, P. ten Dijke, Balancing the activation state of the endothelium via two distinct TGF-β type I receptors, EMBO J. 21 (2002) 1743–1753.
[26] M.J. Goumans, G. Valdimarsdottir, S. Itoh, F. Lebrin, J. Larsson, C. Mummery, S. Karlsson, P. ten Dijke, Activin receptor-like kinase (ALK)1 is an antagonistic mediator of lateral TGFβ/ALK5 signaling, Mol. Cell 12 (2003) 817–828.
[27] F. Lebrin, M.J. Goumans, L. Jonker, R.L. Carvalho, G. Valdimarsdottir, M. Thorikay, C. Mummery, H.M. Arthur, P. ten Dijke, Endoglin promotes endothelial cell proliferation and TGF-βALK1 signal transduction, EMBO J. 23 (2004) 4018–4028.
[28] M.A. Brown, Q. Zhao, K.A. Baker, C. Naik, C. Chen, L. Pukac, M. Singh, T. Tsareva, Y. Parice, A. Mahoney, V. Roschke, I. Sanyal, S. Choe, Crystal structure of BMP-9 and functional interactions with pro-region and receptors, J. Biol. Chem. 280 (2005) 25111–25118.
[29] B. Schmierer, C.S. Hill, Kinetic analysis of Smad nucleocytoplasmic shuttling reveals a mechanism for transforming growth factor beta-dependent nuclear accumulation of Smads, Mol. Cell. Biol. 25 (2005) 9845–9858.
[30] C. Le Roy, J.L. Wrana, Clathrin- and non-clathrin-mediated endocytic regulation of cell signalling, Nat. Rev., Mol. Cell Biol. 6 (2005) 112–126.
[31] H.B. Chen, J. Shen, Y.T. Ip, L. Xu, Identification of phosphatases for Smad in the BMP/DPP pathway, Genes Dev. 20 (2006) 648–653.
[32] X. Lin, X. Duan, Y.Y. Liang, Y. Su, K.H. Wrighton, J. Long, M. Hu, C.M. Davis, J. Wang, F.C. Brunicardi, Y. Shi, Y.G. Chen, A. Meng, X.H. Feng, PPM1A functions as a Smad phosphatase to terminate TGFβ signaling, Cell 125 (2006) 915–928.
[33] A. Moustakas, C.-H. Heldin, Non-Smad TGF-β signals, J. Cell. Sci. 118 (2005) 3573–3584.
[34] S. Markowitz, J. Wang, L. Myeroff, R. Parsons, L. Sun, J. Lutterbaugh,

R.S. Fan, E. Zborowska, K.W. Kinzler, B. Vogelstein, et al., Inactivation of the type II TGF-β receptor in colon cancer cells with microsatellite instability, Science 268 (1995) 1336–1338.
[35] R. Parsons, L.L. Myeroff, B. Liu, J.K. Willson, S.D. Markowitz, K.W. Kinzler, B. Vogelstein, Microsatellite instability and mutations of the transforming growth factor β type II receptor gene in colorectal cancer, Cancer Res. 55 (1995) 5548–5550.
[36] J. Wang, L. Sun, L. Myeroff, X. Wang, L.E. Gentry, J. Yang, J. Liang, E. Zborowska, S. Markowitz, J.K. Willson, et al., Demonstration that mutation of the type II transforming growth factor β receptor inactivates its tumor suppressor activity in replication error-positive colon carcinoma cells, J. Biol. Chem. 270 (1995) 22044–22049.
[37] W.M. Grady, A. Rajput, L. Myeroff, D.F. Liu, K. Kwon, J. Willis, S. Markowitz, Mutation of the type II transforming growth factor-β receptor is coincident with the transformation of human colon adenomas to malignant carcinomas, Cancer Res. 58 (1998) 3101–3104.
[38] P.M. Hempen, L. Zhang, R.K. Bansal, C.A. Iacobuzio-Donahue, K.M. Murphy, A. Maitra, B. Vogelstein, R.H. Whitehead, S.D. Markowitz, J.K. Willson, C.J. Yeo, R.H. Hruban, S.E. Kern, Evidence of selection for clones having genetic inactivation of the activin A type II receptor (ACVR2) gene in gastrointestinal cancers, Cancer Res. 63 (2003) 994–999.
[39] L. Garrigue-Antar, T. Munoz-Antonia, S.J. Antonia, J. Gesmonde, V.F. Vellucci, M. Reiss, Missense mutations of the transforming growth factor β type II receptor in human head and neck squamous carcinoma cells, Cancer Res. 55 (1995) 3982–3987.
[40] P.I. Knaus, D. Lindemann, J.F. DeCoteau, R. Perlman, H. Yankelev, M. Hille, M.E. Kadin, H.F. Lodish, A dominant inhibitory mutant of the type II transforming growth factor β receptor in the malignant progression of a cutaneous T-cell lymphoma, Mol. Cell. Biol. 16 (1996) 3480–3489.
[41] C.D. Lucke, A. Philpott, J.C. Metcalfe, A.M. Thompson, L. Hughes-Davies, P.R. Kemp, R. Hesketh, Inhibiting mutations in the transforming growth factor β type 2 receptor in recurrent human breast cancer, Cancer Res. 61 (2001) 482–485.
[42] M.A. Lynch, R. Nakashima, H. Song, V.L. DeGroff, D. Wang, T. Enomoto, C.M. Weghorst, Mutational analysis of the transforming growth factor β receptor type II gene in human ovarian carcinoma, Cancer Res. 58 (1998) 4227–4232.
[43] W.M. Grady, L.L. Myeroff, S.E. Swinler, A. Rajput, S. Thiagalingam, J.D. Lutterbaugh, A. Neumann, M.G. Brattain, J. Chang, S.J. Kim, K.W. Kinzler, B. Vogelstein, J.K. Willson, S. Markowitz, Mutational inactivation of transforming growth factor β receptor type II in microsatellite stable colon cancers, Cancer Res. 59 (1999) 320–324.
[44] D. Wang, T. Kanuma, H. Mizunuma, F. Takama, Y. Ibuki, N. Wake, A. Mogi, Y. Shitara, S. Takenoshita, Analysis of specific gene mutations in the transforming growth factor-beta signal transduction pathway in human ovarian cancer, Cancer Res. 60 (2000) 4507–4512.
[45] M. Goggins, M. Shekher, K. Turnacioglu, C.J. Yeo, R.H. Hruban, S.E. Kern, Genetic alterations of the transforming growth factor β receptor genes in pancreatic and biliary adenocarcinomas, Cancer Res. 58 (1998) 5329–5332.
[46] T. Chen, W. Yan, R.G. Wells, D.L. Rimm, J. McNiff, D. Leffell, M. Reiss, Novel inactivating mutations of transforming growth factor-β type I receptor gene in head-and-neck cancer metastases, Int. J. Cancer 93 (2001) 653–661.
[47] T. Chen, D. Carter, L. Garrigue-Antar, M. Reiss, Transforming growth factor β type I receptor kinase mutant associated with metastatic breast cancer, Cancer Res. 58 (1998) 4805–4810.
[48] R. Anbazhagan, D.M. Bornman, J.C. Johnston, W.H. Westra, E. Gabrielson, The S387Y mutations of the transforming growth factor-β receptor type I gene is uncommon in metastases of breast cancer and other common types of adenocarcinoma, Cancer Res. 59 (1999) 3363–3364.
[49] G.H. Su, R. Bansal, K.M. Murphy, E. Montgomery, C.J. Yeo, R.H. Hruban, S.E. Kern, ACVR1B (ALK4, activin receptor type 1B) gene mutations in pancreatic carcinoma, Proc. Natl. Acad. Sci. U. S. A. 98 (2001) 3254–3257.
[50] J.R. Howe, J.L. Bair, M.G. Sayed, M.E. Anderson, F.A. Mitros, G.M. Petersen, V.E. Velculescu, G. Traverso, B. Vogelstein, Germline mutations of the gene encoding bone morphogenetic protein receptor 1A in juvenile polyposis, Nat. Genet. 28 (2001) 184–187.
[51] X.P. Zhou, K. Woodford-Richens, R. Lehtonen, K. Kurose, M. Aldred, H. Hampel, V. Launonen, S. Virta, R. Pilarski, R. Salovaara, W.F. Bodmer, B.A. Conrad, M. Dunlop, S.V. Hodgson, T. Iwama, H. Jarvinen, I. Kellokumpu, J.C. Kim, B. Leggett, D. Markie, J.P. Mecklin, K. Neale, R. Phillips, J. Piris, P. Rozen, R.S. Houlston, L.A. Aaltonen, I.P. Tomlinson, C. Eng, Germline mutations in bmpr1a/alk3 cause a subset of cases of juvenile polyposis syndrome and of Cowden and Bannayan–Riley–Ruvalcaba syndromes, Am. J. Hum. Genet. 69 (2001) 704–711.
[52] H. Miyazaki, T. Watabe, T. Kitamura, K. Miyazono, BMP signals inhibit proliferation and in vivo tumor growth of androgen-insensitive prostate carcinoma cells, Oncogene 23 (2004) 9326–9335.
[53] K. Eppert, S.W. Scherer, H. Ozcelik, R. Pirone, P. Hoodless, H. Kim, L.C. Tsui, B. Bapat, S. Gallinger, I.L. Andrulis, G.H. Thomsen, J.L. Wrana, L. Attisano, MADR2 maps to 18q21 and encodes a TGFβ-regulated MAD-related protein that is functionally mutated in colorectal carcinoma, Cell 86 (1996) 543–552.
[54] M.C. Yakicier, M.B. Irmak, A. Romano, M. Kew, M. Ozturk, Smad2 and Smad4 gene mutations in hepatocellular carcinoma, Oncogene 18 (1999) 4879–4883.
[55] T.T. Maliekal, M.L. Antony, A. Nair, R. Paulmurugan, D. Karunagaran, Loss of expression, and mutations of Smad 2 and Smad 4 in human cervical cancer, Oncogene 22 (2003) 4889–4897.
[56] C. Prunier, N. Ferrand, B. Frottier, M. Pessah, A. Atfi, Mechanism for mutational inactivation of the tumor suppressor Smad2, Mol. Cell. Biol. 21 (2001) 3302–3313.
[57] M. Kondo, H. Suzuki, K. Takehara, K. Miyazono, M. Kato, Transforming growth factor-β signaling is differentially inhibited by Smad2D450E and Smad3D407E, Cancer Sci. 95 (2004) 12–17.
[58] E. Dumont, F. Lallemand, C. Prunier, N. Ferrand, A. Guillouzo, B. Clement, A. Atfi, N. Theret, Evidence for a role of Smad3 and Smad2 in stabilization of the tumor-derived mutant Smad2.Q407R, J. Biol. Chem. 278 (2003) 24881–24887.
[59] J. Xu, L. Attisano, Mutations in the tumor suppressors Smad2 and Smad4 inactivate transforming growth factor beta signaling by targeting Smads to the ubiquitin–proteasome pathway, Proc. Natl. Acad. Sci. U. S. A. 97 (2000) 4820–4825.
[60] G.J. Riggins, K.W. Kinzler, B. Vogelstein, S. Thiagalingam, Frequency of Smad gene mutations in human cancers, Cancer Res. 57 (1997) 2578–2580.
[61] T. Arai, Y. Akiyama, S. Okabe, M. Ando, M. Endo, Y. Yuasa, Genomic structure of the human Smad3 gene and its infrequent alterations in colorectal cancers, Cancer Lett. 122 (1998) 157–163.
[62] T. Ikezoe, S. Takeuchi, T. Arai, Y. Yuasa, H. Taguchi, Analysis of the Smad3 gene in hematological malignancies, Int. J. Hematol. 68 (1998) 457–458.
[63] S. Bevan, K. Woodford-Richens, P. Rozen, C. Eng, J. Young, M. Dunlop, K. Neale, R. Phillips, D. Markie, M. Rodriguez-Bigas, B. Leggett, E. Sheridan, S. Hodgson, T. Iwama, D. Eccles, W. Bodmer, R. Houlston, I. Tomlinson, Screening SMAD1, SMAD2, SMAD3, and SMAD5 for germline mutations in juvenile polyposis syndrome, Gut 45 (1999) 406–408.
[64] D. Wang, T. Kanuma, F. Takama, H. Mizumuma, Y. Ibuki, N. Wake, A. Mogi, Y. Shitara, K. Hagiwara, S. Takenoshita, Mutation analysis of the Smad3 gene in human ovarian cancers, Int. J. Oncol. 15 (1999) 949–953.
[65] T.M. Shattuck, J. Costa, M. Bernstein, R.T. Jensen, D.C. Chung, A. Arnold, Mutational analysis of Smad3, a candidate tumor suppressor implicated in TGF-β and menin pathways, in parathyroid adenomas and enteropancreatic endocrine tumors, J. Clin. Endocrinol. Metab. 87 (2002) 3911–3914.
[66] S.U. Han, H.T. Kim, H. Seong do, Y.S. Kim, Y.S. Park, Y.J. Bang, H.K. Yang, S.-J. Kim, Loss of the Smad3 expression increases susceptibility to tumorigenicity in human gastric cancer, Oncogene 23 (2004) 1333–1341.
[67] L.A. Wolfraim, T.M. Fernandez, M. Mamura, W.L. Fuller, R. Kumar, D.E. Cole, S. Byfield, A. Felici, K.C. Flanders, T.M. Walz, A.B.

Roberts, P.D. Aplan, F.M. Balis, J.J. Letterio, Loss of Smad3 in acute T-cell lymphoblastic leukemia, N. Engl. J. Med. 351 (2004) 552–559.
[68] S.A. Hahn, M. Schutte, A.T. Hoque, C.A. Moskaluk, L.T. da Costa, E. Rozenblum, C.L. Weinstein, A. Fischer, C.J. Yeo, R.H. Hruban, S.E. Kern, DPC4, a candidate tumor suppressor gene at human chromosome 18q21.1, Science 271 (1996) 350–353.
[69] M. Schutte, R.H. Hruban, L. Hedrick, K.R. Cho, G.M. Nadasdy, C.L. Weinstein, G.S. Bova, W.B. Isaacs, P. Cairns, H. Nawroz, D. Sidransky, R.A. Casero Jr., P.S. Meltzer, S.A. Hahn, S.E. Kern, DPC4 gene in various tumor types, Cancer Res. 56 (1996) 2527–2530.
[70] M. Nagatake, Y. Takagi, H. Osada, K. Uchida, T. Mitsudomi, S. Saji, K. Shimokata, T. Takahashi, Somatic in vivo alterations of the DPC4 gene at 18q21 in human lung cancers, Cancer Res. 56 (1996) 2718–2720.
[71] S. Thiagalingam, C. Lengauer, F.S. Leach, M. Schutte, S.A. Hahn, J. Overhauser, J.K. Willson, S. Markowitz, S.R. Hamilton, S.E. Kern, K.W. Kinzler, B. Vogelstein, Evaluation of candidate tumour suppressor genes on chromosome 18 in colorectal cancers, Nat. Genet. 13 (1996) 343–346.
[72] S.A. Hahn, D. Bartsch, A. Schroers, H. Galehdari, M. Becker, A. Ramaswamy, I. Schwarte-Waldhoff, H. Maschek, W. Schmiegel, Mutations of the DPC4/Smad4 gene in biliary tract carcinoma, Cancer Res. 58 (1998) 1124–1126.
[73] Y. Shi, A. Hata, R.S. Lo, J. Massagué, N.P. Pavletich, A structural basis for mutational inactivation of the tumour suppressor Smad4, Nature 388 (1997) 87–93.
[74] B.M. Chacko, B.Y. Qin, A. Tiwari, G. Shi, S. Lam, L.J. Hayward, M. De Caestecker, K. Lin, Structural basis of heteromeric Smad protein assembly in TGF-β signaling, Mol. Cell 15 (2004) 813–823.
[75] K. De Bosscher, C.S. Hill, F.J. Nicolas, Molecular and functional consequences of Smad4 C-terminal missense mutations in colorectal tumour cells, Biochem. J. 379 (2004) 209–216.
[76] A. Morén, S. Itoh, A. Moustakas, P. ten Dijke, C.-H. Heldin, Functional consequences of tumorigenic missense mutations in the amino-terminal domain of Smad4, Oncogene 19 (2000) 4396–4404.
[77] D. Maurice, C.E. Pierreux, M. Howell, R.E. Wilentz, M.J. Owen, C.S. Hill, Loss of Smad4 function in pancreatic tumors: C-terminal truncation leads to decreased stability, J. Biol. Chem. 276 (2001) 43175–43181.
[78] M. Liang, Y.Y. Liang, K. Wrighton, D. Ungermannova, X.P. Wang, F.C. Brunicardi, X. Liu, X.-H. Feng, X. Lin, Ubiquitination and Proteolysis of Cancer-Derived Smad4 Mutants by SCF^{Skp2}, Mol. Cell. Biol. 24 (2004) 7524–7537.
[79] J.L. Boulay, G. Mild, A. Lowy, J. Reuter, M. Lagrange, L. Terracciano, U. Laffer, R. Herrmann, C. Rochlitz, SMAD4 is a predictive marker for 5-fluorouracil-based chemotherapy in patients with colorectal cancer, Br. J. Cancer 87 (2002) 630–634.
[80] J.R. Howe, S. Roth, J.C. Ringold, R.W. Summers, H.J. Jarvinen, P. Sistonen, I.P. Tomlinson, R.S. Houlston, S. Bevan, F.A. Mitros, E.M. Stone, L.A. Aaltonen, Mutations in the SMAD4/DPC4 gene in juvenile polyposis, Science 280 (1998) 1086–1088.
[81] K. Woodford-Richens, J. Williamson, S. Bevan, J. Young, B. Leggett, I. Frayling, Y. Thway, S. Hodgson, J.C. Kim, T. Iwama, M. Novelli, D. Sheer, R. Poulsom, N. Wright, R. Houlston, I. Tomlinson, Allelic loss at SMAD4 in polyps from juvenile polyposis patients and use of fluorescence in situ hybridization to demonstrate clonal origin of the epithelium, Cancer Res. 60 (2000) 2477–2482.
[82] W. Friedl, S. Uhlhaas, K. Schulmann, M. Stolte, S. Loff, W. Back, E. Mangold, M. Stern, H.P. Knaebel, C. Sutter, R.G. Weber, S. Pistorius, B. Burger, P. Propping, Juvenile polyposis: massive gastric polyposis is more common in MADH4 mutation carriers than in BMPR1A mutation carriers, Hum. Genet. 111 (2002) 108–111.
[83] C.J. Gallione, G.M. Repetto, E. Legius, A.K. Rustgi, S.L. Schelley, S. Tejpar, G. Mitchell, E. Drouin, C.J. Westermann, D.A. Marchuk, A combined syndrome of juvenile polyposis and hereditary haemorrhagic telangiectasia associated with mutations in MADH4 (SMAD4), Lancet 363 (2004) 852–859.
[84] M. Miyaki, T. Iijima, M. Konishi, K. Sakai, A. Ishii, M. Yasuno, T. Hishima, M. Koike, N. Shitara, T. Iwama, J. Utsunomiya, T. Kuroki, T. Mori, Higher frequency of Smad4 gene mutation in human colorectal cancer with distant metastasis, Oncogene 18 (1999) 3098–3103.
[85] R.E. Wilentz, C.A. Iacobuzio-Donahue, P. Argani, D.M. McCarthy, J.L. Parsons, C.J. Yeo, S.E. Kern, R.H. Hruban, Loss of expression of Dpc4 in pancreatic intraepithelial neoplasia: evidence that DPC4 inactivation occurs late in neoplastic progression, Cancer Res. 60 (2000) 2002–2006.
[86] K.L. Woodford-Richens, A.J. Rowan, P. Gorman, S. Halford, D.C. Bicknell, H.S. Wasan, R.R. Roylance, W.F. Bodmer, I.P. Tomlinson, SMAD4 mutations in colorectal cancer probably occur before chromosomal instability, but after divergence of the microsatellite instability pathway, Proc. Natl. Acad. Sci. U. S. A. 98 (2001) 9719–9723.
[87] X. Liu, J. Yue, R.S. Frey, Q. Zhu, K.M. Mulder, Transforming growth factor beta signaling through Smad1 in human breast cancer cells, Cancer Res. 58 (1998) 4752–4757.
[88] C. Shim, W. Zhang, C.H. Rhee, J.H. Lee, Profiling of differentially expressed genes in human primary cervical cancer by complementary DNA expression array, Clin. Cancer Res. 4 (1998) 3045–3050.
[89] O. Munoz, F. Fend, R. de Beaumont, H. Husson, A. Astier, A.S. Freedman, TGFβ-mediated activation of Smad1 in B-cell non-Hodgkin's lymphoma and effect on cell proliferation, Leukemia 18 (2004) 2015–2025.
[90] A. Gemma, K. Hagiwara, F. Vincent, Y. Ke, A.R. Hancock, M. Nagashima, W.P. Bennett, C.C. Harris, hSmad5 gene, a human hSmad family member: its full length cDNA, genomic structure, promoter region and mutation analysis in human tumors, Oncogene 16 (1998) 951–956.
[91] D.B. Zimonjic, M.E. Durkin, C.L. Keck-Waggoner, S.W. Park, S.S. Thorgeirsson, N.C. Popescu, SMAD5 gene expression, rearrangements, copy number, and amplification at fragile site FRA5C in human hepatocellular carcinoma, Neoplasia 5 (2003) 390–396.
[92] D.P. Hejlik, L.V. Kottickal, H. Liang, J. Fairman, T. Davis, T. Janecki, D. Sexton, W. Perry III, S.V. Tavtigian, D.H. Teng, L. Nagarajan, Localization of SMAD5 and its evaluation as a candidate myeloid tumor suppressor, Cancer Res. 57 (1997) 3779–3783.
[93] J. Zavadil, J. Brezinova, P. Svoboda, Z. Zemanova, K. Michalova, Smad5, a tumor suppressor candidate at 5q31.1, is hemizygously lost and not mutated in the retained allele in human leukemia cell line HL60, Leukemia 11 (1997) 1187–1192.
[94] Y. Jiang, H. Liang, W. Guo, L.V. Kottickal, L. Nagarajan, Differential expression of a novel C-terminally truncated splice form of SMAD5 in hematopoietic stem cells and leukemia, Blood 95 (2000) 3945–3950.
[95] K.H. Cheng, J.F. Ponte, S. Thiagalingam, Elucidation of epigenetic inactivation of SMAD8 in cancer using targeted expressed gene display, Cancer Res. 64 (2004) 1639–1646.
[96] L.G. Horvath, S.M. Henshall, J.G. Kench, J.J. Turner, D. Golovsky, P.C. Brenner, G.F. O'Neill, R. Kooner, P.D. Stricker, J.J. Grygiel, R.L. Sutherland, Loss of BMP2, Smad8, and Smad4 expression in prostate cancer progression, Prostate 59 (2004) 234–242.
[97] D. Wang, T. Kanuma, H. Mizumuma, Y. Ibuki, S. Takenoshita, Mutation analysis of the Smad6 and Smad7 gene in human ovarian cancers, Int. J. Oncol. 17 (2000) 1087–1091.
[98] S. Kawate, S. Ohwada, K. Hamada, T. Koyama, S. Takenoshita, Y. Morishita, K. Hagiwara, Mutational analysis of the Smad6 and Smad7 genes in hepatocellular carcinoma, Int. J. Mol. Med. 8 (2001) 49–52.
[99] T. Fukushima, M. Mashiko, K. Takita, T. Otake, Y. Endo, K. Sekikawa, S. Takenoshita, Mutational analysis of TGF-β type II receptor, Smad2, Smad3, Smad4, Smad6 and Smad7 genes in colorectal cancer, J. Exp. Clin. Cancer Res. 22 (2003) 315–320.
[100] J.L. Boulay, G. Mild, A. Lowy, J. Reuter, M. Lagrange, L. Terracciano, U. Laffer, R. Herrmann, C. Rochlitz, SMAD7 is a prognostic marker in patients with colorectal cancer, Int. J. Cancer 104 (2003) 446–449.
[101] J.M. Cerutti, K.N. Ebina, S.E. Matsuo, L. Martins, R.M. Maciel, E.T. Kimura, Expression of Smad4 and Smad7 in human thyroid follicular carcinoma cell lines, J. Endocrinol. Invest. 26 (2003) 516–521.
[102] Y.H. Kim, H.S. Lee, H.J. Lee, K. Hur, W.H. Kim, Y.J. Bang, S.J. Kim, K.U. Lee, K.J. Choe, H.K. Yang, Prognostic significance of the expression of Smad4 and Smad7 in human gastric carcinomas, Ann. Oncol. 15 (2004) 574–580.
[103] G. Monteleone, G. Del Vecchio Blanco, G. Palmieri, P. Vavassori, I. Monteleone, A. Colantoni, S. Battista, L.G. Spagnoli, M. Romano, M. Borrelli, T.T. MacDonald, F. Pallone, Induction and regulation of Smad7

in the gastric mucosa of patients with Helicobacter pylori infection, Gastroenterology 126 (2004) 674–682.
[104] S.C. Dowdy, A. Mariani, M.M. Reinholz, G.L. Keeney, T.C. Spelsberg, K.C. Podratz, R. Janknecht, Overexpression of the TGF-β antagonist Smad7 in endometrial cancer, Gynecol. Oncol. 96 (2005) 368–373.
[105] J. Kleeff, H. Maruyama, H. Friess, M.W. Buchler, D. Falb, M. Korc, Smad6 suppresses TGF-β-induced growth inhibition in COLO-357 pancreatic cancer cells and is overexpressed in pancreatic cancer, Biochem. Biophys. Res. Commun. 255 (1999) 268–273.
[106] J. Kleeff, T. Ishiwata, H. Maruyama, H. Friess, P. Truong, M.W. Buchler, D. Falb, M. Korc, The TGF-β signaling inhibitor Smad7 enhances tumorigenicity in pancreatic cancer, Oncogene 18 (1999) 5363–5372.
[107] H. Osawa, M. Nakajima, H. Kato, M. Fukuchi, H. Kuwano, Prognostic value of the expression of Smad6 and Smad7, as inhibitory Smads of the TGF-β superfamily, in esophageal squamous cell carcinoma, Anticancer Res. 24 (2004) 3703–3709.
[108] N.B. Arnold, K. Ketterer, J. Kleeff, H. Friess, M.W. Buchler, M. Korc, Thioredoxin is downstream of Smad7 in a pathway that promotes growth and suppresses cisplatin-induced apoptosis in pancreatic cancer, Cancer Res. 64 (2004) 3599–3606.
[109] N. Boyer Arnold, M. Korc, Smad7 abrogates transforming growth factor-beta1-mediated growth inhibition in COLO-357 cells through functional inactivation of the retinoblastoma protein, J. Biol. Chem. 280 (2005) 21858–21866.
[110] C. Chen, X.-F. Wang, L. Sun, Expression of transforming growth factor β (TGFβ) type III receptor restores autocrine TGFβ1 activity in human breast cancer MCF-7 cells, J. Biol. Chem. 272 (1997) 12862–12867.
[111] J.A. Copland, B.A. Luxon, L. Ajani, T. Maity, E. Campagnaro, H. Guo, S.N. LeGrand, P. Tamboli, C.G. Wood, Genomic profiling identifies alterations in TGFβ signaling through loss of TGFβ receptor expression in human renal cell carcinogenesis and progression, Oncogene 22 (2003) 8053–8062.
[112] T. Munoz-Antonia, X. Li, M. Reiss, R. Jackson, S. Antonia, A mutation in the transforming growth factor β type II receptor gene promoter associated with loss of gene expression, Cancer Res. 56 (1996) 4831–4835.
[113] K. Venkatasubbarao, S. Ammanamanchi, M.G. Brattain, D. Mimari, J.W. Freeman, Reversion of transcriptional repression of Sp1 by 5 aza-2′ deoxycytidine restores TGF-β type II receptor expression in the pancreatic cancer cell line MIA PaCa-2, Cancer Res. 61 (2001) 6239–6247.
[114] H. Osada, Y. Tatematsu, A. Masuda, T. Saito, M. Sugiyama, K. Yanagisawa, T. Takahashi, Heterogeneous transforming growth factor (TGF)-β unresponsiveness and loss of TGF-β receptor type II expression caused by histone deacetylation in lung cancer cell lines, Cancer Res. 61 (2001) 8331–8339.
[115] H.T. Zhang, X.F. Chen, M.H. Wang, J.C. Wang, Q.Y. Qi, R.M. Zhang, W.Q. Xu, Q.Y. Fei, F. Wang, Q.Q. Cheng, F. Chen, C.S. Zhu, S.H. Tao, Z. Luo, Defective expression of transforming growth factor β receptor type II is associated with CpG methylated promoter in primary non-small cell lung cancer, Clin. Cancer Res. 10 (2004) 2359–2367.
[116] S.H. Kang, Y.J. Bang, Y.H. Im, H.K. Yang, D.A. Lee, H.Y. Lee, H.S. Lee, N.K. Kim, S.-J. Kim, Transcriptional repression of the transforming growth factor-β type I receptor gene by DNA methylation results in the development of TGF-β resistance in human gastric cancer, Oncogene 18 (1999) 7280–7286.
[117] W. Xie, J.C. Mertens, D.J. Reiss, D.L. Rimm, R.L. Camp, B.G. Haffty, M. Reiss, Alterations of Smad signaling in human breast carcinoma are associated with poor outcome: a tissue microarray study, Cancer Res. 62 (2002) 497–505.
[118] W. Xie, S. Bharathy, D. Kim, B.G. Haffty, D.L. Rimm, M. Reiss, Frequent alterations of Smad signaling in human head and neck squamous cell carcinomas: a tissue microarray analysis, Oncol. Res. 14 (2003) 61–73.
[119] G. Xu, C. Chakraborty, P.K. Lala, Reconstitution of Smad3 restores TGF-β response of tissue inhibitor of metalloprotease-1 upregulation in human choriocarcinoma cells, Biochem. Biophys. Res. Commun. 300 (2003) 383–390.
[120] D.F. Pierce Jr., A.E. Gorska, A. Chytil, K.S. Meise, D.L. Page, R.J. Coffey Jr., H.L. Moses, Mammary tumor suppression by transforming growth factor β 1 transgene expression, Proc. Natl. Acad. Sci. U. S. A. 92 (1995) 4254–4258.
[121] B. Tang, E.P. Böttinger, S.B. Jakowlew, K.M. Bagnall, J. Mariano, M.R. Anver, J.J. Letterio, L.M. Wakefield, Transforming growth factor-β1 is a new form of tumor suppressor with true haploid insufficiency, Nat. Med. 4 (1998) 802–807.
[122] K.B. Ewan, H.A. Oketch-Rabah, S.A. Ravani, G. Shyamala, H.L. Moses, M.H. Barcellos-Hoff, Proliferation of estrogen receptor-α-positive mammary epithelial cells is restrained by transforming growth factor-β1 in adult mice, Am. J. Pathol. 167 (2005) 409–417.
[123] E.P. Böttinger, J.L. Jakubczak, D.C. Haines, K. Bagnall, L.M. Wakefield, Transgenic mice overexpressing a dominant-negative mutant type II transforming growth factor β receptor show enhanced tumorigenesis in the mammary gland and lung in response to the carcinogen 7,12-dimethylbenz-[α]-anthracene, Cancer Res. 57 (1997) 5564–5570.
[124] A.E. Gorska, H. Joseph, R. Derynck, H.L. Moses, R. Serra, Dominant-negative interference of the transforming growth factor β type II receptor in mammary gland epithelium results in alveolar hyperplasia and differentiation in virgin mice, Cell Growth Differ. 9 (1998) 229–238.
[125] E. Forrester, A. Chytil, B. Bierie, M. Aakre, A.E. Gorska, A.R. Sharif-Afshar, W.J. Muller, H.L. Moses, Effect of conditional knockout of the type II TGF-β receptor gene in mammary epithelia on mammary gland development and polyomavirus middle T antigen induced tumor formation and metastasis, Cancer Res. 65 (2005) 2296–2302.
[126] B. Tang, K. de Castro, H.E. Barnes, W.T. Parks, L. Stewart, E.P. Böttinger, D. Danielpour, L.M. Wakefield, Loss of responsiveness to transforming growth factor β induces malignant transformation of nontumorigenic rat prostate epithelial cells, Cancer Res. 59 (1999) 4834–4842.
[127] S. Biswas, A. Chytil, K. Washington, J. Romero-Gallo, A.E. Gorska, P.S. Wirth, S. Gautam, H.L. Moses, W.M. Grady, Transforming growth factor β receptor type II inactivation promotes the establishment and progression of colon cancer, Cancer Res. 64 (2004) 4687–4692.
[128] Y. Zhu, J.A. Richardson, L.F. Parada, J.M. Graff, Smad3 mutant mice develop metastatic colorectal cancer, Cell 94 (1998) 703–714.
[129] M.B. Datto, J.P. Frederick, L. Pan, A.J. Borton, Y. Zhuang, X.-F. Wang, Targeted disruption of Smad3 reveals an essential role in transforming growth factor β-mediated signal transduction, Mol. Cell. Biol. 19 (1999) 2495–2504.
[130] X. Yang, J.J. Letterio, R.J. Lechleider, L. Chen, R. Hayman, H. Gu, A.B. Roberts, C. Deng, Targeted disruption of SMAD3 results in impaired mucosal immunity and diminished T cell responsiveness to TGF-β, EMBO J. 18 (1999) 1280–1291.
[131] A.G. Li, S.L. Lu, M.X. Zhang, C. Deng, X.J. Wang, Smad3 knockout mice exhibit a resistance to skin chemical carcinogenesis, Cancer Res. 64 (2004) 7836–7845.
[132] K. Takaku, J.L. Wrana, E.J. Robertson, M.M. Taketo, No effects of Smad2 (madh2) null mutation on malignant progression of intestinal polyps in Apc(δ716) knockout mice, Cancer Res. 62 (2002) 4558–4561.
[133] T. Hamamoto, H. Beppu, H. Okada, M. Kawabata, T. Kitamura, K. Miyazono, M. Kato, Compound disruption of smad2 accelerates malignant progression of intestinal tumors in apc knockout mice, Cancer Res. 62 (2002) 5955–5961.
[134] K. Takaku, M. Oshima, H. Miyoshi, M. Matsui, M.F. Seldin, M.M. Taketo, Intestinal tumorigenesis in compound mutant mice of both Dpc4 (Smad4) and Apc genes, Cell 92 (1998) 645–656.
[135] K. Takaku, H. Miyoshi, A. Matsunaga, M. Oshima, N. Sasaki, M.M. Taketo, Gastric and duodenal polyps in Smad4 (Dpc4) knockout mice, Cancer Res. 59 (1999) 6113–6117.
[136] W. Li, W. Qiao, L. Chen, X. Xu, X. Yang, D. Li, C. Li, S.G. Brodie, M.M. Meguid, L. Hennighausen, C.-X. Deng, Squamous cell carcinoma and mammary abscess formation through squamous metaplasia in Smad4/Dpc4 conditional knockout mice, Development 130 (2003) 6143–6153.
[137] G. Brodin, P. ten Dijke, K. Funa, C.-H. Heldin, M. Landström, Increased Smad expression and activation are associated with apoptosis in normal

and malignant prostate after castration, Cancer Res. 59 (1999) 2731–2738.
[138] W. He, T. Cao, D.A. Smith, T.E. Myers, X.J. Wang, Smads mediate signaling of the TGFβ superfamily in normal keratinocytes but are lost during skin chemical carcinogenesis, Oncogene 20 (2001) 471–483.
[139] B.J. Jenkins, D. Grail, T. Nheu, M. Najdovska, B. Wang, P. Waring, M. Inglese, R.M. McLoughlin, S.A. Jones, N. Topley, H. Baumann, L.M. Judd, A.S. Giraud, A. Boussioutas, H.J. Zhu, M. Ernst, Hyperactivation of Stat3 in gp130 mutant mice promotes gastric hyperproliferation and desensitizes TGF-β signaling, Nat. Med. 11 (2005) 845–852.
[140] C. Kuang, Y. Xiao, X. Liu, T.M. Stringfield, S. Zhang, Z. Wang, Y. Chen, In vivo disruption of TGF-β signaling by Smad7 leads to premalignant ductal lesions in the pancreas, Proc. Natl. Acad. Sci. U. S. A. 103 (2006) 1858–1863.
[141] S.K. Halder, R.D. Beauchamp, P.K. Datta, Smad7 induces tumorigenicity by blocking TGF-β-induced growth inhibition and apoptosis, Exp. Cell Res. 307 (2005) 231–246.
[142] D. Javelaud, V. Delmas, M. Moller, P. Sextius, J. Andre, S. Menashi, L. Larue, A. Mauviel, Stable overexpression of Smad7 in human melanoma cells inhibits their tumorigenicity in vitro and in vivo, Oncogene 24 (2005) 7624–7629.
[143] J. Massagué, G1 cell-cycle control and cancer, Nature 432 (2004) 298–306.
[144] J.A. Pietenpol, J.T. Holt, R.W. Stein, H.L. Moses, Transforming growth factor β 1 suppression of c-myc gene transcription: role in inhibition of keratinocyte proliferation, Proc. Natl. Acad. Sci. U. S. A. 87 (1990) 3758–3762.
[145] M. Laiho, J.A. DeCaprio, J.W. Ludlow, D.M. Livingston, J. Massagué, Growth inhibition by TGF-β linked to suppression of retinoblastoma protein phosphorylation, Cell 62 (1990) 175–185.
[146] J.A. Pietenpol, R.W. Stein, E. Moran, P. Yaciuk, R. Schlegel, R.M. Lyons, M.R. Pittelkow, K. Munger, P.M. Howley, H.L. Moses, TGF-β 1 inhibition of c-myc transcription and growth in keratinocytes is abrogated by viral transforming proteins with pRB binding domains, Cell 61 (1990) 777–785.
[147] A. Kimchi, X.-F. Wang, R.A. Weinberg, S. Cheifetz, J. Massagué, Absence of TGF-β receptors and growth inhibitory responses in retinoblastoma cells, Science 240 (1988) 196–199.
[148] C.R. Chen, Y. Kang, J. Massagué, Defective repression of c-myc in breast cancer cells: a loss at the core of the transforming growth factor β growth arrest program, Proc. Natl. Acad. Sci. U. S. A. 98 (2001) 992–999.
[149] C.R. Chen, Y. Kang, P.M. Siegel, J. Massague, E2F4/5 and p107 as Smad cofactors linking the TGFbeta receptor to c-myc repression, Cell 110 (2002) 19–32.
[150] J.P. Frederick, N.T. Liberati, D.S. Waddell, Y. Shi, X.-F. Wang, Transforming growth factor β-mediated transcriptional repression of c-myc is dependent on direct binding of Smad3 to a novel repressive Smad binding element, Mol. Cell. Biol. 24 (2004) 2546–2559.
[151] K. Yagi, M. Furuhashi, H. Aoki, D. Goto, H. Kuwano, K. Sugamura, K. Miyazono, M. Kato, c-myc is a downstream target of the Smad pathway, J. Biol. Chem. 277 (2002) 854–861.
[152] M.E. Ewen, C.J. Oliver, H.K. Sluss, S.J. Miller, D.S. Peeper, p53-dependent repression of CDK4 translation in TGF-β-induced G1 cell-cycle arrest, Genes Dev. 9 (1995) 204–217.
[153] S.J. Miller, T. Suthiphongchai, G.P. Zambetti, M.E. Ewen, p53 binds selectively to the 5′ untranslated region of cdk4, an RNA element necessary and sufficient for transforming growth factor β-and p53-mediated translational inhibition of cdk4, Mol. Cell. Biol. 20 (2000) 8420–8431.
[154] K. Polyak, J.Y. Kato, M.J. Solomon, C.J. Sherr, J. Massagué, J.M. Roberts, A. Koff, $p27^{Kip1}$, a cyclin-Cdk inhibitor, links transforming growth factor-β and contact inhibition to cell cycle arrest, Genes Dev. 8 (1994) 9–22.
[155] G.J. Hannon, D. Beach, $p15^{Ink4B}$ is a potential effector of TGF-β-induced cell cycle arrest, Nature 371 (1994) 257–261.
[156] M.B. Datto, Y. Li, J.F. Panus, D.J. Howe, Y. Xiong, X.-F. Wang, Transforming growth factor β induces the cyclin-dependent kinase inhibitor p21 through a p53-independent mechanism, Proc. Natl. Acad. Sci. U. S. A. 92 (1995) 5545–5549.
[157] I. Reynisdottir, K. Polyak, A. Iavarone, J. Massagué, Kip/Cip and Ink4 Cdk inhibitors cooperate to induce cell cycle arrest in response to TGF-β, Genes Dev. 9 (1995) 1831–1845.
[158] J.M. Scandura, P. Boccuni, J. Massagué, S.D. Nimer, Transforming growth factor β-induced cell cycle arrest of human hematopoietic cells requires $p57^{Kip2}$ up-regulation, Proc. Natl. Acad. Sci. U. S. A. 101 (2004) 15231–15236.
[159] A. Iavarone, J. Massagué, Repression of the CDK activator Cdc25A and cell-cycle arrest by cytokine TGF-β in cells lacking the CDK inhibitor p15, Nature 387 (1997) 417–422.
[160] X.H. Feng, X. Lin, R. Derynck, Smad2, Smad3 and Smad4 cooperate with Sp1 to induce $p15^{Ink4B}$ transcription in response to TGF-β, EMBO J. 19 (2000) 5178–5193.
[161] X.-H. Feng, Y.Y. Liang, M. Liang, W. Zhai, X. Lin, Direct interaction of c-Myc with Smad2 and Smad3 to inhibit TGF-β-mediated induction of the CDK inhibitor $p15^{Ink4B}$, Mol. Cell 9 (2002) 133–143.
[162] P. Staller, K. Peukert, A. Kiermaier, J. Seoane, J. Lukas, H. Karsunky, T. Moroy, J. Bartek, J. Massagué, F. Hanel, M. Eilers, Repression of $p15^{Ink4b}$ expression by Myc through association with Miz-1, Nat. Cell Biol. 3 (2001) 392–399.
[163] J. Seoane, C. Pouponnot, P. Staller, M. Schader, M. Eilers, J. Massagué, TGFβ influences Myc, Miz-1 and Smad to control the CDK inhibitor $p15^{Ink4b}$, Nat. Cell Biol. 3 (2001) 400–408.
[164] A. Moustakas, D. Kardassis, Regulation of the human p21/WAF1/Cip1 promoter in hepatic cells by functional interactions between Sp1 and Smad family members, Proc. Natl. Acad. Sci. U. S. A. 95 (1998) 6733–6738.
[165] K. Pardali, A. Kurisaki, A. Morén, P. ten Dijke, D. Kardassis, A. Moustakas, Role of Smad proteins and transcription factor Sp1 in $p21^{WAF1/Cip1}$ regulation by transforming growth factor-β, J. Biol. Chem. 275 (2000) 29244–29256.
[166] M. Miyazaki, M. Sakaguchi, I. Akiyama, Y. Sakaguchi, S. Nagamori, N.H. Huh, Involvement of interferon regulatory factor 1 and S100C/A11 in growth inhibition by transforming growth factor β 1 in human hepatocellular carcinoma cells, Cancer Res. 64 (2004) 4155–4161.
[167] M. Sakaguchi, M. Miyazaki, H. Sonegawa, M. Kashiwagi, M. Ohba, T. Kuroki, M. Namba, N.H. Huh, PKCα mediates TGFβ-induced growth inhibition of human keratinocytes via phosphorylation of S100C/A11, J. Cell Biol. 164 (2004) 979–984.
[168] M. Cordenonsi, S. Dupont, S. Maretto, A. Insinga, C. Imbriano, S. Piccolo, Links between tumor suppressors: p53 is required for TGF-β gene responses by cooperating with Smads, Cell 113 (2003) 301–314.
[169] J. Seoane, H.V. Le, L. Shen, S.A. Anderson, J. Massagué, Integration of Smad and forkhead pathways in the control of neuroepithelial and glioblastoma cell proliferation, Cell 117 (2004) 211–223.
[170] H. Ijichi, M. Otsuka, K. Tateishi, T. Ikenoue, T. Kawakami, F. Kanai, Y. Arakawa, N. Seki, K. Shimizu, K. Miyazono, T. Kawabe, M. Omata, Smad4-independent regulation of p21/WAF1 by transforming growth factor-β, Oncogene 23 (2004) 1043–1051.
[171] L. Levy, C.S. Hill, Smad4 dependency defines two classes of transforming growth factor β (TGF-β) target genes and distinguishes TGF-β-induced epithelial–mesenchymal transition from its antiproliferative and migratory responses, Mol. Cell. Biol. 25 (2005) 8108–8125.
[172] G.F. Claassen, S.R. Hann, A role for transcriptional repression of $p21^{CIP1}$ by c-Myc in overcoming transforming growth factor β-induced cell-cycle arrest, Proc. Natl. Acad. Sci. U. S. A. 97 (2000) 9498–9503.
[173] K. Pardali, M. Kowanetz, C.-H. Heldin, A. Moustakas, Smad pathway-specific transcriptional regulation of the cell cycle inhibitor $p21^{WAF1/Cip1}$, J. Cell. Physiol. 204 (2005) 260–272.
[174] J. Gong, S. Ammanamanchi, T.C. Ko, M.G. Brattain, Transforming growth factor β 1 increases the stability of $p21^{WAF1/CIP1}$ protein and inhibits CDK2 kinase activity in human colon carcinoma FET cells, Cancer Res. 63 (2003) 3340–3346.
[175] F.J. Nicolas, C.S. Hill, Attenuation of the TGF-β-Smad signaling pathway in pancreatic tumor cells confers resistance to TGF-β-induced growth arrest, Oncogene 22 (2003) 3698–3711.

[176] Z. Yan, G.Y. Kim, X. Deng, E. Friedman, Transforming growth factor β 1 induces proliferation in colon carcinoma cells by Ras-dependent, smad-independent down-regulation of $p21^{Cip1}$, J. Biol. Chem. 277 (2002) 9870–9879.

[177] Y. Kang, C.R. Chen, J. Massagué, A self-enabling TGFβ response coupled to stress signaling. Smad engages stress response factor ATF3 for Id1 repression in epithelial cells, Mol. Cell 11 (2003) 915–926.

[178] P.M. Siegel, W. Shu, J. Massagué, Mad Upregulation and Id2 Repression Accompany Transforming Growth Factor (TGF)-β-mediated Epithelial Cell Growth Suppression, J. Biol. Chem. 278 (2003) 35444–35450.

[179] M. Kowanetz, U. Valcourt, R. Bergström, C.-H. Heldin, A. Moustakas, Id2 and Id3 define the potency of cell proliferation and differentiation responses to transforming growth factor β and bone morphogenetic protein, Mol. Cell. Biol. 24 (2004) 4241–4254.

[180] Q.L. Li, K. Ito, C. Sakakura, H. Fukamachi, K. Inoue, X.Z. Chi, K.Y. Lee, S. Nomura, C.W. Lee, S.B. Han, H.M. Kim, W.J. Kim, H. Yamamoto, N. Yamashita, T. Yano, T. Ikeda, S. Itohara, J. Inazawa, T. Abe, A. Hagiwara, H. Yamagishi, A. Ooe, A. Kaneda, T. Sugimura, T. Ushijima, S.C. Bae, Y. Ito, Causal relationship between the loss of RUNX3 expression and gastric cancer, Cell 109 (2002) 113–124.

[181] X.Z. Chi, J.O. Yang, K.Y. Lee, K. Ito, C. Sakakura, Q.L. Li, H.R. Kim, E.J. Cha, Y.H. Lee, A. Kaneda, T. Ushijima, W.J. Kim, Y. Ito, S.C. Bae, RUNX3 suppresses gastric epithelial cell growth by inducing $p21^{WAF1/Cip1}$ expression in cooperation with transforming growth factor β-activated SMAD, Mol. Cell. Biol. 25 (2005) 8097–8107.

[182] D. Noda, S. Itoh, Y. Watanabe, M. Inamitsu, S. Dennler, F. Itoh, S. Koike, D. Danielpour, P. ten Dijke, M. Kato, ELAC2, a putative prostate cancer susceptibility gene product, potentiates TGF-β/Smad-induced growth arrest of prostate cells, Oncogene (2006) 1–10 [Electronic publication ahead of print].

[183] M.C. Birchenall-Roberts, T. Fu, O.S. Bang, M. Dambach, J.H. Resau, C.L. Sadowski, D.C. Bertolette, H.J. Lee, S.J. Kim, F.W. Ruscetti, Tuberous sclerosis complex 2 gene product interacts with human SMAD proteins. A molecular link of two tumor suppressor pathways, J. Biol. Chem. 279 (2004) 25605–25613.

[184] D.R. Alessi, K. Sakamoto, J.R. Bayascas, LKB1-dependent signaling pathways, Annu. Rev. Biochem. 75 (2006) 137–163.

[185] Y. Tang, V. Katuri, A. Dillner, B. Mishra, C.-X. Deng, L. Mishra, Disruption of transforming growth factor-β signaling in ELF β-spectrin-deficient mice, Science 299 (2003) 574–577.

[186] V. Katuri, Y. Tang, C. Li, W. Jogunoori, C.-X. Deng, A. Rashid, A.N. Sidawy, S. Evans, E.P. Reddy, B. Mishra, L. Mishra, Critical interactions between TGF-β signaling/ELF, and E-cadherin/β-catenin mediated tumor suppression, Oncogene 25 (2006) 1871–1886.

[187] Y. Tang, V. Katuri, R. Srinivasan, F. Fogt, R. Redman, G. Anand, A. Said, T. Fishbein, M. Zasloff, E.P. Reddy, B. Mishra, L. Mishra, Transforming growth factor-β suppresses nonmetastatic colon cancer through Smad4 and adaptor protein ELF at an early stage of tumorigenesis, Cancer Res. 65 (2005) 4228–4237.

[188] H.K. Lin, S. Bergmann, P.P. Pandolfi, Cytoplasmic PML function in TGF-β signalling, Nature 431 (2004) 205–211.

[189] S.P. Fink, S.E. Swinler, J.D. Lutterbaugh, J. Massagué, S. Thiagalingam, K.W. Kinzler, B. Vogelstein, J.K. Willson, S. Markowitz, Transforming growth factor-β-induced growth inhibition in a Smad4 mutant colon adenoma cell line, Cancer Res. 61 (2001) 256–260.

[190] M.S. Lee, S.G. Ko, H.P. Kim, Y.B. Kim, S.Y. Lee, S.G. Kim, H.S. Jong, T.Y. Kim, J.W. Lee, Y.J. Bang, Smad2 mediates Erk1/2 activation by TGF-β1 in suspended, but not in adherent, gastric carcinoma cells, Int. J. Oncol. 24 (2004) 1229–1234.

[191] L. Zhang, C.J. Duan, C. Binkley, G. Li, M.D. Uhler, C.D. Logsdon, D.M. Simeone, A transforming growth factor β-induced Smad3/Smad4 complex directly activates protein kinase A, Mol. Cell. Biol. 24 (2004) 2169–2180.

[192] I. Griswold-Prenner, C. Kamibayashi, E.M. Maruoka, M.C. Mumby, R. Derynck, Physical and functional interactions between type I transforming growth factor β receptors and Bα, a WD-40 repeat subunit of phosphatase 2A, Mol. Cell. Biol. 18 (1998) 6595–6604.

[193] C. Petritsch, H. Beug, A. Balmain, M. Oft, TGF-β inhibits p70 S6 kinase via protein phosphatase 2A to induce G1 arrest, Genes Dev. 14 (2000) 3093–3101.

[194] N.A. Bhowmick, M. Ghiassi, M. Aakre, K. Brown, V. Singh, H.L. Moses, TGF-β-induced RhoA and $p160^{ROCK}$ activation is involved in the inhibition of Cdc25A with resultant cell-cycle arrest, Proc. Natl. Acad. Sci. U. S. A. 100 (2003) 15548–15553.

[195] A.K. Kamaraju, A.B. Roberts, Role of Rho/ROCK and p38 MAP kinase pathways in transforming growth factor-β-mediated Smad-dependent growth inhibition of human breast carcinoma cells in vivo, J. Biol. Chem. 280 (2005) 1024–1036.

[196] I. Tachibana, M. Imoto, P.N. Adjei, G.J. Gores, M. Subramaniam, T.C. Spelsberg, R. Urrutia, Overexpression of the TGFβ-regulated zinc finger encoding gene, TIEG, induces apoptosis in pancreatic epithelial cells, J. Clin. Invest. 99 (1997) 2365–2374.

[197] E. Chalaux, T. Lopez-Rovira, J.L. Rosa, G. Pons, L.M. Boxer, R. Bartrons, F. Ventura, A zinc-finger transcription factor induced by TGF-β promotes apoptotic cell death in epithelial Mv1Lu cells, FEBS Lett. 457 (1999) 478–482.

[198] M. Takekawa, K. Tatebayashi, F. Itoh, M. Adachi, K. Imai, H. Saito, Smad-dependent GADD45β expression mediates delayed activation of p38 MAP kinase by TGF-β, EMBO J. 21 (2002) 6473–6482.

[199] J. Yoo, M. Ghiassi, L. Jirmanova, A.G. Balliet, B. Hoffman, A.J. Fornace Jr., D.A. Liebermann, E.P. Böttinger, A.B. Roberts, Transforming growth factor-β-induced apoptosis is mediated by Smad-dependent expression of GADD45b through p38 activation, J. Biol. Chem. 278 (2003) 43001–43007.

[200] M. Ohgushi, S. Kuroki, H. Fukamachi, L.A. O'Reilly, K. Kuida, A. Strasser, S. Yonehara, Transforming growth factor β-dependent sequential activation of Smad, Bim, and caspase-9 mediates physiological apoptosis in gastric epithelial cells, Mol. Cell. Biol. 25 (2005) 10017–10028.

[201] C.W. Jang, C.H. Chen, C.C. Chen, J.Y. Chen, Y.H. Su, R.H. Chen, TGF-β induces apoptosis through Smad-mediated expression of DAP-kinase, Nat. Cell Biol. 4 (2002) 51–58.

[202] H. Valderrama-Carvajal, E. Cocolakis, A. Lacerte, E.H. Lee, G. Krystal, S. Ali, J.J. Lebrun, Activin/TGF-β induce apoptosis through Smad-dependent expression of the lipid phosphatase SHIP, Nat. Cell Biol. 4 (2002) 963–969.

[203] S. Larisch, Y. Yi, R. Lotan, H. Kerner, S. Eimerl, W. Tony Parks, Y. Gottfried, S. Birkey Reffey, M.P. de Caestecker, D. Danielpour, N. Book-Melamed, R. Timberg, C.S. Duckett, R.J. Lechleider, H. Steller, J. Orly, S.J. Kim, A.B. Roberts, A novel mitochondrial septin-like protein, ARTS, mediates apoptosis dependent on its P-loop motif, Nat. Cell Biol. 2 (2000) 915–921.

[204] Y. Gottfried, A. Rotem, R. Lotan, H. Steller, S. Larisch, The mitochondrial ARTS protein promotes apoptosis through targeting XIAP, EMBO J. 23 (2004) 1627–1635.

[205] R.H. Chen, Y.H. Su, R.L. Chuang, T.Y. Chang, Suppression of transforming growth factor-β-induced apoptosis through a phosphatidylinositol 3-kinase/Akt-dependent pathway, Oncogene 17 (1998) 1959–1968.

[206] A.R. Conery, Y. Cao, E.A. Thompson, C.M. Townsend Jr., T.C. Ko, K. Luo, Akt interacts directly with Smad3 to regulate the sensitivity to TGF-β induced apoptosis, Nat. Cell Biol. 6 (2004) 366–372.

[207] I. Remy, A. Montmarquette, S.W. Michnick, PKB/Akt modulates TGF-β signalling through a direct interaction with Smad3, Nat. Cell Biol. 6 (2004) 358–365.

[208] K. Song, H. Wang, T.L. Krebs, D. Danielpour, Novel roles of Akt and mTOR in suppressing TGF-β/ALK5-mediated Smad3 activation, EMBO J. 25 (2006) 58–69.

[209] I. Shin, A.V. Bakin, U. Rodeck, A. Brunet, C.L. Arteaga, Transforming growth factor β enhances epithelial cell survival via Akt-dependent regulation of FKHRL1, Mol. Biol. Cell 12 (2001) 3328–3339.

[210] A. Sánchez-Capelo, Dual role for TGF-β1 in apoptosis, Cytokine Growth Factor Rev. 16 (2005) 15–34.

[211] M. Arsura, G.R. Panta, J.D. Bilyeu, L.G. Cavin, M.A. Sovak, A.A. Oliver, V. Factor, R. Heuchel, F. Mercurio, S.S. Thorgeirsson, G.E.

Sonenshein, Transient activation of NF-κB through a TAK1/IKK kinase pathway by TGF-β1 inhibits AP-1/SMAD signaling and apoptosis: implications in liver tumor formation, Oncogene 22 (2003) 412–425.
[212] M. Arsura, M. Wu, G.E. Sonenshein, TGF β 1 inhibits NF-κB/Rel activity inducing apoptosis of β cells: transcriptional activation of IκBα, Immunity 5 (1996) 31–40.
[213] M. Arsura, M.J. FitzGerald, N. Fausto, G.E. Sonenshein, Nuclear factor-κB/Rel blocks transforming growth factor β1-induced apoptosis of murine hepatocyte cell lines, Cell Growth Differ. 8 (1997) 1049–1059.
[214] M. Schiffer, P. Mundel, A.S. Shaw, E.P. Böttinger, A novel role for the adaptor molecule CD2-associated protein in transforming growth factor-β-induced apoptosis, J. Biol. Chem. 279 (2004) 37004–37012.
[215] J.Y. Yi, I. Shin, C.L. Arteaga, Type I transforming growth factor β receptor binds to and activates phosphatidylinositol 3-kinase, J. Biol. Chem. 280 (2005) 10870–10876.
[216] S.G. Kim, H.S. Jong, T.Y. Kim, J.W. Lee, N.K. Kim, S.H. Hong, Y.J. Bang, Transforming growth factor-β1 induces apoptosis through Fas ligand-independent activation of the Fas death pathway in human gastric SNU-620 carcinoma cells, Mol. Biol. Cell 15 (2004) 420–434.
[217] B.C. Kim, M. Mamura, K.S. Choi, B. Calabretta, S.-J. Kim, Transforming growth factor β 1 induces apoptosis through cleavage of BAD in a Smad3-dependent mechanism in FaO hepatoma cells, Mol. Cell. Biol. 22 (2002) 1369–1378.
[218] G.J. Inman, M.J. Allday, Apoptosis induced by TGF-β 1 in Burkitt's lymphoma cells is caspase 8 dependent but is death receptor independent, J. Immunol. 165 (2000) 2500–2510.
[219] N. Hagimoto, K. Kuwano, I. Inoshima, M. Yoshimi, N. Nakamura, M. Fujita, T. Maeyama, N. Hara, TGF-β 1 as an enhancer of Fas-mediated apoptosis of lung epithelial cells, J. Immunol. 168 (2002) 6470–6478.
[220] R. Perlman, W.P. Schiemann, M.W. Brooks, H.F. Lodish, R.A. Weinberg, TGF-β-induced apoptosis is mediated by the adapter protein Daxx that facilitates JNK activation, Nat. Cell Biol. 3 (2001) 708–714.
[221] T.G. Hofmann, N. Stollberg, M.L. Schmitz, H. Will, HIPK2 regulates transforming growth factor-β-induced c-Jun NH_2-terminal kinase activation and apoptosis in human hepatoma cells, Cancer Res. 63 (2003) 8271–8277.
[222] A. Atfi, M. Buisine, A. Mazars, C. Gespach, Induction of apoptosis by DPC4, a transcriptional factor regulated by transforming growth factor-β through stress-activated protein kinase/c-Jun N-terminal kinase (SAPK/JNK) signaling pathway, J. Biol. Chem. 272 (1997) 24731–24734.
[223] M.E. Engel, M.A. McDonnell, B.K. Law, H.L. Moses, Interdependent SMAD and JNK signaling in transforming growth factor-β-mediated transcription, J. Biol. Chem. 274 (1999) 37413–37420.
[224] Y. Yamamura, X. Hua, S. Bergelson, H.F. Lodish, Critical role of Smads and AP-1 complex in transforming growth factor-β-dependent apoptosis, J. Biol. Chem. 275 (2000) 36295–36302.
[225] M. Landström, N.E. Heldin, S. Bu, A. Hermansson, S. Itoh, P. ten Dijke, C.-H. Heldin, Smad7 mediates apoptosis induced by transforming growth factor β in prostatic carcinoma cells, Curr. Biol. 10 (2000) 535–538.
[226] A. Mazars, F. Lallemand, C. Prunier, J. Marais, N. Ferrand, M. Pessah, G. Cherqui, A. Atfi, Evidence for a role of the JNK cascade in Smad7-mediated apoptosis, J. Biol. Chem. 276 (2001) 36797–36803.
[227] M. Schiffer, M. Bitzer, I.S. Roberts, J.B. Kopp, P. ten Dijke, P. Mundel, E.P. Böttinger, Apoptosis in podocytes induced by TGF-β and Smad7, J. Clin. Invest. 108 (2001) 807–816.
[228] T. Okado, Y. Terada, H. Tanaka, S. Inoshita, A. Nakao, S. Sasaki, Smad7 mediates transforming growth factor-β-induced apoptosis in mesangial cells, Kidney Int. 62 (2002) 1178–1186.
[229] S. Edlund, S. Bu, N. Schüster, P. Aspenström, R. Heuchel, N.E. Heldin, P. ten Dijke, C.-H. Heldin, M. Landström, Transforming growth factor-β1 (TGF-β)-induced apoptosis of prostate cancer cells involves Smad7-dependent activation of p38 by TGF-β-activated kinase 1 and mitogen-activated protein kinase kinase 3, Mol. Biol. Cell 14 (2003) 529–544.
[230] A. Nakao, M. Afrakhte, A. Morén, T. Nakayama, J.L. Christian, R. Heuchel, S. Itoh, M. Kawabata, N.E. Heldin, C.-H. Heldin, P. ten Dijke, Identification of Smad7, a TGFβ-inducible antagonist of TGF-β signalling, Nature 389 (1997) 631–635.
[231] K.Y. Kim, B.C. Kim, Z. Xu, S.J. Kim, Mixed lineage kinase 3 (MLK3)-activated p38 MAP kinase mediates transforming growth factor-β-induced apoptosis in hepatoma cells, J. Biol. Chem. 279 (2004) 29478–29484.
[232] F. Lallemand, A. Mazars, C. Prunier, F. Bertrand, M. Kornprost, S. Gallea, S. Roman-Roman, G. Cherqui, A. Atfi, Smad7 inhibits the survival nuclear factor κB and potentiates apoptosis in epithelial cells, Oncogene 20 (2001) 879–884.
[233] A.B. Glick, W.C. Weinberg, I.H. Wu, W. Quan, S.H. Yuspa, Transforming growth factor β 1 suppresses genomic instability independent of a G1 arrest, p53, and Rb, Cancer Res. 56 (1996) 3645–3650.
[234] A. Glick, N. Popescu, V. Alexander, H. Ueno, E. Böttinger, S.H. Yuspa, Defects in transforming growth factor-β signaling cooperate with a Ras oncogene to cause rapid aneuploidy and malignant transformation of mouse keratinocytes, Proc. Natl. Acad. Sci. U. S. A. 96 (1999) 14949–14954.
[235] E.J. Ehrhart, P. Segarini, M.L. Tsang, A.G. Carroll, M.H. Barcellos-Hoff, Latent transforming growth factor β1 activation in situ: quantitative and functional evidence after low-dose gamma-irradiation, FASEB J. 11 (1997) 991–1002.
[236] K.B. Ewan, R.L. Henshall-Powell, S.A. Ravani, M.J. Pajares, C. Arteaga, R. Warters, R.J. Akhurst, M.H. Barcellos-Hoff, Transforming growth factor-β1 mediates cellular response to DNA damage in situ, Cancer Res. 62 (2002) 5627–5631.
[237] T. Kanamoto, U. Hellman, C.-H. Heldin, S. Souchelnytskyi, Functional proteomics of transforming growth factor-β1-stimulated Mv1Lu epithelial cells: Rad51 as a target of TGFβ1-dependent regulation of DNA repair, EMBO J. 21 (2002) 1219–1230.
[238] A. Dubrovska, T. Kanamoto, M. Lomnytska, C.-H. Heldin, N. Volodko, S. Souchelnytskyi, TGFβ1/Smad3 counteracts BRCA1-dependent repair of DNA damage, Oncogene 24 (2005) 2289–2297.
[239] Y. Katakura, E. Nakata, T. Miura, S. Shirahata, Transforming growth factor β triggers two independent-senescence programs in cancer cells, Biochem. Biophys. Res. Commun. 255 (1999) 110–115.
[240] M.R. Stampfer, J. Garbe, G. Levine, S. Lichtsteiner, A.P. Vasserot, P. Yaswen, Expression of the telomerase catalytic subunit, hTERT, induces resistance to transforming growth factor β growth inhibition in p16INK4A⁻human mammary epithelial cells, Proc. Natl. Acad. Sci. U. S. A. 98 (2001) 4498–4503.
[241] K. Verschueren, J.E. Remacle, C. Collart, H. Kraft, B.S. Baker, P. Tylzanowski, L. Nelles, G. Wuytens, M.T. Su, R. Bodmer, J.C. Smith, D. Huylebroeck, SIP1, a novel zinc finger/homeodomain repressor, interacts with Smad proteins and binds to 5′-CACCT sequences in candidate target genes, J. Biol. Chem. 274 (1999) 20489–20498.
[242] H. Kaji, L. Canaff, J.J. Lebrun, D. Goltzman, G.N. Hendy, Inactivation of menin, a Smad3-interacting protein, blocks transforming growth factor type β signaling, Proc. Natl. Acad. Sci. U. S. A. 98 (2001) 3837–3842.
[243] A.A. Postigo, J.L. Depp, J.J. Taylor, K.L. Kroll, Regulation of Smad signaling through a differential recruitment of coactivators and corepressors by ZEB proteins, EMBO J. 22 (2003) 2453–2462.
[244] B. Hu, D.C. Tack, T. Liu, Z. Wu, M.R. Ullenbruch, S.H. Phan, Role of Smad3 in the regulation of rat telomerase reverse transcriptase by TGFβ, Oncogene 25 (2006) 1030–1041.
[245] M.B. Datto, P.P. Hu, T.F. Kowalik, J. Yingling, X.-F. Wang, The viral oncoprotein E1A blocks transforming growth factor β-mediated induction of p21/WAF1/Cip1 and p15/INK4B, Mol. Cell. Biol. 17 (1997) 2030–2037.
[246] X.-H. Feng, Y. Zhang, R.Y. Wu, R. Derynck, The tumor suppressor Smad4/DPC4 and transcriptional adaptor CBP/p300 are coactivators for Smad3 in TGF-β-induced transcriptional activation, Genes Dev. 12 (1998) 2153–2163.
[247] R. Janknecht, N.J. Wells, T. Hunter, TGF-β-stimulated cooperation of smad proteins with the coactivators CBP/p300, Genes Dev. 12 (1998) 2114–2119.
[248] A. Nishihara, J.I. Hanai, N. Okamoto, J. Yanagisawa, S. Kato, K. Miyazono, M. Kawabata, Role of p300, a transcriptional coactivator, in signalling of TGF-β, Genes Cells 3 (1998) 613–623.
[249] C. Pouponnot, L. Jayaraman, J. Massagué, Physical and functional

interaction of SMADs and p300/CBP, J. Biol. Chem. 273 (1998) 22865–22868.
[250] A. Nishihara, J. Hanai, T. Imamura, K. Miyazono, M. Kawabata, E1A inhibits transforming growth factor-β signaling through binding to Smad proteins, J. Biol. Chem. 274 (1999) 28716–28723.
[251] M.G. Alexandrow, M. Kawabata, M. Aakre, H.L. Moses, Overexpression of the c-Myc oncoprotein blocks the growth-inhibitory response but is required for the mitogenic effects of transforming growth factor β 1, Proc. Natl. Acad. Sci. U. S. A. 92 (1995) 3239–3243.
[252] T. Sasaki, H. Suzuki, K. Yagi, M. Furuhashi, R. Yao, S. Susa, T. Noda, Y. Arai, K. Miyazono, M. Kato, Lymphoid enhancer factor 1 makes cells resistant to transforming growth factor β-induced repression of c-myc, Cancer Res. 63 (2003) 801–806.
[253] E. Labbe, A. Letamendia, L. Attisano, Association of Smads with lymphoid enhancer binding factor 1/T cell-specific factor mediates cooperative signaling by the transforming growth factor-β and wnt pathways, Proc. Natl. Acad. Sci. U. S. A. 97 (2000) 8358–8363.
[254] M. Nishita, M.K. Hashimoto, S. Ogata, M.N. Laurent, N. Ueno, H. Shibuya, K.W. Cho, Interaction between Wnt and TGF-β signalling pathways during formation of Spemann's organizer, Nature 403 (2000) 781–785.
[255] M. Furuhashi, K. Yagi, H. Yamamoto, Y. Furukawa, S. Shimada, Y. Nakamura, A. Kikuchi, K. Miyazono, M. Kato, Axin facilitates Smad3 activation in the transforming growth factor beta signaling pathway, Mol. Cell. Biol. 21 (2001) 5132–5141.
[256] W. Liu, H. Rui, J. Wang, S. Lin, Y. He, M. Chen, Q. Li, Z. Ye, S. Zhang, S.C. Chan, Y.G. Chen, J. Han, S.C. Lin, Axin is a scaffold protein in TGF-β signaling that promotes degradation of Smad7 by Arkadia, EMBO J. 25 (2006) 1646–1658.
[257] M. Kretzschmar, J. Doody, I. Timokhina, J. Massagué, A mechanism of repression of TGFβ/ Smad signaling by oncogenic Ras, Genes Dev. 13 (1999) 804–816.
[258] M.P. de Caestecker, W.T. Parks, C.J. Frank, P. Castagnino, D.P. Bottaro, A.B. Roberts, R.J. Lechleider, Smad2 transduces common signals from receptor serine-threonine and tyrosine kinases, Genes Dev. 12 (1998) 1587–1592.
[259] K. Lehmann, E. Janda, C.E. Pierreux, M. Rytomaa, A. Schulze, M. McMahon, C.S. Hill, H. Beug, J. Downward, Raf induces TGFβ production while blocking its apoptotic but not invasive responses: a mechanism leading to increased malignancy in epithelial cells, Genes Dev. 14 (2000) 2610–2622.
[260] B.A. Roelen, O.S. Cohen, M.K. Raychowdhury, D.N. Chadee, Y. Zhang, J.M. Kyriakis, A.A. Alessandrini, H.-Y. Lin, Phosphorylation of threonine 276 in Smad4 is involved in transforming growth factor-β-induced nuclear accumulation, Am. J. Physiol.: Cell Physiol. 285 (2003) C823–C830.
[261] H. Yamagata, K. Matsuzaki, S. Mori, K. Yoshida, Y. Tahashi, F. Furukawa, G. Sekimoto, T. Watanabe, Y. Uemura, N. Sakaida, K. Yoshioka, Y. Kamiyama, T. Seki, K. Okazaki, Acceleration of Smad2 and Smad3 phosphorylation via c-Jun NH_2-terminal kinase during human colorectal carcinogenesis, Cancer Res. 65 (2005) 157–165.
[262] M.J. Calonge, J. Massagué, Smad4/DPC4 silencing and hyperactive Ras jointly disrupt transforming growth factor-β antiproliferative responses in colon cancer cells, J. Biol. Chem. 274 (1999) 33637–33643.
[263] B.J. Park, J.I. Park, D.S. Byun, J.H. Park, S.G. Chi, Mitogenic conversion of transforming growth factor-β1 effect by oncogenic Ha-Ras-induced activation of the mitogen-activated protein kinase signaling pathway in human prostate cancer, Cancer Res. 60 (2000) 3031–3038.
[264] Z. Yan, X. Deng, E. Friedman, Oncogenic Ki-ras confers a more aggressive colon cancer phenotype through modification of transforming growth factor-β receptor III, J. Biol. Chem. 276 (2001) 1555–1563.
[265] M. Oft, J. Peli, C. Rudaz, H. Schwarz, H. Beug, E. Reichmann, TGF-β1 and Ha-Ras collaborate in modulating the phenotypic plasticity and invasiveness of epithelial tumor cells, Genes Dev. 10 (1996) 2462–2477.
[266] E. Janda, K. Lehmann, I. Killisch, M. Jechlinger, M. Herzig, J. Downward, H. Beug, S. Grünert, Ras and TGFβ cooperatively regulate epithelial cell plasticity and metastasis: dissection of Ras signaling pathways, J. Cell Biol. 156 (2002) 299–313.
[267] I. Yakymovych, P. ten Dijke, C.-H. Heldin, S. Souchelnytskyi, Regulation of Smad signaling by protein kinase C, FASEB J. 15 (2001) 553–555.
[268] S. Akiyoshi, H. Inoue, J. Hanai, K. Kusanagi, N. Nemoto, K. Miyazono, M. Kawabata, c-Ski acts as a transcriptional co-repressor in transforming growth factor-beta signaling through interaction with smads, J. Biol. Chem. 274 (1999) 35269–35277.
[269] K. Luo, S.L. Stroschein, W. Wang, D. Chen, E. Martens, S. Zhou, Q. Zhou, The Ski oncoprotein interacts with the Smad proteins to repress TGFβ signaling, Genes Dev. 13 (1999) 2196–2206.
[270] Y. Sun, X. Liu, E.N. Eaton, W.S. Lane, H.F. Lodish, R.A. Weinberg, Interaction of the Ski oncoprotein with Smad3 regulates TGF-beta signaling, Mol. Cell 4 (1999) 499–509.
[271] W. Xu, K. Angelis, D. Danielpour, M.M. Haddad, O. Bischof, J. Campisi, E. Stavnezer, E.E. Medrano, Ski acts as a co-repressor with Smad2 and Smad3 to regulate the response to type β transforming growth factor, Proc. Natl. Acad. Sci. U. S. A. 97 (2000) 5924–5929.
[272] H. Suzuki, K. Yagi, M. Kondo, M. Kato, K. Miyazono, K. Miyazawa, c-Ski inhibits the TGF-β signaling pathway through stabilization of inactive Smad complexes on Smad-binding elements, Oncogene 23 (2004) 5068–5076.
[273] C. Prunier, M. Pessah, N. Ferrand, S.R. Seo, P. Howe, A. Atfi, The oncoprotein Ski acts as an antagonist of transforming growth factor-β signaling by suppressing Smad2 phosphorylation, J. Biol. Chem. 278 (2003) 26249–26257.
[274] S.L. Stroschein, W. Wang, S. Zhou, Q. Zhou, K. Luo, Negative feedback regulation of TGF-β signaling by the SnoN oncoprotein, Science 286 (1999) 771–774.
[275] Y. Sun, X. Liu, E. Ng-Eaton, H.F. Lodish, R.A. Weinberg, SnoN and Ski protooncoproteins are rapidly degraded in response to transforming growth factor beta signaling, Proc. Natl. Acad. Sci. U. S. A. 96 (1999) 12442–12447.
[276] J. He, S.B. Tegen, A.R. Krawitz, G.S. Martin, K. Luo, The transforming activity of Ski and SnoN is dependent on their ability to repress the activity of Smad proteins, J. Biol. Chem. 278 (2003) 30540–30547.
[277] M. Mizuide, T. Hara, T. Furuya, M. Takeda, K. Kusanagi, Y. Inada, M. Mori, T. Imamura, K. Miyazawa, K. Miyazono, Two short segments of Smad3 are important for specific interaction of Smad3 with c-Ski and SnoN, J. Biol. Chem. 278 (2003) 531–536.
[278] N. Ueki, M.J. Hayman, Direct interaction of Ski with either Smad3 or Smad4 is necessary and sufficient for Ski-mediated repression of transforming growth factor-β signaling, J. Biol. Chem. 278 (2003) 32489–32492.
[279] T. Shinagawa, H.D. Dong, M. Xu, T. Maekawa, S. Ishii, The sno gene, which encodes a component of the histone deacetylase complex, acts as a tumor suppressor in mice, EMBO J. 19 (2000) 2280–2291.
[280] T. Shinagawa, T. Nomura, C. Colmenares, M. Ohira, A. Nakagawara, S. Ishii, Increased susceptibility to tumorigenesis of ski-deficient heterozygous mice, Oncogene 20 (2001) 8100–8108.
[281] J.A. Reed, E. Bales, W. Xu, N.A. Okan, D. Bandyopadhyay, E.E. Medrano, Cytoplasmic localization of the oncogenic protein Ski in human cutaneous melanomas in vivo: functional implications for transforming growth factor β signaling, Cancer Res. 61 (2001) 8074–8078.
[282] A.R. Krakowski, J. Laboureau, A. Mauviel, M.J. Bissell, K. Luo, Cytoplasmic SnoN in normal tissues and nonmalignant cells antagonizes TGF-β signaling by sequestration of the Smad proteins, Proc. Natl. Acad. Sci. U. S. A. 102 (2005) 12437–12442.
[283] S. Bonni, H.R. Wang, C.G. Causing, P. Kavsak, S.L. Stroschein, K. Luo, J.L. Wrana, TGF-beta induces assembly of a Smad2–Smurf2 ubiquitin ligase complex that targets SnoN for degradation, Nat. Cell Biol. 3 (2001) 587–595.
[284] S.L. Stroschein, S. Bonni, J.L. Wrana, K. Luo, Smad3 recruits the anaphase-promoting complex for ubiquitination and degradation of SnoN, Genes Dev. 15 (2001) 2822–2836.
[285] Y. Wan, X. Liu, M.W. Kirschner, The anaphase-promoting complex mediates TGF-β signaling by targeting SnoN for destruction, Mol. Cell 8 (2001) 1027–1039.
[286] F. Zhang, M. Lundin, A. Ristimaki, P. Heikkila, J. Lundin, J. Isola, H.

Joensuu, M. Laiho, Ski-related novel protein N (SnoN), a negative controller of transforming growth factor-β signaling, is a prognostic marker in estrogen receptor-positive breast carcinomas, Cancer Res. 63 (2003) 5005–5010.

[287] M. Fukuchi, M. Nakajima, Y. Fukai, T. Miyazaki, N. Masuda, M. Sohda, R. Manda, K. Tsukada, H. Kato, H. Kuwano, Increased expression of c-Ski as a co-repressor in transforming growth factor-β signaling correlates with progression of esophageal squamous cell carcinoma, Int. J. Cancer 108 (2004) 818–824.

[288] J.S. Edmiston, W.A. Yeudall, T.D. Chung, D.A. Lebman, Inability of transforming growth factor-β to cause SnoN degradation leads to resistance to transforming growth factor-β-induced growth arrest in esophageal cancer cells, Cancer Res. 65 (2005) 4782–4788.

[289] Q. Zhu, S. Pearson-White, K. Luo, Requirement for the SnoN oncoprotein in transforming growth factor β-induced oncogenic transformation of fibroblast cells, Mol. Cell. Biol. 25 (2005) 10731–10744.

[290] M. Fukuchi, Y. Fukai, N. Masuda, T. Miyazaki, M. Nakajima, M. Sohda, R. Manda, K. Tsukada, H. Kato, H. Kuwano, High-level expression of the Smad ubiquitin ligase Smurf2 correlates with poor prognosis in patients with esophageal squamous cell carcinoma, Cancer Res. 62 (2002) 7162–7165.

[291] M. Kurokawa, K. Mitani, K. Irie, T. Matsuyama, T. Takahashi, S. Chiba, Y. Yazaki, K. Matsumoto, H. Hirai, The oncoprotein Evi-1 represses TGF-β signalling by inhibiting Smad3, Nature 394 (1998) 92–96.

[292] P. Sun, P. Dong, K. Dai, G.J. Hannon, D. Beach, p53-independent role of MDM2 in TGF-β1 resistance, Science 282 (1998) 2270–2272.

[293] C.H. Yam, W.Y. Siu, T. Arooz, C.H. Chiu, A. Lau, X.Q. Wang, R.Y. Poon, MDM2 and MDMX inhibit the transcriptional activity of ectopically expressed SMAD proteins, Cancer Res. 59 (1999) 5075–5078.

[294] M. Kadakia, T.L. Brown, M.M. McGorry, S.J. Berberich, MdmX inhibits Smad transactivation, Oncogene 21 (2002) 8776–8785.

[295] S.W. Blain, J. Massagué, Different sensitivity of the transforming growth factor-β cell cycle arrest pathway to c-Myc and MDM-2, J. Biol. Chem. 275 (2000) 32066–32070.

[296] R. Derynck, D.V. Goeddel, A. Ullrich, J.U. Gutterman, R.D. Williams, T.S. Bringman, W.H. Berger, Synthesis of messenger RNAs for transforming growth factors a and β and the epidermal growth factor receptor by human tumors, Cancer Res. 47 (1987) 707–712.

[297] B.A. Arrick, A.R. Lopez, F. Elfman, R. Ebner, C.H. Damsky, R. Derynck, Altered metabolic and adhesive properties and increased tumorigenesis associated with increased expression of transforming growth factor β 1, J. Cell Biol. 118 (1992) 715–726.

[298] M.S. Anscher, W.P. Peters, H. Reisenbichler, W.P. Petros, R.L. Jirtle, Transforming growth factor β as a predictor of liver and lung fibrosis after autologous bone marrow transplantation for advanced breast cancer, N. Engl. J. Med. 328 (1993) 1592–1598.

[299] B.I. Dalal, P.A. Keown, A.H. Greenberg, Immunocytochemical localization of secreted transforming growth factor-β 1 to the advancing edges of primary tumors and to lymph node metastases of human mammary carcinoma, Am. J. Pathol. 143 (1993) 381–389.

[300] R.A. Walker, S.J. Dearing, B. Gallacher, Relationship of transforming growth factor β 1 to extracellular matrix and stromal infiltrates in invasive breast carcinoma, Br. J. Cancer 69 (1994) 1160–1165.

[301] N. Ito, S. Kawata, S. Tamura, Y. Shirai, S. Kiso, H. Tsushima, Y. Matsuzawa, Positive correlation of plasma transforming growth factor-β 1 levels with tumor vascularity in hepatocellular carcinoma, Cancer Lett. 89 (1995) 45–48.

[302] Y. Hasegawa, S. Takanashi, Y. Kanehira, T. Tsushima, T. Imai, K. Okumura, Transforming growth factor-β1 level correlates with angiogenesis, tumor progression, and prognosis in patients with nonsmall cell lung carcinoma, Cancer 91 (2001) 964–971.

[303] V. Ivanovic, A. Melman, B. Davis-Joseph, M. Valcic, J. Geliebter, Elevated plasma levels of TGF-β 1 in patients with invasive prostate cancer, Nat. Med. 1 (1995) 282–284.

[304] P. Wikstrom, P. Stattin, I. Franck-Lissbrant, J.E. Damber, A. Bergh, Transforming growth factor β1 is associated with angiogenesis, metastasis, and poor clinical outcome in prostate cancer, Prostate 37 (1998) 19–29.

[305] S.F. Shariat, M. Shalev, A. Menesses-Diaz, I.Y. Kim, M.W. Kattan, T.M. Wheeler, K.M. Slawin, Preoperative plasma levels of transforming growth factor β_1 (TGF-β_1) strongly predict progression in patients undergoing radical prostatectomy, J. Clin. Oncol. 19 (2001) 2856–2864.

[306] H. Tsushima, S. Kawata, S. Tamura, N. Ito, Y. Shirai, S. Kiso, Y. Imai, H. Shimomukai, Y. Nomura, Y. Matsuda, Y. Matsuzawa, High levels of transforming growth factor β 1 in patients with colorectal cancer: association with disease progression, Gastroenterology 110 (1996) 375–382.

[307] K.S. Shim, K.H. Kim, W.S. Han, E.B. Park, Elevated serum levels of transforming growth factor-β1 in patients with colorectal carcinoma: its association with tumor progression and its significant decrease after curative surgical resection, Cancer 85 (1999) 554–561.

[308] H. Tsushima, N. Ito, S. Tamura, Y. Matsuda, M. Inada, I. Yabuuchi, Y. Imai, R. Nagashima, H. Misawa, H. Takeda, Y. Matsuzawa, S. Kawata, Circulating transforming growth factor β 1 as a predictor of liver metastasis after resection in colorectal cancer, Clin. Cancer Res. 7 (2001) 1258–1262.

[309] K. Krasagakis, D. Tholke, B. Farthmann, J. Eberle, U. Mansmann, C.E. Orfanos, Elevated plasma levels of transforming growth factor (TGF)-β1 and TGF-β2 in patients with disseminated malignant melanoma, Br. J. Cancer 77 (1998) 1492–1494.

[310] A. Ghellal, C. Li, M. Hayes, G. Byrne, N. Bundred, S. Kumar, Prognostic significance of TGF β 1 and TGF β 3 in human breast carcinoma, Anticancer Res. 20 (2000) 4413–4418.

[311] M.J. Puchner, J.A. Koppen, S. Zapf, C. Knabbe, M. Westphal, The influence of tamoxifen on the secretion of transforming growth factor-β2 (TGF-β2) in glioblastomas: in vitro and in vivo findings, Anticancer Res. 22 (2002) 45–51.

[312] S. Ananth, B. Knebelmann, W. Gruning, M. Dhanabal, G. Walz, I.E. Stillman, V.P. Sukhatme, Transforming growth factor β1 is a target for the von Hippel–Lindau tumor suppressor and a critical growth factor for clear cell renal carcinoma, Cancer Res. 59 (1999) 2210–2216.

[313] M. Laitinen, L. Jortikka, T. Halttunen, J. Nevalainen, A.J. Aho, A. Marttinen, T.S. Lindholm, Measurement of total and local bone morphogenetic protein concentration in bone tumours, Int. Orthop. 21 (1997) 188–193.

[314] M. Raida, M. Sarbia, J.H. Clement, S. Adam, H.E. Gabbert, K. Hoffken, Expression, regulation and clinical significance of bone morphogenetic protein 6 in esophageal squamous-cell carcinoma, Int. J. Cancer 83 (1999) 38–44.

[315] J. Dai, J. Keller, J. Zhang, Y. Lu, Z. Yao, E.T. Keller, Bone morphogenetic protein-6 promotes osteoblastic prostate cancer bone metastases through a dual mechanism, Cancer Res. 65 (2005) 8274–8285.

[316] S. Yang, C. Zhong, B. Frenkel, A.H. Reddi, P. Roy-Burman, Diverse biological effect and Smad signaling of bone morphogenetic protein 7 in prostate tumor cells, Cancer Res. 65 (2005) 5769–5777.

[317] E.M. Langenfeld, Y. Kong, J. Langenfeld, Bone morphogenetic protein 2 stimulation of tumor growth involves the activation of Smad-1/5, Oncogene 25 (2006) 685–692.

[318] M.W. Helms, J. Packeisen, C. August, B. Schittek, W. Boecker, B.H. Brandt, H. Buerger, First evidence supporting a potential role for the BMP/SMAD pathway in the progression of oestrogen receptor-positive breast cancer, J. Pathol. 206 (2005) 366–376.

[319] D.R. Welch, A. Fabra, M. Nakajima, Transforming growth factor β stimulates mammary adenocarcinoma cell invasion and metastatic potential, Proc. Natl. Acad. Sci. U. S. A. 87 (1990) 7678–7682.

[320] H.L. Chang, N. Gillett, I. Figari, A.R. Lopez, M.A. Palladino, R. Derynck, Increased transforming growth factor β expression inhibits cell proliferation in vitro, yet increases tumorigenicity and tumor growth of Meth A sarcoma cells, Cancer Res. 53 (1993) 4391–4398.

[321] W. Cui, D.J. Fowlis, S. Bryson, E. Duffie, H. Ireland, A. Balmain, R.J. Akhurst, TGFβ1 inhibits the formation of benign skin tumors, but enhances progression to invasive spindle carcinomas in transgenic mice, Cell 86 (1996) 531–542.

[322] G. Portella, S.A. Cumming, J. Liddell, W. Cui, H. Ireland, R.J. Akhurst, A. Balmain, Transforming growth factor β is essential for spindle cell conversion of mouse skin carcinoma in vivo: implications for tumor invasion, Cell Growth Differ. 9 (1998) 393–404.
[323] B.H. Weeks, W. He, K.L. Olson, X.-J. Wang, Inducible expression of transforming growth factor β1 in papillomas causes rapid metastasis, Cancer Res. 61 (2001) 7435–7443.
[324] R.S. Muraoka-Cook, H. Kurokawa, Y. Koh, J.T. Forbes, L.R. Roebuck, M.H. Barcellos-Hoff, S.E. Moody, L.A. Chodosh, C.L. Arteaga, Conditional overexpression of active transforming growth factor β1 in vivo accelerates metastases of transgenic mammary tumors, Cancer Res. 64 (2004) 9002–9011.
[325] G. Han, S.L. Lu, A.G. Li, W. He, C.L. Corless, M. Kulesz-Martin, X.-J. Wang, Distinct mechanisms of TGF-β1-mediated epithelial-to-mesenchymal transition and metastasis during skin carcinogenesis, J. Clin. Invest. 115 (2005) 1714–1723.
[326] L.Y. Bourguignon, P.A. Singleton, H. Zhu, B. Zhou, Hyaluronan promotes signaling interaction between CD44 and the transforming growth factor β receptor I in metastatic breast tumor cells, J. Biol. Chem. 277 (2002) 39703–39712.
[327] A. Wicki, F. Lehembre, N. Wick, B. Hantusch, D. Kerjaschki, G. Christofori, Tumor invasion in the absence of epithelial–mesenchymal transition: podoplanin-mediated remodeling of the actin cytoskeleton, Cancer Cell 9 (2006) 261–272.
[328] M. Oft, K.H. Heider, H. Beug, TGFβ signaling is necessary for carcinoma cell invasiveness and metastasis, Curr. Biol. 8 (1998) 1243–1252.
[329] J.J. Yin, K. Selander, J.M. Chirgwin, M. Dallas, B.G. Grubbs, R. Wieser, J. Massagué, G.R. Mundy, T.A. Guise, TGF-β signaling blockade inhibits PTHrP secretion by breast cancer cells and bone metastases development, J. Clin. Invest. 103 (1999) 197–206.
[330] J.A. McEarchern, J.J. Kobie, V. Mack, R.S. Wu, L. Meade-Tollin, C.L. Arteaga, N. Dumont, D. Besselsen, E. Seftor, M.J. Hendrix, E. Katsanis, E.T. Akporiaye, Invasion and metastasis of a mammary tumor involves TGF-β signaling, Int. J. Cancer 91 (2001) 76–82.
[331] B. Tang, M. Vu, T. Booker, S.J. Santner, F.R. Miller, M.R. Anver, L.M. Wakefield, TGF-β switches from tumor suppressor to prometastatic factor in a model of breast cancer progression, J. Clin. Invest. 112 (2003) 1116–1124.
[332] P.M. Siegel, W. Shu, R.D. Cardiff, W.J. Muller, J. Massagué, Transforming growth factor β signaling impairs Neu-induced mammary tumorigenesis while promoting pulmonary metastasis, Proc. Natl. Acad. Sci. U. S. A. 100 (2003) 8430–8435.
[333] R.S. Muraoka-Cook, I. Shin, J.Y. Yi, E. Easterly, M.H. Barcellos-Hoff, J.M. Yingling, R. Zent, C.L. Arteaga, Activated type I TGFβ receptor kinase enhances the survival of mammary epithelial cells and accelerates tumor progression, Oncogene 25 (2005) 3403–3423.
[334] R.S. Muraoka, Y. Koh, L.R. Roebuck, M.E. Sanders, D. Brantley-Sieders, A.E. Gorska, H.L. Moses, C.L. Arteaga, Increased malignancy of Neu-induced mammary tumors overexpressing active transforming growth factor β1, Mol. Cell. Biol. 23 (2003) 8691–8703.
[335] F. Tian, S.D. Byfield, W.T. Parks, C.H. Stuelten, D. Nemani, Y.E. Zhang, A.B. Roberts, Smad-binding defective mutant of transforming growth factor β type I receptor enhances tumorigenesis but suppresses metastasis of breast cancer cell lines, Cancer Res. 64 (2004) 4523–4530.
[336] F. Tian, S. DaCosta Byfield, W.T. Parks, S. Yoo, A. Felici, B. Tang, E. Piek, L.M. Wakefield, A.B. Roberts, Reduction in Smad2/3 signaling enhances tumorigenesis but suppresses metastasis of breast cancer cell lines, Cancer Res. 63 (2003) 8284–8292.
[337] Y. Kang, W. He, S. Tulley, G.P. Gupta, I. Serganova, C.R. Chen, K. Manova-Todorova, R. Blasberg, W.L. Gerald, J. Massagué, Breast cancer bone metastasis mediated by the Smad tumor suppressor pathway, Proc. Natl. Acad. Sci. U. S. A. 102 (2005) 13909–13914.
[338] M. Deckers, M. van Dinther, J. Buijs, I. Que, C. Lowik, G. van der Pluijm, P. ten Dijke, The tumor suppressor Smad4 is required for transforming growth factor β-induced epithelial to mesenchymal transition and bone metastasis of breast cancer cells, Cancer Res. 66 (2006) 2202–2209.
[339] H. Azuma, S. Ehata, H. Miyazaki, T. Watabe, O. Maruyama, T. Imamura, T. Sakamoto, S. Kiyama, Y. Kiyama, T. Ubai, T. Inamoto, S. Takahara, Y. Itoh, Y. Otsuki, Y. Katsuoka, K. Miyazono, S. Horie, Effect of Smad7 expression on metastasis of mouse mammary carcinoma JygMC(A) cells, J. Natl. Cancer Inst. 97 (2005) 1734–1746.
[340] Y. Kang, P.M. Siegel, W. Shu, M. Drobnjak, S.M. Kakonen, C. Cordon-Cardo, T.A. Guise, J. Massagué, A multigenic program mediating breast cancer metastasis to bone, Cancer Cell 3 (2003) 537–549.
[341] J.P. Thiery, Epithelial–mesenchymal transitions in development and pathologies, Curr. Opin. Cell Biol. 15 (2003) 740–746.
[342] M. Zeisberg, R. Kalluri, The role of epithelial-to-mesenchymal transition in renal fibrosis, J. Mol. Med. 82 (2004) 175–181.
[343] E.D. Hay, The mesenchymal cell, its role in the embryo, and the remarkable signaling mechanisms that create it, Dev. Dyn. 233 (2005) 706–720.
[344] M.A. Huber, N. Kraut, H. Beug, Molecular requirements for epithelial–mesenchymal transition during tumor progression, Curr. Opin. Cell Biol. 17 (2005) 548–558.
[345] P.J. Miettinen, R. Ebner, A.R. Lopez, R. Derynck, TGF-β induced transdifferentiation of mammary epithelial cells to mesenchymal cells: involvement of type I receptors, J. Cell Biol. 127 (1994) 2021–2036.
[346] C. Caulin, F.G. Scholl, P. Frontelo, C. Gamallo, M. Quintanilla, Chronic exposure of cultured transformed mouse epidermal cells to transforming growth factor-β 1 induces an epithelial–mesenchymal transdifferentiation and a spindle tumoral phenotype, Cell Growth Differ. 6 (1995) 1027–1035.
[347] D. Danielpour, Transdifferentiation of NRP-152 rat prostatic basal epithelial cells toward a luminal phenotype: regulation by glucocorticoid, insulin-like growth factor-I and transforming growth factor-β, J. Cell. Sci. 112 (Pt. 2) (1999) 169–179.
[348] V. Ellenrieder, S.F. Hendler, W. Boeck, T. Seufferlein, A. Menke, C. Ruhland, G. Adler, T.M. Gress, Transforming growth factor β1 treatment leads to an epithelial–mesenchymal transdifferentiation of pancreatic cancer cells requiring extracellular signal-regulated kinase 2 activation, Cancer Res. 61 (2001) 4222–4228.
[349] J. Zavadil, M. Bitzer, D. Liang, Y.C. Yang, A. Massimi, S. Kneitz, E. Piek, E.P. Böttinger, Genetic programs of epithelial cell plasticity directed by transforming growth factor-β, Proc. Natl. Acad. Sci. U. S. A. 98 (2001) 6686–6691.
[350] J. Gotzmann, H. Huber, C. Thallinger, M. Wolschek, B. Jansen, R. Schulte-Hermann, H. Beug, W. Mikulits, Hepatocytes convert to a fibroblastoid phenotype through the cooperation of TGF-β1 and Ha-Ras: steps towards invasiveness, J. Cell. Sci. 115 (2002) 1189–1202.
[351] M. Grande, A. Franzén, J.O. Karlsson, L.E. Ericson, N.E. Heldin, M. Nilsson, Transforming growth factor-β and epidermal growth factor synergistically stimulate epithelial to mesenchymal transition (EMT) through a MEK-dependent mechanism in primary cultured pig thyrocytes, J. Cell. Sci. 115 (2002) 4227–4236.
[352] F. Valdes, A.M. Alvarez, A. Locascio, S. Vega, B. Herrera, M. Fernandez, M. Benito, M.A. Nieto, I. Fabregat, The epithelial mesenchymal transition confers resistance to the apoptotic effects of transforming growth factor β in fetal rat hepatocytes, Mol. Cancer Res. 1 (2002) 68–78.
[353] Y. Li, J. Yang, C. Dai, C. Wu, Y. Liu, Role for integrin-linked kinase in mediating tubular epithelial to mesenchymal transition and renal interstitial fibrogenesis, J. Clin. Invest. 112 (2003) 503–516.
[354] M. Sato, Y. Muragaki, S. Saika, A.B. Roberts, A. Ooshima, Targeted disruption of TGF-β1/Smad3 signaling protects against renal tubulointerstitial fibrosis induced by unilateral ureteral obstruction, J. Clin. Invest. 112 (2003) 1486–1494.
[355] K.A. Brown, M.E. Aakre, A.E. Gorska, J.O. Price, S.E. Eltom, J.A. Pietenpol, H.L. Moses, Induction by transforming growth factor-β1 of epithelial to mesenchymal transition is a rare event in vitro, Breast Cancer Res. 6 (2004) R215–R231.
[356] S. Saika, S. Kono-Saika, Y. Ohnishi, M. Sato, Y. Muragaki, A. Ooshima, K.C. Flanders, J. Yoo, M. Anzano, C.Y. Liu, W.W. Kao, A.B. Roberts,

Smad3 signaling is required for epithelial–mesenchymal transition of lens epithelium after injury, Am. J. Pathol. 164 (2004) 651–663.
[357] R.C. Bates, A.M. Mercurio, The epithelial–mesenchymal transition (EMT) and colorectal cancer progression, Cancer Biol. Ther. 4 (2005) 365–370.
[358] U. Valcourt, M. Kowanetz, H. Niimi, C.-H. Heldin, A. Moustakas, TGF-β and the Smad signaling pathway support transcriptomic reprogramming during epithelial–mesenchymal cell transition, Mol. Biol. Cell 16 (2005) 1987–2002.
[359] B.C. Willis, J.M. Liebler, K. Luby-Phelps, A.G. Nicholson, E.D. Crandall, R.M. du Bois, Z. Borok, Induction of epithelial–mesenchymal transition in alveolar epithelial cells by transforming growth factor-β1: potential role in idiopathic pulmonary fibrosis, Am. J. Pathol. 166 (2005) 1321–1332.
[360] E. Piek, A. Moustakas, A. Kurisaki, C.-H. Heldin, P. ten Dijke, TGF-β type I receptor/ALK-5 and Smad proteins mediate epithelial to mesenchymal transdifferentiation in NMuMG breast epithelial cells, J. Cell. Sci. 112 (1999) 4557–4568.
[361] S. Saika, K. Ikeda, O. Yamanaka, K.C. Flanders, Y. Ohnishi, Y. Nakajima, Y. Muragaki, A. Ooshima, Adenoviral gene transfer of BMP-7, Id2, or Id3 suppresses injury-induced epithelial-to-mesenchymal transition of lens epithelium in mice, Am. J. Physiol.: Cell Physiol. 290 (2006) C282–C289.
[362] M. Zeisberg, A.A. Shah, R. Kalluri, Bone morphogenic protein-7 induces mesenchymal to epithelial transition in adult renal fibroblasts and facilitates regeneration of injured kidney, J. Biol. Chem. 280 (2005) 8094–8100.
[363] M. Oft, R.J. Akhurst, A. Balmain, Metastasis is driven by sequential elevation of H-ras and Smad2 levels, Nat. Cell Biol. 4 (2002) 487–494.
[364] S. Itoh, M. Thorikay, M. Kowanetz, A. Moustakas, F. Itoh, C.-H. Heldin, P. ten Dijke, Elucidation of Smad requirement in transforming growth factor-β type I receptor-induced responses, J. Biol. Chem. 278 (2003) 3751–3761.
[365] K. Kurisaki, A. Kurisaki, U. Valcourt, A.A. Terentiev, K. Pardali, P. ten Dijke, C.-H. Heldin, J. Ericsson, A. Moustakas, Nuclear factor YY1 inhibits transforming growth factor β-and bone morphogenetic protein-induced cell differentiation, Mol. Cell. Biol. 23 (2003) 4494–4510.
[366] M. Takeda, M. Mizuide, M. Oka, T. Watabe, H. Inoue, H. Suzuki, T. Fujita, T. Imamura, K. Miyazono, K. Miyazawa, Interaction with Smad4 is indispensable for suppression of BMP signaling by c-Ski, Mol. Biol. Cell 15 (2004) 963–972.
[367] M. Jechlinger, S. Grunert, I.H. Tamir, E. Janda, S. Ludemann, T. Waerner, P. Seither, A. Weith, H. Beug, N. Kraut, Expression profiling of epithelial plasticity in tumor progression, Oncogene 22 (2003) 7155–7169.
[368] L. Xie, B.K. Law, M.E. Aakre, M. Edgerton, Y. Shyr, N.A. Bhowmick, H.L. Moses, Transforming growth factor β-regulated gene expression in a mouse mammary gland epithelial cell line, Breast Cancer Res. 5 (2003) R187–R198.
[369] D. LaGamba, A. Nawshad, E.D. Hay, Microarray analysis of gene expression during epithelial–mesenchymal transformation, Dev. Dyn. 234 (2005) 132–142.
[370] N.A. Bhowmick, M. Ghiassi, A. Bakin, M. Aakre, C.A. Lundquist, M.E. Engel, C.L. Arteaga, H.L. Moses, Transforming growth factor-β1 mediates epithelial to mesenchymal transdifferentiation through a RhoA-dependent mechanism, Mol. Biol. Cell 12 (2001) 27–36.
[371] A.V. Bakin, C. Rinehart, A.K. Tomlinson, C.L. Arteaga, p38 mitogen-activated protein kinase is required for TGFβ-mediated fibroblastic transdifferentiation and cell migration, J. Cell. Sci. 115 (2002) 3193–3206.
[372] L. Xie, B.K. Law, A.M. Chytil, K.A. Brown, M.E. Aakre, H.L. Moses, Activation of the Erk pathway is required for TGF-β1-induced EMT in vitro, Neoplasia 6 (2004) 603–610.
[373] M. Davies, M. Robinson, E. Smith, S. Huntley, S. Prime, I. Paterson, Induction of an epithelial to mesenchymal transition in human immortal and malignant keratinocytes by TGF-β1 involves MAPK, Smad and AP-1 signalling pathways, J. Cell. Biochem. 95 (2005) 918–931.
[374] M.A. Huber, N. Azoitei, B. Baumann, S. Grünert, A. Sommer, H. Pehamberger, N. Kraut, H. Beug, T. Wirth, NF-κB is essential for epithelial–mesenchymal transition and metastasis in a model of breast cancer progression, J. Clin. Invest. 114 (2004) 569–581.
[375] J.H. Shim, C. Xiao, A.E. Paschal, S.T. Bailey, P. Rao, M.S. Hayden, K.Y. Lee, C. Bussey, M. Steckel, N. Tanaka, G. Yamada, S. Akira, K. Matsumoto, S. Ghosh, TAK1, but not TAB1 or TAB2, plays an essential role in multiple signaling pathways in vivo, Genes Dev. 19 (2005) 2668–2681.
[376] N.A. Bhowmick, R. Zent, M. Ghiassi, M. McDonnell, H.L. Moses, Integrin β1 signaling is necessary for transforming growth factor-β activation of p38 MAPK and epithelial plasticity, J. Biol. Chem. 276 (2001) 46707–46713.
[377] R.C. Bates, D.I. Bellovin, C. Brown, E. Maynard, B. Wu, H. Kawakatsu, D. Sheppard, P. Oettgen, A.M. Mercurio, Transcriptional activation of integrin β6 during the epithelial–mesenchymal transition defines a novel prognostic indicator of aggressive colon carcinoma, J. Clin. Invest. 115 (2005) 339–347.
[378] B. Ozdamar, R. Bose, M. Barrios-Rodiles, H.R. Wang, Y. Zhang, J.L. Wrana, Regulation of the polarity protein Par6 by TGFβ receptors controls epithelial cell plasticity, Science 307 (2005) 1603–1609.
[379] R. Vogelmann, M.D. Nguyen-Tat, K. Giehl, G. Adler, D. Wedlich, A. Menke, TGFβ-induced downregulation of E-cadherin-based cell–cell adhesion depends on PI3-kinase and PTEN, J. Cell. Sci. 118 (2005) 4901–4912.
[380] A.B. Hjelmeland, M.D. Hjelmeland, Q. Shi, J.L. Hart, D.D. Bigner, X.-F. Wang, C.D. Kontos, J.N. Rich, Loss of phosphatase and tensin homologue increases transforming growth factor β-mediated invasion with enhanced SMAD3 transcriptional activity, Cancer Res. 65 (2005) 11276–11281.
[381] M. Kondo, E. Cubillo, K. Tobiume, T. Shirakihara, N. Fukuda, H. Suzuki, K. Shimizu, K. Takehara, A. Cano, M. Saitoh, K. Miyazono, A role for Id in the regulation of TGF-β-induced epithelial–mesenchymal transdifferentiation, Cell Death Differ. 11 (2004) 1092–1101.
[382] J. Perk, A. Iavarone, R. Benezra, Id family of helix–loop–helix proteins in cancer, Nat. Rev., Cancer 5 (2005) 603–614.
[383] H. Peinado, F. Portillo, A. Cano, Transcriptional regulation of cadherins during development and carcinogenesis, Int. J. Dev. Biol. 48 (2004) 365–375.
[384] J. Comijn, G. Berx, P. Vermassen, K. Verschueren, L. van Grunsven, E. Bruyneel, M. Mareel, D. Huylebroeck, F. van Roy, The two-handed E box binding zinc finger protein SIP1 downregulates E-cadherin and induces invasion, Mol. Cell 7 (2001) 1267–1278.
[385] C. Vandewalle, J. Comijn, B. De Craene, P. Vermassen, E. Bruyneel, H. Andersen, E. Tulchinsky, F. Van Roy, G. Berx, SIP1/ZEB2 induces EMT by repressing genes of different epithelial cell–cell junctions, Nucleic Acids Res. 33 (2005) 6566–6578.
[386] L.A. Romano, R.B. Runyan, Slug is an essential target of TGFβ2 signaling in the developing chicken heart, Dev. Biol. 223 (2000) 91–102.
[387] H. Peinado, M. Quintanilla, A. Cano, Transforming growth factor β-1 induces snail transcription factor in epithelial cell lines: mechanisms for epithelial mesenchymal transitions, J. Biol. Chem. 278 (2003) 21113–21123.
[388] A. Eger, A. Stockinger, J. Park, E. Langkopf, M. Mikula, J. Gotzmann, W. Mikulits, H. Beug, R. Foisner, β-Catenin and TGFβ signalling cooperate to maintain a mesenchymal phenotype after FosER-induced epithelial to mesenchymal transition, Oncogene 23 (2004) 2672–2680.
[389] C. Martinez-Alvarez, M.J. Blanco, R. Perez, M.A. Rabadan, M. Aparicio, E. Resel, T. Martinez, M.A. Nieto, Snail family members and cell survival in physiological and pathological cleft palates, Dev. Biol. 265 (2004) 207–218.
[390] A. Nawshad, D. LaGamba, E.D. Hay, Transforming growth factor β (TGFβ) signalling in palatal growth, apoptosis and epithelial mesenchymal transformation (EMT), Arch. Oral Biol. 49 (2004) 675–689.
[391] S. Thuault, U. Valcourt, M. Petersen, G. Manfioletti, C.-H Heldin, A. Moustakas, Transforming growth factor-β employs HMGA2 to elicit epithelial–mesenchymal transition, J. Cell Biol. 174 (2006) 175–183.

[392] J. Zavadil, L. Cermak, N. Soto-Nieves, E.P. Böttinger, Integration of TGF-β/Smad and Jagged1/Notch signalling in epithelial-to-mesenchymal transition, EMBO J. 23 (2004) 1155–1165.
[393] M. Jechlinger, A. Sommer, R. Moriggl, P. Seither, N. Kraut, P. Capodiecci, M. Donovan, C. Cordon-Cardo, H. Beug, S. Grünert, Autocrine PDGFR signaling promotes mammary cancer metastasis, J. Clin. Invest. 116 (2006) 1561–1570.
[394] J. Gotzmann, A.N. Fischer, M. Zojer, M. Mikula, V. Proell, H. Huber, M. Jechlinger, T. Waerner, A. Weith, H. Beug, W. Mikulits, A crucial function of PDGF in TGF-β-mediated cancer progression of hepatocytes, Oncogene 25 (2006) 3170–3185.
[395] C. Prunier, P.H. Howe, Disabled-2 (Dab2) is required for transforming growth factor β-induced epithelial to mesenchymal transition (EMT), J. Biol. Chem. 280 (2005) 17540–17548.
[396] Y. Maehara, Y. Kakeji, A. Kabashima, Y. Emi, A. Watanabe, K. Akazawa, H. Baba, S. Kohnoe, K. Sugimachi, Role of transforming growth factor-β 1 in invasion and metastasis in gastric carcinoma, J. Clin. Oncol. 17 (1999) 607–614.
[397] C. Prunier, A. Mazars, V. Noe, E. Bruyneel, M. Mareel, C. Gespach, A. Atfi, Evidence that Smad2 is a tumor suppressor implicated in the control of cellular invasion, J. Biol. Chem. 274 (1999) 22919–22922.
[398] G. Subramanian, R.E. Schwarz, L. Higgins, G. McEnroe, S. Chakravarty, S. Dugar, M. Reiss, Targeting endogenous transforming growth factor β receptor signaling in SMAD4-deficient human pancreatic carcinoma cells inhibits their invasive phenotype, Cancer Res. 64 (2004) 5200–5211.
[399] X. Shen, J. Li, P.P. Hu, D. Waddell, J. Zhang, X.-F. Wang, The activity of guanine exchange factor NET1 is essential for transforming growth factor-β-mediated stress fiber formation, J. Biol. Chem. 276 (2001) 15362–15368.
[400] A.V. Bakin, A. Safina, C. Rinehart, C. Daroqui, H. Darbary, D.M. Helfman, A critical role of tropomyosins in TGF-β regulation of the actin cytoskeleton and cell motility in epithelial cells, Mol. Biol. Cell 15 (2004) 4682–4694.
[401] P. Michl, A.R. Ramjaun, O.E. Pardo, P.H. Warne, M. Wagner, R. Poulsom, C. D'Arrigo, K. Ryder, A. Menke, T. Gress, J. Downward, CUTL1 is a target of TGFβ signaling that enhances cancer cell motility and invasiveness, Cancer Cell 7 (2005) 521–532.
[402] G. Giannelli, E. Fransvea, F. Marinosci, C. Bergamini, S. Colucci, O. Schiraldi, S. Antonaci, Transforming growth factor-β1 triggers hepatocellular carcinoma invasiveness via α3β1 integrin, Am. J. Pathol. 161 (2002) 183–193.
[403] N. Dumont, A.V. Bakin, C.L. Arteaga, Autocrine transforming growth factor-β signaling mediates Smad-independent motility in human cancer cells, J. Biol. Chem. 278 (2003) 3275–3285.
[404] D. Zicha, E. Genot, G.A. Dunn, I.M. Kramer, TGFβ1 induces a cell-cycle-dependent increase in motility of epithelial cells, J. Cell. Sci. 112 (Pt. 4) (1999) 447–454.
[405] S.M. Kakonen, K.S. Selander, J.M. Chirgwin, J.J. Yin, S. Burns, W.A. Rankin, B.G. Grubbs, M. Dallas, Y. Cui, T.A. Guise, Transforming growth factor-β stimulates parathyroid hormone-related protein and osteolytic metastases via Smad and mitogen-activated protein kinase signaling pathways, J. Biol. Chem. 277 (2002) 24571–24578.
[406] M.J. Bissell, D. Radisky, Putting tumours in context, Nat. Rev., Cancer 1 (2001) 46–54.
[407] O.W. Petersen, H.L. Nielsen, T. Gudjonsson, R. Villadsen, F. Rank, E. Niebuhr, M.J. Bissell, L. Ronnov-Jessen, Epithelial to mesenchymal transition in human breast cancer can provide a nonmalignant stroma, Am. J. Pathol. 162 (2003) 391–402.
[408] P. Micke, A. Östman, Exploring the tumour environment: cancer-associated fibroblasts as targets in cancer therapy, Expert Opin. Ther. Targets 9 (2005) 1217–1233.
[409] T.P. Mäkelä, R. Alitalo, Y. Paulsson, B. Westermark, C.-H. Heldin, K. Alitalo, Regulation of platelet-derived growth factor gene expression by transforming growth factor β and phorbol ester in human leukemia cell lines, Mol. Cell. Biol. 7 (1987) 3656–3662.
[410] O. Ishikawa, E.C. LeRoy, M. Trojanowska, Mitogenic effect of transforming growth factor β 1 on human fibroblasts involves the induction of platelet-derived growth factor a receptors, J. Cell. Physiol. 145 (1990) 181–186.
[411] F. Strutz, M. Zeisberg, A. Renziehausen, B. Raschke, V. Becker, C. van Kooten, G. Muller, TGF-β 1 induces proliferation in human renal fibroblasts via induction of basic fibroblast growth factor (FGF-2), Kidney Int. 59 (2001) 579–592.
[412] A. Igarashi, H. Okochi, D.M. Bradham, G.R. Grotendorst, Regulation of connective tissue growth factor gene expression in human skin fibroblasts and during wound repair, Mol. Biol. Cell 4 (1993) 637–645.
[413] A.K. Scaffidi, N. Petrovic, Y.P. Moodley, M. Fogel-Petrovic, K.M. Kroeger, R.M. Seeber, K.A. Eidne, P.J. Thompson, D.A. Knight, $\alpha_v\beta_3$ Integrin interacts with the transforming growth factor β (TGFβ) type II receptor to potentiate the proliferative effects of TGFβ1 in living human lung fibroblasts, J. Biol. Chem. 279 (2004) 37726–37733.
[414] O. De Wever, W. Westbroek, A. Verloes, N. Bloemen, M. Bracke, C. Gespach, E. Bruyneel, M. Mareel, Critical role of N-cadherin in myofibroblast invasion and migration in vitro stimulated by colon-cancer-cell-derived TGF-β or wounding, J. Cell. Sci. 117 (2004) 4691–4703.
[415] M.P. Lewis, K.A. Lygoe, M.L. Nystrom, W.P. Anderson, P.M. Speight, J.F. Marshall, G.J. Thomas, Tumour-derived TGF-β1 modulates myofibroblast differentiation and promotes HGF/SF-dependent invasion of squamous carcinoma cells, Br. J. Cancer 90 (2004) 822–832.
[416] N.A. Bhowmick, A. Chytil, D. Plieth, A.E. Gorska, N. Dumont, S. Shappell, M.K. Washington, E.G. Neilson, H.L. Moses, TGF-β signaling in fibroblasts modulates the oncogenic potential of adjacent epithelia, Science 303 (2004) 848–851.
[417] N. Cheng, N.A. Bhowmick, A. Chytil, A.E. Gorksa, K.A. Brown, R. Muraoka, C.L. Arteaga, E.G. Neilson, S.W. Hayward, H.L. Moses, Loss of TGF-β type II receptor in fibroblasts promotes mammary carcinoma growth and invasion through upregulation of TGF-α-, MSP-and HGF-mediated signaling networks, Oncogene 24 (2005) 5053–5068.
[418] P. Carmeliet, Angiogenesis in life, disease and medicine, Nature 438 (2005) 932–936.
[419] M.C. Dickson, J.S. Martin, F.M. Cousins, A.B. Kulkarni, S. Karlsson, R.J. Akhurst, Defective haematopoiesis and vasculogenesis in transforming growth factor-β 1 knock out mice, Development 121 (1995) 1845–1854.
[420] M. Oshima, H. Oshima, M.M. Taketo, TGF-β receptor type II deficiency results in defects of yolk sac hematopoiesis and vasculogenesis, Dev. Biol. 179 (1996) 297–302.
[421] D.Y. Li, L.K. Sorensen, B.S. Brooke, L.D. Urness, E.C. Davis, D.G. Taylor, B.B. Boak, D.P. Wendel, Defective angiogenesis in mice lacking endoglin, Science 284 (1999) 1534–1537.
[422] J. Larsson, M.J. Goumans, L.J. Sjostrand, M.A. van Rooijen, D. Ward, P. Leveen, X. Xu, P. ten Dijke, C.L. Mummery, S. Karlsson, Abnormal angiogenesis but intact hematopoietic potential in TGF-β type I receptor-deficient mice, EMBO J. 20 (2001) 1663–1673.
[423] S.P. Oh, T. Seki, K.A. Goss, T. Imamura, Y. Yi, P.K. Donahoe, L. Li, K. Miyazono, P. ten Dijke, S. Kim, E. Li, Activin receptor-like kinase 1 modulates transforming growth factor-β 1 signaling in the regulation of angiogenesis, Proc. Natl. Acad. Sci. U. S. A. 97 (2000) 2626–2631.
[424] K.A. McAllister, 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, et al., Endoglin, a TGF-β binding protein of endothelial cells, is the gene for hereditary haemorrhagic telangiectasia type 1, Nat. Genet. 8 (1994) 345–351.
[425] D.W. Johnson, J.N. Berg, M.A. Baldwin, C.J. Gallione, I. Marondel, S.J. Yoon, T.T. Stenzel, M. Speer, M.A. Pericak-Vance, A. Diamond, A.E. Guttmacher, C.E. Jackson, L. Attisano, R. Kucherlapati, M.E. Porteous, D.A. Marchuk, Mutations in the activin receptor-like kinase 1 gene in hereditary haemorrhagic telangiectasia type 2, Nat. Genet. 13 (1996) 189–195.
[426] P. Bertolino, M. Deckers, F. Lebrin, P. ten Dijke, Transforming growth factor-β signal transduction in angiogenesis and vascular disorders, Chest 128 (2005) 585S–590S.
[427] B. Enholm, K. Päävonen, A. Ristimaki, V. Kumar, Y. Gunji, J. Klefström, L. Kivinen, M. Laiho, B. Olofsson, V. Joukov, U. Eriksson, K. Alitalo,

Comparison of VEGF, VEGF-B, VEGF-C and Ang-1 mRNA regulation by serum, growth factors, oncoproteins and hypoxia, Oncogene 14 (1997) 2475–2483.
[428] C. Benckert, S. Jonas, T. Cramer, Z. Von Marschall, G. Schafer, M. Peters, K. Wagner, C. Radke, B. Wiedenmann, P. Neuhaus, M. Hocker, S. Rosewicz, Transforming growth factor β 1 stimulates vascular endothelial growth factor gene transcription in human cholangiocellular carcinoma cells, Cancer Res. 63 (2003) 1083–1092.
[429] T. Sanchez-Elsner, L.M. Botella, B. Velasco, A. Corbi, L. Attisano, C. Bernabeu, Synergistic cooperation between hypoxia and transforming growth factor-β pathways on human vascular endothelial growth factor gene expression, J. Biol. Chem. 276 (2001) 38527–38535.
[430] M.S. Pepper, Transforming growth factor-β: vasculogenesis, angiogenesis, and vessel wall integrity, Cytokine Growth Factor Rev. 8 (1997) 21–43.
[431] N. Ueki, M. Nakazato, T. Ohkawa, T. Ikeda, Y. Amuro, T. Hada, K. Higashino, Excessive production of transforming growth-factor β 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 (1992) 189–196.
[432] J.A. Tuxhorn, S.J. McAlhany, F. Yang, T.D. Dang, D.R. Rowley, Inhibition of transforming growth factor-β activity decreases angiogenesis in a human prostate cancer-reactive stroma xenograft model, Cancer Res. 62 (2002) 6021–6025.
[433] S.L. Lu, D. Reh, A.G. Li, J. Woods, C.L. Corless, M. Kulesz-Martin, X.J. Wang, Overexpression of transforming growth factor β1 in head and neck epithelia results in inflammation, angiogenesis, and epithelial hyperproliferation, Cancer Res. 64 (2004) 4405–4410.
[434] Q. Yu, I. Stamenkovic, Cell surface-localized matrix metalloproteinase-9 proteolytically activates TGF-β and promotes tumor invasion and angiogenesis, Genes Dev. 14 (2000) 163–176.
[435] T. Gohongi, D. Fukumura, Y. Boucher, C.O. Yun, G.A. Soff, C. Compton, T. Todoroki, R.K. Jain, Tumor–host interactions in the gallbladder suppress distal angiogenesis and tumor growth: involvement of transforming growth factor β1, Nat. Med. 5 (1999) 1203–1208.
[436] K.Y. Kim, S.Y. Jeong, J. Won, P.D. Ryu, M.J. Nam, Induction of angiogenesis by expression of soluble type II transforming growth factor-β receptor in mouse hepatoma, J. Biol. Chem. 276 (2001) 38781–38786.
[437] C. Go, P. Li, X.J. Wang, Blocking transforming growth factor β signaling in transgenic epidermis accelerates chemical carcinogenesis: a mechanism associated with increased angiogenesis, Cancer Res. 59 (1999) 2861–2868.
[438] I. Schwarte-Waldhoff, O.V. Volpert, N.P. Bouck, B. Sipos, S.A. Hahn, S. Klein-Scory, J. Luttges, G. Kloppel, U. Graeven, C. Eilert-Micus, A. Hintelmann, W. Schmiegel, Smad4/DPC4-mediated tumor suppression through suppression of angiogenesis, Proc. Natl. Acad. Sci. U. S. A. 97 (2000) 9624–9629.
[439] S. Breit, K. Ashman, J. Wilting, J. Rossler, E. Hatzi, T. Fotsis, L. Schweigerer, The N-myc oncogene in human neuroblastoma cells: down-regulation of an angiogenesis inhibitor identified as activin A, Cancer Res. 60 (2000) 4596–4601.
[440] E. Panopoulou, C. Murphy, H. Rasmussen, E. Bagli, E.K. Rofstad, T. Fotsis, Activin A suppresses neuroblastoma xenograft tumor growth via antimitotic and antiangiogenic mechanisms, Cancer Res. 65 (2005) 1877–1886.
[441] A. Schramm, V. von Schuetz, H. Christiansen, W. Havers, M. Papoutsi, J. Wilting, L. Schweigerer, High activin A-expression in human neuroblastoma: suppression of malignant potential and correlation with favourable clinical outcome, Oncogene 24 (2005) 680–687.
[442] E.M. Langenfeld, J. Langenfeld, Bone morphogenetic protein-2 stimulates angiogenesis in developing tumors, Mol. Cancer Res. 2 (2004) 141–149.
[443] M. Raida, J.H. Clement, R.D. Leek, K. Ameri, R. Bicknell, D. Niederwieser, A.L. Harris, Bone morphogenetic protein 2 (BMP-2) and induction of tumor angiogenesis, J. Cancer Res. Clin. Oncol. 131 (2005) 741–750.
[444] S. Tsunawaki, M. Sporn, A. Ding, C. Nathan, Deactivation of macrophages by transforming growth factor-β, Nature 334 (1988) 260–262.
[445] S. Bodmer, K. Strommer, K. Frei, C. Siepl, N. de Tribolet, I. Heid, A. Fontana, Immunosuppression and transforming growth factor-β in glioblastoma. Preferential production of transforming growth factor-β 2, J. Immunol. 143 (1989) 3222–3229.
[446] W. von Bernstorff, M. Voss, S. Freichel, A. Schmid, I. Vogel, C. Johnk, D. Henne-Bruns, B. Kremer, H. Kalthoff, Systemic and local immunosuppression in pancreatic cancer patients, Clin. Cancer Res. 7 (2001) 925s–932s.
[447] G. Torre-Amione, R.D. Beauchamp, H. Koeppen, B.H. Park, H. Schreiber, H.L. Moses, D.A. Rowley, A highly immunogenic tumor transfected with a murine transforming growth factor type β 1 cDNA escapes immune surveillance, Proc. Natl. Acad. Sci. U. S. A. 87 (1990) 1486–1490.
[448] L. Gorelik, R.A. Flavell, Immune-mediated eradication of tumors through the blockade of transforming growth factor-β signaling in T cells, Nat. Med. 7 (2001) 1118–1122.
[449] C. Becker, M.C. Fantini, C. Schramm, H.A. Lehr, S. Wirtz, A. Nikolaev, J. Burg, S. Strand, R. Kiesslich, S. Huber, H. Ito, N. Nishimoto, K. Yoshizaki, T. Kishimoto, P.R. Galle, M. Blessing, S. Rose-John, M.F. Neurath, TGF-β suppresses tumor progression in colon cancer by inhibition of IL-6 trans-signaling, Immunity 21 (2004) 491–501.
[450] M.L. Chen, M.J. Pittet, L. Gorelik, R.A. Flavell, R. Weissleder, H. von Boehmer, K. Khazaie, Regulatory T cells suppress tumor-specific CD8 T cell cytotoxicity through TGF-β signals in vivo, Proc. Natl. Acad. Sci. U. S. A. 102 (2005) 419–424.
[451] S. Huber, C. Schramm, H.A. Lehr, A. Mann, S. Schmitt, C. Becker, M. Protschka, P.R. Galle, M.F. Neurath, M. Blessing, Cutting edge: TGF-β signaling is required for the in vivo expansion and immunosuppressive capacity of regulatory $CD4^{+}CD25^{+}$T cells, J. Immunol. 173 (2004) 6526–6531.
[452] D.A. Thomas, J. Massagué, TGF-β directly targets cytotoxic T cell functions during tumor evasion of immune surveillance, Cancer Cell 8 (2005) 369–380.
[453] J.M. Yingling, K.L. Blanchard, J.S. Sawyer, Development of TGF-β signalling inhibitors for cancer therapy, Nat. Rev., Drug Discov. 3 (2004) 1011–1022.
[454] T.F. Witham, L. Villa, T. Yang, I.F. Pollack, H. Okada, P.D. Robbins, W.H. Chambers, Expression of a soluble transforming growth factor-β (TGFβ) receptor reduces tumorigenicity by regulating natural killer (NK) cell activity against 9L gliosarcoma in vivo, J. Neuro-Oncol. 64 (2003) 63–69.
[455] J. Adnane, F.A. Bizouarn, Z. Chen, J. Ohkanda, A.D. Hamilton, T. Munoz-Antonia, S.M. Sebti, Inhibition of farnesyltransferase increases TGFβ type II receptor expression and enhances the responsiveness of human cancer cells to TGFβ, Oncogene 19 (2000) 5525–5533.
[456] A. Miyajima, T. Asano, M. Hayakawa, Captopril restores transforming growth factor-β type II receptor and sensitivity to transforming growth factor-β in murine renal cell cancer cells, J. Urol. 165 (2001) 616–620.
[457] N.R. Murray, C. Weems, L. Chen, J. Leon, W. Yu, L.A. Davidson, L. Jamieson, R.S. Chapkin, E.A. Thompson, A.P. Fields, Protein kinase C betaII and TGFβRII in ω-3 fatty acid-mediated inhibition of colon carcinogenesis, J. Cell Biol. 157 (2002) 915–920.
[458] S. Ammanamanchi, M.G. Brattain, Restoration of transforming growth factor-β signaling through receptor RI induction by histone deacetylase activity inhibition in breast cancer cells, J. Biol. Chem. 279 (2004) 32620–32625.
[459] B.K. Law, A. Chytil, N. Dumont, E.G. Hamilton, M.E. Waltner-Law, M.E. Aakre, C. Covington, H.L. Moses, Rapamycin potentiates transforming growth factor β-induced growth arrest in nontransformed, oncogene-transformed, and human cancer cells, Mol. Cell. Biol. 22 (2002) 8184–8198.
[460] N. Suh, A.B. Roberts, S. Birkey Reffey, K. Miyazono, S. Itoh, P. ten Dijke, E.H. Heiss, A.E. Place, R. Risingsong, C.R. Williams, T. Honda, G.W. Gribble, M.B. Sporn, Synthetic triterpenoids enhance transforming growth factor β/Smad signaling, Cancer Res. 63 (2003) 1371–1376.
[461] N.J. Laping, E. Grygielko, A. Mathur, S. Butter, J. Bomberger, C. Tweed,

W. Martin, J. Fornwald, R. Lehr, J. Harling, L. Gaster, J.F. Callahan, B.A. Olson, Inhibition of transforming growth factor (TGF)-β1-induced extracellular matrix with a novel inhibitor of the TGF-β type I receptor kinase activity: SB-431542, Mol. Pharmacol. 62 (2002) 58–64.

[462] S.B. Peng, L. Yan, X. Xia, S.A. Watkins, H.B. Brooks, D. Beight, D.K. Herron, M.L. Jones, J.W. Lampe, W.T. McMillen, N. Mort, J.S. Sawyer, J.M. Yingling, Kinetic characterization of novel pyrazole TGF-β receptor I kinase inhibitors and their blockade of the epithelial–mesenchymal transition, Biochemistry 44 (2005) 2293–2304.

[463] I. Yakymovych, U. Engström, S. Grimsby, C.-H. Heldin, S. Souchelnytskyi, Inhibition of transforming growth factor-β signaling by low molecular weight compounds interfering with ATP- or substrate-binding sites of the TGF β type I receptor kinase, Biochemistry 41 (2002) 11000–11007.

[464] G.J. Inman, F.J. Nicolas, J.F. Callahan, J.D. Harling, L.M. Gaster, A.D. Reith, N.J. Laping, C.S. Hill, SB-431542 is a potent and specific inhibitor of transforming growth factor-β superfamily type I activin receptor-like kinase (ALK) receptors ALK4, ALK5, and ALK7, Mol. Pharmacol. 62 (2002) 65–74.

[465] M. Uhl, S. Aulwurm, J. Wischhusen, M. Weiler, J.Y. Ma, R. Almirez, R. Mangadu, Y.W. Liu, M. Platten, U. Herrlinger, A. Murphy, D.H. Wong, W. Wick, L.S. Higgins, M. Weller, SD-208, a novel transforming growth factor β receptor I kinase inhibitor, inhibits growth and invasiveness and enhances immunogenicity of murine and human glioma cells in vitro and in vivo, Cancer Res. 64 (2004) 7954–7961.

[466] M. Tojo, Y. Hamashima, A. Hanyu, T. Kajimoto, M. Saitoh, K. Miyazono, M. Node, T. Imamura, The ALK-5 inhibitor A-83-01 inhibits Smad signaling and epithelial-to-mesenchymal transition by transforming growth factor-β, Cancer Sci. 96 (2005) 791–800.

[467] M.D. Lacher, M.I. Tiirikainen, E.F. Saunier, C. Christian, M. Anders, M. Oft, A. Balmain, R.J. Akhurst, W.M. Korn, Transforming growth factor-β receptor inhibition enhances adenoviral infectability of carcinoma cells via up-regulation of Coxsackie and Adenovirus Receptor in conjunction with reversal of epithelial–mesenchymal transition, Cancer Res. 66 (2006) 1648–1657.

[468] C.L. Arteaga, T. Carty-Dugger, H.L. Moses, S.D. Hurd, J.A. Pietenpol, Transforming growth factor beta 1 can induce estrogen-independent tumorigenicity of human breast cancer cells in athymic mice, Cell Growth Differ. 4 (1993) 193–201.

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

[470] X. Lei, A. Bandyopadhyay, T. Le, L. Sun, Autocrine TGFβ supports growth and survival of human breast cancer MDA-MB-231 cells, Oncogene 21 (2002) 7514–7523.

[471] M.A. Rowland-Goldsmith, H. Maruyama, K. Matsuda, T. Idezawa, M. Ralli, S. Ralli, M. Korc, Soluble type II transforming growth factor-β receptor attenuates expression of metastasis-associated genes and suppresses pancreatic cancer cell metastasis, Mol. Cancer Ther. 1 (2002) 161–167.

[472] R.S. Muraoka, N. Dumont, C.A. Ritter, T.C. Dugger, D.M. Brantley, J. Chen, E. Easterly, L.R. Roebuck, S. Ryan, P.J. Gotwals, V. Koteliansky, C.L. Arteaga, Blockade of TGF-β inhibits mammary tumor cell viability, migration, and metastases, J. Clin. Invest. 109 (2002) 1551–1559.

[473] Y.A. Yang, O. Dukhanina, B. Tang, M. Mamura, J.J. Letterio, J. MacGregor, S.C. Patel, S. Khozin, Z.Y. Liu, J. Green, M.R. Anver, G. Merlino, L.M. Wakefield, Lifetime exposure to a soluble TGF-β antagonist protects mice against metastasis without adverse side effects, J. Clin. Invest. 109 (2002) 1607–1615.

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

[475] F. Matsuno, Y. Haruta, M. Kondo, H. Tsai, M. Barcos, 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 (1999) 371–382.

[476] Q. Cui, S.K. Lim, B. Zhao, F.M. Hoffmann, Selective inhibition of TGF-β responsive genes by Smad-interacting peptide aptamers from FoxH1, Lef1 and CBP, Oncogene 24 (2005) 3864–3874.

[477] K.H. Schlingensiepen, R. Schlingensiepen, A. Steinbrecher, P. Hau, U. Bogdahn, B. Fischer-Blass, P. Jachimczak, Targeted tumor therapy with the TGF-β2 antisense compound AP 12009, Cytokine Growth Factor Rev. 17 (2006) 129–139.

[478] R. Schlingensiepen, M. Goldbrunner, M.N. Szyrach, G. Stauder, P. Jachimczak, U. Bogdahn, F. Schulmeyer, P. Hau, K.H. Schlingensiepen, Intracerebral and intrathecal infusion of the TGF-β2-specific antisense phosphorothioate oligonucleotide AP 12009 in rabbits and primates: toxicology and safety, Oligonucleotides 15 (2005) 94–104.

[479] M.A. Friese, J. Wischhusen, W. Wick, M. Weiler, G. Eisele, A. Steinle, M. Weller, RNA interference targeting transforming growth factor-β enhances NKG2D-mediated antiglioma immune response, inhibits glioma cell migration and invasiveness, and abrogates tumorigenicity in vivo, Cancer Res. 64 (2004) 7596–7603.