Published in final edited form as:

Gastroenterology. 2011 October ; 141(4): 1167–1178. doi:10.1053/j.gastro.2011.07.048.

Transforming Growth Factor- β in the Gastrointestinal and Hepatic Tumor Microenvironment

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Abstract

Transforming growth factor (TGF)- β is a multifunctional cytokine that has important roles in tumor formation, progression, and metastasis. TGF- β is overproduced, and its signaling is deregulated, in a variety of human tumors, including colorectal, gastric, pancreatic, and liver. Therapeutics are being developed to block TGF- β signaling. However, TGF- β also functions as a tumor suppressor in premalignant cells. It is not clear how its function changes from that of a tumor suppressor to a tumor promoter; improvements are needed in our understanding of TGF- β functions in tumor development before we can design inhibitors for use as anticancer therapies. TGF- β regulates not only different tumor-cell autonomous signaling pathways, but also interactions between tumor and host cells, through paracrine mechanisms. We review recent findings about how TGF- β is regulated and its roles in the tumor microenvironment and metastasis, with a focus on gastrointestinal cancers. Improved understanding of TGF- β regulation and how it mediates interaction between cancer epithelial cells, immune cells, and fibroblasts will provide important insights into tumor development and progression.

Keywords

Cancer Cell Signaling; Proliferation; T β R; Apoptosis

Transforming growth factor (TGF)- β signals through a complex network of transduction pathways and has important roles in embryonic development, cell proliferation, differentiation, angiogenesis, and wound healing. TGF- β belongs to a family of dimeric peptide growth factors that includes bone morphogenetic proteins (BMPs), activins, and inhibins.¹ The isoforms of the TGF- β ligand are TGF- β 1, TGF- β 2, and TGF- β 3. TGF- β 1 is expressed in epithelial, endothelial, hematopoietic, and connective tissue cells; TGF- β 2 is expressed in epithelial and neuronal cells; and TGF- β 3 is expressed primarily in mesenchymal cells.¹ There is 70%–80% homology among TGF- β ligand isoforms, which have different binding affinities to their receptors in a tissue-specific fashion. TGF- β ligand is synthesized as a large precursor molecule that contains a latent TGF- β binding protein region, which is stored in the extracellular matrix. Latent TGF- β can be activated by

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Conflicts of interest The authors disclose no conflicts.

thrombospondin-1 and the integrin $\alpha v\beta 6$ to release the mature TGF- β protein, which can bind TGF- β receptors and stimulate a response.¹

The TGF- β ligand signals through type I and II TGF- β receptors (T β RI and T β RII, respectively). There are 7 T β RI and 5 T β RII receptors that provide a receptor system for the whole family of TGF- β ligands. These receptors contain a serine/threonine kinase domain within the cytoplasmic domain. TGF- β ligand signals through T β RII, which recruits and phosphorylates the T β RI kinase domain. The membrane-bound T β RIII (β -glycan) assists TGF- β direct ligation to the T β RII by forming a high-affinity ternary complex.¹ Recruitment and phosphorylation of T β RI then allows for the activation and phosphorylation of the downstream mediators Smad2 and Smad3. Phosphorylated Smad2 and Smad3 combine with Smad4 and enter the nucleus.¹ This Smad complex recruits co-activators, repressors, and chromatin remodeling factors to regulatory regions of target genes in specific cells in a context-dependent fashion.¹ Smad7 is a negative regulator of the Smad signaling pathway² (Figure 1).

In addition to Smad-dependent signaling, the binding of TGF- β to its receptors activates many noncanonical signaling pathways, such as the phosphoinositol-3 kinase (PI3K), mitogen-activated protein kinase, and small guanosine triphosphatase pathways. These pathways most often are implicated in tumor cell motility and migration.¹ Other mediators that belong to the TGF- β family, based on their structure and function, include BMPs, which signal through Smad1, Smad5, or Smad8, and regulate skeletal development, and activins and inhibins, which regulate reproductive physiology¹ (for a complete review see Derynck and Miyazono³).

The Dual Role of TGF- β : Challenges in Cancer Biology

TGF- β controls many different cell functions and regulates several important processes during tumor progression, including proliferative signaling, evasion of growth suppressors, resistance to cell death, replicative immortality, angiogenesis, invasion, and metastasis.⁴ TGF- β functions as a tumor suppressor and a tumor promoter. It inhibits proliferation of normal stromal, hematopoietic, and epithelial cells by inducing apoptosis, cell-cycle control, immune surveillance, and suppression of cytokine, chemokine, and inflammation. Recent studies have indicated that regulation of senescence⁵⁻⁷ and autophagy (degradation of a cell's own components by the lysosomal machinery in response to environmental stress) are also among its anti-oncogenic functions.⁸ Human pancreatic, colon, and gastric tumor cells express mutant forms or down-regulate TGF- β signaling components. In mice, conditional knockout of Tgfbr2 promotes development of more aggressive tumors of several types.^{9,10} In mice, the conditional knockout of Tgfbr2 or its downstream target Smad4 in several gastrointestinal (GI) tumor models has resulted in the development of much more aggressive tumors.¹⁰⁻¹³ More detailed information about TGF- β signaling alterations will be discussed in the section Alteration of TGF- β Signaling in Gastrointestinal Cancers, and Recent Progress in Tumor Microenvironment Studies.

TGF- β promotes metastasis through immunosuppression, induction of the epithelial to mesenchymal transition (EMT) (a process of transdifferentiation in which epithelial cells

acquire mesenchymal phenotypes), and increased tumor angiogenesis (Figure 2). TGF- β is produced in large quantities by many tumors. Aggressive, proliferative glioma cells have high TGF- β activity, associated with poor prognosis of patients.¹⁴ In mice with mammary tumors, increased TGF- β signaling, via expression of constitutively active forms of TGF- β 1 or T β RI in conjunction with c-Neu or c-Neu and polyoma virus middle T antigen (PyVmT), increase metastasis to lung¹⁵; systemic inhibition of TGF- β signaling suppressed development of pulmonary metastases.¹⁵ TGF- β signaling also promotes development of osteolytic bone metastases.¹⁶

TGF- β exerts a growth inhibition function in premalignant cells but promotes tumor metastasis in later stages of cancer. The mechanisms underlying the dual role of TGF- β described earlier are very complex and intricate.^{1,17,18} Diverse autonomous tumor-cell signaling pathways have significant roles with changes in the signal intensity and connectivity of Smad-dependent and Smad-independent pathways.^{19–21} Smad-dependent pathways might mediate growth-inhibitory effects of TGF- β signaling, whereas Smad-independent pathways could mediate the tumor-promoting effect of the TGF- β signaling. Smad-independent pathways might synergize with the oncogenic amplification of *MYC* and activating mutations of *RAS*, along with inactivating mutations in retinoblastoma or cyclin-dependent kinase inhibitors.^{20,21}

However, studies have contradicted this model. TGF- β signaling through Smads promotes metastasis to lung by inducing angiopoietin-like 4.²² TGF- β signaling to Smad is also active in aggressive, highly proliferative glioma cells and associated with a poor prognosis in patients with gliomas.¹⁴ Interestingly, mutant forms of p53 can promote Smad-mediated metastasis.²³ In addition, ubiquitination tightly controls Smad4 activity to regulate TGF- β signaling.²⁴ Phosphorylation of Smad1 and Smad5 by activin receptor-like kinase 5, the L45 loop motif of T β RI, mediates the protumor activity of TGF- β in breast cancer.²⁵ These data point to the very diverse and complex role of TGF- β signaling.

TGF- β regulation of tumor progression is context-dependent.¹ TGF- β can play an inhibitory or promoting role in the same biological processes (eg, apoptosis and cell-cycle progression). This often is mediated through different signaling molecules. For example, TGF- β may activate the apoptotic pathway through death-associated protein kinase, growth arrest, and DNA-damage-inducible b protein.^{26,27} However, TGF- β also governs anti-apoptotic effects by inducing the activity of the differentially expressed in chondrocytes 1.²⁸ In cell cycle regulation, TGF- β inhibits cyclin-dependent kinase (CDK) that are important in G1 phase progression. TGF- β also induces the expression of p15^{Ink4b} and p21^{Waf1}, inhibitors for cyclin D-CDK4/6 and cyclin E/A-CDK2 complexes respectively.^{29,30} TGF- β also represses expression of c-myc, a transcription factor that promotes cell proliferation.³¹ On the other hand, TGF- β promotes cell-cycle progression through induction of platelet-derived growth factor A or B.¹⁴ TGF- β signaling can induce angiogenic factors such as connective tissue growth factor and vascular endothelial growth factor (VEGF) in epithelial cells and the fibro-blasts that promote proliferation of these epithelial cells.³² This TGF- β functional change from tumor suppressor to tumor promoter has been well recognized.^{33,34} Host immune and inflammatory cells might contribute to this process.³⁴ If we are to develop

modifiers of TGF- β activity as therapeutics, we need to improve our understanding of these processes.

TGF- β , a Master Regulator of Inflammation and Tumor Microenvironment

Inflammation and the significant effect of the tumor microenvironment on tumor progression are identified as the hallmarks of cancer.⁴ The tumor microenvironment comprises inflammatory cells, including Tie2+, VEGFR1+, CD11b+, and F4/80+ populations; innate and adaptive immune cells, including natural killer (T) cells, T cells, and B cells; as well as cancer-associated fibroblasts and endothelial progenitor cells (Figure 3).^{34,35} These stromal cells collectively create an environment that promotes tumor progression by providing growth factors, pro-angiogenic factors, proteases, and adhesion molecules that facilitate tumor cell proliferation, angiogenesis, invasion, and metastasis.^{36–39} This dynamic tumor microenvironment provides a selective pressure for tumor cell variants that give rise genomic instability, genomic heterogeneity, and epigenetic alteration.⁴⁰

Inflammation and tumor microenvironment are critical players in GI cancer progression. Mice with p120-catenin deletion developed in oral, esophageal, and forestomach squamous cell carcinoma. The tumors produce increased levels of cytokines and showed significant immune cell recruitment. The infiltrating myeloid cells, which constitute the majority, activate fibroblasts. Together they contribute to squamous cell carcinoma progression.⁴¹ Mice with overexpression of interleukin (IL)-1 β in the stomach developed spontaneous gastric inflammation and cancer that were infiltrated with myeloid-derived suppressive cells (MDSCs).⁴² MDSCs promote tumor progression and metastasis. In a mouse model of pancreatic ductal adenocarcinomas, from the inception of preinvasive disease to invasive cancer, a prominent infiltration of immune-suppressive cells such as MDSCs, T-regulatory cells, and tumor-associated macrophages appeared early during pancreatic tumorigenesis, and persisted through invasive cancer.⁴³

TGF- β is a well-known immune and inflammation regulator (Figure 4; for review, see Yang et al³⁴ and Flavell et al⁴⁴). The evidence of TGF- β regulation of the tumor microenvironment comes from studies with a specific deletion of *Tgfb2* or *Smad4* in a variety of epithelial cells including mammary,³⁷ pancreatic,¹⁰ gastric,¹² intestinal,¹¹ colon,^{13,45} and head-and-neck squamous cell carcinoma.⁴⁶ In mammary epithelial cells, TGF- β signaling acts as a tumor suppressor. Deletion or attenuation of TGF- β signaling increases chemokine signaling, including that of C-X-C motif chemokine ligand 5 (CXCL5) through C-X-C chemokine receptor type 2 (CXCR2) and stromal-derived factor (SDF)-1 through CXCR4, which recruits immune cells such as Gr-1+CD11b+ cells into the tumor microenvironment. Gr-1+CD11b+ cells produce high levels of matrix metalloproteinases and TGF- β , which promote tumor invasion and metastasis.³⁷ Disruption of TGF- β signaling correlated with reduced relapse-free survival times of patients with breast cancer—especially among patients whose tumors were estrogen-receptor positive.¹⁸ Deletion of *Smad4* from colon tumors in mice increased recruitment of CCR1+ myeloid cells (CD34+), which promoted tumor invasion.¹¹ Deletion of *Smad4* from head and neck epithelia of mice induced spontaneous formation of tumors, characterized by increased inflammation and genomic instability.⁴⁷ Levels of inflammatory molecules such as IL-1 β , macrophage

inflammatory protein 2 (MIP-2), SDF-1, and CXCR4 are increased in head-and-neck tumors that lack TGF- β signaling.⁴⁶ In TGF- β 1-deficient mice, inflammation causes precancerous lesions to progress to colon cancer.⁴⁸

TGF- β signal transduction in stromal fibroblasts has an important role in suppressing tumorigenesis in adjacent epithelium, through negative regulation of hepatocyte growth factor, macrophage stimulating protein 1, and TGF- α .^{49,50} Deletion of TGF- β signaling in a subset of fibroblasts (via Cre expression, controlled by a fibroblast-specific protein 1 promoter) leads to prostate intraepithelial neoplasia and invasive squamous cell carcinoma in the forestomach.⁴⁹ Interestingly, heterogeneous TGF- β signaling in stromal cells, some of which contain a dominant-negative form of T β RII whereas others contain wild-type TGF- β , promotes prostate tumor invasion and metastasis. This occurs through increased expression of TGF- β 1, SDF1, CXCL12, fibroblast growth factor, and BMP.⁵¹ TGF- β and SDF-1, through an autocrine signaling loop and cross-communication, convert resident human mammary fibroblasts into tumor-promoting myofibroblasts during the course of tumor progression.⁵² Similar to the deletion of TGF- β signaling in fibroblasts, selective deletion of *Smad4* from T cells promotes development of carcinomas throughout the GI tract,⁵³ which is accompanied by increased expression of inflammatory factors (such as IL-5, IL-6, and IL-13) and infiltration by inflammatory cells.⁵³ Further studies and new mouse models are needed to investigate stromal cell-type-specific TGF- β signaling and its interactions with TGF- β signaling by epithelial cells.

These results show that TGF- β , the tumor suppressor, also functions as an inflammation suppressor in tumor progression. Disruption of TGF- β signaling in either epithelial or stromal cells increases inflammatory responses that promote tumor initiation, progression, and metastasis. One important mechanism by which TGF- β regulates inflammation involves controlling activation of the transcription factor nuclear factor- κ B, the master regulator of the inflammation reaction. For example, in the gut, TGF- β 1 prevents the activation of nuclear factor- κ B through Smad7.⁵⁴ Mice deficient in *Smad3* develop colon cancer after infection with *Helicobacter* infection because of increased inflammation.⁵⁵ In TGF- β 1-deficient mice, inflammation causes precancerous lesions to progress to colon cancer.⁴⁸ TGF- β also regulates production of IL-1 to control chronic inflammation,⁵⁶ and the production of chemokines and chemokine receptors that recruit inflammatory cells. These chemokines include SDF-1 (or CXCL12), a key regulator of hematopoietic stem and progenitor cell trafficking between the peripheral circulation and targeted tumor tissues. SDF-1 mediates its effects on chemotaxis through its receptor, CXCR4, which is highly expressed on putative stem and progenitor cells.^{36,39}

Effects of Tumor-Associated Inflammation and TGF- β Production on Metastasis

Tumor-infiltrating stromal cells have significant effects on tumor epithelial cells. They produce growth factors, chemokines, and inflammatory mediators that promote tumor cell invasion and metastasis.⁵⁷ TGF- β is an important mediator of these interactions.

Interestingly, TGF- β is expressed mostly in stromal cells such as smooth muscle actin-positive fibroblasts or MDSCs, rather than malignant epithelial cells.^{37,58,59}

Promoting the EMT and Maintaining Cancer Stem Cells

Increased TGF- β production in the tumor microenvironment can induce EMT, a well-known transdifferentiation program that enables epithelial cells to generate a mesenchymal phenotype.⁶⁰ TGF- β induces EMT, allowing carcinoma cells to lose polarity and cell-cell contacts and acquire characteristics of fibroblasts that allow the cell to invade surrounding tissues and eventually metastasize.^{60,61} EMT often is observed at the invasive front of tumors, as is TGF- β .⁶² TGF- β -induced EMT often coincides with lost expression of E-cadherin, a cell-cell adhesion protein that is down-regulated in many tumor types.⁶⁰ EMT involves a number of factors mediated by TGF- β , including SNAIL, Ras, and BMP7.^{63,64} An autocrine signaling network of TGF- β /ZEB/miR-200 regulates the transition between epithelial and mesenchymal states.⁶⁵ However, in contrast with the conventional review of TGF- β promotion of EMT, factors such as thyroid transcription factor-1 and metformin can inhibit the induction of the EMT by TGF- β .^{66,67} Interestingly, cells that undergo EMT express not only proteins such as TWIST, SNAIL, SIP1, ZEB2, vimentin, and fibronectin, but also markers of cancer stem cells (CSCs) (eg, CD44+/CD24-) and have many of their properties.⁶⁸ TWIST, SNAIL, ZEB and SIP1 are transcriptional factors implicated in EMT process. TGF- β signaling is required for the self-renewal capacities of glioma stem cells via induction of Sox4 and leukemia inhibitory factor.^{69,70} The inhibitor of T β RI kinase causes CSCs to lose mesenchymal traits and acquire a more epithelial phenotype, suggesting a role of the TGF- β pathway in EMT and CSC generation.⁷¹ An inhibitor of T β RI reduced the capacity of human glioma initiating cells to initiate tumors. This reduction was associated with down-regulation of CD44, the inhibitors of DNA-binding 1 and 3, and transcription factors required for glioma-initiating cell function.⁷² However, contradicting results also have been reported. For example, TGF- β suppresses CSCs and reduces the size of the side population fraction and associated transcription markers.⁷³⁻⁷⁵ The side population, which shows stem cell-like characteristics, is distinct from the main population based on markers used in flow cytometry. The study of CSCs remains a very controversial area largely owing to uncertainty surrounding surface marker identification and lack of functional studies.^{60,68} Functional studies of TGF- β regulation of CSCs are needed.

Regulation of Distant Organ Environment for Tumor Metastasis

It is not clear how or why tumor cells metastasize to specific organs; studies indicate the modulation of the premetastatic distant organ and premetastatic niche formation are very important aspects of metastatic processes.⁷⁶⁻⁸⁰ Tenascin C, an extracellular matrix protein of stem cell niches, and lysyl oxidase, which regulates cross-links of collagen IV, have been found recently to be important mediators.^{78,80} Immature Gr-1+CD11b+ myeloid cells significantly decrease production of interferon- γ and increase production of inflammatory mediators, growth factors, and T-helper 2 cytokines in lung, before metastases develop. In addition, Gr-1+CD11b+ cells produce large quantities of matrix metal-loproteinase-9 and promote vascular remodeling that favors metastasis.⁷⁹ In lung tissue before metastatic cell arrival, TGF- β regulates the production of the chemoattractants S100A8 and S100A9, which

attract Mac1⁺ myeloid cells that activate mitogen-activated protein kinase p38 in tumor cells.^{81,82} This activation promotes invasive activity by inducing cells to form pseudopodia (invadopodia). TGF- β also induces tumor cells to produce angiopoietin-like 4, which disrupts vascular endothelial cell–cell junctions, increases the permeability of lung capillaries, and facilitates the transendothelial passage of tumor cells.²² Other studies have reported the importance of TGF- β signaling in metastasis to distant organs.^{51,83}

Alteration of TGF- β Signaling in GI Cancers, and Progress in Studies of Tumor Microenvironment

Alteration in TGF- β signaling is common in GI cancers including oral, esophageal, gastric, hepatic, biliary, colon, and pancreatic cancers (Table 1).⁸⁴ Many of these tumor types have increased expression of TGF- β and decreased responses to TGF- β -mediated growth inhibition. Mutations or loss of expression of TGF- β receptors and Smad proteins mediate the decreased responses to TGF- β .

TGF- β Expression

Increased expression of TGF- β is observed commonly in esophageal cancers.^{85,86} High levels of TGF- β 1 have been detected in the gastric mucosa of gastric cancer patients and their first-degree relatives.⁸⁷ The expression of TGF- β 1, TGF- β 2, and TGF- β 3 also are increased markedly in hepatocellular carcinoma (HCC).⁸⁸ Overexpression of TGF- β 2 and TGF- β 3 in cholangiocarcinoma promotes tumor cell proliferation.⁸⁹ In addition, overexpression of TGF- β contributes significantly to the development of pancreatic cancer.⁹⁰

TGF- β Receptors

Decreased expression or loss of TGF- β -receptor expression correlates with cancer progression or poor prognosis.⁹¹ It is observed in patients with primary gastric tumors,⁹² HCC,^{93,94} esophageal adenocarcinoma,⁹⁵ or oral squamous cell carcinoma,⁹⁶ as well as colorectal cancer.⁹⁷ The reduced T β RII expression in HCCs was correlated with larger tumor size, poor differentiation, portal vein invasion, intrahepatic metastasis, shorter recurrence-free survival, and TGF- β resistance.^{93,98} Frameshift mutations at coding nucleotide repeats in *Tgfbr2* occur during late stages of development of gastric tumors with microsatellite instability.^{84,99} *Tgfbr2* mutations also have been found in HCC,^{93,94} and in more than 20% of colon cancers. There is an even higher percentage of *Tgfbr2* frameshift mutations (70%–90%) in colon tumors with microsatellite instability.^{100,101} Mutations in *Tgfbr1* and *Tgfbr2* also are observed in pancreatic tumors.^{84,102}

Expression and Mutation of Smads

Smad4 messenger RNA expression often is reduced or absent in oral¹⁰³ and esophageal squamous cell carcinoma,¹⁰⁴ the reduced level correlates with tumor progression.¹⁰⁴ Haploid loss of *Smad4* initiates gastric polyposis and carcinogenesis in mice.¹⁰⁵ The gastric polyposis resembles juvenile polyposis, a disease with inactivated germ-line mutations of *Smad4*. In mice, targeted deletion of *Smad4* and Phosphatase and tensin homolog (*pten*) in

liver leads to the development of cholangiocellular carcinoma.¹⁰⁶ The mutation of *Smad2* and *Smad4* is a frequent event; *Smad4* mutations are detected in 30% of colon tumors, *Smad2* mutations are detected in approximately 5% of colon tumors,^{84,101} and are associated with poor prognosis in patients with surgically resected pancreatic cancer.^{84,107} In addition, mutations in *Smad2* and *Smad4* are found in HCC.¹⁰⁸ Interestingly, increased copy numbers of *Smad7* were observed in and is associated with a worse prognosis in patients with colorectal cancer.¹⁰⁹

Alteration of TGF- β Signaling, Inflammation, and Tumor Microenvironment

Pancreatic ductal adenocarcinomas have increased proliferation of fibroblasts and pancreatic stellate cells that produce fibronectin and collagens. These changes in the stroma are facilitated by the abundance of fibroblast growth factors, epidermal growth factor, and TGF- β .⁵⁸ Interestingly, in gastric tumors, active TGF- β 1 is expressed most highly in smooth muscle actin-positive fibroblasts, but not in malignant epithelial cells.⁵⁹ *Smad4* haploinsufficiency alters expression of inflammatory factors in the mesenchymal and epithelial compartments of intestinal tumors.¹¹⁰ In mice, deletion of *Tgfb2* from different epithelial cells (pancreatic,¹⁰ intestinal,¹¹¹ and colon⁴⁵) increases tumor progression and metastasis, which are accompanied by significant increases in inflammatory cells. Deletion of *Smad4* from intestinal tumor cells increases recruitment of CCR1+ myeloid cells (CD34+), which promote tumor invasion.¹¹ Targeted disruption of SMAD3 induces defects in mucosal immunity that result in early death from infiltration of T cells and the presence of bacterial abscesses in the GI tract.¹¹²

In addition, TGF- β significantly regulates tumor-associated fibroblast function. TGF- β 1 induces the transition of stromal fibroblasts to myofibroblasts in the tumor microenvironment. This is mediated by MicroRNA (Mir)-21, which down-regulates the expression of programmed cell death 4 (PDCD4). PDCD4 protein in turn negatively regulates several factors of myofibroblasts.¹¹³ Loss of *Tgfb2* from fibroblasts promotes invasive squamous cell carcinoma in the forestomach, through up-regulation of signaling networks mediated by TGF- α , macrophage stimulating protein, and hepatocyte growth factor.¹¹⁴ One interesting study showed that disruption of TGF- β signaling in T cells led to the development of carcinomas throughout the GI tract, accompanied by increased expression of inflammatory factors (IL-5, IL-6, and IL-13) and infiltration by inflammatory cells.⁵³ T-cell-specific deletion of furin, an activator of the TGF- β latent molecule, led to colitis and intestinal inflammation.¹¹⁵ There fore, alterations in TGF- β signaling frequently are found in GI cancers and significantly affect the tumor microenvironment.

TGF- β Targeting Challenges and Opportunities

Because TGF- β is overexpressed by tumors and promotes tumor development, it is an attractive therapeutic target. Several inhibitory approaches have been developed and have shown efficacy in preclinical and clinical studies.^{15,44,116} These include blocking production of TGF- β ligands with antisense molecules, small-molecule inhibitors of the kinase activity of T β RI and T β RII, monoclonal antibodies that block TGF- β signaling, and soluble forms of T β RII and T β RIII that function as ligand traps (Figure 5). In addition, epigenetic

therapies such as histone deacetylase inhibitors and combined therapies of small-molecule inhibitor with immune-stimulating vaccine represent future potential therapeutic approaches.^{117,118}

Antisense molecules reduce TGF- β secretion into the tumor microenvironment.¹¹⁹ AP12009 (trabedersen), an 18-oligomer antisense phosphorothioate oligodeoxynucleotide, prevents production of TGF- β 2 and is used to treat patients with pancreatic adenocarcinomas and colon cancers,^{120,121} especially those with an advanced stage.¹²² Small-molecule inhibitors of TGF- β -receptor kinases have been designed and tested in mouse models of several types of cancer.^{116,123} LY2109761, a dual inhibitor of T β RI and T β RII, significantly decreased metastasis of pancreatic and colorectal tumors.^{124,125} LY2109761 additionally blocks the paracrine interactions between HCC and endothelial cells, and inhibited secretion of VEGF and neoangiogenesis, without affecting physiological angiogenic development of normal blood vessels.¹²⁶ Several other inhibitors, including LY364947 and SB-431542, have been used to treat pancreatic adenocarcinomas, diffuse-type gastric cancers,¹²⁷ and colon cancers.^{128,129} Mechanisms involve the induction of cytotoxic T-lymphocyte activity and attenuating the EMT. Small-molecule inhibitors of the TGF- β pathway therefore also have potential as therapeutics for GI cancers.

Neutralizing antibody to minimize the interactions between ligands and receptors is another therapeutic strategy, such as 2G7 and 1D11 (Genzyme, Inc, Cambridge, MA), which reduce the biological activity of all forms of TGF- β ligands. Treatment with 2G7 or 1D11 significantly suppressed lung metastasis through increased natural killer cell and cytotoxic T-lymphocyte activity,^{130,131} as well as decreased numbers of Gr-1+ myeloid cells in the tumor microenvironment.¹³² Anti-T β RII antibodies TR1 (anti-human) and MT1 (anti-mouse) suppress primary tumor growth and metastasis.¹³² In addition to neutralizing antibodies, soluble T β RII/T β RIII and T β RII:Fc fusion proteins function as ligand traps by minimizing ligand-receptor interactions. Soluble T β RII reduced pulmonary, pancreatic, and liver metastasis of pancreatic ductal adenocarcinomas.¹³³ Similarly, expression of soluble T β RIII inhibited growth of human colon carcinoma tumors.¹³⁴ Transgenic mice that express soluble T β RII:Fc fusion protein did not develop metastases in the lung, liver, spleen, or pancreas.¹³⁵ However, none of these agents have entered clinical trials, partially because of safety concerns.¹¹⁶

These current TGF- β -targeting approaches do not address the problem of the dual functions of TGF- β . There are many challenges to developing therapeutic reagents against TGF- β , including patient selection, timing of treatment, and the indicative biomarkers. Interestingly, the efficacy of TGF- β antagonists do not seem to depend on a direct effect on tumor cells, rather, these reagents act on the tumor environment and host factors. These features might affect patient selection for trials. For example, patients who produce high levels of TGF- β after radiation or chemotherapy might be more likely to respond to antagonists.¹³⁶ Improved understanding of TGF- β regulation of inflammation in tumorigenesis might help in selection of patients and developing biomarkers for clinical trials.

Strategies are being developed to target the tumor microenvironment. One approach is to convert tumor-associated macrophages, tumor-associated neutrophils from M2 or N2 to M1

or N1 to stimulate host immune response.^{137,138} TGF- β promotes the formation of tumor-associated macrophages and tumor-associated neutrophils. Epidermal growth factor–receptor inhibitors are identified as a potential strategy to target tumor microenvironment.^{139,140} Chemokine signaling pathways that recruit specific stromal cells also might be targeted, such as CXCL5/CXCR2 for MDSC recruitment.^{37,141} Inhibition of this axis showed attenuated tumor growth and metastasis.^{37,141} Growth factors such as epidermal growth factor, VEGF, and connective tissue growth factor are required for sustained growth of host stromal cells and might be targeted.¹⁴² Breast, prostate, and lung tumors all metastasize to bone, which has unique characteristics that provide a favorable microenvironment for cancer cells.¹⁴³ Cancer cells interact with osteoclasts through several pathways that might be targeted therapeutically, including signaling via receptor activator of nuclear factor kappa-B ligand (RANKL) or parathyroid hormone-related protein (PTHrP).^{16,143} Tumor necrosis factor- α , produced by recruited macrophages, activates Akt signaling and glycogen synthase kinase 3 (GSK3) β phosphorylation in epithelial cells and promotes Wnt signaling to β -catenin and gastric carcinogenesis.¹⁴⁴ Further elucidation of the interactions between epithelial and stromal cells could identify novel targets for GI cancers.

Biomarkers in TGF- β -targeted therapy may deserve further research. Blocking TGF- β decreases the suppression of host immune surveillance. This effect of TGF- β antagonism is particularly important because it may be combined with antitumor immunotherapy. TGF- β antagonism also abrogates the tumor-promoting effect of Gr-1+CD11b+ cells in the tumor microenvironment by neutralizing high levels of TGF- β 1 that these cells produce.³⁷ Recent studies have shown that immune/inflammatory responses are reliable markers for predicting clinical outcomes in HCC¹⁴⁵ and colorectal tumors.¹⁴⁶ A recent study using T-cell-based gene expression profiling to identify biomarkers for TGF- β -targeted therapy also supports this idea.¹⁴⁷

Conclusions and Outlook

Understanding the mechanisms underlying the dual role of the TGF- β signaling pathway is critical for the development of specific and efficient TGF- β -targeted therapy. One daunting task is to delineate the roles of TGF- β in specific tumor types and in different stages of cancer progression. It will be necessary to conduct comparative studies in different preclinical mouse models with certain models showing the antitumor effect of TGF- β inhibition and others showing the protumor effect of TGF- β inhibition. The mechanisms behind the varied responses in these models would provide valuable information for future studies in the field. In addition, the use of tumor genomic expression profiling and the detection of patient TGF- β response biomarkers may prove very important in selecting appropriate patients for anti-TGF- β therapeutics, monitoring the bioactivity of new drug candidates, and predicting therapy outcomes and adverse events.

Acknowledgments

The authors thank Mr Bo Sun, a summer intern in our laboratory, for his excellent editorial assistance.

Abbreviations used in this paper:

BMP	bone morphogenetic protein
CSC	cancer stem cells
EMT	epithelial to mesenchymal transition
GI	gastrointestinal
HCC	hepatocellular carcinoma
IL	interleukin
MDSC	myeloid-derived suppressive cell
SDF	stromal-derived factor
TβRI	type I TGF- β receptor
TβRII	type II TGF- β receptor
TGF	transforming growth factor
VEGF	vascular endothelial growth factor

References

1. Massague J TGFbeta in Cancer. Cell 2008;134:215–230. [PubMed: 18662538]
2. Nakao A, Afrakhte M, Moren A, et al. Identification of Smad7, a TGFbeta-inducible antagonist of TGF-beta signalling. Nature 1997;389:631–635. [PubMed: 9335507]
3. Derynck R, Miyazono K. The TGF-beta family. Cold Spring Harbor, NY: Cold Spring Harbor Monograph Series 50; 2007.
4. Hanahan DW. Hallmarks of cancer: the next generation. Cell 2011;144:646–674. [PubMed: 21376230]
5. Senturk S, Mumcuoglu M, Gursoy-Yuzugullu O, et al. Transforming growth factor-beta induces senescence in hepatocellular carcinoma cells and inhibits tumor growth. Hepatology 2010;52: 966–974. [PubMed: 20583212]
6. Cipriano R, Kan CE, Graham J, et al. TGF- β signaling engages an ATM-CHK2-p53-independent RAS-induced senescence and prevents malignant transformation in human mammary epithelial cells. Proc Natl Acad Sci U S A 2011;108:8668–8673. [PubMed: 21555587]
7. Reimann M, Lee S, Loddenkemper C, et al. Tumor stroma-derived TGF-beta limits myc-driven lymphomagenesis via Suv39h1-dependent senescence. Cancer Cell 2010;17:262–272. [PubMed: 20227040]
8. Kiyono K, Suzuki HI, Matsuyama H, et al. Autophagy is activated by TGF-beta and potentiates TGF-beta-mediated growth inhibition in human hepatocellular carcinoma cells. Cancer Res 2009;69: 8844–8852. [PubMed: 19903843]
9. Forrester E, Chytil A, Brier B, et al. Effect of conditional knockout of the type II TGF-beta receptor gene in mammary epithelia on mammary gland development and polyomavirus middle T antigen induced tumor formation and metastasis. Cancer Res 2005; 65:2296–2302. [PubMed: 15781643]
10. Ijichi H, Chytil A, Gorska AE, et al. Aggressive pancreatic ductal adenocarcinoma in mice caused by pancreas-specific blockade of transforming growth factor-beta signaling in cooperation with active Kras expression. Genes Dev 2006;20:3147–3160. [PubMed: 17114585]
11. Kitamura T, Kometani K, Hashida H, et al. SMAD4-deficient intestinal tumors recruit CCR1+ myeloid cells that promote invasion. Nat Genet 2007;39:467–475. [PubMed: 17369830]

12. Takaku K, Miyoshi H, Matsunaga A, et al. Gastric and duodenal polyps in Smad4 (Dpc4) knockout mice. *Cancer Res* 1999;59: 6113–6117. [PubMed: 10626800]
13. Takaku K, Oshima M, Miyoshi H, et al. Intestinal tumorigenesis in compound mutant mice of both Dpc4 (Smad4) and Apc genes. *Cell* 1998;92:645–656. [PubMed: 9506519]
14. Bruna A, Darken RS, Rojo F, et al. High TGFbeta-Smad activity confers poor prognosis in glioma patients and promotes cell proliferation depending on the methylation of the PDGF-B gene. *Cancer Cell* 2007;11:147–160. [PubMed: 17292826]
15. Arteaga CL. Inhibition of TGFbeta signaling in cancer therapy. *Curr Opin Genet Dev* 2006;16:30–37. [PubMed: 16377175]
16. Mundy GR. Metastasis to bone: causes, consequences and therapeutic opportunities. *Nat Rev Cancer* 2002;2:584–593. [PubMed: 12154351]
17. Roberts AB, Wakefield LM. The two faces of transforming growth factor beta in carcinogenesis. *Proc Natl Acad Sci U S A* 2003; 100:8621–8623. [PubMed: 12861075]
18. Bieri B, Chung CH, Parker JS, et al. Abrogation of TGF-beta signaling enhances chemokine production and correlates with prognosis in human breast cancer. *J Clin Invest* 2009;119: 1571–1582. [PubMed: 19451693]
19. Wieser R The transforming growth factor-beta signaling pathway in tumorigenesis. *Curr Opin Oncol* 2001;13:70–77. [PubMed: 11148690]
20. Siegel PM, Massague J. Cytostatic and apoptotic actions of TGF-beta in homeostasis and cancer. *Nat Rev Cancer* 2003;3: 807–821. [PubMed: 14557817]
21. Ikushima H, Miyazono K. TGFbeta signalling: a complex web in cancer progression. *Nat Rev Cancer* 2010;10:415–424. [PubMed: 20495575]
22. Padua D, Zhang XH, Wang Q, et al. TGFbeta primes breast tumors for lung metastasis seeding through angiopoietin-like 4. *Cell* 2008;133:66–77. [PubMed: 18394990]
23. Adorno M, Cordenonsi M, Montagner M, et al. A Mutant-p53/Smad complex opposes p63 to empower TGFbeta-induced metastasis. *Cell* 2009;137:87–98. [PubMed: 19345189]
24. Dupont S, Mamidi A, Cordenonsi M, et al. FAM/USP9x, a deubiquitinating enzyme essential for TGFbeta signaling, controls Smad4 monoubiquitination. *Cell* 2009;136:123–135. [PubMed: 19135894]
25. Liu IM, Schilling SH, Knouse KA, et al. TGFbeta-stimulated Smad1/5 phosphorylation requires the ALK5 L45 loop and mediates the pro-migratory TGFbeta switch. *EMBO J* 2009;28:88–98. [PubMed: 19096363]
26. Jang CW, Chen CH, Chen CC, et al. TGF-beta induces apoptosis through Smad-mediated expression of DAP-kinase. *Nat Cell Biol* 2002;4:51–58. [PubMed: 11740493]
27. Takekawa M, Tatebayashi K, Itoh F, et al. Smad-dependent GADD45beta expression mediates delayed activation of p38 MAP kinase by TGF-beta. *EMBO J* 2002;21:6473–6482. [PubMed: 12456654]
28. Ehata S, Hanyu A, Hayashi M, et al. Transforming growth factor-beta promotes survival of mammary carcinoma cells through induction of antiapoptotic transcription factor DEC1. *Cancer Res* 2007;67:9694–9703. [PubMed: 17942899]
29. Hannon GJ, Beach D. p15INK4B is a potential effector of TGF-beta-induced cell cycle arrest. *Nature* 1994;371:257–261. [PubMed: 8078588]
30. Datto MB, Li Y, Panus JF, et al. Transforming growth factor beta induces the cyclin-dependent kinase inhibitor p21 through a p53-independent mechanism. *Proc Natl Acad Sci U S A* 1995; 92:5545–5549. [PubMed: 7777546]
31. Yagi K, Furuhashi M, Aoki H, et al. c-myc is a downstream target of the Smad pathway. *J Biol Chem* 2002;277:854–861. [PubMed: 11689553]
32. Sanchez-Elsner T, Botella LM, Velasco B, et al. Synergistic cooperation between hypoxia and transforming growth factor-beta pathways on human vascular endothelial growth factor gene expression. *J Biol Chem* 2001;276:38527–38535. [PubMed: 11486006]
33. Inman GJ. Switching TGFbeta from a tumor suppressor to a tumor promoter. *Curr Opin Genet Dev* 2011;21:93–99. [PubMed: 21251810]

34. Yang L, Pang Y, Moses HL. TGF-beta and immune cells: an important regulatory axis in the tumor microenvironment and progression. *Trends Immunol* 2010;31:220–227. [PubMed: 20538542]
35. Augsten M, Hagglof C, Pena C, et al. A digest on the role of the tumor microenvironment in gastrointestinal cancers. *Cancer Microenviron* 2010;3:167–176. [PubMed: 21209782]
36. Du R, Lu KV, Petritsch C, et al. HIF1alpha induces the recruitment of bone marrow-derived vascular modulatory cells to regulate tumor angiogenesis and invasion. *Cancer Cell* 2008;13: 206–220. [PubMed: 18328425]
37. Yang L, Huang J, Ren X, et al. Abrogation of TGF beta signaling in mammary carcinomas recruits Gr-1+CD11b+ myeloid cells that promote metastasis. *Cancer Cell* 2008;13:23–35. [PubMed: 18167337]
38. Yang L, Debusk LM, Fukuda K, et al. Expansion of myeloid immune suppressor Gr+CD11b+ cells in tumor-bearing host directly promotes tumor angiogenesis. *Cancer Cell* 2004;6:409–421. [PubMed: 15488763]
39. Balkwill F, Coussens LM. Cancer: an inflammatory link. *Nature* 2004;431:405–406. [PubMed: 15385993]
40. Bristow RG, Hill RP. Hypoxia and metabolism. Hypoxia, DNA repair and genetic instability. *Nat Rev Cancer* 2008;8:180–192. [PubMed: 18273037]
41. Stairs DB, Bayne LJ, Rhoades B, et al. Deletion of p120-catenin results in a tumor microenvironment with inflammation and cancer that establishes it as a tumor suppressor gene. *Cancer Cell* 2011;19:470–483. [PubMed: 21481789]
42. Tu S, Bhagat G, Cui G, et al. Overexpression of interleukin-1beta induces gastric inflammation and cancer and mobilizes myeloid-derived suppressor cells in mice. *Cancer Cell* 2008;14:408–419. [PubMed: 18977329]
43. Clark CE, Hingorani SR, Mick R, et al. Dynamics of the immune reaction to pancreatic cancer from inception to invasion. *Cancer Res* 2007;67:9518–9527. [PubMed: 17909062]
44. Flavell RA, Sanjabi S, Wrzesinski SH, et al. The polarization of immune cells in the tumour environment by TGFbeta. *Nat Rev Immunol* 2010;10:554–567. [PubMed: 20616810]
45. Biswas S, Chytil A, Washington K, et al. Transforming growth factor beta receptor type II inactivation promotes the establishment and progression of colon cancer. *Cancer Res* 2004;64: 4687–4692. [PubMed: 15256431]
46. Lu SL, Herrington H, Reh D, et al. Loss of transforming growth factor-beta type II receptor promotes metastatic head-and-neck squamous cell carcinoma. *Genes Dev* 2006;20:1331–1342. [PubMed: 16702406]
47. Bornstein S, White R, Malkoski S, et al. Smad4 loss in mice causes spontaneous head and neck cancer with increased genomic instability and inflammation. *J Clin Invest* 2009;119: 3408–3419. [PubMed: 19841536]
48. Engle SJ, Ormsby I, Pawlowski S, et al. Elimination of colon cancer in germ-free transforming growth factor beta 1-deficient mice. *Cancer Res* 2002;62:6362–6366. [PubMed: 12438215]
49. Bhowmick NA, Chytil A, Plieth D, et al. TGF-beta signaling in fibroblasts modulates the oncogenic potential of adjacent epithelia. *Science* 2004;303:848–851. [PubMed: 14764882]
50. Cheng N, Bhowmick NA, Chytil A, et al. Loss of TGF-beta type II receptor in fibroblasts promotes mammary carcinoma growth and invasion through upregulation of TGF-alpha-, MSP- and HGF-mediated signaling networks. *Oncogene* 2005;24:5053–5068. [PubMed: 15856015]
51. Franco OE, Jiang M, Strand DW, et al. Altered TGF-beta signaling in a subpopulation of human stromal cells promotes prostatic carcinogenesis. *Cancer Res* 2011;71:1272–1281. [PubMed: 21303979]
52. Kojima Y, Acar A, Eaton EN, et al. Autocrine TGF-beta and stromal cell-derived factor-1 (SDF-1) signaling drives the evolution of tumor-promoting mammary stromal myofibroblasts. *Proc Natl Acad Sci USA* 2010;107:20009–14. [PubMed: 21041659]
53. Kim BG, Li C, Qiao W, et al. Smad4 signalling in T cells is required for suppression of gastrointestinal cancer. *Nature* 2006;441:1015–1019. [PubMed: 16791201]
54. Monteleone G, Mann J, Monteleone I, et al. A failure of transforming growth factor-beta1 negative regulation maintains sustained NF-kappaB activation in gut inflammation. *J Biol Chem* 2004;279:3925–3932. [PubMed: 14600158]

55. Maggio-Price L, Treuting P, Zeng W, et al. Helicobacter infection is required for inflammation and colon cancer in SMAD3-deficient mice. *Cancer Res* 2006;66:828–838. [PubMed: 16424015]
56. Lu T, Tian L, Han Y, et al. Dose-dependent cross-talk between the transforming growth factor-beta and interleukin-1 signaling pathways. *Proc Natl Acad Sci U S A* 2007;104:4365–4370. [PubMed: 17360530]
57. Yang L TGFbeta and cancer metastasis: an inflammation link. *Cancer Metastasis Rev* 2010;29:263–271. [PubMed: 20437081]
58. Korc M Pancreatic cancer-associated stroma production. *Am J Surg* 2007;194:S84–S86. [PubMed: 17903452]
59. Hawinkels LJ, Verspaget HW, van Duijn W, et al. Tissue level, activation and cellular localisation of TGF-beta1 and association with survival in gastric cancer patients. *Br J Cancer* 2007;97: 398–404. [PubMed: 17637685]
60. Polyak K, Weinberg RA. Transitions between epithelial and mesenchymal states: acquisition of malignant and stem cell traits. *Nat Rev Cancer* 2009;9:265–273. [PubMed: 19262571]
61. Kalluri R, Weinberg RA. The basics of epithelial-mesenchymal transition. *J Clin Invest* 2009;119:1420–1428. [PubMed: 19487818]
62. Ao M, Williams K, Bhowmick NA, et al. Transforming growth factor-beta promotes invasion in tumorigenic but not in nontumorigenic human prostatic epithelial cells. *Cancer Res* 2006;66: 8007–8016. [PubMed: 16912176]
63. Zeisberg M, Hanai J, Sugimoto H, et al. BMP-7 counteracts TGF-beta1-induced epithelial-to-mesenchymal transition and reverses chronic renal injury. *Nat Med* 2003;9:964–968. [PubMed: 12808448]
64. Horiguchi K, Shirakihara T, Nakano A, et al. Role of Ras signaling in the induction of snail by transforming growth factor-beta. *J Biol Chem* 2009;284:245–253. [PubMed: 19010789]
65. Gregory PA, Bracken CP, Smith E, et al. An autocrine TGF- β /ZEB/miR-200 signaling network regulates establishment and maintenance of epithelial-mesenchymal transition. *Mol Biol Cell* 2011;22:1686–1698. [PubMed: 21411626]
66. Saito RA, Watabe T, Horiguchi K, et al. Thyroid transcription factor-1 inhibits transforming growth factor-beta-mediated epithelial-to-mesenchymal transition in lung adenocarcinoma cells. *Cancer Res* 2009;69:2783–2791. [PubMed: 19293183]
67. Cufi S, Vazquez-Martin A, Oliveras-Ferraro C, et al. Metformin against TGFbeta-induced epithelial-to-mesenchymal transition (EMT): from cancer stem cells to aging-associated fibrosis. *Cell Cycle* 2010;9:4461–4468. [PubMed: 21088486]
68. Mani SA, Guo W, Liao MJ, et al. The epithelial-mesenchymal transition generates cells with properties of stem cells. *Cell* 2008;133:704–715. [PubMed: 18485877]
69. Ikushima H, Todo T, Ino Y, et al. Autocrine TGF-beta signaling maintains tumorigenicity of glioma-initiating cells through Sry related HMG-box factors. *Cell Stem Cell* 2009;5:504–514. [PubMed: 19896441]
70. Penuelas S, Anido J, Prieto-Sanchez RM, et al. TGF-beta increases glioma-initiating cell self-renewal through the induction of LIF in human glioblastoma. *Cancer Cell* 2009;15:315–327. [PubMed: 19345330]
71. Shipitsin M, Campbell LL, Argani P, et al. Molecular definition of breast tumor heterogeneity. *Cancer Cell* 2007;11:259–273. [PubMed: 17349583]
72. Anido J, Saez-Borderias A, Gonzalez-Junca A, et al. TGF-beta receptor inhibitors target the CD44(high)/Id1(high) glioma-initiating cell population in human glioblastoma. *Cancer Cell* 2010;18: 655–668. [PubMed: 21156287]
73. Tang B, Yoo N, Vu M, et al. Transforming growth factor-beta can suppress tumorigenesis through effects on the putative cancer stem or early progenitor cell and committed progeny in a breast cancer xenograft model. *Cancer Res* 2007;67:8643–8652. [PubMed: 17875704]
74. Hirschmann-Jax C, Foster AE, Wulf GG, et al. A distinct “side population” of cells in human tumor cells: implications for tumor biology and therapy. *Cell Cycle* 2005;4:203–205. [PubMed: 15655356]

75. Ehata S, Johansson E, Katayama R, et al. Transforming growth factor-beta decreases the cancer-initiating cell population within diffuse-type gastric carcinoma cells. *Oncogene* 2011;30:1693–1705. [PubMed: 21132002]
76. Kaplan RN, Riba RD, Zacharoulis S, et al. VEGFR1-positive haematopoietic bone marrow progenitors initiate the pre-metastatic niche. *Nature* 2005;438:820–827. [PubMed: 16341007]
77. Psaila B, Lyden D. The metastatic niche: adapting the foreign soil. *Nat Rev Cancer* 2009;9:285–293. [PubMed: 19308068]
78. Erler JT, Bennewith KL, Cox TR, et al. Hypoxia-induced lysyl oxidase is a critical mediator of bone marrow cell recruitment to form the premetastatic niche. *Cancer Cell* 2009;15:35–44. [PubMed: 19111879]
79. Yan HH, Pickup M, Pang Y, et al. Gr-1+CD11b+ myeloid cells tip the balance of immune protection to tumor promotion in the premetastatic lung. *Cancer Res* 2010;70:6139–6149. [PubMed: 20631080]
80. Oskarsson T, Acharyya S, Zhang XH, et al. Breast cancer cells produce tenascin C as a metastatic niche component to colonize the lungs. *Nat Med* 2011;17:867–874. [PubMed: 21706029]
81. Peinado H, Lavotshkin S, Lyden D. The secreted factors responsible for pre-metastatic niche formation: old sayings and new thoughts. *Semin Cancer Biol* 2011;21:139–146. [PubMed: 21251983]
82. Hiratsuka S, Watanabe A, Aburatani H, et al. Tumour-mediated upregulation of chemoattractants and recruitment of myeloid cells predetermines lung metastasis. *Nat Cell Biol* 2006;8: 1369–1375. [PubMed: 17128264]
83. Javelaud D, Mohammad KS, McKenna CR, et al. Stable overexpression of Smad7 in human melanoma cells impairs bone metastasis. *Cancer Res* 2007;67:2317–2324. [PubMed: 17332363]
84. Akhurst RJ, Derynck R. TGF-beta signaling in cancer—a double-edged sword. *Trends Cell Biol* 2001;11:S44–S51. [PubMed: 11684442]
85. Gholamin M, Moaven O, Memar B, et al. Overexpression and interactions of interleukin-10, transforming growth factor beta, and vascular endothelial growth factor in esophageal squamous cell carcinoma. *World J Surg* 2009;33:1439–1445. [PubMed: 19440651]
86. Zhou Q, Dong Wang L, et al. Changes of TGFbeta1 and TGFbetaR2 expression in esophageal precancerous and cancerous lesions: a study of a high-risk population in Henan, northern China. *Dis Esophagus* 2002;15:74–79. [PubMed: 12060047]
87. Ebert MP, Yu J, Miehke S, et al. Expression of transforming growth factor beta-1 in gastric cancer and in the gastric mucosa of first-degree relatives of patients with gastric cancer. *British J Cancer* 2000;82:1795–1800.
88. Abou-Shady M, Baer HU, Friess H, et al. Transforming growth factor betas and their signaling receptors in human hepatocellular carcinoma. *Am J Surg* 1999;177:209–215. [PubMed: 10219856]
89. Lee KT, Liu TS. Expression of transforming growth factor betas and their signaling receptors in stone-containing intrahepatic bile ducts and cholangiocarcinoma. *World J Surg* 2003;27:1143–1148. [PubMed: 12917766]
90. Truty MJ, Urrutia R. Basics of TGF-beta and pancreatic cancer. *Pancreatology* 2007;7:423–435. [PubMed: 17898532]
91. Fukai Y, Fukuchi M, Masuda N, et al. Reduced expression of transforming growth factor-beta receptors is an unfavorable prognostic factor in human esophageal squamous cell carcinoma. *Int J Cancer* 2003;104:161–166. [PubMed: 12569570]
92. Kang SH, Bang YJ, Im YH, et al. Transcriptional repression of the transforming growth factor-beta type I receptor gene by DNA methylation results in the development of TGF-beta resistance in human gastric cancer. *Oncogene* 1999;18:7280–7286. [PubMed: 10602482]
93. Mamiya T, Yamazaki K, Masugi Y, et al. Reduced transforming growth factor-beta receptor II expression in hepatocellular carcinoma correlates with intrahepatic metastasis. *Lab Invest* 2010; 90:1339–1345. [PubMed: 20531292]
94. Furuta K, Misao S, Takahashi K, et al. Gene mutation of transforming growth factor beta1 type II receptor in hepatocellular carcinoma. *Int J Cancer* 1999;81:851–853. [PubMed: 10362128]

95. Mendelson J, Song S, Li Y, et al. Dysfunctional transforming growth factor-beta signaling with constitutively active notch signaling in Barrett's esophageal adenocarcinoma. *Cancer* 2011; 117:3691–3702. [PubMed: 21305538]
96. Meng W, Xia Q, Wu L, et al. Downregulation of TGF-beta receptor types II and III in oral squamous cell carcinoma and oral carcinoma-associated fibroblasts. *BMC Cancer* 2011;11:88. [PubMed: 21352603]
97. Pasche B, Wisinski KB, Sadim M, et al. Constitutively decreased TGFBR1 allelic expression is a common finding in colorectal cancer and is associated with three TGFBR1 SNPs. *J Exp Clin Cancer Res* 2010;29:57. [PubMed: 20500843]
98. Musch A, Rabe C, Paik MD, et al. Altered expression of TGF-beta receptors in hepatocellular carcinoma—effects of a constitutively active TGF-beta type I receptor mutant. *Digestion* 2005;71: 78–91. [PubMed: 15775675]
99. Kim JJ, Baek MJ, Kim L, et al. Accumulated frameshift mutations at coding nucleotide repeats during the progression of gastric carcinoma with microsatellite instability. *Lab Invest* 1999;79: 1113–1120. [PubMed: 10496529]
100. Calin GA, Gafa R, Tibiletti MG, et al. Genetic progression in microsatellite instability high (MSI-H) colon cancers correlates with clinico-pathological parameters: a study of the TGRbetaR2, BAX, hMSH3, hMSH6, IGFIR and BLM genes. *Int J Cancer* 2000;89:230–235. [PubMed: 10861498]
101. Bellam N, Pasche B. Tgf-beta signaling alterations and colon cancer. *Cancer Treat Res* 2010;155:85–103. [PubMed: 20517689]
102. Goggins M, Shekher M, Turnacioglu K, et al. Genetic alterations of the transforming growth factor beta receptor genes in pancreatic and biliary adenocarcinomas. *Cancer Res* 1998;58:5329–5332. [PubMed: 9850059]
103. Paterson IC, Davies M, Stone A, et al. TGF-beta1 acts as a tumor suppressor of human malignant keratinocytes independently of Smad 4 expression and ligand-induced G(1) arrest. *Oncogene* 2002;21:1616–1624. [PubMed: 11896591]
104. Fukuchi M, Masuda N, Miyazaki T, et al. Decreased Smad4 expression in the transforming growth factor-beta signaling pathway during progression of esophageal squamous cell carcinoma. *Cancer* 2002;95:737–743. [PubMed: 12209716]
105. Xu X, Brodie SG, Yang X, et al. Haploid loss of the tumor suppressor Smad4/Dpc4 initiates gastric polyposis and cancer in mice. *Oncogene* 2000;19:1868–1874. [PubMed: 10773876]
106. Xu X, Kobayashi S, Qiao W, et al. Induction of intrahepatic cholangiocellular carcinoma by liver-specific disruption of Smad4 and Pten in mice. *J Clin Invest* 2006;116:1843–1852. [PubMed: 16767220]
107. Blackford A, Serrano OK, Wolfgang CL, et al. SMAD4 gene mutations are associated with poor prognosis in pancreatic cancer. *Clin Cancer Res* 2009;15:4674–4679. [PubMed: 19584151]
108. Yakicier MC, Irmak MB, Romano A, et al. Smad2 and Smad4 gene mutations in hepatocellular carcinoma. *Oncogene* 1999; 18:4879–4883. [PubMed: 10490821]
109. Boulay JL, Mild G, Lowy A, et al. SMAD7 is a prognostic marker in patients with colorectal cancer. *Int J Cancer* 2003;104:446–449. [PubMed: 12584741]
110. Alberici P, Gaspar C, Franken P, et al. Smad4 haploinsufficiency: a matter of dosage. *Pathogenetics* 2008;1:2. [PubMed: 19014666]
111. Munoz NM, Upton M, Rojas A, et al. Transforming growth factor beta receptor type II inactivation induces the malignant transformation of intestinal neoplasms initiated by Apc mutation. *Cancer Res* 2006;66:9837–9844. [PubMed: 17047044]
112. Yang X, Letterio JJ, Lechleider RJ, et al. Targeted disruption of SMAD3 results in impaired mucosal immunity and diminished T cell responsiveness to TGF-beta. *EMBO J* 1999;18:1280–1291. [PubMed: 10064594]
113. Yao Q, Cao S, Li C, et al. Micro-RNA-21 regulates TGF-beta-induced myofibroblast differentiation by targeting PDCD4 in tumor-stroma interaction. *Int J Cancer* 2011;128:1783–1792. [PubMed: 20533548]
114. Bhowmick NA, Neilson EG, Moses HL. Stromal fibroblasts in cancer initiation and progression. *Nature* 2004;432:332–337. [PubMed: 15549095]

115. Pesu M, Watford WT, Wei L, et al. T-cell-expressed proprotein convertase furin is essential for maintenance of peripheral immune tolerance. *Nature* 2008;455:246–250. [PubMed: 18701887]
116. Kelly RJ, Morris JC. Transforming growth factor-beta: a target for cancer therapy. *J Immunotoxicol* 2010;7:15–26. [PubMed: 19916703]
117. Chowdhury S, Ammanamanchi S, Howell GM. Epigenetic targeting of transforming growth factor beta receptor II and implications for cancer therapy. *Mol Cell Pharmacol* 2009;1:57–70. [PubMed: 20414468]
118. Terabe M, Ambrosino E, Takaku S, et al. Synergistic enhancement of CD8+ T cell-mediated tumor vaccine efficacy by an anti-transforming growth factor-beta monoclonal antibody. *Clin Cancer Res* 2009;15:6560–6569. [PubMed: 19861451]
119. Muraoka-Cook RS, Kurokawa H, Koh Y, et al. Conditional over expression of active transforming growth factor beta1 in vivo accelerates metastases of transgenic mammary tumors. *Cancer Res* 2004;64:9002–9011. [PubMed: 15604265]
120. Trabedersen Vallieres L, a TGFbeta2-specific antisense oligonucleotide for the treatment of malignant gliomas and other tumors overexpressing TGFbeta2. *IDrugs* 2009;12:445–453. [PubMed: 19579166]
121. Schlingensiepen KH, Jaschinski F, Lang SA, et al. TGF-beta2 gene silencing with trabedersen (AP 12009) in pancreatic cancer. *Cancer Sci* 2011;102:1193–1200. [PubMed: 21366804]
122. Schlingensiepen KH, Fischer-Blass B, Schmaus S, et al. Anti-sense therapeutics for tumor treatment: the TGF-beta2 inhibitor AP 12009 in clinical development against malignant tumors. *Recent Results Cancer Res* 2008;177:137–150. [PubMed: 18084956]
123. Giannelli G, Mazzocca A, Fransvea E, et al. Inhibiting TGF-beta signaling in hepatocellular carcinoma. *Biochim Biophys Acta* 2011;1815:214–223. [PubMed: 21129443]
124. Melisi D, Ishiyama S, Scialabba GM, et al. LY2109761, a novel transforming growth factor beta receptor type I and type II dual inhibitor, as a therapeutic approach to suppressing pancreatic cancer metastasis. *Mol Cancer Ther* 2008;7:829–840. [PubMed: 18413796]
125. Zhang B, Halder SK, Zhang S, et al. Targeting transforming growth factor-beta signaling in liver metastasis of colon cancer. *Cancer Lett* 2009;277:114–120. [PubMed: 19147275]
126. Mazzocca A, Fransvea E, Lavezzari G, et al. Inhibition of transforming growth factor beta receptor I kinase blocks hepatocellular carcinoma growth through neo-angiogenesis regulation. *Hepatology* 2009;50:1140–1151. [PubMed: 19711426]
127. Kano MR, Bae Y, Iwata C, et al. Improvement of cancer-targeting therapy, using nanocarriers for intractable solid tumors by inhibition of TGF-beta signaling. *Proc Natl Acad Sci U S A* 2007;104: 3460–3465. [PubMed: 17307870]
128. Tanaka H, Shinto O, Yashiro M, et al. Transforming growth factor beta signaling inhibitor, SB-431542, induces maturation of dendritic cells and enhances anti-tumor activity. *Oncol Rep* 2010; 24:1637–1643. [PubMed: 21042762]
129. Halder SK, Beauchamp RD, Datta PK. A specific inhibitor of TGF-beta receptor kinase, SB-431542, as a potent antitumor agent for human cancers. *Neoplasia* 2005;7:509–521. [PubMed: 15967103]
130. Arteaga CL, Hurd SD, Winnier AR, et al. Anti-transforming growth factor (TGF)-beta antibodies inhibit breast cancer cell tumorigenicity and increase mouse spleen natural killer cell activity. Implications for a possible role of tumor cell/host TGF-beta interactions in human breast cancer progression. *J Clin Invest* 1993;92:2569–2576. [PubMed: 7504687]
131. Nam JS, Suchar AM, Kang MJ, et al. Bone sialoprotein mediates the tumor cell-targeted prometastatic activity of transforming growth factor beta in a mouse model of breast cancer. *Cancer Res* 2006;66:6327–6335. [PubMed: 16778210]
132. Zhong Z, Carroll KD, Policarpio D, et al. Anti-transforming growth factor beta receptor II antibody has therapeutic efficacy against primary tumor growth and metastasis through multieffects on cancer, stroma, and immune cells. *Clin Cancer Res* 2010;16: 1191–1205. [PubMed: 20145179]
133. Rowland-Goldsmith MA, Maruyama H, Matsuda K, et al. Soluble type II transforming growth factor-beta receptor attenuates expression of metastasis-associated genes and suppresses pancreatic cancer cell metastasis. *Mol Cancer Ther* 2002;1:161–167. [PubMed: 12467210]

134. Bandyopadhyay A, Zhu Y, Malik SN, et al. Extracellular domain of TGFbeta type III receptor inhibits angiogenesis and tumor growth in human cancer cells. *Oncogene* 2002;21:3541–3551. [PubMed: 12032856]
135. Yang YA, Dukhanina O, Tang B, et al. Lifetime exposure to a soluble TGF-beta antagonist protects mice against metastasis without adverse side effects. *J Clin Invest* 2002;109:1607–1615. [PubMed: 12070308]
136. Biswas S, Guix M, Rinehart C, et al. Inhibition of TGF-beta with neutralizing antibodies prevents radiation-induced acceleration of metastatic cancer progression. *J Clin Invest* 2007;117:1305–1313. [PubMed: 17415413]
137. Sica A, Allavena P, Mantovani A. Cancer related inflammation: the macrophage connection. *Cancer Lett* 2008;267:204–215. [PubMed: 18448242]
138. Fridlender ZG, Sun J, Kim S, et al. Polarization of tumor-associated neutrophil phenotype by TGF-beta: “N1” versus “N2” TAN. *Cancer Cell* 2009;16:183–194. [PubMed: 19732719]
139. Joyce JA, Pollard JW. Microenvironmental regulation of metastasis. *Nat Rev Cancer* 2009;9:239–252. [PubMed: 19279573]
140. Condeelis J, Pollard JW. Macrophages: obligate partners for tumor cell migration, invasion, and metastasis. *Cell* 2006;124: 263–266. [PubMed: 16439202]
141. Cataisson C, Ohman R, Patel G, et al. Inducible cutaneous inflammation reveals a protumorigenic role for keratinocyte CXCR2 in skin carcinogenesis. *Cancer Res* 2009;69:319–328. [PubMed: 19118017]
142. Hofmeister V, Schrama D, Becker JC. Anti-cancer therapies targeting the tumor stroma. *Cancer Immunol Immunother* 2008;57: 1–17. [PubMed: 17661033]
143. Guise TA, Chirgwin JM. Transforming growth factor-beta in osteolytic breast cancer bone metastases. *Clin Orthop Relat Res* 2003:S32–S38. [PubMed: 14600590]
144. Oguma K, Oshima H, Aoki M, et al. Activated macrophages promote Wnt signalling through tumour necrosis factor-alpha in gastric tumour cells. *EMBO J* 2008;27:1671–1681. [PubMed: 18511911]
145. Budhu A, Forgues M, Ye QH, et al. Prediction of venous metastases, recurrence, and prognosis in hepatocellular carcinoma based on a unique immune response signature of the liver microenvironment. *Cancer Cell* 2006;10:99–111. [PubMed: 16904609]
146. Galon J, Costes A, Sanchez-Cabo F, et al. Type, density, and location of immune cells within human colorectal tumors predict clinical outcome. *Science* 2006;313:1960–1964. [PubMed: 17008531]
147. Classen S, Muth C, Debey-Pascher S, et al. Application of T cell-based transcriptomics to identify three candidate biomarkers for monitoring anti-TGFbetaR therapy. *Pharmacogenet Genomics* 2010;20:147–156. [PubMed: 20084050]

Figure 1.

Simplified TGF- β signaling pathways. TGF- β , a multifunctional cytokine, signals through TGF- β receptors. Canonical signaling goes through phosphorylation of Smad2 and Smad3, which then combine with Smad4 to enter into the nucleus. TGF- β mediates growth inhibition mainly through this pathway. Smad7 negatively regulates this pathway through inhibition of Smad2 and Smad3 phosphorylation. TGF- β binding to its receptors also activates noncanonical signaling pathways such as mitogen-activated protein kinase and PI3 kinase pathways, as well as small guanosine triphosphatase. These pathways are implicated in EMT, tumor cell motility, and migration. cyD, cyclin D; cyE, cyclin E; GSK3, glycogen synthase kinase 3; MEKK1, mitogen-activated protein kinase kinase 1; MKK4,

mitogen-activated protein kinase kinase 4; MLK3, mixed lineage kinase 3; mTOR, mammalian target of rapamycin; myc, myelocytomatosis oncogene; PI3K, phosphatidylinositol 3-kinases; Rb, retinoblastoma; RhoA, ras homolog gene family, member A; ROCK, rho-associated protein kinase; TAK1, transforming growth factor β -activated kinase 1.

Figure 2.

TGF- β signaling exerts both anti-oncogenic and pro-oncogenic activities in carcinogenesis. For anti-oncogenic properties, TGF- β inhibits cell growth and induces apoptosis, autophagy, and senescence. TGF- β signaling also suppresses inflammation and growth factor expression. The genetic evidence for the anti-oncogenic role of TGF- β includes the fact that T β Rs and other components of this pathway often are mutated or down-regulated. Dysregulation of cell-cycle progression, promotion of EMT/mesenchymal to epithelial transition (MET), induction of angiogenesis, an increase in expression of proteases and extracellular matrix deposition, and a decrease in immune surveillance, as well as stem cell maintenance are pro-oncogenic hallmarks of TGF- β .

Figure 3. TGF- β is secreted in an autocrine and paracrine fashion by a variety of cells including tumor cells, immune cells, and fibroblasts in the tumor microenvironment. It promotes EMT/MET, reduces tumor cell apoptosis, and enhances tumor angiogenesis and decreases immune surveillance. Caf, cancer associated fibroblasts; MET, mesenchymal to epithelial transition; NK, natural killer, TAM, tumor associated macrophages; Treg, T-regulatory cell.

Figure 4.

TGF- β is a master regulator of the host immune cell function. TGF- β inhibits T-helper 1 (Th1), M1, and N1, but promotes Th2, M2, and N2 differentiation and development in the tumor microenvironment. The mechanisms regulated by TGF- β are listed in the figure. In addition, TGF- β suppresses cytotoxic T-lymphocyte (CTL), natural killer (NK), and dendritic cells (DC) function. TGF- β induces Foxp3 expression and generates Tregs. TGF- β , together with IL-6, is also responsible for the proinflammatory Th17 cell production (ROR γ t).^{34,44} M, macrophage; N, neutrophil; ROR γ t, RAR-related orphan receptor gamma 2; STAT, signal transducer and activator of transcription; T-bet; T-cell-specific T-box transcription factor.

Figure 5. Current strategies in TGF- β -targeted therapy: antisense-molecule-mediated silencing of TGF- β ligands, small-molecule inhibitors targeting T β RI/II kinase activity, ligand traps using monoclonal TGF- β /T β RII-neutralizing antibodies, and soluble T β RII/T β RIII receptors. mRNA, messenger RNA.

Table 1.

Alterations of TGF- β Signaling Molecules in Gastrointestinal Cancers

GI cancers	TGF- β	Tgfr1	Tgfr2	Smad2/3	Smad4	References
Oral	—	—	Reduced expression	—	Reduced expression	96,103
Esophagus	Increased expression of TGF- β 1	Reduced expression	Reduced expression	Reduced expression	Reduced expression	86,91,95,104
Gastric	Increased expression of TGF- β 1	Reduced expression	<i>Tgfr2</i> mutation	—	<i>Smad4</i> mutation	87,92,105
Liver	Increased expression of TGF- β 1	<i>Tgfr1</i> mutation	Reduced expression; <i>Tgfr2</i> mutation	<i>Smad2</i> mutation	<i>Smad4</i> mutation	88,93,94,98,108
Pancreas	Increased expression of TGF- β 1 and TGF- β 2	<i>Tgfr1</i> mutation	<i>Tgfr2</i> mutation	—	<i>Smad4</i> mutation	90,102,107
Colon	Increased expression of TGF- β 1	<i>Tgfr1</i> mutation	<i>Tgfr2</i> mutation	<i>Smad2</i> mutation	<i>Smad4</i> mutation	97,100,101
Biliary	Increased expression of all TGF- β s	<i>Tgfr1</i> mutation	<i>Tgfr2</i> mutation	—	<i>Smad4</i> mutation	89,102,106