

Role of Transforming Growth Factor Beta in Human Cancer

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A B S T R A C T

Transforming growth factor beta (TGF- β) is a ubiquitous and essential regulator of cellular and physiologic processes including proliferation, differentiation, migration, cell survival, angiogenesis, and immunosurveillance. Alterations in the TGF- β signaling pathway, including mutation or deletion of members of the signaling pathway and resistance to TGF- β -mediated inhibition of proliferation are frequently observed in human cancers. Although these alterations define a tumor suppressor role for the TGF- β pathway in human cancer, TGF- β also mediates tumor-promoting effects, either through differential effects on tumor and stromal cells or through a fundamental alteration in the TGF- β responsiveness of the tumor cells themselves. TGF- β and members of the TGF- β signaling pathway are being evaluated as prognostic or predictive markers for cancer patients. Ongoing advances in understanding the TGF- β signaling pathway will enable targeting of this pathway for the chemoprevention and treatment of human cancers.

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INTRODUCTION

The complex process of tumor formation in humans has been distilled to a series of stochastic events that occur in virtually all human cancers.¹ These hallmarks include seven functions that cancer cells acquire: the ability to become resistant to growth inhibitory factors, proliferate in the absence of exogenous growth factors, invade and metastasize, achieve limitless replication potential, evade apoptosis, recruit a blood supply through angiogenesis, and evade destruction by the immune system. Acquisition of these functions is facilitated by a general property of human cancer cells: their genomic instability. Each of these functions is regulated through signal transduction pathways that normally control cellular homeostasis. Thus, human tumorigenesis can be viewed as a disruption of these pathways, through genetic, epigenetic, or somatic alterations. One of these signal transduction pathways, the transforming growth factor beta (TGF- β) pathway, has a defined yet complex

role in mediating or regulating each of these hallmarks (Table 1). Despite the prominent role of this pathway in tumorigenesis, targeting of the TGF- β pathway has been hampered by insufficient understanding of the mechanisms regulating the pathway in vivo and by the heterogeneity of alterations that occur in the pathway in human cancers. This review highlights the role of the TGF- β pathway in these hallmarks of human cancer and the specific role of the pathway in the four most common human cancers (cancers of the breast, colon, lung, and prostate). The review concludes with a discussion of the utility of assessing the pathway for diagnostic, prognostic, or predictive purposes, and potential strategies for targeting the pathway for the chemoprevention or treatment of human cancers.

TGF- β SIGNALING PATHWAY

The TGF- β signaling pathway has been the focus of several recent reviews.⁹⁻¹¹ Three TGF- β isoforms are expressed in mammals

Table 1. TGF- β and the Hallmarks of Cancer

Hallmark	Effect of TGF- β	Example
Resistance to growth-inhibitory factors	Loss of TGF- β -induced growth inhibition	About half of human pancreatic cancers are not growth inhibited by TGF- β because of mutation of the <i>Smad4</i> gene ²
Proliferation in absence of exogenous growth factors	TGF- β stimulates proliferation of some cancer cells	In colon carcinoma cells, TGF- β stimulates proliferation through a Ras-dependent mechanism ³
Invasion and metastasis	TGF- β promotes invasiveness and metastasis	In prostate cancer cells, exogenous TGF- β increases secretion of plasminogen activator, the expression of which promotes extracellular matrix degradation and is correlated with a more invasive phenotype ⁴
Limitless replicative potential	Loss of TGF- β -induced repression of <i>hTERT</i>	TGF- β induces telomere shortening followed by senescence in lung cancer cells ⁵
Evasion of apoptosis	Loss of TGF- β -mediated apoptosis	Blocking TGF- β signaling inhibits tamoxifen-induced apoptosis in human breast cancer cells ⁶
Induction of angiogenesis	TGF- β induces angiogenesis	Neutralizing TGF- β antibody decreases the angiogenesis and tumorigenesis of TGF- β -insensitive renal cell carcinoma cells in an animal model ⁷
Immune system evasion	TGF- β is a potent immunosuppressant	In animal models increased TGF- β expression allows highly immunogenic cancer cells to escape immune surveillance and form tumors ⁸

Abbreviation: TGF- β , transforming growth factor β .

(TGF- β 1, TGF- β 2, and TGF- β 3) and each is encoded by a unique gene and expressed in both a tissue-specific and developmentally regulated fashion. TGF- β 1 is the most abundant and universally expressed isoform; most studies have either examined or been performed with exogenous TGF- β 1. TGF- β is secreted into the extracellular matrix as a latent protein complex bound to a latency-associated protein and one of the four isoforms of latent TGF- β binding protein. Activation of TGF- β , which is required for biologic activity, occurs through poorly understood mechanisms likely involving proteolytic processing of the associated proteins and release of the TGF- β ligand. Once activated, the TGF- β ligands regulate cellular processes by binding to three high-affinity cell surface receptors: the type I TGF- β receptor (T β RI), type II TGF- β receptor (T β RII), and type III TGF- β receptor (T β RIII, also referred to as betaglycan). Where expressed, T β RIII is the most abundant TGF- β receptor and classically functions by binding the TGF- β ligand and transferring it to its signaling receptors, T β RI and T β RII.¹² T β RI and T β RII contain serine/threonine protein kinases in their intracellular domains. T β RI initiates intracellular signaling by phosphorylating a family of transcription factors, the Smads. Smad2 and Smad3 are the receptor-activated Smads for TGF- β because they are phosphorylated by T β RI. Smad4 is a common partner for all of the receptor-activated Smads. Smad6 and Smad7 are inhibitory Smads that block the phosphorylation of Smad2 or Smad3, thus inhibiting TGF- β signaling.

A general mechanism for TGF- β signaling has been elucidated (Fig 1).^{13,14} The TGF- β ligand either binds to T β RIII, which presents TGF- β to T β RII, or binds to T β RII directly. Once bound to TGF- β , T β RII recruits, binds, and

transphosphorylates T β RI, thereby stimulating its protein kinase activity. The activated T β RI phosphorylates Smad2 or Smad3, which binds to Smad4. The resulting Smad complex translocates into the nucleus and interacts in a cell-specific manner with transcription factors to regulate specifically the transcription of a multitude of TGF- β -responsive genes. TGF- β signaling is regulated by the level and duration of TGF- β receptor activation, with continuous nucleocytoplasmic shuttling of Smads permitting them to monitor the levels of activated receptors continuously.¹⁵ In addition, TGF- β signaling may be regulated by internalization of the receptors, with some studies suggesting that receptor internalization is required for signaling,¹⁶⁻¹⁸ and others suggesting a role for internalization in downregulation of signaling.^{18,19}

Although T β RI, T β RII, Smad2, Smad3, and Smad4 comprise the core Smad-dependent TGF- β signaling pathway, Smad-independent signaling through mitogen-activated protein kinase (MAPK) signaling pathways,²⁰⁻²² Rho guanosine triphosphatases,²³ PI-3 kinase/Akt,²⁴ and protein phosphatase 2A²⁵ has been reported; precise molecular mechanisms by which the TGF- β signaling pathway signals to these pathways have not been established.

ROLE OF TGF- β IN THE HALLMARKS OF CANCER BIOLOGY

Resistance to Antiproliferative Signals and Independence From Exogenous Growth Signals

Cellular proliferation is normally regulated by the concerted action of both mitogenic growth signals and antiproliferative signals that converge on regulators of the cell cycle.

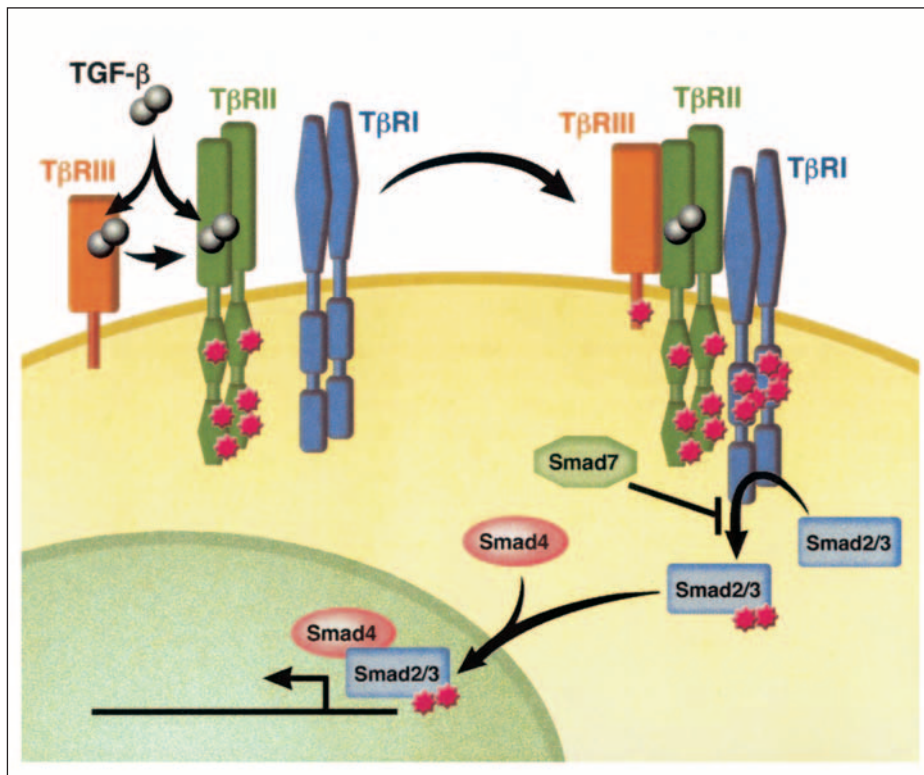


Fig 1. The transforming growth factor beta (TGF- β) signaling pathway. TGF- β binds type II TGF- β receptor (T β RII), directly or through T β RIII, inducing association of T β RII with T β RI. T β RII then phosphorylates and activates T β RI, which then phosphorylates Smad2 or Smad3. Phosphorylated Smad2 or Smad3 associates with Smad4 and they translocate into the nucleus, where they activate transcription of target genes. Smad7 inhibits TGF- β signaling by preventing the activation of Smad2 or Smad3 by T β RI.

To proliferate in a dysregulated manner, cancer cells become resistant to these antiproliferative signals and proliferate in the absence of exogenous mitogenic growth signals. TGF- β is an important regulator of cellular proliferation. Although first identified and named for its ability to stimulate the proliferation and transformation of mesenchymal cells,²⁶ TGF- β potently inhibits epithelial, endothelial, and hematopoietic cell proliferation. Although the precise mechanism for this growth-inhibitory effect remains elusive, TGF- β is able to prevent progression through the cell cycle first by inducing expression of the cyclin kinase inhibitors p15^{INK4b},²⁷ p21^{CIP1},²⁸ and p27^{KIP1},²⁹ which block cyclin and cyclin-dependent kinases from phosphorylating the retinoblastoma protein (Rb), thus allowing the hypophosphorylated form of Rb to bind and sequester the E2F transcription factor, and second by directly suppressing *c-myc* expression.³⁰ Although the growth-inhibitory effect of TGF- β is believed to be mediated by the Smad-dependent TGF- β signaling pathway, there have been reports of TGF- β -mediated inhibition of proliferation in Smad4 null cells.²² Furthermore, the growth-inhibitory effect of TGF- β can be mediated by Smad-independent pathways, including the MAPK pathways³¹ and the PP2A/p70S6 kinase pathway.²⁵

Virtually all epithelial-derived tumors (> 85% of all human cancers) become resistant to the growth-inhibitory effects of TGF- β . In some cancers, including colon and

pancreatic cancers, mechanisms for resistance are well defined,^{32,33} with defects in the Smad proteins (predominately Smad4, originally cloned as deleted in pancreatic cancer 4 [*DPC4*]) or in TGF- β receptors, (predominately T β RII, which is a target for mismatch repair errors in hereditary nonpolyposis colon cancer). However, in most human cancers, including cancers of the breast, lung, and prostate, mechanisms for resistance to TGF- β -mediated inhibition of proliferation remain poorly defined. Proposed alternative mechanisms for resistance to TGF- β include decreased expression of T β RI,³⁴ T β RII,³⁵ or T β RIII³⁶ on the cell surface; increased expression of the inhibitory Smad, Smad7³⁷; repression of TGF- β signaling by a variety of oncoproteins including p53,³⁸ Myc,³⁹ E1A,⁴⁰ Ras,⁴¹ Ski/SnoN,^{42,43} and Evi-1⁴⁴; reduced expression or inactivation of tumor suppressors that directly regulate the TGF- β signaling pathway including Menin,⁴⁵ Disabled-2,⁴⁶ and RUNX3⁴⁷; and activation of other signaling pathways including protein kinase C (PKC).⁴⁸

Cancer cells become independent of exogenous mitogenic growth signals either through overexpression of the mitogenic growth factors themselves or constitutive activation of the signaling pathway downstream of these growth factors. Similar to its effect on mesenchymal cells, TGF- β is able to stimulate the proliferation of a number of epithelial-derived cancer cell lines, including colon, pancreatic, and prostate cancer cells.^{3,49,50} Although the precise mechanism

by which this is accomplished has not been defined, TGF- β is able to increase the production of mitogenic growth factors, including platelet-derived growth factor,⁵¹ fibroblast growth factor,⁵² TGF- α ,⁵³ and connective tissue growth factor,⁵⁴ and to increase expression of the platelet-derived growth factor receptor,⁵⁵ and the epithelial growth factor receptor.⁵⁶ In addition, TGF- β can activate Smad-independent pathways, including the Ras/Raf/MAPK pathway,^{20-22,57,58} which often mediates the proliferative signal of growth factors. Indeed, it is in conjunction with oncogenic forms of Ras (Ha-Ras or Ki-Ras), that TGF- β drives Smad-independent proliferation of human colon and prostate cancer cells.^{3,49}

Tissue Invasion and Metastasis

Solid tumors exhibit their lethal effects by invading into surrounding tissues and metastasizing to distant sites in the body. The process by which cancer cells invade and metastasize involves complex interactions between the cancer cells and their extracellular environment. TGF- β is a potent regulator of cellular adhesion, motility, and the extracellular matrix. TGF- β regulates the adhesive properties of cells by decreasing the expression of E-cadherin⁵⁹ to decrease cell adhesion, by increasing the expression of invasion-associated integrins including $\alpha_{III}\beta_1$ integrin,⁶⁰ and by increasing the expression of integrin-binding proteins including fibulin-5.⁶¹ TGF- β also directly increases the motility of epithelial cells⁶² and breast cancer cells.⁶³

TGF- β normally stimulates the production of the extracellular matrix by directly increasing the production of extracellular matrix proteins, including collagen and fibronectin; decreasing the production of enzymes that degrade the extracellular matrix, including collagenase, heparinase, and stromelysin; and increasing the production of proteins that inhibit enzymes that degrade the extracellular matrix, including plasminogen activator inhibitor-1 and tissue inhibitor of metalloprotease.⁶⁴ However, during tumorigenesis, TGF- β frequently stimulates the proteolytic activity of cancer cells by increasing the expression of matrix-degrading enzymes.⁴

Taken together, by decreasing the adhesiveness and increasing the motility and proteolytic activity of cancer cells, increased levels of TGF- β result in more invasive cancer cells, which may represent one of the tumor-promoting activities of TGF- β .⁶⁵ Indeed, exogenous TGF- β 1 increases the invasiveness and metastatic behavior of breast cancer cells in vivo, even while inhibiting their proliferation in vitro.⁶⁶

Limitless Replication Potential

Human somatic cells have a finite replicative potential; after a limited number of cell divisions, they become senescent and cease dividing. Inactivation of p53 or other tumor suppressors can overcome this obstacle for a limited number of cell divisions, after which the cells enter crisis, char-

acterized by chromosomal abnormalities and cell death. In contrast, cancer cells do not undergo senescence or crisis, but are instead immortalized. This immortalization is achieved through two mechanisms that maintain the ends of chromosomes (telomeres) as they are replicated through successive cell divisions: either upregulation of the enzyme telomerase (approximately 90%), which adds repeats of nucleotides to maintain telomere length, or by alternative lengthening of telomeres (approximately 10%), which maintains telomeres through a recombination-based system.¹

Cancer cells upregulate telomerase through activating transcription of the catalytic component of human telomerase, *hTERT*.⁶⁷ Somatic cells have numerous mechanisms to suppress telomerase expression and mechanisms that relieve this suppression during tumorigenesis are not fully understood. Both autocrine and exogenous TGF- β potently suppress *hTERT* expression at the transcriptional level.⁵ A recent screen for negative regulators of telomerase identified three genes in known tumor suppressor/oncogene pathways: *Mad1*, *Menin*, and *SIP1/ZEB-2*.⁶⁸ *Mad1*, *Menin*, and *Sip1* are all direct transcriptional targets of the TGF- β signaling pathway.^{45,69,70} *Mad1* and *Menin* are both transcriptional repressors and function by directly binding the *hTERT* promoter, whereas *Sip1* is necessary for TGF- β -induced *hTERT* repression.⁶⁸ Thus, the TGF- β signaling pathway downregulates *hTERT* expression through at least three distinct mechanisms, suggesting that TGF- β may mediate its tumor suppressor effects, in part, through suppressing telomerase expression. *hTERT* expression has also been demonstrated to directly induce resistance to the growth inhibitory effects of TGF- β in human mammary epithelial cells lacking p16^{INK4}, suggesting that there is significant coregulation between the TGF- β signaling pathway and the pathways regulating telomerase function.⁷¹

Evasion of Apoptosis

In addition to regulation of cellular proliferation, cell number is also controlled by regulated apoptosis, or programmed cell death. Cancer cells become refractory to this regulatory signal and thus do not undergo apoptosis under appropriate conditions (ie, DNA damage). Although TGF- β has been shown to promote, suppress, or have no effect on apoptosis,⁷² in most cases, the TGF- β signaling pathway is proapoptotic. For example, TGF- β induces apoptosis in epithelial cells,⁷³ endothelial cells,⁷⁴ hematopoietic stem cells,⁷⁵ lymphocytes,⁷⁶ hepatocytes,⁷⁷ and neurons,⁷⁸ as well as in breast cancer,⁶ gastric cancer,⁴⁷ hepatic cancer,⁷⁹ lymphoma,⁸⁰ ovarian cancer,⁸¹ and prostate cancer cells.⁸²

The mechanisms by which TGF- β induces and regulates apoptosis are cell and context specific. TGF- β -induced apoptosis is frequently mediated by the Smad-dependent pathway.^{83,84} However, the inhibitor Smad, Smad7, has been shown to promote TGF- β -induced

apoptosis in prostate carcinoma cells and lung epithelial cells,^{82,85} and Smad-independent pathways, including Daxx-mediated JNK activation,⁸⁶ may also be involved.

TGF- β -induced apoptosis may occur through both p53-dependent⁸⁷ and p53-independent mechanisms,⁷⁵ and involves caspase activation,⁸⁸ upregulation of proapoptotic factors (ie, Bax), and/or downregulation of antiapoptotic factors (ie, Bcl-2 and Bcl-x_L).⁸⁷ The TGF- β signaling pathway also interacts with other pathways that regulate apoptosis. For example, TGF- β is able to enhance Fas-induced apoptosis under conditions in which TGF- β alone does not induce apoptosis,⁸⁹ whereas activation of the PI-3 kinase/Akt pathway is able to inhibit TGF- β -mediated apoptosis.⁹⁰ Recent studies have identified a direct interaction of Akt with Smad3 that prevents Smad3 phosphorylation and Smad3 nuclear translocation, and inhibits both Smad3-mediated transcriptional events and apoptosis.^{91,92} Furthermore, the ratio of Smad3 to Akt correlates with the sensitivity of cells to TGF- β -mediated apoptosis, providing a potential explanation for the variable apoptotic response of cells to TGF- β .^{91,92}

Resistance to TGF- β -induced apoptosis may be an essential component of tumorigenesis, particularly for cancers arising from tissues in which TGF- β is a prominent regulator of apoptosis, including hepatocellular carcinoma and prostate cancer. In addition, the ability of TGF- β to induce apoptosis in lymphocytes may be a critical component for the immunosuppressive effect of TGF- β during tumorigenesis.

Induction of Angiogenesis

Limited by the diffusion of nutrients and oxygen, solid tumors require a blood supply to grow beyond 1 to 2 mm in diameter. Solid tumors obtain this blood supply through the formation of new blood vessels (ie, angiogenesis). TGF- β is one of several cytokines that coordinate to regulate angiogenesis. TGF- β can function either as a proangiogenic or antiangiogenic factor in vitro; however, the preponderance of evidence supports a proangiogenic role for TGF- β in vivo.⁹³ Several lines of evidence support a prominent role for the TGF- β signaling pathway in stimulating angiogenesis. First, targeted deletion of members of this pathway in mice, including TGF- β 1, T β R1, and T β R2, all result in aberrant angiogenesis.⁹⁴⁻⁹⁶ Second, two endothelial-specific TGF- β receptors, endoglin (a type III receptor in the TGF- β family) and ALK-1 (a type I receptor in the TGF- β family), are essential for angiogenesis as demonstrated by their mutation in the human vascular disorder hereditary hemorrhagic telangiectasia^{97,98} and by the embryonic lethal phenotype due to defects in angiogenesis exhibited by mice in which their expression has been abolished.^{99,100} Third, expression of endoglin on endothelial cells is dramatically increased during tumor-induced angiogenesis.¹⁰¹ Finally,

TGF- β induces the expression of vascular endothelial growth factor, which then directly promotes angiogenesis.¹⁰²

TGF- β signaling in endothelial cells is unique in that TGF- β can activate two distinct pathways: the classical Smad-dependent pathway through T β R2 and T β R1 (also known as ALK-5) to activate Smads 2 and 3, and the pathway through T β R2 and ALK-1 to activate Smads 1, 5, and 8, which are usually activated by the TGF- β superfamily members, the bone morphogenetic proteins.¹⁰² These two pathways have opposing effects on endothelial cell proliferation and migration. The balance of signaling between these pathways regulates endothelial cell biology through the activation (increased endothelial cell proliferation and migration) and maturation (decreased endothelial cell proliferation and migration) phases of angiogenesis.^{103,104} These opposing pathways likely explain the ability of TGF- β to mediate proangiogenic or antiangiogenic effects in vitro. Endoglin is a likely candidate to regulate the balance of TGF- β signaling through these pathways in endothelial cells, inhibiting the ALK-5 pathway¹⁰⁵ while activating the ALK-1 pathway.¹⁰⁶

Evasion of the Immune System

Cancer cells express tumor-specific antigens that normally would be recognized by the immune system and lead to destruction of the cancer cell; during tumorigenesis, most cancer cells acquire the ability to evade this immunosurveillance. Although there are multiple mechanisms by which cancer cells evade an immune response, a major mechanism is active cancer cell-mediated immunosuppression via secretion of TGF- β , which is a potent immunosuppressive cytokine.¹⁰⁷ A role for TGF- β in cancer cell-mediated immunosuppression is supported by the following observations: cancer cells are able to produce and secrete TGF- β ,¹⁰⁸ which is able to immunosuppress cancer patients in the absence of cytotoxic treatment¹⁰⁹; elevated levels of TGF- β have been found in a number of experimental models and in specimens from patients with cancer¹¹⁰; increasing expression of TGF- β 1 in animal models allows immunogenic cell lines to escape immunosurveillance and form tumors⁸; and anticancer cell immune responses can be augmented by blocking the TGF- β signaling pathway in T cells.¹¹¹

The immunosuppressive effects of TGF- β have been demonstrated both in vitro and in vivo,¹¹² and are mediated predominantly through effects on T cells and antigen presenting cells (APCs). TGF- β is produced by T cells and blocks production of interleukin 2 (IL-2) to inhibit IL-2-dependent proliferation of T cells.¹¹³ TGF- β also inhibits the differentiation of T cells, and prevents naïve T cells from acquiring effector (cytotoxic or helper) functions.¹¹⁴ TGF- β may also mediate some of its immunosuppressive effects on T cells through CD4⁺CD25⁺ regulatory T cells, which both secrete TGF- β 1 and express cell surface-bound TGF- β 1.¹¹⁵ These in vitro effects of TGF- β on T cells have

been validated in murine models. TGF- β 1-deficient mice develop a severe autoimmune phenotype leading to death by 3 weeks, in part, from overactive T cells,¹¹⁶ and T-cell-specific abrogation of TGF- β signaling in mice results in spontaneous T-cell activation and the development of an autoimmune disease of the lung and colon.¹¹⁷ TGF- β also has potent effects on APCs. Macrophages secrete TGF- β , which inhibits tissue macrophage activation.¹¹⁸ TGF- β also is required for differentiation of dendritic cells from precursors, primarily by protecting their viability.¹¹⁹ In vivo, TGF- β 1-deficient mice have a complete absence of Langerhans cells in the epidermis, although they express functional precursors, suggesting that TGF- β is required for normal Langerhans cell development and/or migration to the epidermis.¹²⁰

Genome Instability

There are a number of DNA monitoring and repair systems that are caretakers of the genome,¹²¹ including mitotic checkpoints, the p53 system, and mismatch repair; cancer cells frequently bypass these systems. Thus, a single alteration in one or more of these pathways creates an environment in which other mutations can occur at increased frequency, allowing cancers to form at a more appreciable rate. The TGF- β signaling pathway has prominent effects on a number of these caretaker systems. For example, TGF- β 1 decreases the expression of Rad51 and decreases Rad51-mediated DNA repair efficiency to promote DNA instability.¹²² TGF- β has also been shown to regulate the function of p53, with decreased TGF- β signaling resulting in decreased radiation-induced activation of p53.¹²³ TGF- β may also directly regulate genome stability through less-defined pathways. Keratinocytes from *Tgfb1*-null animals undergo more *N*-phosphonoacetyl-L-aspartate-induced gene amplification, and when transduced with Ha-Ras, have more aneuploidy and accumulation of chromosomal aberrations than keratinocytes from wild-type littermates. Importantly, exogenous TGF- β 1 suppresses gene amplification, aneuploidy, and chromosome breaks in the *Tgfb1*-null keratinocytes and in human tumor cell lines, independent of p53 and Rb status, suggesting that the TGF- β signaling pathway directly mediates genomic stability.¹²⁴

CROSS-TALK OF THE TGF- β PATHWAY WITH OTHER SIGNAL TRANSDUCTION PATHWAYS REGULATING TUMORIGENESIS

In addition to the role the TGF- β signaling pathway has in directly mediating and regulating these hallmarks of cancer, the TGF- β signaling pathway and other prominent signaling pathways cross-talk to regulate tumor biology. For example, the Wnt and TGF- β pathways cooperate to suppress colon¹²⁵ and pancreatic¹²⁶ tumorigenesis through several direct interactions between these pathways: Axin, a negative

regulator of the Wnt pathway, activates TGF- β signaling through binding Smad3,¹²⁷ whereas the HMG box transcription factor, lymphoid enhancer binding factor 1/T-cell-specific factor (LEF1/TCF), a mediator of Wnt effects, interacts directly with Smad3 to coordinately regulate LEF1/TCF target genes,¹²⁸ and β -catenin and LEF1/TCF both interact directly with Smad4 to regulate target genes during development.¹²⁹

The PKC pathway has also been shown to interact with the TGF- β pathway to regulate tumorigenesis. In murine colon cancer models, elevated expression of PKC β II elevates cyclooxygenase-2 expression and represses T β R β II expression to increase susceptibility to colon cancer.¹³⁰

SYNTHESIS: TGF- β AS A TUMOR SUPPRESSOR AND TUMOR PROMOTER

TGF- β has the potential to function as a tumor suppressor (via its effects on proliferation, replication potential, and apoptosis) and as a tumor promoter (via its effects on migration, invasion, angiogenesis, and the immune system; Fig 2). Indeed, in animal models, evidence for TGF- β in mediating each of these roles has been established. The tumor suppressor role is evident in that hemizygous *Tgfb1*-null animals, which express 10% to 30% of wild-type TGF- β 1 levels, develop an increased number of chemically induced tumors,¹³¹ and hemizygous Smad4-null animals when mated with hemizygous adenomatous polyposis coli-null animals develop more invasive colonic tumors.¹²⁵ The tumor-promoting effects of TGF- β have been demonstrated by the ability of agents that block TGF- β signaling (dominant negative T β R β II or neutralizing TGF- β antibodies) to inhibit the invasiveness of cancer cell lines in vitro and their metastatic ability in vivo,¹³² and by the ability of TGF- β to directly stimulate the motility of cancer cells.⁶³

TGF- β has also been demonstrated to have this dichotomous function in human cancers. The tumor suppressor role is supported by the loss or mutation of members of the TGF- β signaling pathway in human cancers, particularly colon and pancreatic cancers,^{32,33} with resulting resistance to TGF- β -mediated effects correlating to malignant progression.¹³³ The tumor-promoting effect is supported by the elevated levels of TGF- β found in patients in the latter stages of cancers, with this increased production associated with increased invasiveness and a poorer prognosis for these patients.^{65,134,135}

How can this dichotomy of function be resolved? The prevailing theory suggests that during tumorigenesis TGF- β functions as both a tumor suppressor and as a tumor promoter, mediating tumor suppressor functions early on, and tumor promoting functions later in the course of disease (Fig 2). Several studies using animal models support both a tumor-suppressor and a tumor-promoter role for

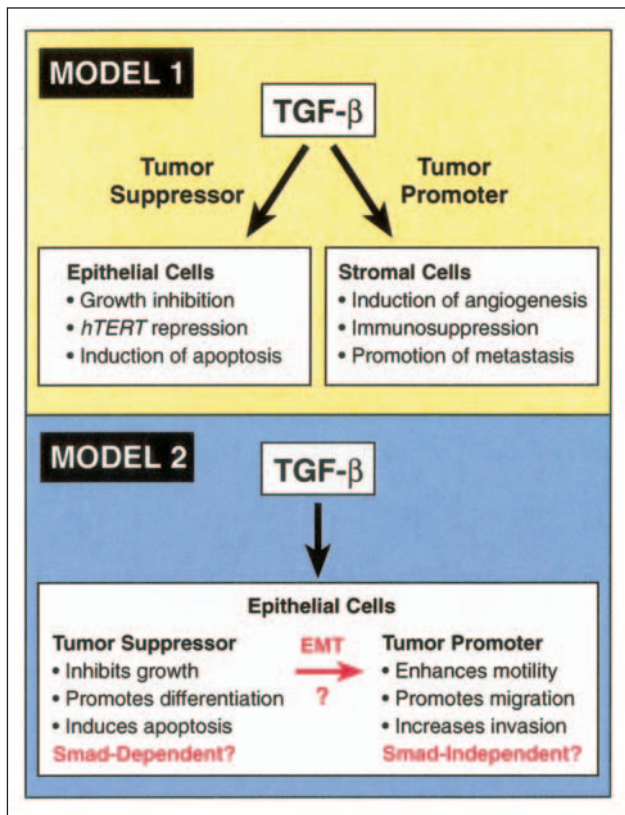


Fig 2. Transforming growth factor beta (TGF- β) as a tumor suppressor and tumor promoter. In one model TGF- β accomplishes this by continuing to act as a tumor promoter through effects on stromal cells even after the epithelial-derived cancer cells have become resistant to the tumor suppressor effects of TGF- β . In another model, TGF- β switches from a tumor suppressor to a tumor promoter because of a fundamental change in the response of the epithelial-derived cancer cells to TGF- β . EMT, epithelial to mesenchymal transition.

TGF- β during tumorigenesis. When TGF- β 1 overexpression is targeted to the keratinocytes of mice and their skin is exposed to chemical carcinogens, TGF- β initially inhibits the formation of benign skin tumors, consistent with its tumor-suppressor action. However, in the benign tumors that form, progression to invasive spindle carcinomas is increased, consistent with its tumor-promoting action.¹³⁶ In addition, introduction of a dominant negative T β R β II (to block TGF- β signaling) into a series of human breast-derived cell lines representing different stages in breast cancer progression cooperates with other oncogenic stimuli to make premalignant cells tumorigenic and low-grade tumorigenic cells more aggressive, while decreasing the metastatic potential of high-grade tumorigenic cells.¹³⁷ Finally, transgenic mice expressing activated T β R β I under control of the mouse mammary tumor virus promoter crossed with mice expressing activated Neu receptor have an increased primary tumor latency but enhanced frequency of lung metastases, whereas transgenic mice expressing dominant negative T β R β II crossed with mice expressing activated Neu

receptor have decreased primary tumor latency, but significantly fewer lung metastases.¹³⁸

The precise mechanism for the dichotomous function of TGF- β in human cancers remains poorly defined. In one proposed scenario, TGF- β mediates tumor suppressor functions on precancerous epithelial cells by inhibiting proliferation and telomerase, and stimulating differentiation and apoptosis as appropriate (Fig 2). During carcinogenesis, the epithelial-derived cancer cells, through mechanisms defined here and others yet to be discerned, become resistant to these tumor-suppressor effects of TGF- β . The cancer cells then increase their production of TGF- β , which promotes tumorigenesis, predominately through effects on the stroma (altering the extracellular matrix and adhesion molecules to increase metastasis; increasing angiogenesis; and inducing immunosuppression).

However, another potential scenario is that the tumor-suppressor and tumor-promotor effects are both mediated by effects on the epithelial-derived cancer cells themselves, which undergo a fundamental alteration in their TGF- β responsiveness whereby they become resistant to the tumor-suppressor effects of TGF- β (growth inhibition, differentiation, antiapoptotic, suppression of telomerase) but remain responsive to the tumor-promotor effects (enhanced motility, migration, and invasion; Fig 2).^{132,139} This fundamental alteration in TGF- β responsiveness may occur as the cancer cells undergo an epithelial to mesenchymal transition (EMT). During EMT, epithelial cells lose their epithelial phenotype (strong cell-cell contact, nonmotility) and adopt a mesenchymal cell phenotype (reduced cell-cell contact, increased motility, and invasion of surrounding tissues).¹⁴⁰ EMT is a well-established process during development and, when examined, has been documented to occur in 24% to 45% of human breast cancers,¹³² 39% to 60% of gastric cancers,¹⁴¹ and 74% of renal cell cancers.¹³² TGF- β is able to induce EMT in a number of cancer models either by itself or in cooperation with oncogenic Ras (Ha-Ras), and an intact TGF- β signaling pathway is necessary for cancer cell invasion and metastasis in at least one model of breast EMT.¹³² EMT is an attractive model for the fundamental alteration in TGF- β responsiveness because TGF- β is known to have strikingly disparate effects on epithelial cells and mesenchymal cells. The mechanism by which TGF- β induces EMT in vivo has been not been established, but studies in vitro have indicated that this proceeds through both Smad-dependent and Smad-independent pathways.^{24,142}

Recent studies have implicated T β R β III in EMT and as a potential mediator of the differential effects of TGF- β on epithelial and mesenchymal cells. Specifically, T β R β III has been shown to have an essential nonredundant role in TGF- β signaling, mediating the effects of TGF- β on EMT in chick embryonic heart development,¹⁴³ and T β R β III is downregulated during EMT in a human breast epithelial

cell model.¹⁴⁴ In addition, T β RIII has been demonstrated to enhance TGF- β signaling in mesenchymal cells,¹⁴⁵ but to inhibit TGF- β signaling in epithelial cells.¹⁴⁶ T β RIII also has an emerging role in regulating tumorigenesis, with decreased expression of T β RIII reported in human breast cancer cell lines¹⁴⁷ and renal cell cancer cell lines.¹⁴⁸ In concordance with this, re-expression of T β RIII in breast cancer cell lines and renal cell cancer cell lines that lack T β RIII expression suppresses their tumorigenicity *in vivo*.^{147,148} Taken together with recent reports supporting a substantial role for T β RIII in regulating TGF- β signaling,¹⁹ these observations suggest an emerging role for T β RIII in mediating and/or regulating the effects of TGF- β , including the dichotomous effects during tumorigenesis.

TGF- β SIGNALING IN HUMAN CANCERS

Because TGF- β has a prominent role in epithelial cell function and epithelial-derived tumors represent the vast majority of human cancers, we focus on the four most prevalent human cancers: cancers of the breast, colon, lung, and prostate, which together comprise more than 50% of the new occurrences and cancer deaths in the United States each year (Table 2).

Breast Cancer

TGF- β has an important role in normal mammary biology as a potent regulator of mammary epithelial proliferation, mammary ductal and alveolar development, and postlactation involution of the mammary gland.¹⁵⁹ The TGF- β signaling pathway also has an important role in human mammary carcinogenesis. Data to support this include: evidence that TGF- β can act directly on breast epithelial cells to potently inhibit their growth¹⁶⁰; mutation in or loss of expression of members of the TGF- β signaling pathway including T β RII and T β RI in some human breast cancers¹⁴⁹; demonstration that most human breast cancers

develop resistance to the antiproliferative effects of TGF- β despite expression of Smad3, Smad4, T β RI, and T β RII¹⁵⁰; decreased breast cancer formation in human patients with elevated levels of TGF- β ¹⁶¹; evidence that the chemopreventive and therapeutic effects of the antiestrogen agent, tamoxifen, may be mediated through potent induction of TGF- β ¹⁶²; demonstration of increased TGF- β levels in human breast cancers, with production increasing with advancing stages of tumor progression,¹⁶³ decreasing after surgical resection,¹⁶⁴ and persistently elevated levels after surgical resection correlating with lymph node metastasis or residual tumor¹⁶⁴; and elevated TGF- β levels conferring a poorer prognosis for human breast cancer patients.¹⁶⁵ These results suggest that although resistance to the growth-inhibitory effects of TGF- β is a key cellular event during mammary carcinogenesis, mechanisms for this resistance remain to be defined. In addition, TGF- β clearly has a dichotomous function in human breast cancer, and this dichotomous function has been demonstrated in animal models as discussed previously.

Colon Cancer

TGF- β inhibits the proliferation of normal intestinal epithelial cells¹⁶⁶ and regulates the proliferation and differentiation of normal colonic epithelium.¹⁶⁷ Although colonic cells from normal epithelia or well-differentiated adenomas are TGF- β sensitive, cells lines derived from poorly differentiated, invasive colon carcinomas are not.¹⁶⁸ In colon cancers, loss of TGF- β sensitivity is frequently accounted for by loss or mutation of known components of the TGF- β signaling pathway, notably T β RII and Smad4. Colorectal carcinomas are either replication error (RER) –positive tumors, characterized by microsatellite instability due to defects in mismatch repair (12%); or RER-negative tumors, with no defects in mismatch repair (88%).¹⁶⁹ RER-positive colon cancers are a prominent characteristic of the familial colon cancer syndrome, hereditary nonpolyposis

Table 2. Mutation of Components of the TGF- β Signaling Pathway in Cancer

Cancer	T β RII Gene	Smad2 Gene	Smad4 Gene
Breast	Frequently downregulated, rarely mutated ¹⁴⁹	No mutations detected in approximately 100% ¹⁵⁰	No mutations detected in approximately 100% ¹⁵⁰
Colon	Mutated in 58%-82% of RER-positive tumors and 15% of RER-negative tumors ^{33,151}	Mutated in 6% ¹⁵³	Mutated in 20% invasive tumors and 5% noninvasive tumors ¹⁵²
Lung	Frequently downregulated, particularly in SCLC, but rarely mutated ³⁵	Mutated in 2% NSCLC; normal in almost all SCLC ¹⁵⁵	Mutated in 7% NSCLC; normal in almost all SCLC ¹⁵⁴
Pancreatic	Mutated in 4% ¹⁵⁶	No mutations detected in ~100% ³²	Mutated in 50% ²
Prostate	T β RII protein not detected in 24% ¹³⁵	No mutations detected in ~100% ¹⁵⁷	No mutations detected in approximately 100% ¹⁵⁸

Abbreviations: TGF- β , transforming growth factor β ; T β RII, TGF- β type II receptor; RER, replication error; SCLC, small-cell lung cancer; NSCLC, non-small-cell lung cancer.

colorectal cancer; however, the majority of RER-positive colon cancers are sporadic. A polyadenine tract in the coding sequence of the *TBR1* gene makes it a target for mismatch repair defect frameshift mutations. These *TBR1* mutations, which result in a nonfunctional receptor, have been detected in 58% to 82% of RER-positive colorectal carcinomas.¹⁵¹ Tumors with these *TBR1* mutations also have loss of heterozygosity at the *TBR1* locus, supporting a tumor-suppressor role of *TBR1*. *TBR1* is rendered non-functional by homozygous mutations at other sites in 15% of RER-negative colorectal carcinomas³³ and re-expression of wild-type *TBR1* decreases tumorigenicity of *TBR1*-mutant colon cancer cells,¹⁷⁰ supporting a causal role for this mutation in colon carcinogenesis.

The *Smad4* gene is mutated or deleted in 20% of invasive colorectal carcinomas, whereas it is altered in only 5% of adenomas and noninvasive carcinomas,¹⁵² consistent with loss of TGF- β sensitivity occurring in the later stages of colorectal carcinogenesis. Inactivating mutations in the *Smad2* gene are less common, but have been detected in 6% of colorectal carcinomas.¹⁵³ A critical role for the TGF- β signaling pathway in colon cancer is further supported by the finding of *Smad4* mutations in a subset of patients with familial juvenile polyposis, an autosomal dominant disease characterized by a predisposition to hamartomatous polyps and cancers of the GI tract.¹⁷¹

Although these mutations in colon cancers and colon cancer syndromes define a tumor-suppressor role for the TGF- β signaling pathway, there is evidence that TGF- β can also have a tumor-promoting role in colon cancer. In some colon cancer models, TGF- β increases tumor invasion and metastasis to increase tumorigenicity.¹³² In addition, the fact that RER-positive tumors (the majority of which have *TBR1* mutations) are less aggressive clinically despite being more poorly differentiated¹⁷² is consistent with the importance of TGF- β sensitivity for invasiveness and metastatic potential of colon cancers.

Lung Cancer

TGF- β inhibits the proliferation and induces the differentiation of normal bronchial epithelial cells.¹⁷³ Most non-small-cell lung carcinomas (NSCLCs) become resistant to the growth-inhibitory effects of TGF- β , sometimes as a result of decreased expression of *TBR1*,³⁵ or from mutation of the *Smad4* (7%¹⁵⁴) or *Smad2* (2%¹⁵⁵) genes. In some cases, decreased expression of *TBR1* has been linked to aberrant histone deacetylation.¹⁷⁴ However, in the majority of NSCLC patients, mechanisms for resistance to TGF- β are poorly defined. In contrast, although the majority of small-cell lung carcinomas (SCLCs) are also resistant to the growth-inhibitory effects of TGF- β , in SCLCs this is nearly all accounted for by their decreased or absent *TBR1* expression.¹⁷⁵ Although rare mutations of the *TBR1* gene in SCLC have been defined, in most cases this decreased

expression occurs through poorly understood mechanisms.¹⁷⁵ In both SCLC and NSCLC tumors, TGF- β expression is frequently upregulated,³⁵ with elevated plasma levels of TGF- β conferring a poorer prognosis for patients with lung cancer.¹⁷⁶

Prostate Cancer

TGF- β mediates inhibition of proliferation, differentiation, and apoptosis (both androgen-deprivation induced and androgen independent) of normal prostate epithelial cells to potentially inhibit prostate growth.¹⁷⁷ During tumorigenesis, prostate cancer cells become resistant to the antiproliferative effects of TGF- β , while still exhibiting some TGF- β responses, including TGF- β -mediated stimulation of motility.¹⁷⁸ Although decreased or absent *TBR1* or *TBR1* expression has been noted in approximately 30% of prostate cancer specimens,¹³⁵ the vast majority of prostate cancers become resistant to the antiproliferative effects of TGF- β without defined mutations or deletions of members of the classical Smad-dependent signaling pathway.

Consistent with an essential role in prostate cancer tumorigenesis, loss of TGF- β receptor expression correlates with advanced Gleason score and tumor stage, and thus predicts a poor prognosis for these patients.¹⁷⁹ In addition, elevated urinary or plasma TGF- β levels have been consistently associated with a worse prognosis for patients with prostate cancer.¹³⁵ Because bone is a rich source of TGF- β , TGF- β may also regulate the blastic bone metastases characteristic of prostate cancer by mediating the ability of prostate cancer cells to migrate and invade into the bone.¹⁸⁰

TGF- β AS A DIAGNOSTIC, PROGNOSTIC, OR PREDICTIVE TOOL IN CANCER DETECTION AND TREATMENT

Given that TGF- β has important roles in tumor suppression and tumor progression, measurements of TGF- β ligand (in serum, urine, and tissue), TGF- β mRNA (in tissue), or TGF- β receptor levels may serve as diagnostic, prognostic, or predictive tools. Although increased TGF- β 1 protein levels are found in the serum of patients with invasive breast cancer,¹⁶³ colorectal cancer,¹⁸¹ hepatocellular carcinoma,¹⁸² lung cancer,¹⁸³ metastatic melanoma,¹⁸⁴ and prostate cancer,¹⁸⁵ and urinary excretion of TGF- β 1 is increased in patients with hepatocellular carcinoma,¹⁸⁶ a diagnostic role for elevated TGF- β levels has not yet been established. However, a recent study demonstrated that elevated serum levels of TGF- β 1 were a more sensitive indicator of small hepatocellular carcinomas than alpha-fetoprotein levels, suggesting that measuring serum TGF- β 1 levels may be clinically useful for diagnosing these tumors.¹⁸² In addition, elevated expression of the endothelial-specific TGF- β receptor endoglin on tumor-associated vasculature does have the potential to enhance

detection of solid tumors that may not be identified by other means.¹⁸⁷

Several studies have revealed the potential clinical prognostic or predictive utility of TGF- β or TGF- β receptor levels. For example, increased TGF- β 1 serum protein levels are predictive of liver metastasis after surgery for colon cancer,¹⁸⁸ prostate cancer progression after radical prostatectomy,¹⁸⁹ bladder cancer recurrence after radical cystectomy,¹⁹⁰ and lymph node metastases and peritoneal recurrence after gastric cancer surgery.¹⁹¹ High serum TGF- β 1 concentrations also correlate with the development of fibrosis in postradiation therapy breast cancer patients,¹⁹² and with the development of chronic graft-versus-host disease, idiopathic interstitial pneumonitis, and veno-occlusive disease in hematopoietic stem-cell transplantation patients.^{193,194} Early increases in serum TGF- β 2 concentrations also predict a clinical response to tamoxifen in breast cancer patients.¹⁶¹ In non-small-cell lung cancer patients, plasma TGF- β 1 levels may be useful to select patients for radiation therapy dose escalation, thus increasing response rates without increasing toxicity.¹⁹⁵ *TBR11* mutations in colon cancers with microsatellite instability¹⁹⁶ and *SMAD4* diploidy¹⁹⁷ are predictive of a significantly better prognosis after adjuvant chemotherapy, whereas loss of *TBR11* expression in renal cell cancers¹⁹⁸ and tumor microvessel density of breast,¹⁹⁹ colon,²⁰⁰ and lung²⁰¹ cancer specimens quantified using an antibody to endoglin predicts a worse prognosis. Finally, a TGF- β 1 polymorphism (T29-C), has been associated with a higher level of serum TGF- β 1 and with a decreased risk of breast cancer.²⁰²

THE TGF- β SIGNALING PATHWAY AS A THERAPEUTIC TARGET

In human cancers, TGF- β promotes tumorigenesis through both decreased TGF- β signaling during early tumorigenesis and increased TGF- β signaling in advanced, progressive disease. In addition, TGF- β has complex and often opposing context-specific effects on its cellular targets, mediating these cellular effects through several TGF- β -specific pathways and through cross-talk with other signaling pathways. Finally, the TGF- β signaling pathway has a complex role in other human diseases including cardiovascular disease and fibrotic disease.⁶⁴ Although each of these factors represents a fundamental challenge to targeting the TGF- β pathway, numerous preclinical strategies have been tried with an encouraging degree of success.

In clinical scenarios involving decreased TGF- β activity, attempts to restore or increase TGF- β signaling could be used as a chemoprevention strategy, as a postsurgical adjuvant therapy, or as a therapy for early-stage disease. Indeed, the effects of the chemopreventive agents tamoxifen and retinoids may be mediated through their ability to increase

serum TGF- β concentrations.^{203,204} Increased understanding of TGF- β ligand activation and the generation of agents that could increase activation may also lead to potential chemoprevention agents.

Many human cancers become resistant to the antiproliferative effects of TGF- β through decreased receptor expression (as opposed to mutation or deletion of the receptor). In these cases, increasing expression of the receptors may be a reasonable therapeutic target. Indeed, increasing expression of *TBR11* through use of the histone deacetylase inhibitor MS-275,²⁰⁵ the angiotensin-converting enzyme inhibitor captopril,²⁰⁶ the farnesyltransferase inhibitor FTI-277,²⁰⁷ and indirectly, through induction of Sp1 by DNA methyl transferase inhibitor 5-aza-2'-deoxycytidine,²⁰⁸ are all able to restore sensitivity to TGF- β . Given that the ubiquitin/proteasome pathway has a role in regulating TGF- β receptor expression,²⁰⁹ the use of proteasome inhibitors such as bortezomib (Velcade; Millennium Pharmaceuticals, Cambridge, MA), could also have a potential role in increasing TGF- β receptor expression. These agents potentially could be used in conjunction with standard adjuvant therapy for colon cancer and SCLCs, both of which frequently have decreased *TBR11* levels.

In clinical scenarios involving increased TGF- β activity, attempts to decrease or abrogate TGF- β signaling could be used as a therapy for advanced or metastatic disease. Attempts to block the effects of excessive TGF- β activity have involved agents that inhibit TGF- β binding to its receptors including natural TGF- β inhibitors (eg, decorin),²¹⁰ neutralizing TGF- β antibodies,⁷ and soluble extracellular domains of *TBR11* (s*TBR11*)²¹¹ or *TBR111* (s*TBR111*).²¹² Several neutralizing TGF- β antibodies are currently being developed, including humanized monoclonal TGF- β 1- and TGF- β 2-specific antibodies,²¹³ as well as antibodies that recognize all three isoforms of TGF- β (pan-TGF- β antibodies).⁷ Although isoform-specific antibodies may be better tolerated, the contribution of the individual isoforms to the effects of TGF- β on tumorigenesis in vivo has not been firmly established, making selection of a specific isoform target difficult. With regard to agents using the soluble extracellular domains of receptors, s*TBR111* has several advantages over s*TBR11*. First, s*TBR111* is a naturally occurring protein generated by ectodomain shedding of *TBR111*,²¹⁴ and as such, an immune response should not be generated. Second, s*TBR111* binds to all three TGF- β isoforms with high affinity, whereas s*TBR11* binds to only TGF- β 1 and TGF- β 3. Third, s*TBR111* has two TGF- β binding sites as opposed to one TGF- β binding site in s*TBR11*.

Attempts to block the effects of excessive TGF- β activity for the treatment of cancer have also included agents that directly inhibit TGF- β signaling, including overexpression of the inhibitory Smad or Smad7,⁸² or of dominant-negative *TBR11* (truncation mutant lacking the kinase domain),¹³⁷ and most recently, small molecule inhibitors of *TBR1* kinase

activity.^{215,216} These small molecule inhibitors of T β R1 exhibit a significant degree of specificity for type I TGF- β superfamily receptors over other cellular serine and threonine kinases (ie, PKC, ERK, JNK, or p38 MAPK).^{215,216} However, the inhibitor for T β R1 (ALK-5) also inhibits other type I TGF- β superfamily receptors including ALK-4 (a type I receptor for the TGF- β superfamily member, activin) and ALK-7,²¹⁵ increasing the likelihood of nonspecific adverse effects.

Although global blockade of TGF- β signaling might be expected to have an undesirable adverse effect profile, expression of a soluble chimeric protein of the extracellular domain of T β R2 and the Fc portion of the murine or human immunoglobulin G1 heavy chain (Fc: T β R2) has been shown to inhibit mammary tumor viability and block metastasis in murine models, without significant adverse effects such as autoimmune disease or tumor promotion, even with lifelong exposure.^{217,218}

Application of targeted therapies to block elevated TGF- β signaling in advanced and metastatic cancers may initially be used in humans to block specific tumor-promoting effects of TGF- β including proangiogenic and immunosuppressive functions *in vivo*. With regard to the proangiogenic effects of TGF- β , because the expression of the endothelial-specific TGF- β receptor endoglin is up-regulated during tumor-induced angiogenesis, antiendoglin antibodies coupled to toxins and radionuclides have been used to selectively target the tumor vasculature in animal models with marked success.²¹⁹

Given that the TGF- β signaling pathway has a defined role in tumor-induced immunosuppression,¹⁰⁷ inhibitors of this pathway may be used to improve natural immunosurveillance of tumor cells or to enhance the effectiveness of active or passive immunotherapy strategies. Indeed, many of the aforementioned strategies to block the TGF- β signaling pathway have been demonstrated to improve the ability of the immune system to destroy tumors in animal models. For example, neutralizing antibodies to TGF- β combined with IL-2 therapy to stimulate the immune system were able to decrease the number of metastases in a murine B16 melanoma model,²²⁰ whereas vaccinating with resected cancer cells containing antisense to TGF- β 1 ligand (to decrease TGF- β 1 production) successfully increased the ability of these vaccines to eradicate cancer cells.²²¹ More recently, blockade of the TGF- β signaling pathway specifically in CD4⁺ and CD8⁺ T cells through expression of dominant negative T β R2 has been shown to increase the ability of these T cells to produce specific anticancer cell (thymoma and melanoma) cytotoxic responses and to eradicate these cancer cells *in vivo*.²²² Taken together, these studies provide proof of principle that targeting the TGF- β signaling pathway represents a viable method for improving immunotherapy strategies for human cancers.

Another potential strategy for blocking the TGF- β signaling pathway would be to specifically target defined path-

ways mediating the immunosuppressive effects of TGF- β using RNA-mediated interference (RNAi) technology. RNAi is an evolutionarily conserved process that produces small (21 to 23 nucleotide) interfering RNA molecules, which then result in specific degradation of homologous RNA, downregulating the mRNA and subsequently the resulting protein expression. RNAi is able to specifically and potentially abrogate expression of targeted proteins in mammalian cells.²²³ Such an approach could be used to engineer T cells for use in immunotherapy that are specifically resistant to the immunosuppressive effects of TGF- β through stable expression of RNAi constructs. This approach could initially be used to target members of the TGF- β pathway known to be necessary for T-cell function—T β R2 and Smad3. This approach has the advantages that it could be applied to any component of the TGF- β signaling pathway or other pathways involved in immunosuppression, it could target specific arms of these pathways (ie, the immunosuppressive arm), it could be used to target more than one pathway simultaneously, and it could be expressed in various components of the immune system (T cells and APC) to modulate the TGF- β signaling pathway in numerous immunotherapy or vaccine approaches.

FUTURE DIRECTIONS

Given that TGF- β has numerous and often opposing cellular effects, as a tumor promoter and a tumor suppressor, and as an inhibitor and stimulator of cellular proliferation, apoptosis, and angiogenesis, a major challenge remains in more precisely defining TGF- β signaling pathways, including specific pathways involved in mediating the specific and context-dependent effects of TGF- β . Although Smad-mediated signaling is well established as the predominant TGF- β signaling pathway, the significance of contributions by other signaling pathways (ie, MAP kinase, Rho, and PI-3 kinase/Akt pathways) and mechanisms for this signaling remain to be established. Once these pathways and other potential signaling pathways downstream of TGF- β are defined, and the contributions of these pathways to the specific cellular and context-dependent effects of TGF- β are established, more specific targeting of this pathway will be possible. Concurrently, the ability to define the alterations occurring in the TGF- β signaling pathways at a molecular level in an individual's tumor will allow the matching of targeted therapies developed with these alterations to make individualized cancer treatment a less toxic and more effective reality.

Authors' Disclosures of Potential Conflicts of Interest

The authors indicated no potential conflicts of interest.

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