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Transforming Growth Factor β: Tumor Suppressor or Promoter? Are Host Immune Cells the Answer?

Li Yang and **Harold L. Moses**
Department of Cancer Biology, the Vanderbilt-Ingram Cancer Center, Vanderbilt University School of Medicine, Nashville, Tenessee

Abstract

Therapies targeting transforming growth factor β (TGFβ) signaling using neutralizing antibodies and small molecular inhibitors are in multiple clinical trails. However, TGFβ is known to work as both a tumor suppressor and a tumor promoter, and current knowledge does not provide sufficient information on what factors mediate this switch in function and when this switch occurs. Recent advances in multiple disciplines suggest that immune cells from the tumor host mayprovide the answer.

Transforming Growth Factor β: A Tumor Suppressor or a Tumor Promoter?

The transforming growth factor (TGF)-β ligands (TGFβ1, TGFβ2, and TGFβ3) signal through the type I and type II TGFβ receptors (TβRI and TβRII, respectively). Canonical signaling proceeds with phosphorylation of Smad2 and Smad3, which then combine with Smad4 to enter the nucleus to modulate transcription in cooperation with other transcription factors, coactivators, and corepressors. In addition, TGFβ binding to its receptors activates many noncanonical signaling pathways (Fig. 1).

Alterations in TGFβ signaling have significant effects on tumor initiation and progression. However, TGFβ can function both as a tumor suppressor and as a tumor promoter, which is extensively reviewed by Bierie and Moses (1). The mechanisms determining when and how TGFβ switches from a tumor suppressor to a tumor promoter are a great challenge in the field. A variety of drugs including neutralizing antibodies and small molecular inhibitors have been developed to inhibit TGFβ signaling. However, the therapeutic effects of systemic inhibition of TGFβ signaling are likely dependent on the context and stage of tumor progression.

The evidence supporting TGFβ as a tumor suppressor comes from both human studies and mouse models. In a number of human cancers, mutations of the genes encoding TβRI and TβRII (*Tgfbr1* and *Tgfbr2*, respectively) or decreased expression and phosphorylation of other components of this pathway have been reported. In mouse models, conditional knockout of *Tgfbr2* in combination with expression of $Kras^{G12D}$ in pancreatic cancer (2) or with *APC* mutation in intestinal carcinomas (3) resulted in the development of much more aggressive tumors. Conditional deletion of *Tgfbr2* in mammary epithelial cells that also express the polyoma middle T antigen (PyVmT) under the mouse mammary tumor virus promoter resulted in a shortened tumor latency and increased metastases (4). This observation is also true in

Requests for reprints: Li Yang or Harold Moses, Vanderbilt-Ingram Cancer Center, 612 PRB, 2220 Pierce Avenue, Nashville, TN 37232. Phone: 615-936-1507; Fax: 615-936-1790; E-mail: li.yang@vanderbilt.edu or hal.moses@vanderbilt.edu.

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several other mouse models including colon cancer and head and neck squamous cell carcinoma. Abrogation of TGFβ signaling specifically in stratified epithelia leads to destabilization of epithelial homeostasis and results in the development of spontaneous squamous cell carcinomas in the anogenital region (5). TGFβ suppresses tumor progression through inhibition of cell cycle progression; increased apoptosis; and suppression of expression of growth factors, cytokines, and chemokines.

TGFβ is often produced in large quantities by many tumor types and is known to be pro-oncogenic. A recent clinical study showed that high TGFβ activity is present in aggressive, highly proliferative gliomas and is associated with a poor prognosis in glioma patients (6). In colon cancer, mutation of the gene for the type II receptor in cancers with high levels of microsatellite instability points to a favorable outcome after adjuvant chemotherapy with fluorouracil-based regimens for stage III colon cancer (7). In mouse models as mentioned above, abrogation of TGFβ signaling in PyVmT mammary carcinomas enhances metastasis (4), and paradoxically, enhancement of TGFβ signaling by expression of a constitutively active TGFβ1 or TβRI in mammary epithelial cells in conjunction with c-Neu or PyVmT expression increases pulmonary metastases (8). Systemic inhibition of TGFβ signaling suppresses pulmonary metastases, indicating that TGFβ is a tumor promoter (8). Studies using genetically modified cancer cells and mouse tumor models also support TGFβ signaling in breast cancer bone metastases. The mechanisms of TGFβ as tumor promoter include dysregulation of cyclin-dependent kinase inhibitors; alteration in cytoskeletal architecture, which is often implicated in epithelial to mesenchymal transition; increases in proteases and extracellular matrix formation; decreased immunosurveillance; and increased angiogenesis.

What factors mediate this switch of TGFβ signaling in function from tumor suppressor to tumor promoter? When exactly does this switch occur? It has been postulated that changes in signal intensity and connectivity of Smad-dependent pathways and Smad-independent pathways may underlie the complex transition. In this hypothesis, whereas Smad-dependent signaling mediates the growth inhibition of TGFβ signaling, the Smad-independent pathways likely contribute to the tumor-promoting effect of the TGFβ signaling cascade. This includes oncogenic events such as amplification of *MYC*, activating mutations of *RAS*, and inactivating mutations of downstream effectors (retinoblastoma and cyclin-dependent kinase inhibitors). However, there are also data showing that Smad-dependent pathways are involved in tumor progression. For example, Smad signaling is responsible for lung metastasis through induction of angiopoietin-like 4 (9). High TGFβ-Smad activity is present in aggressive, highly proliferative gliomas and confers poor prognosis in glioma patients (6).

Effect of TGFβ on the Immune System of Tumor Host

One of the significant effects of TGFβ is to inhibit immunosurveillance mechanisms in the tumor host (10). TGFβ markedly and directly suppresses the transcription of genes encoding multiple key proteins of the "cytotoxic program" of $CD8^+$ CTL, such as perforin and granzymes, cytotoxins that act through the granule exocytosis pathway (11). This inhibition of CD8 CTL is mediated by Gr-1CD11b myeloid cells through the production of TGFβ, which is regulated by natural killer T cells that secrete interleukin (IL)-3. Very interestingly, this induction of TGFβ production by myeloid cells plays a greater role in suppressing the immune response than production of TGFβ by the tumor itself because blocking production by these myeloid cells (by eliminating these cells or by blocking the upstream signal from natural killer T cells or IL-13) abrogated the suppression although the TGFβ production by the tumor itself was not affected (12). TGFβ also alters the polarization of the $CD8^+$ cells in tumor-bearing mice, resulting in elevated IL-17, which suppressed apoptosis of tumor cells (13). TGFβ, coordinated with IL-21, induces $CD4^+CD25^+$ regulatory T cells, which counterbalance the effect of IL-6 that promotes proinflammatory IL-17–producing T cells (14). In addition,

TGFβ is responsible for $CD4^+CD25^+$ regulatory T-cell inhibition of natural killer cell functions (15).

Very interestingly, publications from different laboratories, including our own, show that $Gr\text{-}1^+CD11b^+$ immature myeloid cells are the major source for high level of TGFβ in the tumor host (12,16,17). These $Gr\text{-}1^+CD11b^+$ cells have been known as myeloid immune suppressor cells or myeloid-derived suppressor cells since 1979. They inhibit natural killer cell, B-cell, and T-cell functions through the production of arginase and reactive oxygen species; they also inhibit functional maturation of dendritic cells and promote type II macrophage development, and thus represent one mechanism of tumor escape from immune system control and compromise the efficacy of cancer immunotherapy (18). There are two major subpopulations of these cells: mononuclear cells (precursor for macrophages) and low-density polymorphonuclear cells (immature neutrophils), and both populations suppressed antigen-specific T-cell responses, but through distinct effector molecules and signaling pathways (19). Recently, $Gr\text{-}1^+CD11b^+$ cells were found to directly disrupt the binding of specific peptide-MHC dimers to CD8-expressing T cells through nitration of tyrosines in a T-cell receptor-CD8 complex. This process makes CD8-expressing T cells unable to bind peptide-MHC and to respond to the specific peptide, although they retain their ability to respond to nonspecific stimulation (20).

TGFβ Orchestrates the Inflammatory Reaction in the Tumor Microenvironment

Host-derived inflammatory cells infiltrate into tumor tissues and provide growth factors, proangiogenic factors, proteases, as well as adhesion molecules that facilitate tumor cell proliferation, angiogenesis, invasion, and metastasis. One of the known mechanisms through which TGFβ mediates inflammatory reaction is the chemokines and chemokine receptors [e.g., CXC chemokine (CXCR)-4 and stromal cell–derived factor 1 (SDF-1)] and the recently reported CXC chemokine ligand 5 (CXCL5; ref. 17). These molecules play vital roles in the recruitment of host inflammatory cells into the tumor microenvironment. In addition, TGFβ also mediates nuclear factor-κB signaling, a master regulator of inflammation reaction. Tumor-infiltrating receptor activator of nuclear factor-κB ligand–expressing cells activate nuclear IκB kinase α and inhibit the transcription of tumor metastasis suppressor Maspin, thereby promoting tumor metastasis (21). TGFβ1 negatively regulates nuclear factor-κB activation in the gut through Smad7 (22). Inflammation by *Helicobacter* infection in Smad3-deficient mice caused the development of colon cancer (23). Furthermore, TGFβ cross-talks with inflammatory pathways through the modulation of IL-1 (24). In addition to epithelial cells, TGFβ signaling in stromal cells has significant effect on tumor development and growth. Loss of the TGFβ type II receptor in fibroblasts promotes mammary carcinoma growth and invasion through up-regulation of TGFα-, macrophage-stimulating protein–,and hepatocyte growth factor–mediated signaling networks (25). TGFβ signaling also regulates endothelial cells, resulting in a significant effect on tumor angiogenesis.

In the distant premetastatic lung, TGFβ is one of the factors produced by tumor cells responsible for the production of the chemoattractants S100A8 and S100A9, which attract $Mac1^+$ myeloid cells (26). Through this mechanism, tumor cells also activate mitogen-activated protein kinase p38 to acquire migratory activity with pseudopodia for invasion (invadopodia; ref. 26).

$Gr\text{-}1^+CD11b^+$ Myeloid Cells in the Switch of TGFβ Signaling from Tumor Suppressor to Tumor Promoter

$Gr\text{-}1^+CD11b^+$ myeloid cells are significantly overproduced in the bone marrow and spleens of tumor-bearing mice as well as in the peripheral blood of cancer patients. In addition to the

immunosuppressive effect on tumor host, Gr-1$^+$CD11b$^+$ cells also infiltrate into tumors and promote tumor angiogenesis and metastasis by producing high levels of matrix metalloproteinases (MMP) and TGFβ (17,27). Recently, these cells have been found to mediate tumor refractoriness to anti–vascular endothelial growth factor treatment (28).

Recent work from our laboratory showed that deletion of *Tgfbr2* in mammary carcinoma cells results in increased CXCL5/CXCR2 and SDF-1/CXCR4 chemokine signals that enhance Gr-1$^+$CD11b$^+$ myeloid cell infiltration into the invasive front of the tumors (17). Once there, these Gr-1$^+$CD11b$^+$ cells directly promote tumor invasion and metastasis through increased production and function of MMPs. In addition, Gr-1$^+$CD11b$^+$ cell infiltration also results in increased TGFβ production in the tumor microenvironment. Our data show that autologous TGFβ signaling in mammary epithelial cells acts as a tumor suppressor, and when it is deleted or altered, it results in Gr-1$^+$CD11b$^+$ cell recruitment. This leads to increased MMP and TGFβ production, which enhances tumor invasion and metastasis. Thus, the switch of TGFβ signaling from tumor suppressor to tumor promoter involves an additional component, which is the recruitment of Gr-1$^+$CD11b$^+$ cells in the tumor microenvironment. This is supported by a recent publication in which CCR1$^+$ myeloid cells (CD34$^+$) are shown to be recruited to colon cancers with deletion of Smad4 and promote tumor invasion (29). Indeed, inflammatory cells (CD45 and BM8-positive cells) have been observed in head and neck tumors lacking TGFβ signaling (30). In TGFβ1-deficient mice, inflammation causes precancerous lesions to progress to colon cancer (31).

However, contradictory to the above observations, overexpression of TGFβ1 in head and neck epithelia results in inflammation, angiogenesis, and epithelial hyperproliferation (32). It is unclear what underlies these different observations, and whether there are different mechanisms of chemokine/chemokine receptor mechanisms involved for deletion of TGFβ signaling versus increased TGFβ signaling.

Are Immune Cells from Tumor Host the Answer for TGFβ Antagonism Therapy?

Although changes in signal intensity and connectivity of Smad-dependent and Smad-independent pathways have been postulated as a mechanism for the TGFβ switch from suppressor to promoter, the factors that initiate such changes in signaling are not known. The above studies and results suggest that tumor-infiltrating bone marrow–derived Gr-1$^+$CD11b$^+$ cells change the dynamics in the primary tumor microenvironment, which results in changes in the signaling cascade in tumor cells. These studies also point out that TGFβ produced by Gr-1$^+$CD11b$^+$ cells is a significant component of the tumor-promoting effect of TGFβ signaling, affecting the tumor microenvironment and host immune system (Fig. 2). In fact, in preclinical mouse models, the efficacy of the anti-TGFβ antibody 1D11 in suppressing metastasis was dependent on a synergistic combination of effects on both the tumor parenchyma and microenvironment. This includes enhancement of the CD8$^+$ T-cell–mediated antitumor immune response, increased infiltration of natural killer cells and T cells at the metastatic site, and increased expression of an NKG2D ligand (Rae1γ) and of a death receptor (TNFRSF1A) on tumor cells (33). This line of understanding of TGFβ is particularly important because the therapeutic effect of TGFβ antagonism is largely dependent on when TGFβ switches from tumor suppressor to tumor promoter. Our studies suggest that Gr-1$^+$CD11b$^+$ cells may be used as a biomarker for patient selection in ongoing phase I/II clinical trials of TGFβ therapy. This is supported by recent findings that the immune/inflammatory responses are reliable markers in human hepatocellular carcinoma (34) and colorectal tumors predicting clinical outcome (35). These studies also point out a novel therapeutic strategy for advanced cancer, which is the prevention of the recruitment of MMP-expressing cells by chemokine receptor antagonists.

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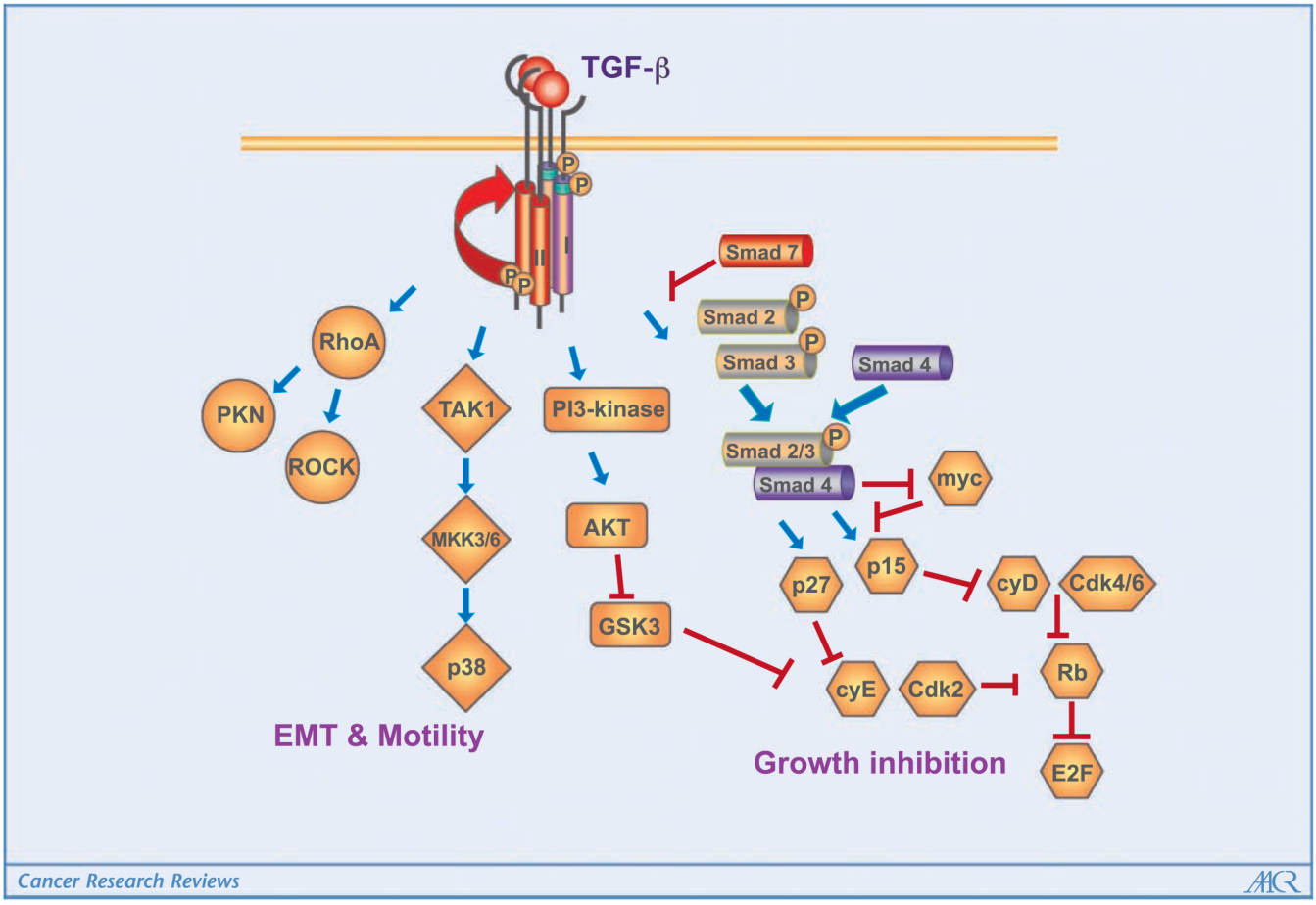

Figure 1.

The TGFβ ligands signal through the type I and type II TGFβ receptors. Canonical signaling proceeds with phosphorylation of Smad2 and Smad3, which then combine with Smad4 to enter the nucleus to mediate growth inhibition. Smad7 is a negative mediator in this process. In addition, TGFβ binding to its receptors activates many noncanonical signaling pathways and regulates tumor cell migration and metastasis.

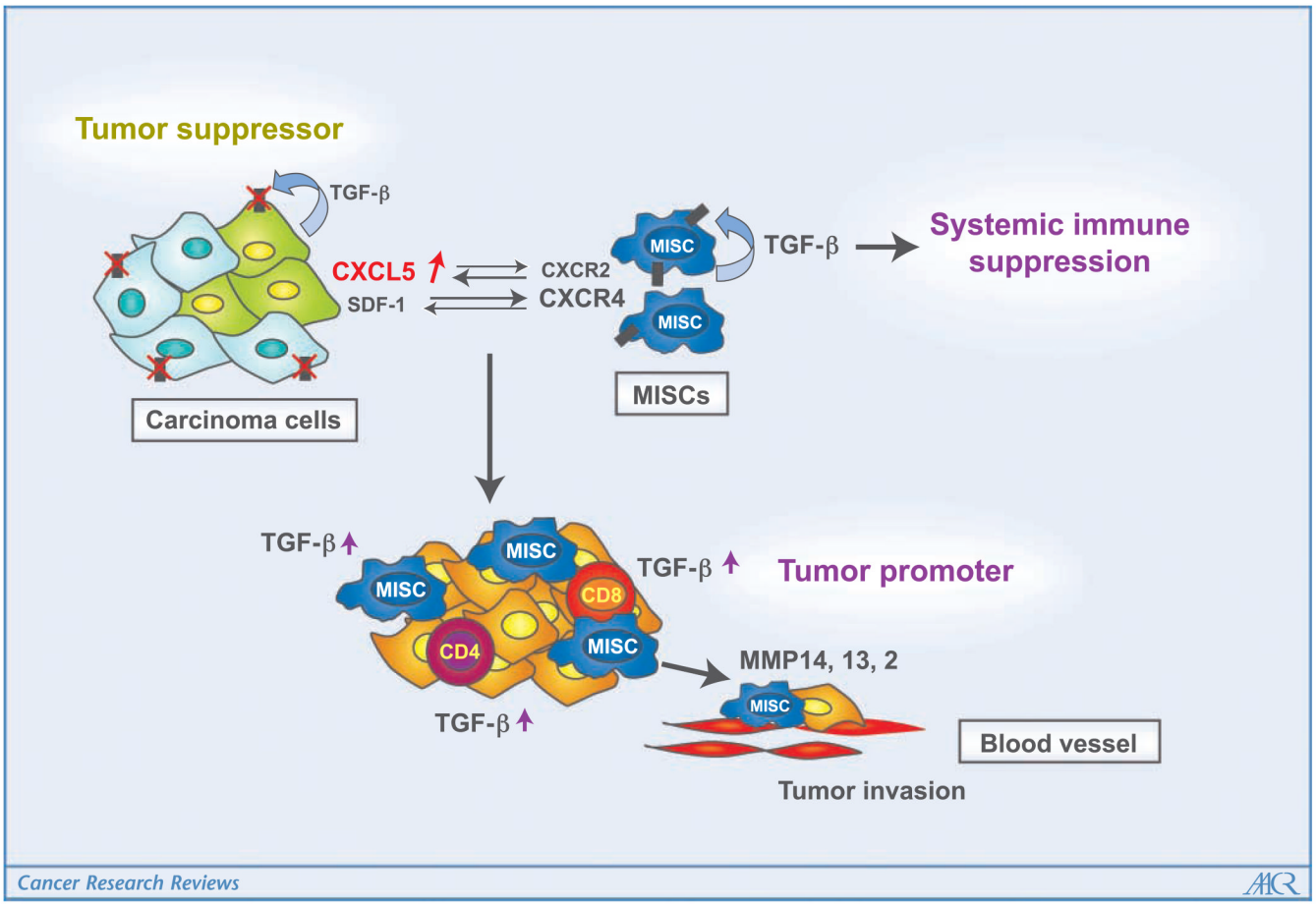

Figure 2.

How TGFβ signaling switches from being tumor suppressive to tumor promoting is unknown. Host-derived immature myeloid Gr-1^{+}CD11b^{+} cells are recruited into the tumor microenvironment with deletion of the type II TGFβ receptor gene in mammary carcinomas through CXCL5/CXCR2 and SDF-1/CXCR4. In addition, Gr-1^{+}CD11b^{+} cells express high levels of MMPs and TGFβ1, which promote tumor invasion and immune suppression. The effects of Gr-1^{+}CD11b^{+} cells on the tumor microenvironment and host immunosurveillance constitute a tumor-promoting mechanism of TGFβ signaling.