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TGF β and cancer metastasis: an inflammation link

Li Yang

Laboratory of Cancer Biology and Genetics, Center for Cancer Research, NCI, National Institutes of Health, Building 37, Room 3134C, 37 Convent Drive, MSC 4258, Bethesda, MD 20876-4258, USA

Abstract

Dysregulated transforming growth factor beta (TGF β) signaling is observed in a variety of human cancers. TGF β is produced in large quantities by many tumor types and is known to be pro-oncogenic. Therapeutic strategies directed against TGF β signaling using neutralizing antibodies and small molecular inhibitors have been developed. However, TGF β is also found to function as a tumor suppressor. This switch from a tumor suppressor in premalignant stages of tumorigenesis to a tumor promoter in later stages of the disease poses great challenges in TGF β -targeted cancer therapy. It remains unclear what mechanisms underlie the dual role of TGF β and what factors mediate the switch. In the past, most work on dissecting underlying mechanisms was focused on differential regulation of signaling pathways by tumor cell autonomous TGF β signaling. Recent progress in elucidating TGF β effects on host immune/inflammatory reactions in the tumor microenvironment and distant organs brings exciting new perspectives to the field.

Keywords

TGF β ; Tumor suppressor; Metastasis; Immune; Inflammation; Tumor microenvironment

1 TGF β and cancer metastasis: challenges in clinical application of TGF β -targeted therapy

Alterations in transforming growth factor beta (TGF β) signaling are common in human cancers. TGF β is often produced in large quantities in many tumors. High TGF β activity is present in aggressive, highly proliferative gliomas and is associated with a poor prognosis in patients [1]. *Tgfb β 2* mutation in colon cancers is associated with a favorable outcome after adjuvant chemotherapy [2]. In mouse models, enhancement of TGF β signaling by expression of a constitutively active TGF β 1 or T β RI in mammary epithelial cells in conjunction with c-Neu or PyVmT expression increases pulmonary metastases [3], and consistently, systemic inhibition of TGF β signaling suppresses pulmonary metastases [3]. TGF β signaling is also a well-known mediator in the “vicious cycle” of osteolytic bone metastases [4]. TGF β promotes tumor progression by many mechanisms. Among these is dysregulation of cyclindependent kinase inhibitors, alteration in cytoskeletal architecture which is often implicated in epithelial to mesenchymal transition increases in proteases and

extracellular matrix formation, decreases in immune surveillance, and increases in angiogenesis.

However, TGF β also functions as a tumor suppressor in early tumor development. In a number of human cancers, mutations in the genes encoding T β RI and T β RII (*Tgfbri* and *Tgfbr2*, respectively) or decreases in expression and phosphorylation of other components of this pathway have been reported. In mouse models, the conditional knockout of *Tgfbr2* in different tumor models has resulted in the development of much more aggressive tumors [5–8]. These data support TGF β as a tumor suppressor, through which TGF β inhibits cell cycle progression, increases apoptosis, and suppresses the expression of growth factors, cytokines, and chemokines.

This dual role of TGF β poses a great challenge for TGF β -targeted therapy. In fact, multiple clinical trials are underway with various approaches, including neutralizing antibodies and small molecular inhibitors (Fig. 1). Apparently, the therapeutic effects of TGF β antagonism treatment rest on our understanding of the mechanisms by which TGF β switches from a tumor suppressor to a tumor promoter. The goal of developing therapies is to abolish the tumor-promoting effect of TGF β while maintaining its tumor-suppressive properties. It is clear that the function of TGF β as a tumor suppressor or a tumor promoter depends on context and stage of tumor progression [9], but what factors mediate this switch of TGF β signaling in function from tumor suppressor to tumor promoter? When does this switch occur?

A good body of literature support that the diverse autonomous tumor cell signaling pathways play significant roles, which is often characterized by changes in signal intensity and connectivity of SMAD-dependent and independent pathways. The TGF β ligands (TGF β 1, 2, and 3) signal through the type I and type II TGF β receptors (T β RI and T β RII, respectively). Canonical signaling proceeds with phosphorylation of Smad2 and Smad3, which then combined with Smad4 to enter the nucleus and modulate transcription in cooperation with other transcription factors, co-activators and co-repressors. Additionally, TGF β binding to its receptors activates many non-canonical signaling pathways including PI3-kinase, MAP kinase, and small GTPase pathways (Fig. 2).

It is postulated that SMAD-dependent pathways mediate the growth inhibition of TGF β signaling, whereas the SMAD-independent pathways likely contribute to the tumor-promoting effect of the TGF β signaling cascade, synergizing with the oncogenic amplification of *MYC*, activating mutations of *RAS*, as well as inactivating mutations of downstream effectors such as retinoblastoma and cyclin-dependent kinase inhibitors. However, recent data dispute this hypothesis. Recently, Smad signaling was found to be responsible for lung metastasis through induction of angiopoietin-like 4 (ANGPTL4) by TGF β [10]. High TGF β -Smad activity is present in aggressive, highly proliferative gliomas and confers poor prognosis in patients with gliomas [1]. Interestingly, mutant-p53 may promote Smad-mediated tumor metastasis [11]. In addition, it has also been found that ubiquitination tightly controls Smad4 activity [12]. Phosphorylation of Smad1 and Smad5 through ALK5, the L45 loop motif of the type I TGF receptor, mediates the pro-tumor activity of the TGF β “switch” in breast cancer [13]. These data show a very diverse and

complex role of TGF β signaling. Yet, the host immune/inflammatory cells may hold the answer [14].

2 Tumor microenvironment: an indispensable participant in metastasis

Tumor metastasis accounts for the majority of cancer-associated death in patients. There are very few effective treatment options. During metastasis, tumor cells must disseminate, intravasate into circulation at the primary tumor site, travel through the vascular systems, arrest in capillary beds, and subsequently extravasate into the organ parenchyma. In a hostile distant organ, they must escape host immune surveillance in order to survive and grow. Evidence from recent years strongly suggests that the tumor microenvironment is an indispensable participant in this process. It provides a favorable microenvironment for tumor cells to not only escape from host immune surveillance, but also induce the formation of new blood vessels and invade the vasculature. Tumor is described as unhealed chronic inflamed wound, filled with various inflammatory cells (Fig. 3) including Tie2+, VEGFR1+, CD11b+, and F4/80+ subpopulations, as well as the innate and adaptive immune cells—natural killer T cells (NKT), T, and B cells. There is also an abundance of cancer-associated fibroblasts and endothelial progenitor cells [15]. These stromal cells collectively create an environment that favors tumor progression by providing growth factors, proangiogenic factors, proteases, and adhesion molecules that facilitate tumor cell proliferation, angiogenesis, invasion, and metastasis [15–19]. This very dynamic tumor microenvironment likely serves as a selective pressure for tumor cell variants through genomic instability, genomic heterogeneity, and epigenetic alterations [20].

Tumor-infiltrating lymphoid cells include cytotoxic T lymphocytes (CTL), regulatory T cells (Treg), NKT, and B cells. CTL proliferation and function is usually suppressed under tumor conditions through various mechanisms: suppression of the transcription of genes encoding multiple key proteins such as perforin, granzymes, and cytotoxins that act through the granule exocytosis pathway [21, 22]; loss of the colocalization of the T-cell receptor and CD8 [23]; the development of other T-cell types such as tumor-infiltrating gamma/delta T cells that suppress T and dendritic cell function through Toll-like receptor 8 ligands, MyD88, TRAF6, IKK α , IKK β , and p38 α molecules [24]; and aberrant expression of stress-inducible NKG2D ligands (MIC) by later stage tumor cells, which causes sustained NKG2D costimulation and population expansion of immunosuppressive T cells [25]. CD4+CD25+ Tregs are induced in the tumor microenvironment. TGF β is well known to induce Foxp3 expression and generates these Treg cells [26, 27]. Proinflammatory CD4+ T cells play a tumor-promoting role in the HPV16 mouse model (a transgenic model of multi-stage squamous carcinogenesis induced by human papillomavirus oncogenes), and deletion of CD4+ T cells results in a delayed neoplastic progression with a lower incidence of tumors [28]. IL-4-expressing CD4+ T lymphocytes regulate phenotype and function of CD11b+F4/80+ macrophages, which in turn enhance EGFR signaling in mammary epithelial cells promoting invasion and metastasis [29]. Chronic activation of B cells is indicated in potentiating carcinoma development. Genetic elimination of mature T and B lymphocytes in a transgenic mouse model of K14-HPV16 limits neoplastic progression to development of epithelial hyperplasias, which can be corrected by adoptive transfer of B lymphocytes or serum from HPV16 mice [30]. In fact, B cells, humoral immunity, and activating Fc γ Rs are

required for establishing chronic inflammatory programs that promote *de novo* carcinogenesis [31]. TGF β (and/or IL-10) is responsible for Breg-mediated immune suppression [32, 33]. B-cell depletion could therapeutically enhance antitumor immune responses by decreasing IL-10 production [32]. Complement C5a in a tumor microenvironment recruits myeloid-derived suppressor cells that suppress CTL function, promoting tumor growth [34]. The NKT cell is a new natural killer cell type that produces IL-13, which signals through the IL-4R-STAT6 pathway to regulate the production of TGF β in Gr-1+CD11b+ myeloid cells [35–37].

Tumor-infiltrating myeloid cells include three major cell types: Gr-1+CD11b+ immature myeloid cells, tumor-associated macrophages (TAM), and tumor-associated neutrophils (TAN). Gr-1+CD11b+ cells are a heterogeneous set of immature myeloid cells, while TAM and TAN are well differentiated. It remains to be seen how these three populations of cells are interrelated in phenotype and function under tumor conditions and in the tumor microenvironment. Gr-1+CD11b+ cells are also called myeloid immune suppressor cells (MISCs) or immune-derived suppressor cells. They are well known for their immune suppressive effects [38–41]. In addition, Gr-1+CD11b+ myeloid cells produce MMP9 and TGF β [18, 19], which has a profound impact on tumor progression and metastasis through modulation of tumor vascularization and tumor cell invasion [18, 19]. TAMs are identified as Mac-1 (CD11b+) and/or F4/80+. They have been shown to promote tumor progression and metastasis [42–48] through elevated CSF-1 production and enhanced EGF signaling in cancer cells [43]. TAN, identified as CD11b+Ly6G+ cells, have only recently been reported. TGF β regulates N1–N2 polarization of neutrophils [49]. This N1–N2 polarization of neutrophils may mirror the M1–M2 polarization of macrophages that are defined by interferon- γ and IL-4 production as Th1 and Th2 cells, respectively [50].

In summary, a variety of immune cells infiltrate into tumors constituting the tumor microenvironment. The outcome of tumor development to some extent is determined by the type of immune response. CTL, Th1 cells, and NK cells likely mediate anti-tumor immunity through the production of IL-2 and IFN γ , which inhibit tumor development and progression. The probable outcome of the immune response involving B cells and activation of humoral immunity and/or a Th2 polarized response is likely promoting tumor development and progression via production of IL-4, -5, -6, -10, and -13, as well as GM-CSF [51].

3 TGF β is a key regulator of inflammation and tumor microenvironment

Recent evidence strongly suggests that TGF β is one of the critical regulators in the inflammatory reaction that orchestrates the tumor microenvironment. Specific deletion of *Tgfb2* in different types of epithelial cells including mammary [7], pancreatic [5], intestinal [6], colon [52], and head-and-neck squamous cell carcinomas [53] results in increased tumor progression and metastasis, which is mediated through inflammation and the tumor microenvironment [14, 19, 54]. Interestingly, this complete abrogation of TGF β signaling correlates with reduced relapse-free survival in four human breast cancer data sets, especially in patients with estrogen receptor-positive tumors [55]. Deletion of SMAD4, the down-stream mediator of TGF β signaling, in a colon cancer model results in an increased recruitment of CCR1+ myeloid cells (CD34+) that promote tumor invasion [56].

In addition to epithelial cells, TGF β signaling in stromal cells also plays a significant role in tumor development and growth. Loss of the TGF β type II receptor in a subset of fibroblasts (FSP promoter driving cre expression) leads to intraepithelial dysplasia in prostate cancer and invasive squamous cell carcinoma in the fore-stomach through upregulation of TGF α , MSP- and HGF-mediated signaling networks [57]. This is often accompanied by increased abundance of stromal cells and inflammatory cell infiltration. Deletion of TGF β signaling in T lymphocytes results in the development of carcinomas throughout the GI tract, which is also accompanied by increased inflammatory gene expression of IL-5, IL-6, and IL-13 resulting in inflammatory cell infiltration [58].

The effects of TGF β on inflammation and tumor microenvironment may be regulated through nuclear factor kappa B (NF- κ B), a master regulator of inflammatory reactions and a critical mediator in tumor development and progression. For instance, TGF β 1 negatively regulates NF- κ B activation through Smad7 [59], a critical mediator of TGF β signaling thus blocking proinflammatory TNF signals [60]. Mice deficient in SMAD3 develop colon cancer due to increased inflammation caused by *Helicobacter* infection [61]. In TGF β 1-deficient mice, inflammation causes precancerous lesions to progress into colon cancer [62]. Recently, it was found that tumor-infiltrating RANKL (receptor activator of NF- κ B ligand)-expressing cells activate nuclear IKK α and inhibit the transcription of the tumor metastasis suppressor Maspin, thereby promoting tumor metastasis [63]. Additionally, TGF β cross talks with inflammatory pathways through the modulation of IL-1 [64]. These findings demonstrate that TGF β , a tumor suppressor, also functions as an inflammation suppressor. When it is deleted in the epithelial or stromal compartment, it causes an increased inflammatory reaction that promotes tumor development and progression. But, contradictory to the above observations, over-expression of TGF β 1 in head and neck epithelia results in inflammation, angiogenesis, and epithelial hyperproliferation [65]. It is unclear what underlies these different observations, and whether there are different chemokine/chemokine receptor mechanisms involved in deletion of TGF β signaling versus increased TGF β signaling.

Our recent work suggests that TGF β regulates the production of chemokine/chemokine receptors that are important for inflammatory cell recruitment. This includes stromal derived factor 1 (SDF-1 or CXCL12), key regulators 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 [15, 16]. In studies carried out in our lab, we found that TGF β also suppresses expression of CXCL1 and CXCL5. Deleted or diminished TGF β signaling in tumor epithelial cells significantly elevates the expression of CXCL1 and CXCL5 [19, 54]. CXCL5 interacts with the CXCR2 chemokine receptor to recruit Gr-1+CD11b+ myeloid suppressor cells to the tumor microenvironment [19]. As myeloid progenitor cells, Gr-1+CD11b+ cells express high levels of CXCR4, which interacts with SDF-1 in the tumor microenvironment to mediate the recruitment of these myeloid cells (Fig. 3). In the tumor microenvironment, these myeloid cells produce large quantities of matrix metalloproteases including MMP2, MMP13, and MMP14. Interestingly, tumors that lack TGF β signaling produce significantly higher levels of TGF β 1 than the control tumors, probably due to

increased infiltration of Gr-1+CD11b+ myeloid cells. The increased production of MMPs and TGF β 1 enhances tumor invasion and metastasis (Fig. 4).

Aside from the tumor microenvironment, a recent report also demonstrates an important role of TGF β in the distant premetastatic lung. In the premetastatic lungs of tumorbearing mice, TGF β regulates the production of chemoattractants S100A8 and S100A9, which attract Mac1+ myeloid cells and activate mitogen-activated protein kinase p38 in tumor cells. This activation confers invasive activity via the development of pseudopodia (invadopodia) [66]. In addition, TGF β also induces tumor cells to produce angiopoietin-like 4 that disrupts vascular endothelial cell–cell junctions and decreases the integrity of lung capillaries, facilitating trans-endothelial passage of tumor cells [10].

4 Other tumor suppressors in inflammation and the tumor microenvironment

Do tumor suppressors other than TGF β function as inflammatory suppressors? p53 is a very important tumor suppressor, regulating cell cycle, check point control, DNA damage/repair, and induction of apoptosis [67]. It is the most commonly mutated gene in human cancers [68]. p53 mutations in mice are associated with inflammation, bile duct injury, and fibrosis, which is similar to intrahepatic cholangiocarcinoma, a lethal human malignancy of the biliary epithelium [69]. p53 is also frequently mutated in ulcerative colitis-associated dysplastic lesions and colorectal cancer. The increased frequency of specific p53 mutations in non-cancerous ulcerative colitis may confer susceptibility to the development of colorectal cancer through an increased level of nitric oxide in the microenvironment [70, 71]. Combined loss of p53 and Atr, an essential genome maintenance regulator, leads to severe defects in hair follicle regeneration and deterioration of the intestinal epithelium. This is associated with increased inflammatory Mac1+Gr1+ cell infiltration [72]. Head and neck squamous cell carcinomas with mutated p53 show differentially expressed gene signatures compared to those with wild-type p53. NF- κ B phospho-p65 is inversely associated with p53 [73].

Pten, a tumor suppressor with lipid and protein phosphatase activity, is a major regulator of PI3K signaling [74]. Genetic inactivation of Pten in stromal fibroblasts of mouse mammary glands accelerates the initiation, progression, and malignant transformation of mammary epithelial tumors, which is associated with the massive remodeling of extracellular matrix (ECM), innate immune cell infiltration, and increased angiogenesis [75]. This loss of Pten also leads to increased expression, phosphorylation, and recruitment of Ets2, a protein that modulates gene expression associated with inflammation, angiogenesis, and ECM [75].

APC tumor suppressor gene is mutated in the Min mouse in which multiple benign polyps develop in the intestine. A compound heterozygote of APC and Smad4 also leads to intestinal polyps which develop into more malignant tumors characterized by extensive stromal cell proliferation, submucosal invasion, and cell-type heterogeneity. These tumors show increased CD34+ immature myeloid cell infiltration, elevated matrix metalloproteinases MMP9 and MMP2, as well as increased CC-chemokine receptor CCR1 [56, 76].

Semaphorin 3B (SEMA3B), a putative tumor suppressor, is expressed at high levels in many invasive and metastatic human cancers. SEMA3B suppresses primary tumor growth but also induces the production of IL-8 by tumor cells, which in turn, leads to the recruitment of tumor-associated macrophages and metastatic dissemination to the lung [77]. SEMA3B seems to possess dual functions in tumor development. It inhibits primary tumor growth, but enhances metastatic dissemination.

In addition to tumor suppressor, metastasis suppressor is also a very interesting class of molecules that are important in tumor host interaction. A metastasis suppressor is defined as a molecule whose expression results in the inhibition of a cancer cell's ability to metastasize while having little or no effect on primary tumor growth [78]. More than 20 of them have been found, including Ecadherin, Nm23, BRMS1, and others. The majority of disseminated cells, which should be fully malignant, persist in a potentially dormant state for a prolonged period. They do not proliferate immediately at secondary sites. While genetic mutations or epigenetic changes may be required for a cell or group of cells to separate and survive at distance from the primary tumor, the microenvironment within secondary tissues plays a substantial role in influencing whether disseminated cells survive and proliferate. Studying these suppressor proteins helps to dissect interactions among cancer cells and their microenvironment hopefully identifying targets for inhibiting metastatic growth and prolonged disease-free survival [79].

5 Anti-metastasis effect of TGF β antagonists dependent on host inflammation/inflammation responses

Increasing evidence supports that tumor metastasis is a coordinated event between tumor cells and host cells through inflammation. In fact, inflammatory cells produce much more TGF β than tumor cells [19, 36]. Gr-1+CD11b+ cells infiltrate into tumors in response to a loss of TGF β signaling, yet tumor cells with deletion of *Tgfb2* produced more TGF β than their control counterpart [19]. Indeed, recent developments in the field point out that the efficacy of TGF β antagonist therapy may not derive from a direct effect on tumor cells as was originally thought, but rather through mechanisms acting on the host compartment. Blocking the presence of TGF β may reduce the suppression of host immune surveillance. This effect is particularly important as TGF β antagonists may be combined with antitumor immunotherapy. Secondly, TGF β antagonism may abrogate the tumor-promoting effect of Gr-1+CD11b+ cells in the tumor microenvironment by neutralizing the high levels of TGF β 1 that they produce [19]. Third, TGF β antagonism could be very effective in blocking the "vicious cycle" of bone metastasis as TGF β signaling is a key player in the differentiation of osteoclasts that destroy the bone [4]. Lastly, TGF β antagonism may be effective for patients directly after radiation or chemotherapy because there is a surge in TGF β production after these treatments [80].

TGF β is a critical regulator of the tumor microenvironment and distant premetastatic organ conditions. TGF β exerts systemic immune suppression and significantly inhibits host tumor immune surveillance [26, 27, 81]. Neutralizing TGF β in preclinical mouse models enhances CD8+ T-cell and natural killer cell-mediated anti-tumor immune response [82–84]. It also

increases neutrophil-attracting chemokines and results in the recruitment and activation of neutrophils with an anti-tumor phenotype [49]. In addition to the systemic effects, TGF β also regulates the infiltration of inflammatory/immune cells and cancer-associated fibroblasts into the tumor microenvironment, which affects the signaling cascade in tumor cells. This emerging understanding of TGF β regulation on the interface between tumor cells and host immune cells may provide new insight for the development of successful TGF β antagonist treatments. Perhaps profiling of the inflammatory Gr-1+CD11b+ cells in the tumor microenvironment could be used as a biomarker for patient selection in the ongoing phase I/II clinical trials of TGF β -based therapy. This is supported by recent findings indicating that these immune/inflammatory responses are reliable markers for predicting clinical outcome in human hepatocellular carcinoma [85] and colorectal tumors [86], as well as breast cancer [87]. These studies also point out a novel therapeutic strategy for advanced cancers.

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Fig. 1. Strategies in TGFβ-targeted therapy. A variety of antagonists against TGFβ have been developed for cancer therapy, which include neutralizing antibodies to eliminate TGFβ ligands (recombinant TβRIII, TGFβ antibody, sTβRII:Fc fusion protein), or to block ligand-receptor binding (TGFβ receptor antibody), as well as small molecule kinase inhibitors against the TGFβ receptors. The challenge for a successful cancer therapy relies on how to precisely aim at the tumor-promoting activity of TGFβ but spare its tumor-suppressing function

Fig. 2.

Simplified TGFβ signaling pathways. The TGFβ ligand transduces its signal through type I and type II TGFβ receptors. Canonical signaling leads to phosphorylation of Smad2 and Smad3, which then combine with Smad4, and translocate into the nucleus and mediate growth inhibition. Smad7 functions as a negative mediator in this process that blocks Smad2/3 phosphorylation. TGFβ also activates many non-canonical signaling pathways through interaction with the same receptors. It includes activation of small Rho GTPases, p38 kinase, and PI3 kinase pathways. These are important in regulating tumor cell migration and metastasis. In addition, bone morphogenetic proteins (*BMPs*) signal through Smad1, Smad5, or Smad8, which interact with Smad4 and activate or repress targeted gene transcription important for tissue and organ development. Smad6 and 7 are natural antagonists in TGFβ signaling pathway

Fig. 3.

Host cell infiltration during tumorigenesis. A variety of host bone marrow-derived cells, which include CD8⁺ CTL (cytotoxic T lymphocyte), natural killer (*NK*), regulatory T cell (*Treg*) and B cell lymphocyte, as well as macrophages, neutrophils, and Gr-1+CD11b⁺ cells or MDSCs, infiltrate into tumors and create a microenvironment that favors tumor growth and progression

Fig. 4.

Potential mechanisms by which TGFβ signaling switches from a tumor suppressor to a tumor promoter. TGFβ functions through the type II receptor on tumor cells to exhibit growth inhibition of carcinoma cells. TβRII deletion/mutation or downregulation leads to an increased CXCL1-CXCL5/CXCR2 and SDF-1/CXCR4 chemokine/chemokine receptor signaling, and subsequent recruitment of host-derived immature myeloid Gr-1+CD11b+ cells into tumors. These infiltrating myeloid cells produce large quantity of TGFβ1 and MMPs that suppress the host immune system and concurrently promote tumor invasion. The effect of Gr-1+CD11b+ cells on the tumor microenvironment and host immune surveillance constitutes a tumor-promoting mechanism of TGF β signaling