

Review

Transforming growth factor- β and breast cancer Tumor promoting effects of transforming growth factor- β

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Received: 3 December 1999
Revisions requested: 3 January 2000
Revisions received: 19 January 2000
Accepted: 26 January 2000
Published: 21 February 2000

Breast Cancer Res 2000, 2:125–132

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Abstract

The transforming growth factor (TGF)- β s are potent growth inhibitors of normal epithelial cells. In established tumor cell systems, however, the preponderant experimental evidence suggests that TGF- β s can foster tumor–host interactions that indirectly support the viability and/or progression of cancer cells. The timing of this ‘TGF- β switch’ during the progressive transformation of epithelial cells is not clear. More recent evidence also suggests that autocrine TGF- β signaling is operative in some tumor cells, and can also contribute to tumor invasiveness and metastases independent of an effect on nontumor cells. The dissociation of antiproliferative and matrix associated effects of autocrine TGF- β signaling at a transcriptional level provides for a mechanism(s) by which cancer cells can selectively use this signaling pathway for tumor progression. Data in support of the cellular and molecular mechanisms by which TGF- β signaling can accelerate the natural history of tumors will be reviewed in this section.

Keywords: transforming growth factor (TGF)- β , TGF- β receptors, epithelial-to-mesenchymal transition, angiogenesis

Introduction

Although the transforming growth factor (TGF)- β s can be tumor suppressive [1], there is increasing evidence that TGF- β secretion by tumor cells and/or stromal cells within the peritumoral microenvironment can contribute to tumor maintenance and progression. How, then, can TGF- β s be both tumor suppressive and tumor promoting? This apparent paradox is reconciled by a study showing that, in a mouse skin model of chemical carcinogenesis, targeted expression of TGF- β 1 in suprabasal keratinocytes appears to have dual effects. It suppresses the formation of benign skin tumors, but once tumors develop, it enhances their progression to a highly invasive spindle cell phenotype [2**]. These results suggest that the effects of TGF- β 1 are

biphasic: TGF- β 1 acts early as a tumor suppressor, probably by inhibiting the proliferation of nontransformed cells, and it acts later as a tumor promoter by eliciting an epithelial-to-mesenchymal transition (EMT). Additional experiments have suggested that upregulation of TGF- β 3 in the spindle carcinomas was responsible for maintenance of this invasive phenotype [2**]. This is consistent with TGF- β 3 expression at sites in mouse embryos where epithelial–mesenchymal interactions are important, like the lung and palatal shelves [3,4], and also the abnormal lung development and cleft palate observed in TGF- β 3 null mice [5]. Also consistent with an early tumor suppressive effect is the recent observation that *tgf- β 1*^{-/-} mice develop an accelerated progression of epithelial hyperplasia to

CTL = cytotoxic T lymphocyte; EMT = epithelial-to-mesenchymal transition; JNK = c-Jun *N*-terminal kinase; MMP = matrix metalloproteases; PAI-1 = plasminogen activator inhibitor; PTHrP = parathyroid hormone-related protein; T β RI = TGF- β receptor type I; T β RII = TGF- β receptor type II; TGF- β = transforming growth factor- β .

colonic adenomas and cancers [6*]. The existence of dual effects for TGF- β s in tumor progression follows the observation that TGF- β -induced growth inhibitory responses and extracellular matrix responses may represent distinct processes in certain cell types. For example, overexpression of the antagonistic Smad, *Smad7*, in pancreatic carcinoma cell lines not only suppresses TGF- β 1-mediated growth inhibition, but enhances the ability of TGF- β 1 to induce matrix associated transcriptional responses [7*].

The progression of epithelial tumors to an invasive metastatic state is often associated with EMT, downregulation of cellular adhesion molecules, elevated expression of metalloproteases, and increased motility and angiogenesis, all of which can be modulated by TGF- β s. It is therefore not surprising that the TGF- β s can also promote tumorigenesis by modulating these critical processes. In support of this view, elevated levels of TGF- β are often observed in advanced carcinomas, and have been correlated with disease progression in several studies [8–13]. This suggests that secreting higher levels of TGF- β may provide an advantage to tumor cells. Both autocrine and paracrine signaling may be involved in conferring this selective advantage. While mutations in various components of the TGF- β signaling pathway have been observed in some carcinomas, particularly colorectal cancers [14,15], an intact TGF- β signaling pathway is often retained in other malignancies as some tumors can exhibit increased invasiveness in response to exogenous TGF- β [16,17*,18,19,20*,21]. Moreover, in a recent study of a large cohort of human breast tumors, loss or low levels of the type II TGF- β receptor (T β RII) correlated with high tumor grade, but 60% of *in situ* and invasive breast carcinomas retained robust levels of T β RII expression by immunohistochemistry [22]. Finally, although *Smad4* is frequently inactivated in pancreatic cancers [23,24], the *Smad* genes, which encode proteins that transduce TGF- β signals, are rarely mutated in most human carcinomas [25–30]. This suggests that after cells lose their sensitivity to TGF- β growth inhibition, autocrine TGF- β signaling may potentially promote tumor progression. In addition, TGF- β s produced in excess by tumor cells may act in a paracrine fashion on the peritumoral stroma, tumor neovessels, or the immune system, indirectly fostering tumor progression.

Autocrine effects

Epithelial-to-mesenchymal transition

Similar to keratinocytes [2**], TGF- β 1 can also induce a rapid and reversible EMT in melanoma cells [31], and in both nontumor [32] and Ha-Ras transformed [17*] mammary epithelial cells *in vitro*. In Ha-Ras mammary tumors, EMT appears to be initiated by TGF- β produced by peritumoral host cells and later maintained by autocrine TGF- β 1 as the converted tumor cells themselves begin to secrete TGF- β 1. The Ha-Ras tumor cells obtained after

EMT *in vitro* or *in vivo* display loss of epithelial polarity, downregulation of E-cadherin, disruption of cell–cell adhesion, and invasive properties in several *in vitro* assays [17*]. Supporting the importance of autocrine TGF- β for the tumorigenesis of Ha-Ras mammary cells, introduction of dominant negative T β RII into these cells retarded tumor formation and prevented EMT *in vivo*; moreover, introduction of the same construct into highly invasive murine colon carcinoma cells reconstituted an epithelial phenotype *in vitro*, and inhibited both tumor outgrowth and the establishment of metastases [20*]. In colon cancer cells of low invasive potential and with naturally occurring mutations in the T β RII gene, re-expression of T β RII function restored tumor cell invasiveness [20*]. In another study, expression of dominant negative T β RII in clones derived from a metastatic squamous carcinoma cell line prevented their spontaneous progression to a spindle phenotype *in vivo* [21]. Furthermore, approximately 90% of colon cancers with microsatellite instability have inactivating mutations of T β RII [33], and this instability is significantly correlated with longer patient survival [34], suggesting that complete loss of T β RII in carcinomas may limit systemic metastases. Taken together, these results suggest that EMT, local tumor growth, and metastatic progression can be sustained by autocrine TGF- β signaling.

When tumors are grown in nude mice, TGF- β s made by host cells can induce responses in tumor cells with intact TGF- β signaling, with the net effect of these tumor–host interactions being deleterious to the host. For example, MDA-231 human breast tumor cells secrete parathyroid hormone-related protein (PTHrP) in response to exogenous TGF- β 1, metastasize to bone when injected into nude mice, and induce osteolysis and hypercalcemia, resulting in host death. Transfection of these cells with dominant negative T β RII blocks TGF- β 1-mediated stimulation of PTHrP production. Mice injected with these cells exhibited less osteolysis, higher body weight, lower serum calcium and PTHrP levels, and longer survival than mice injected with control MDA-231 cells [35*]. On the contrary, accelerated osteolysis and reduced host survival were observed when mice were injected with tumor cells transfected with a constitutively active T β RI, suggesting a possible role for TGF- β -mediated responses in the pathogenesis of some adverse paraneoplastic syndromes.

Several recent studies have contributed to our understanding of the biochemical mechanisms by which transformed cells can lose autocrine growth inhibition but retain TGF- β -mediated responses that contribute to tumor progression. For example, oncogenic activation of the Ras pathway, acting via MAP kinases, causes phosphorylation of Smad2 and Smad3 at specific Erk consensus sites in the linker region between their DNA binding and transcriptional activation domains. This results in loss of nuclear accumulation of Smad2/3 and silencing of TGF- β -mediated

antiproliferative responses [36**]. In nontransformed mammary cells, introduction of mutant Ras not only blocks growth inhibition by TGF- β , but also subverts this pathway into one that can stimulate epithelial-to-mesenchymal transdifferentiation [17*,20*]. In MDCK epithelial cells, transfection of the missense mutations Smad2.D450E and Smad2.P445H, reported in primary colorectal and lung carcinomas, does not abolish TGF- β -mediated growth arrest. Instead, it increases both basal and TGF- β stimulated invasiveness, neither of which is prevented by overexpression of the inhibitory Smad7 [37*]. This suggests the existence of *Smad* 'gain-of-function' mutations that enhance malignant progression by mechanisms independent of T β RI and Smad phosphorylation. Another study has shown that Smad7 mRNA levels are increased in human pancreatic cancers compared with normal pancreas [7*]. Stable transfection of COLO-357 human pancreatic cancer cells with a Smad7 expression vector results in loss of TGF- β 1-mediated growth inhibition and p21/Cip1 promoter activity. However, TGF- β 1-induced plasminogen activator inhibitor-1 (PAI-1) promoter activity is maintained and, more importantly, basal PAI-1 promoter activity, PAI-1 mRNA levels, anchorage independent colony growth, and tumorigenicity in nude mice, are all increased in the Smad7 transfected clones [7*]. This result suggests another potential mechanism, the overexpression of Smad7, for the segregation between antiproliferative and matrix associated TGF- β responses. In addition, overexpression of Smad4 in colon carcinoma cells does not reconstitute TGF- β -mediated antiproliferative responses [38*,39], but inhibits cell adhesion and spreading, reduces the levels of urokinase plasminogen activator and PAI-1, and prolongs tumor latency [39], suggesting an additional function for Smad4 in restraining genes involved in peritumor proteolysis and invasion. This is further supported by reports of homozygous deletion of *T β RI* or homozygous missense mutations of *T β RII* [40,41], each coexisting with deletions of *Smad4* in individual tumors. The coexistence of these mutations in the same tumors would not be expected if the function of these two gene products (T β RII and Smad4 or T β RI and Smad4) was limited to a single common signal transduction pathway. Taken together, these studies suggest, first, that the threshold for loss of TGF- β antimitogenic effects is lower than that required to lose responses associated with cell adhesion, invasion, and metastases; second, that not one but multiple biochemical mechanisms can contribute to the enhancement or unmasking of the tumor promoting effects of autocrine TGF- β ; and, third, that some of these mechanisms may be independent of Smad function or T β RI phosphorylation. The identification of Smad dependent and independent genes causally involved in these TGF- β -mediated tumor promoting effects requires further research. Of note, Hocevar *et al* [42*] recently reported c-Jun N-terminal kinase (JNK) dependent TGF- β -induced fibronectin expression in cell lines lacking the *Smad4* gene or protein expression.

Increased motility

TGF- β can stimulate the motility of many cell types *in vitro* [43–45], therefore suggesting that TGF- β production *in vivo* may enhance migration of tumor cells and metastatic potential. Indeed, cyclosporine treatment of lung adenocarcinoma cells results in increased cell motility and anchorage independent growth *in vitro*, as well as increased metastases *in vivo*, all of which can be blocked with neutralizing TGF- β 1 antibodies [46]. These results suggest that *in vivo* tumor progression by cyclosporine is dependent on autocrine TGF- β 1. In prostate cancer cells, TGF- β 1 stimulates motility without affecting cell proliferation, suggesting that the effects on motility and proliferation may occur via different biochemical pathways [43].

Whether blockade of the Smad pathway, critical for TGF- β -mediated antimitogenic effects [47,48], is also critical for the effects of TGF- β s on cell motility is not clear. Some evidence suggests that the latter may follow alternative signaling pathways, perhaps in cooperation with activated oncogenes. Atfi *et al* [49] reported recently that inactivating components of the JNK pathway, which regulates AP-1 activity via c-Jun, inhibits TGF- β -mediated induction of 3TP-Lux, a reporter construct that contains Smad and AP-1 binding elements. Dominant negative mutants of RhoA, Rac1, and Cdc42, GTPases that mediate cell shape, cytoskeletal organization, and motility, abolish TGF- β -mediated transcription of AP-1 [49,50], suggesting that the Rho family of GTPases and the JNK pathway are essential components of TGF- β signaling responses. TGF- β 1 can also upregulate integrin linked kinase [31], a protein associated with fibronectin production and increased cell motility. In another study, TGF- β 1 treatment of NMuMG mouse mammary epithelial cells increased the expression of N-cadherin [51], which has been shown to increase motility of squamous cancer cells [52].

Paracrine effects

Induction of metalloproteases

Matrix metalloproteases (MMPs) play a critical role in the proteolytic degradation of basement membrane that is required for tumor invasion [53]. The expression of several MMPs, including MMP-2 [54] and MMP-9 [18,31,55], can be induced by TGF- β . Moreover, TGF- β 1 has been shown to selectively induce MMP-9 activity in a subset of metastatic but not primary mouse prostate tumors, implying that this TGF- β 1-induced response may be an important selection step in tumor progression [18]. There is also evidence that TGF- β increases MT-MMP-1 and MMP-9 expression in metastatic melanoma [31]. Although MMPs are listed separately, recent data implicate them strongly in the process of tumor-induced neovascularization [56], thereby suggesting that their upregulation might be an integral component of the TGF- β -mediated angiogenic processes discussed next.

Tumor angiogenesis

It is generally accepted that solid tumors require an adequate blood supply in order to grow beyond a few millimeters in size. TGF- β s, particularly TGF- β 1, have been shown to regulate new blood vessel formation both *in vitro* and *in vivo* by a combination of responses that include increased production and facilitation of vascular endothelial growth factor, facilitation of basic fibroblast growth factor mediated capillary sprouting, inhibition of endothelial cell migration, and increased production of extracellular matrix, among others (reviewed in [57]). In most cells, T β RI/ALK-5 is the signaling receptor for TGF- β . However, in endothelial cells, it has been suggested that ALK-1 may also function as a type I receptor for TGF- β [58]. In addition to the type I, II, and III TGF- β receptors, endoglin is another integral membrane protein that binds TGF- β 1 and TGF- β 3, and is highly expressed in endothelial cells [59]. Although TGF- β effects appear to be mediated mostly by the receptor specific Smad2 and Smad3 proteins [47,48], there is evidence that Smad5 is involved in TGF- β signaling in hematopoietic cells [60]. Targeted disruption of genes encoding various components of the TGF- β signaling pathway, including TGF- β 1 itself [61], its receptors, T β RII [62], ALK-1 [63], and endoglin [64], and one of its signal transducers, Smad5 [65], has each revealed that these proteins play an important role in vascular development. The phenotype of the TGF- β 1 and T β RII knockout mice is virtually indistinguishable and is characterized by defective endothelial differentiation resulting in abnormal capillary tube formation [61,62]. In contrast, disruption of ALK-1, endoglin, or Smad5 does not affect endothelial differentiation or vasculogenesis, but instead they each affect angiogenesis. In addition, *endoglin*^{-/-} and *Smad5*^{-/-} mice exhibit impaired vascular smooth muscle cell development. These results are consistent with previous reports demonstrating that TGF- β can regulate smooth muscle cell differentiation and migration *in vitro* [66], thus contributing to pericyte recruitment and vessel stabilization. This hypothesis, as it applies to tumor angiogenesis, is somewhat challenged by the notion that the majority of intratumoral neovessels seem to lack periendothelial smooth muscle cells [67], suggesting that there may be additional roles for the TGF- β s in tumor angiogenesis. In that light, Higaki and Shimokado [68] recently reported TGF- β 1-mediated stimulation of phosphatidylinositol-3 kinase activity and amino acid uptake in vascular smooth muscle cells, suggesting a direct anti-apoptotic role for TGF- β . Elucidation of the paracrine mechanisms driving TGF- β -mediated tumor angiogenesis requires further investigation.

Further supporting the role of TGF- β s in tumor angiogenesis, administration of a neutralizing TGF- β 1 antibody to nude mice harboring CHO cell xenografts transfected with ectopic TGF- β 1 inhibits both tumor growth and intratumor microvessel density [69]. In addition, a monoclonal anti-

body that blocks TGF- β 1, TGF- β 2, and TGF- β 3 has been shown to suppress the growth of TGF- β 1-overexpressing renal cancer xenografts [70]. In this study, the TGF- β blocking monoclonal-abrogated factor VIII staining in the xenografts, suggesting an antitumor mechanism that targets endothelial cells [70]. Furthermore, TGF- β 1 and PAI-1 have been shown to inhibit the conversion of plasminogen to the anti-angiogenic molecule angiostatin in medium conditioned by human pancreatic cancer cells [71]. This suggests an additional pro-angiogenic mechanism for TGF- β by interfering with the production of endogenous inhibitors of endothelial cell proliferation. Finally, high levels of TGF- β 1 mRNA correlate strongly with high microvessel density in breast tumors, and each of these factors is associated with poor patient outcome [72].

Host immunosuppression

TGF- β 1 and TGF- β 2 are potent immunosuppressants [73]. Thus, elevated levels of TGF- β s secreted by tumors could potentially inhibit immune effector cells and favor tumor progression. In support of this idea, Torre Amione *et al* [74] demonstrated that, unlike parental tumor cells, fibrosarcoma cells transfected to express 10 ng/ml TGF- β 1 *in vitro* are unable to induce cytotoxic T lymphocyte (CTL) responses and can escape immune recognition. Likewise, EMT6 mammary tumor cells, which produce high levels of TGF- β 1, can inhibit CTLs *in vivo*. Transfection of these cells with interleukin-2, a known T cell growth factor, can reverse this TGF- β 1 effect and induce tumor rejection [75]. This result suggests that, by dampening the generation of tumor reactive T cells, TGF- β can promote tumor viability. There is also evidence that overexpression of the soluble T β RII extracellular domain in thymoma cells can prevent the progression of unmodified thymoma cells when injected near the primary tumor inoculation site [76], further suggesting that secretion of soluble T β RII by these cells is sufficient to restore tumor specific cellular immunity and mediate partial tumor rejection. Overall, these results are consistent with the phenotype of *TGF- β 1* null mice that die shortly after birth as a result of widespread inflammation and multiorgan T cell infiltration and necrosis [77].

In addition to inhibiting CTL responses, TGF- β s can modulate other immune functions that may favor tumor progression. For example, CHO cells transfected with an expression vector encoding latent TGF- β 1, when injected into nude mice, can decrease mouse spleen natural killer activity and rapidly form tumors [78]. Antagonizing TGF- β s by intraperitoneal injection of an antibody that neutralizes TGF- β 1, TGF- β 2, and TGF- β 3 has the opposite effect. It prevents tumor and metastases formation by MDA-231 human breast carcinoma cells, and markedly increases natural killer activity of mouse splenocytes [79]. Consistent with this TGF- β -mediated immunosuppressive effect, reduced immune function has been observed in animals

bearing TGF- β overexpressing tumors [80] as well as in patients with glioblastoma, a common type of brain tumor that frequently overexpresses TGF- β 2 [81].

The cited studies suggest that tumor cell secreted TGF- β s may block the efferent function of immune effectors at sites of tumor implantation. Other reports, however, suggest tumor cell TGF- β s may modify the afferent component of the immune response and confer antitumor immunity. Stable infection of breast and glioma tumor cells with antisense TGF- β 1 and antisense TGF- β 2 retroviruses, respectively, has been shown to restore the immunogenicity of these tumor cells when injected into immunocompetent animals. Furthermore, they induce a partial rejection of unmodified, less immunogenic established wild type tumor cells [82,83]. In both of these studies, *in vitro* and *in vivo* CTL activity was markedly increased in medium conditioned by antisense TGF- β -infected cells and/or in mice injected with tumor cells bearing the antisense compared with tumor cells infected with a control vector. These studies have therapeutic implications for the use of an antisense TGF- β based approach as a means of adoptive immunotherapy against TGF- β overproducing tumors.

Alternative views and conclusions

A tumor permissive role for the TGF- β s may not apply to all solid tumors. Indeed, transfection of an antisense TGF- β 1 expression vector into FET and CBS well-differentiated human colon cancer cells has been shown to enhance tumor formation in nude mice [84,85], supporting the notion that, in some fully transformed cells, endogenous TGF- β 1 can continue to mediate a tumor suppressor function. In a recent report, mice bearing transplanted gallbladder Mz-Cha-2 tumors showed inhibition of angiogenesis and leukocyte-endothelial cell interactions at a distant cranial site and threefold higher levels of circulating TGF- β 1 compared with tumor free mice [86]. This reduction in microvessel density and leukocyte rolling were reversed by systemic administration of a TGF- β 1 neutralizing antibody, suggesting a negative role for TGF- β 1 in early neovascularization. Moreover, in a recent survey of 104 *in situ* and invasive primary breast carcinomas, 40/45 (89%) tumors with low invasive potential and low proliferation rate exhibited high levels of T β RII by immunohistochemistry [22]. Whether autocrine TGF- β signaling is causally associated with the observed low proliferation and invasiveness in this subset of breast tumors is a question that remains unclear.

Nonetheless, the potential tumor promoting effects of TGF- β provide novel molecular targets for interventions aimed at altering the natural history of solid tumors. The lack of an obvious physiological role for TGF- β signaling in postdevelopmental normal physiological states suggests that these interventions may in fact be tumor specific and

spare the tumor host from undue toxicity. Several approaches have been proposed, including the use of blocking antibodies against TGF- β 1, TGF- β 2, and TGF- β 3, using the soluble ectodomains of the type II and III TGF- β receptors, which would sequester TGF- β isoforms at tumor sites and prevent binding to cognate receptors [87,88], and, finally, using adenovirus encoding inhibitors of TGF- β signaling [89], to name a few. The theoretical and logistical strengths and limitations of these approaches are beyond the scope of this review. Nonetheless, these represent tools that, if effective in blocking TGF- β action, will allow us to address the net effect of autocrine/paracrine TGF- β signaling at early and late stages of transformation and cancer progression.

References

Articles of particular interest have been highlighted as:

- of special interest
- of outstanding interest

1. Markowitz SD, Roberts AB: **Tumor suppressor activity of the TGF-beta pathway in human cancers.** *Cytokine Growth Factor Rev* 1996, **7**:93-102.
2. Cui W, Fowles DJ, Bryson S, *et al*: **TGFbeta1 inhibits the formation of benign skin tumors, but enhances progression to invasive spindle carcinomas in transgenic mice.** *Cell* 1996, **86**:531-542.
This is the first study which demonstrates that TGF- β 1 may actually have a dual role and be both tumor suppressive and tumor promoting. Mice expressing active TGF- β 1 under the control of the K14 promoter, specific to suprabasal skin keratinocytes, are more resistant to the induction of benign skin tumors than controls. However, their conversion to invasive, spindle cell carcinomas is markedly increased.
3. Schmid P, Cox D, Bilbe G, Maier R, McMaster GK: **Differential expression of TGF beta 1, beta 2 and beta 3 genes during mouse embryogenesis.** *Development* 1991, **111**:117-130.
4. Millan FA, Denhez F, Kondaiah P, Akhurst RJ: **Embryonic gene expression patterns of TGF beta 1, beta 2 and beta 3 suggest different developmental functions in vivo.** *Development* 1991, **111**:131-143.
5. Kaartinen V, Voncken JW, Shuler C, *et al*: **Abnormal lung development and cleft palate in mice lacking TGF-beta 3 indicates defects of epithelial-mesenchymal interaction.** *Nat Genet* 1995, **11**:415-421.
6. Engle SJ, Hoying JB, Boivin GP, Ormsby I, Gartside PS, Doetschman T: **Transforming growth factor beta1 suppresses nonmetastatic colon cancer at an early stage of tumorigenesis.** *Cancer Res* 1999, **59**:3379-3386.
Tgfb1^{-/-} *Rag2*^{-/-} mice rapidly develop carcinomas of the cecum and colon. In contrast, the colon from *Tgfb*^{+/+} and *Tgfb*^{+/-} *Rag*^{-/-} mice remains hyperplastic with almost no progression to carcinoma. Data in the paper suggest that loss of TGF- β 1 contributes to tumor formation by mechanisms other than loss of growth control, increased inflammation, or genetic instability.
7. Kleeff J, Ishiwata T, Maruyama H, *et al*: **The TGF-beta signaling inhibitor smad7 enhances tumorigenicity in pancreatic cancer.** *Oncogene* 1999, **18**:5363-5372.
Stable transfection of ectopic Smad7 in COLO-357 human pancreatic cancer cells abrogates TGF- β -mediated growth inhibition and transcription via the p21 promoter, but potentiates PAI-1 mRNA induction, 3TP-luciferase activity, and tumorigenicity in nude mice, highlighting a dissociation between the growth and matrix effects of TGF- β .
8. Walker RA, Dearing SJ: **Transforming growth factor beta 1 in ductal carcinoma in situ and invasive carcinomas of the breast.** *Eur J Cancer* 1992, **28**:641-644.

9. Gorsch SM, Memoli VA, Stukel TA, Gold LI, Arrick BA: **Immunohistochemical staining for transforming growth factor beta 1 associates with disease progression in human breast cancer.** *Cancer Res* 1992, **52**:6949–6952.
 10. Friess H, Yamanaka Y, Buchler M, *et al*: **Enhanced expression of transforming growth factor beta isoforms in pancreatic cancer correlates with decreased survival.** *Gastroenterology* 1993, **105**: 1846–1856.
 11. Reed JA, McNutt NS, Prieto VG, Albino AP: **Expression of transforming growth factor-beta 2 in malignant melanoma correlates with the depth of tumor invasion. Implications for tumor progression.** *Am J Pathol* 1994, **145**:97–104.
 12. Ito N, Kawata S, Tamura S, *et al*: **Positive correlation of plasma transforming growth factor-beta 1 levels with tumor vascularity in hepatocellular carcinoma.** *Cancer Lett* 1995, **89**:45–48.
 13. Wikstrom P, Stattin P, Franck-Lissbrant I, Damber JE, Bergh A: **Transforming growth factor beta1 is associated with angiogenesis, metastasis, and poor clinical outcome in prostate cancer.** *Prostate* 1998, **37**:19–29.
 14. Hata A, Shi Y, Massague J: **TGF-beta signaling and cancer: structural and functional consequences of mutations in Smads.** *Mol Med Today* 1998, **4**:257–262.
 15. Dumont N: **Genetic and epigenetic contributions to colorectal cancer.** *APMIS* 1999, **107**:711–722.
 16. Welch DR, Fabra A, Nakajima M: **Transforming growth factor beta stimulates mammary adenocarcinoma cell invasion and metastatic potential.** *Proc Natl Acad Sci USA* 1990, **87**:7678–7682.
 17. Oft M, Peli J, Rudaz C, Schwarz H, Beug H, Reichmann E: **TGF-beta1 and Ha-Ras collaborate in modulating the phenotypic plasticity and invasiveness of epithelial tumor cells.** *Genes Dev* 1996, **10**: 2462–2477.
- Ras-transformed mouse mammary epithelial cells lose the growth inhibitory response to TGF- β and undergo a TGF- β -induced EMT that is maintained by the upregulated expression of TGF- β in an autocrine fashion.
18. Sehgal I, Baley PA, Thompson TC: **Transforming growth factor beta1 stimulates contrasting responses in metastatic versus primary mouse prostate cancer-derived cell lines *in vitro*.** *Cancer Res* 1996, **56**:3359–3365.
 19. Farina AR, Coppa A, Tiberio A, *et al*: **Transforming growth factor-beta1 enhances the invasiveness of human MDA-MB-231 breast cancer cells by up-regulating urokinase activity.** *Int J Cancer* 1998, **75**:721–730.
 20. Oft M, Heider KH, Beug H: **TGFbeta signaling is necessary for carcinoma cell invasiveness and metastasis.** *Curr Biol* 1998, **8**: 1243–1252.
- Inhibition of autocrine TGF- β signaling, by expression of dominant negative T β RII constructs, can revert the metastatic, invasive phenotype of tumor cells *in vitro* and *in vivo*, thus implying that the contribution of TGF- β signaling to invasion is a cell autonomous process.
21. Portella G, Cumming SA, Liddell J, *et al*: **Transforming growth factor beta is essential for spindle cell conversion of mouse skin carcinoma *in vivo*: implications for tumor invasion.** *Cell Growth Diff* 1998, **9**:393–404.
 22. Gobbi H, Arteaga CL, Jenson RA, *et al*: **Loss of expression of transforming growth factor beta type II receptor correlates with high tumor grade in human breast *in situ* and invasive carcinomas.** *Histopathology* 2000, in press.
 23. Hahn SA, Schutte M, Hoque AT, *et al*: **DPC4, a candidate tumor suppressor gene at human chromosome 18q21.1.** *Science* 1996, **271**:350–353.
 24. Jonson T, Gorunova L, Dawiskiba S, *et al*: **Molecular analyses of the 15q and 18q SMAD genes in pancreatic cancer.** *Genes Chromosomes Cancer* 1999, **24**:62–71.
 25. Kim SK, Fan Y, Papadimitrakopoulou V, *et al*: **DPC4, a candidate tumor suppressor gene, is altered infrequently in head and neck squamous cell carcinoma.** *Cancer Res* 1996, **56**:2519–2521.
 26. Lei J, Zou TT, Shi YQ, *et al*: **Infrequent DPC4 gene mutation in esophageal cancer, gastric cancer and ulcerative colitis-associated neoplasms.** *Oncogene* 1996, **13**:2459–2462.
 27. Schutte M, Hruban RH, Hedrick L, *et al*: **DPC4 gene in various tumor types.** *Cancer Res* 1996, **56**:2527–2530.
 28. Riggins GJ, Kinzler KW, Vogelstein B, Thiagalingam S: **Frequency of Smad gene mutations in human cancers.** *Cancer Res* 1997, **57**:2578–2580.
 29. Arai T, Akiyama Y, Okabe S, Ando M, Endo M, Yuasa Y: **Genomic structure of the human Smad3 gene and its infrequent alterations in colorectal cancers.** *Cancer Lett* 1998, **122**:157–163.
 30. Latil A, Pesche S, Valeri A, Fournier G, Cussenot O, Lidereau R: **Expression and mutational analysis of the MADR2/Smad2 gene in human prostate cancer.** *Prostate* 1999, **40**:225–231.
 31. Janji B, Melchior C, Gouon V, Vallar L, Kieffer N: **Autocrine TGF-beta-regulated expression of adhesion receptors and integrin-linked kinase in HT-144 melanoma cells correlates with their metastatic phenotype.** *Int J Cancer* 1999, **83**:255–262.
 32. Miettinen PJ, Ebner R, Lopez AR, Derynck R: **TGF-beta induced transdifferentiation of mammary epithelial cells to mesenchymal cells: involvement of type I receptors.** *J Cell Biol* 1994, **127**:2021–2036.
 33. Parsons R, Myeroff LL, Liu B, *et al*: **Microsatellite instability and mutations of the transforming growth factor beta type II receptor gene in colorectal cancer.** *Cancer Res* 1995, **55**:5548–5550.
 34. Thibodeau SN, Bren G, Schaid D: **Microsatellite instability in cancer of the proximal colon.** *Science* 1993, **260**:816–819.
 35. Yin JJ, Selander K, Chirgwin JM, *et al*: **TGF-beta signaling blockade inhibits PTHrP secretion by breast cancer cells and bone metastases development.** *J Clin Invest* 1999, **103**:197–206.
- This paper reports a lethal bone metastases model of MDA-231 human breast tumor cells in athymic mice. Expression of ectopic dominant negative T β RII into MDA-231 cells inhibits TGF- β -induced PTHrP production by tumor cells, tumor cell mediated osteolysis, and prolongs mouse survival. On the contrary, forced expression of a constitutively active T β RI expression vector accelerates these processes.
36. Kretzschmar M, Doody J, Timokhina I, Massague J: **A mechanism of repression of TGFbeta/Smad signaling by oncogenic Ras.** *Genes Dev* 1999, **13**:804–816.
- Ras, acting via Erk MAP kinases, causes phosphorylation at sites in the linker region of Smad2 and Smad3, which, in turn, inhibit Smad accumulation in the nucleus and TGF- β -mediated growth inhibition. Expression of a Ras-resistant Smad3 mutant, lacking the MAPK phosphorylation sites, restored transcriptional and cellular responses to exogenous TGF- β in Ras-transformed cells.
37. Prunier C, Mazars A, Noe V, *et al*: **Evidence that Smad2 is a tumor suppressor implicated in the control of cellular invasion.** *J Biol Chem* 1999, **274**:22919–22922.
- The tumor-derived missense mutation Smad2.D450E is not a 'loss of function mutation'; that is, it does not confer resistance to TGF- β -mediated growth inhibition. Rather unexpectedly, it behaves as a 'gain of function' mutation by promoting increased tumor cell invasiveness. Overexpression of Smad7 does not block this effect on invasion, suggesting that Smad2.D450E action does not require T β RI phosphorylation.
38. Calonge MJ, Massague J: **Smad4/DPC4 silencing and hyperactive ras jointly disrupt transforming growth factor-beta antiproliferative responses in colon cancer cells.** *J Biol Chem* 1999, **274**:33637–33643.
- Forced expression of Smad4 does not rescue TGF- β -mediated antiproliferative and transcriptional responses in SW480.7 colon cancer cells, which contain an activated Ki-ras oncogene. Cotransfection of Smad4 with a Ras

phosphorylation-resistant Smad3, however, restores TGF- β actions, suggesting that both loss of Smad4 function plus hyperactive Ras signaling partner in abrogating TGF- β antiproliferative responses.

39. Schwarte-Waldhoff I, Klein S, Blass-Kampmann S, *et al*: **DPC4/SMAD4 mediated tumor suppression of colon carcinoma cells is associated with reduced urokinase expression.** *Oncogene* 1999, **18**:3152–3158.
40. Goggins M, Shekher M, Turnacioglu K, Yeo CJ, Hruban RH, Kern SE: **Genetic alterations of the transforming growth factor beta receptor genes in pancreatic and biliary adenocarcinomas.** *Cancer Res* 1998, **58**:5329–5332.
41. Grady WM, Myeroff LL, Swinler SE, *et al*: **Mutational inactivation of transforming growth factor beta receptor type II in microsatellite stable colon cancers.** *Cancer Res* 1999, **59**:320–324.
42. Hocevar BA, Brown TL, Howe PH: **TGF-beta induces fibronectin synthesis through a c-Jun N-terminal kinase-dependent, Smad4 independent pathway.** *EMBO J* 1999, **18**:1345–1356.
First report of a TGF- β -regulated gene independent of Smad4 function.
43. Morton DM, Barrack ER: **Modulation of transforming growth factor beta 1 effects on prostate cancer cell proliferation by growth factors and extracellular matrix.** *Cancer Res* 1995, **55**:2596–2602.
44. Hasina R, Matsumoto K, Matsumoto-Taniura N, Kato I, Sakuda M, Nakamura T: **Autocrine and paracrine motility factors and their involvement in invasiveness in a human oral carcinoma cell line.** *Br J Cancer* 1999, **80**:1708–1717.
45. Zicha D, Genot E, Dunn GA, Kramer IM: **TGFbeta1 induces a cell-cycle-dependent increase in motility of epithelial cells.** *J Cell Sci* 1999, **112**:447–454.
46. Hojo M, Morimoto T, Maluccio M, *et al*: **Cyclosporine induces cancer progression by a cell-autonomous mechanism.** *Nature* 1999, **397**:530–534.
47. Liu X, Sun Y, Constantinescu SN, Karam E, Weinberg RA, Lodish HF: **Transforming growth factor beta-induced phosphorylation of Smad3 is required for growth inhibition and transcriptional induction in epithelial cells.** *Proc Natl Acad Sci USA* 1997, **94**:10669–10674.
48. Datto MB, Frederick JP, Pan L, Borton AJ, Zhuang Y, Wang XF: **Targeted disruption of Smad3 reveals an essential role in transforming growth factor beta-mediated signal transduction.** *Mol Cell Biol* 1999, **19**:2495–2504.
49. Atfi A, Djelloul S, Chastre E, Davis R, Gespach C: **Evidence for a role of Rho-like GTPases and stress-activated protein kinase/c-Jun N-terminal kinase (SAPK/JNK) in transforming growth factor beta-mediated signaling.** *J Biol Chem* 1997, **272**:1429–1432.
50. Engel ME, Datta PK, Moses HL: **RhoB is stabilized by transforming growth factor beta and antagonizes transcriptional activation.** *J Biol Chem* 1998, **273**:9921–9926.
51. Knudsen KA, Frankowski C, Johnson KR, Wheelock MJ: **A role for cadherins in cellular signaling and differentiation.** *J Cell Biochem Suppl* 1998, **30–31**:168–176.
52. Islam S, Carey TE, Wolf GT, Wheelock MJ, Johnson KR: **Expression of N-cadherin by human squamous carcinoma cells induces a scattered fibroblastic phenotype with disrupted cell-cell adhesion.** *J Cell Biol* 1996, **135**:1643–1654.
53. Liotta LA, Stetler-Stevenson WG: **Tumor invasion and metastasis: an imbalance of positive and negative regulation.** *Cancer Res* 1991, **51**(suppl):S5054–S5059.
54. Overall CM, Wrana JL, Sodek J: **Transcriptional and post-transcriptional regulation of 72-kDa gelatinase/type IV collagenase by transforming growth factor-beta 1 in human fibroblasts. Comparisons with collagenase and tissue inhibitor of matrix metalloproteinase gene expression.** *J Biol Chem* 1991, **266**:14064–14071.
55. Samuel SK, Hurta RA, Kondaiah P, *et al*: **Autocrine induction of tumor protease production and invasion by a metallothionein-regulated TGF-beta 1 (Ser223, 225).** *EMBO J* 1992, **11**:1599–1605.
56. Coussens LM, Raymond WW, Bergers G, *et al*: **Inflammatory mast cells up-regulate angiogenesis during squamous epithelial carcinogenesis.** *Genes Dev* 1999, **13**:1382–1397.
57. Pepper MS: **Transforming growth factor-beta: vasculogenesis, angiogenesis, and vessel wall integrity.** *Cytokine Growth Factor Rev* 1997, **8**:21–43.
58. Lux A, Attisano L, Marchuk DA: **Assignment of transforming growth factor beta1 and beta3 and a third new ligand to the type I receptor ALK-1.** *J Biol Chem* 1999, **274**:9984–9992.
59. Barbara NP, Wrana JL, Letarte M: **Endoglin is an accessory protein that interacts with the signaling receptor complex of multiple members of the transforming growth factor-beta superfamily.** *J Biol Chem* 1999, **274**:584–594.
60. Bruno E, Horrigan SK, Van Den Berg D, *et al*: **The Smad5 gene is involved in the intracellular signaling pathways that mediate the inhibitory effects of transforming growth factor-beta on human hematopoiesis.** *Blood* 1998, **91**:1917–1923.
61. Dickson MC, Martin JS, Cousins FM, Kulkarni AB, Karlsson S, Akhurst RJ: **Defective haematopoiesis and vasculogenesis in transforming growth factor-beta 1 knock out mice.** *Development* 1995, **121**:1845–1854.
62. Oshima M, Oshima H, Taketo MM: **TGF-beta receptor type II deficiency results in defects of yolk sac hematopoiesis and vasculogenesis.** *Dev Biol* 1996, **179**:297–302.
63. Oh SP, Seki T, Goss KA, *et al*: **ALK-1 modulates TGF-beta1 signaling during vascular development.** In *Proceedings of the Third International TGF-Beta Conference: Biological Mechanisms and Clinical Applications*. September 20–22 1999, NIH, Bethesda, MD.
64. Li DY, Sorensen LK, Brooke BS, *et al*: **Defective angiogenesis in mice lacking endoglin.** *Science* 1999, **284**:1534–1537.
65. Yang X, Castilla LH, Xu X, *et al*: **Angiogenesis defects and mesenchymal apoptosis in mice lacking SMAD5.** *Development* 1999, **126**:1571–1580.
66. Hirschi KK, Rohovsky SA, D'Amore PA: **PDGF, TGF-beta, and heterotypic cell-cell interactions mediate endothelial cell-induced recruitment of 10T1/2 cells and their differentiation to a smooth muscle fate [published erratum appears in J Cell Biol 1998, 141(5):1287].** *J Cell Biol* 1998, **141**:805–814.
In response to activated TGF- β 1, 10T1/2 fibroblasts migrate toward endothelial cells and acquire a smooth muscle phenotype. This effect is blocked by TGF- β neutralizing antibodies, suggesting that TGF- β s are critical for heterotypic cell-cell interactions involved in pericyte recruitment to nascent capillaries and in microvessel wall integrity.
67. Benjamin LE, Golijanin D, Itin A, Podes D, Keshet E: **Selective ablation of immature blood vessels in established human tumors follows vascular endothelial growth factor withdrawal.** *J Clin Invest* 1999, **103**:159–165.
68. Higaki M, Shimokado K: **Phosphatidylinositol 3-kinase is required for growth factor induced amino acid uptake by vascular smooth muscle cells.** *Arterioscler Thromb Vasc Biol* 1999, **19**:2127–2132.
69. Ueki N, Nakazato M, Ohkawa T, *et al*: **Excessive production of transforming growth factor beta 1 can play an important role in the development of tumorigenesis by its action for angiogenesis: validity of neutralizing antibodies to block tumor growth.** *Biochim Biophys Acta* 1992, **1137**:189–196.
70. Ananth S, Knebelmann B, Gruning W, *et al*: **Transforming growth factor beta1 is a target for the von Hippel-Lindau tumor suppressor and a critical growth factor for clear cell renal carcinoma.** *Cancer Res* 1999, **59**:2210–2216.

71. O'Mahony CA, Albo D, Tuszynski GP, Berger DH: **Transforming growth factor-beta 1 inhibits generation of angiostatin by human pancreatic cancer cells.** *Surgery* 1998, **124**:388–393.
72. Relf M, LeJeune S, Scott PA, *et al*: **Expression of the angiogenic factors vascular endothelial cell growth factor, acidic and basic fibroblast growth factor, tumor growth factor beta-1, platelet-derived endothelial cell growth factor, placenta growth factor, and pleiotrophin in human primary breast cancer and its relation to angiogenesis.** *Cancer Res* 1997, **57**:963–969.
73. Letterio JJ, Roberts AB: **Regulation of immune responses by TGF-beta.** *Annu Rev Immunol* 1998, **16**:137–161.
74. Torre-Amione G, Beauchamp RD, Koeppen H, *et al*: **A highly immunogenic tumor transfected with a murine transforming growth factor type beta 1 cDNA escapes immune surveillance.** *Proc Natl Acad Sci USA* 1990, **87**:1486–1490.
75. McAdam AJ, Felcher A, Woods ML, *et al*: **Transfection of transforming growth factor-beta producing tumor EMT6 with interleukin-2 elicits tumor rejection and tumor reactive cytotoxic T-lymphocytes.** *J Immunother Emphasis Tumor Immunol* 1994, **15**:155–164.
76. Won J, Kim H, Park EJ, Hong Y, Kim SJ, Yun Y: **Tumorigenicity of mouse thymoma is suppressed by soluble type II transforming growth factor beta receptor therapy.** *Cancer Res* 1999, **59**:1273–1277.
77. Shull MM, Ormsby I, Kier AB, *et al*: **Targeted disruption of the mouse transforming growth factor-beta 1 gene results in multifocal inflammatory disease.** *Nature* 1992, **359**:693–699.
78. Wallick SC, Figari IS, Morris RE, Levinson AD, Palladino MA: **Immunoregulatory role of transforming growth factor beta (TGF-beta) in development of killer cells: comparison of active and latent TGF-beta 1.** *J Exp Med* 1990, **172**:1777–1784.
79. Arteaga CL, Hurd SD, Winnier AR, Johnson MD, Fendly BM, Forbes JT: **Anti-transforming growth factor (TGF)-beta antibodies inhibit breast cancer cell tumorigenicity and increase mouse spleen natural killer cell activity. Implications for a possible role of tumor cell/host TGF-beta interactions in human breast cancer progression.** *J Clin Invest* 1993, **92**:2569–2576.
80. Li XF, Takiuchi H, Zou JP, *et al*: **Transforming growth factor-beta (TGF-beta) mediated immunosuppression in the tumor-bearing state: enhanced production of TGF-beta and a progressive increase in TGF-beta susceptibility of anti-tumor CD4⁺ T cell function.** *Jpn J Cancer Res* 1993, **84**:315–325.
81. Kuppner MC, Hamou MF, Bodmer S, Fontana A, de Tribolet N: **The glioblastoma derived T-cell suppressor factor/transforming growth factor beta 2 inhibits the generation of lymphokine-activated killer (LAK) cells.** *Int J Cancer* 1988, **42**:562–567.
82. Fakhrai H, Dorigo O, Shawler DL, *et al*: **Eradication of established intracranial rat gliomas by transforming growth factor beta antisense gene therapy.** *Proc Natl Acad Sci USA* 1996, **93**:2909–2914.
83. Park JA, Wang E, Kurt RA, Schluter SF, Hersh EM, Akporiaye ET: **Expression of an antisense transforming growth factor-beta1 transgene reduces tumorigenicity of EMT6 mammary tumor cells.** *Cancer Gene Ther* 1997, **4**:42–50.
84. Wu SP, Theodorescu D, Kerbel RS, *et al*: **TGF-beta 1 is an autocrine-negative growth regulator of human colon carcinoma FET cells *in vivo* as revealed by transfection of an antisense expression vector.** *J Cell Biol* 1992, **116**:187–196.
85. Wu SP, Sun LZ, Willson JK, Humphrey L, Kerbel R, Brattain MG: **Repression of autocrine transforming growth factor beta 1 and beta 2 in quiescent CBS colon carcinoma cells leads to progression of tumorigenic properties.** *Cell Growth Diff* 1993, **4**:115–123.
86. Gohongi T, Fukumura D, Boucher Y, *et al*: **Tumor-host interactions in the gallbladder suppress distal angiogenesis and tumor growth: involvement of transforming growth factor beta1.** *Nat Med* 1999, **5**:1203–1208.
87. Bandyopadhyay A, Zhu Y, Cibull ML, Bao L, Chen C, Sun L: **A soluble transforming growth factor beta type III receptor suppresses tumorigenicity and metastasis of human breast cancer MDA-MB-231 cells.** *Cancer Res* 1999, **59**:5041–5046.
88. George J, Roulot D, Koteliensky VE, Bissell DM: ***In vivo* inhibition of rat stellate cell activation by soluble transforming growth factor beta type II receptor: a potential new therapy for hepatic fibrosis.** *Proc Natl Acad Sci USA* 1999, **96**:12719–12724.
89. Nakao A, Fujii M, Matsumura R, *et al*: **Transient gene transfer and expression of Smad7 prevents bleomycin-induced lung fibrosis in mice.** *J Clin Invest* 1999, **104**:5–11.

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Sponsorship: This review was supported by US Army DAMD17-98-1-8263 Pre-doctoral Training Award (to ND), PHS grant R01 CA62212, US Army DAMD17-98-1-8262 IDEA grant, a Department of Veteran Affairs Clinical Investigator Award (to CLA), and a Vanderbilt-Ingram Cancer Center support grant CA68485

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