TGF-β antagonists: Why suppress a tumor suppressor?

Commentary

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Tumor metastasis is the major determinant of cancer patient survival. This ultimate phase in tumorigenesis depends on the ability of a tumor cell to invade the stroma, migrate in and out of blood or lymphatic vessels, and survive and re-establish itself at a secondary site. A large number of papers have provided strong evidence for a role of TGF-β in tumor invasion and/or metastasis (1–6). Now, two papers in this issue of the *JCI* highlight this clinically significant action of TGF-β in tumorigenesis and provide very encouraging results regarding both the efficacy and the low toxicity of a soluble TGF-β receptor antagonist that effectively reduces tumor spread (7, 8).

Positive and negative effects of TGF-β signaling in cancer

TGF-β is a potent growth inhibitor of all epithelial and hematopoietic cells and can also induce apoptosis (1–3). For this reason, much emphasis has been placed on elucidating TGF-β signaling pathways, particularly those responsible for growth inhibition (summarized in Figure 1). After activation of the TGFβ type II/TGFβ type I (TβRII/TβRI) receptor complex, TGF-βs signal predominantly via the Smad pathway, although the activated receptor complex can also signal independently of Smads, via phosphatidylinositol 3-kinase (PI3K), protein phosphatase 2A/p70 S6 kinase (PP2A/p70S6K), and various mitogen-activated protein kinase (MAPK) pathways. There is also interplay between these pathways, such that activation of the Ras pathway or other non-Smad pathways can modulate signaling via Smads (1–6).

Homozygous mutations or deletions in the genes for Smad4, TβRII, or Smad2 are observed in some human tumors (1–3), suggesting a significant role for TGF-β signaling in tumor suppression. Nevertheless, only a minority of tumors show this type of genetic aberration, and the most commonly deleted such gene, *MADH4* (encoding Smad4), is not essential for all TGF-β activities (1–3). Some authors have suggested that the tumor-suppressing function of *MADH4* can be attributed to its antiangiogenic effect (not necessarily mediated by TGF-β), rather than to growth inhibition (9).

The tumor-suppressive effects of TGF-β have been clearly demonstrated in transgenic mouse models. Hemizygous or homozygous *Tgfb1*-null animals show an increased incidence of chemically or spontaneously induced tumors, respectively (1–3, 10, 11). Similarly, targeting a dominant negative TβRII to mammary or skin epithelia also enhances tumorigenicity, whereas TGF-β1–overexpressing mice have a decreased incidence of tumors (1–3). This tumor-suppressive function of TGF-β has raised concerns about the use of TGF-β antagonists to treat cancer, despite the increasingly strong evidence that TGF-β1 can promote tumor metastasis.

It is widely accepted that during multistage tumorigenesis, TGF-β growth-inhibitory and apoptotic effects are lost, frequently by subversion of the normal signaling pathway due to activation of other signaling molecules including PI3K and Ras (1–3). Meanwhile, other TGF-β responses prevail, unrelated to

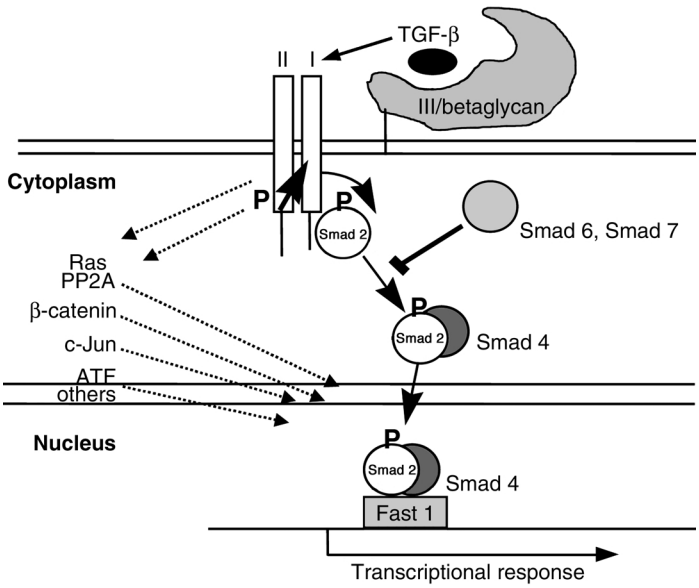

Figure 1
The TGF-β signaling pathway. TGF-βs bind and activate the TGF-β receptor complex, which transmits signal predominantly via activation and nuclear translocation of Smad proteins. However, several Smad-independent signaling pathways are also activated by this receptor complex, and the outcome of Smad signaling can be modified by interaction with other signaling pathways (1).

growth inhibition and favoring tumorigenesis (Figure 2; refs. 1–3). Moreover, as tumor cells progress, they secrete ever-increasing quantities of TGF-β1 (1–3, 6). TGF-β activity is increased partly by autostimulation of the *Tgfb1* gene, but also through transcriptional activation by Ras and other effectors, as well as by the action of proteases that activate the latent TGF-β in the ECM (1–3, 6).

In response to elevated TGF-β levels, the tumor cell becomes more migratory and invasive. Indeed, in cooperation with activated Ras, TGF-β1 can induce a complete epithelioid-to-fibroblastoid transition in both mammary and keratinocyte-derived tumors (1–3, 6), and it can drive metastasis of epithelioid tumors (6–8, 12). TGF-β can also stimulate tumor angiogenesis, alter the stromal environment, and cause local and systemic immunosuppression, all of which contribute to tumor progression and metastasis (1–3).

As discussed in the two articles in this issue of the *JCI* (7, 8), the concept of using soluble protein antagonists that bind and inactivate extracellular TGF-β was first tested over a decade ago using decorin, a natural inhibitor of TGF-β, in a therapeutic model for fibrosis (8). More recently, the chimeric Fc:TβRII protein used in the current studies has proved attractive because of its high affinity for TGF-β, its ready purification by protein A affinity chromatography, and its effectiveness in a number of models of fibrosis.

Early attempts to demonstrate the efficacy of this approach involved stably transfected glioma (13), thymoma (14), pancreatic (15), or metastatic breast tumor cell lines (16) carrying cDNAs for soluble forms of decorin (13), TβRII (14, 15), or TβRIII (16). Each demonstrated tumor suppression after subsequent injection of the modified tumor cell line into mice. In the first two cases (13, 14), this was attributed to re-acquisition of tumor-specific cellular immunity, whereas the effects on the pancreas and breast cancer lines included suppression of invasion (15), angiogenesis (15), and lung metastasis (16).

Efficacy and toxicity

The articles in this issue of the *JCI* (7, 8) have pushed the story two steps further, firstly by applying soluble Fc:TβRII as an injectable drug to prove efficacy in suppression of breast tumor metastasis in vivo (7), and secondly by screening for any adverse effects on the mice after lifetime exposure to high-level circulating Fc:TβRII (8). Muraoka et al. (7), using the MMTV-PyV mT transgenic model of mammary tumorigenesis, show that twice-weekly intraperitoneal injection of Fc:TβRII reduces lung metastasis tenfold. Fc:TβRII treatment also inhibits metastasis of two metastatic mammary cell lines. In all three cases, Fc:TβRII has no effect on proliferative rate of the primary tumor cells. Yang et al. (8) take a different approach, focusing on possible adverse effects in transgenic mice that stably express soluble Fc:TβRII. Circulating Fc:TβRII, which is found at about 1 mg/ml in the blood, not only reduces metastasis formation of melanoma cells injected into the tail vein of the mice but also reduces metastasis to the lung from endogenous mammary tumors that arise when the mice are crossed onto the MMTV-Neu transgenic model of mammary carcinogenesis. Both groups find that Fc:TβRII leads to no changes in tumor latency, yield, or size.

Taken together, the two papers (7, 8) show that soluble Fc:TβRII is efficacious in reducing tumor metastasis, whether delivered genetically from within the neoplastic cell or administered as an injectable circulating drug. Both groups also addressed the mechanisms of action of Fc:TβRII in attenuating metastatic spread. In the MMTV-PyV mT model, Muraoka et al. (7) specifically exclude an effect on TGF-β–induced angiogenesis. In their model, Fc:TβRII appears to decrease tumor cell intravasation and/or decrease survival of tumor cells in the circulation, since the number of circulating tumor cells is lower in the Fc:TβRII-treated mice than in controls (7). In support of this mechanism, Smad2 activation has recently been shown to drive tumor cell extravasation in a skin tumor model (6). Consistent with an effect on tumor intravasation, the Fc:TβRII-treated mammary tumor cells are altered toward a more differentiated, less motile/migratory phenotype than is seen in untreated tumor cells. Production of active matrix metalloproteinase 2 (MMP2) and MMP9, proteases thought to be important in tumor invasion, migration, and intravasation, is diminished and apoptosis is elevated in response to Fc:TβRII (7).

In the injectable melanoma model, Yang et al. (8) argue, the effect of Fc:TβRII on metastasis is likely indirect, possibly including decreased angiogenesis and/or elevated immunosuppression. Although metastasis is diminished in the Fc:TβRII transgenic mice following tail vein injections of melanoma cells, the initial appearance of micro-metastases

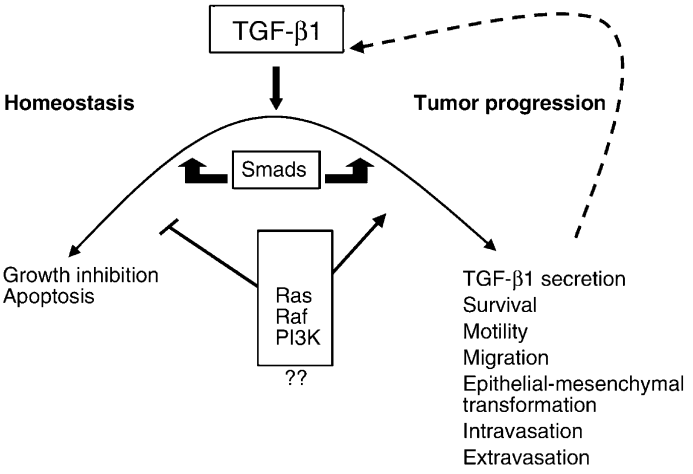

Figure 2
The balance between the autocrine homeostatic and tumor-progressing activities of TGF-β is perturbed by activation of oncogenic signaling pathways. As tumor progression proceeds, the homeostatic branch of TGF-β action becomes increasingly compromised, and tumors secrete more TGF-β1, thus exacerbating tumor progression.

is no different from that seen in wild-type mice. Since TGF-β has multiple actions that can drive tumor metastasis, the exact mechanisms involved are probably context-specific, depending on the tumor type, genetic constitution, and the exact stage of carcinogenesis of the tumor. However, the exciting take-home message is that soluble TGF-β antagonists can significantly decrease metastasis in models of breast cancer and melanoma, as previously suggested for thymoma (13), glioma (14), and pancreatic (15) and mammary carcinoma (16).

The complete absence of TGF-β1 in mice leads to death resulting from uncontrolled systemic inflammation, and even a T cell–specific deficit in TGF-β causes lethal immune system defects (discussed in ref. 8). Therefore, the apparent absence of side effects, even after lifetime exposure to approximately 1 mg/ml circulating Fc:TβRII (8), is particularly encouraging. No immune phenotype was seen in the study of Yang et al. (8), apart from a minimal increase in memory T cells, and a nonsignificant increase in lymphocytic infiltration into organs of aged mice. The authors employed several techniques to demonstrate that Fc:TβRII at this level does not completely block all TGF-β1 bioactivity in vivo (8). Indeed, although circulating TGF-β levels are reduced in the Fc:TβRII transgenics to probably ≤10% of the wild-type level, these animals still thrive in the laboratory. It would be interesting to examine how the mice fare when challenged with other environmental hazards such as foreign antigens and pathogens.

Also heartening is the finding by both groups that soluble Fc:TβRII had no tumor-promoting action in vivo. Conversely, mice in which TGF-β activity is diminished globally, as a result of hemizygosity for *Tgfb1*, are tumor-prone (10, 11), as are animals in which this factor is ablated in a tissue-specific manner using dominant negative (DN) TβRII. The basis of this discrepancy is uncertain, but it may be that Fc:TβRII preferentially targets circulating TGF-β1 because it is too bulky to gain access to the more functionally important TGF-β tightly bound to the cell surface or ECM. In addition, different thresholds of TGF-β activity required for the growth-suppressing and the metastasis-promoting effects of TGF-β could help account for the tumor incidence seen in DN-TβRII transgenic strains. Growth inhibition, for example, is more sensitive than other TGF-β responses to decreases in the level of TβRII (3, 17). Moreover, recent studies in a skin carcinogenesis model do indeed show that a high threshold of Smad2 activation must be surpassed in order to drive metastatic spread (6).

Nevertheless, since *Tgfb1*$^{+/-}$ animals have an increased incidence of chemically induced tumorigenesis (10), one might expect a similar phenotype in Fc:TβRII mice (8), but this is not the case. The explanation probably lies in the different models used. The tumors and cell lines studied by Muraoka et al. (7) have already lost growth sensitivity to TGF-β, as assessed by BrdU incorporation, so tumor-suppressive effects of TGF-β would not be expected. The TGF-β growth sensitivity of MMTV-Neu tumor cells has not been studied, but transfection of normal differentiated thyroid cells with ErbB2 (Neu) attenuates the growth-inhibitory response to TGF-β, suggesting that Neu does indeed attenuate growth sensitivity to TGF-β (18). In this context, chemical carcinogenesis studies on Fc:TβRII transgenic mice are warranted to uncover any tumor-promoting effects of Fc:TβRII, especially in view of the fact that a soluble TβRII transfected into a hepatoma cell line has been shown to promote tumor development (19).

Despite these reservations, Fc:TRII clearly is highly efficacious in reducing metastasis and is of exceptionally low toxicity in mice. Indeed, many drugs for treatment of both malignant and nonmalignant conditions, such as cyclosporin, have tumor-promoting activity (12), and most cancer drugs show general cytotoxicity levels orders of magnitude higher than does this soluble TβRII receptor.

Future developments in anti–TGF-β drug design

Pharmaceutical companies have avoided TGF-β agonists or antagonists, partly because of fear of nonspecificity and consequent side effects. The articles in this issue of the *JCI* (7, 8) might cause them to reconsider this decision. TGF-β antagonists such as Fc:TβRII could prove as useful clinically as Herceptin (20), an anti-Neu antibody used for the treatment of Neu-positive breast tumors. They would also be expected to have a wider range of applications, since metastasis of many tumor types may be inhibited by their use.

Small-molecule inhibitors of TGF-β action could also be of value and should offer better drug specificity than the fusion protein described here. Their design will depend on a greater understanding of the cross-talk between the intracellular signaling pathways that propagate TGF-β metastatic versus homeostatic signals in different cell and tumor types (Figure 2). However, it should be possible to design and select small-molecule drugs that specifically inhibit the invasion/metastasis branch of TGF-β action, while leaving growth-inhibitory and apoptotic pathways intact. Inhibition of the Ras/Raf and/or PI3K pathways, in addition to blocking the cell survival and mitogenic effects of these pathways, might also attenuate the adverse effects of TGF-β (1). Hence, combination therapies using metastasis inhibitors that target TGF-β, as well as specific Ras/Raf and/or PI3K inhibitors, might be particularly efficacious and safe.

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