

TGF- β and cancer

Michael Reiss

Department of Medicine (Medical Oncology) and Yale Cancer Center, Yale University School of Medicine, 333 Cedar Street, New Haven, CT 06520, CT, USA

ABSTRACT – The relationships between transforming growth factor- β (TGF- β) and cancer are varied and complex. The paradigm that is emerging from the experimental evidence accumulated over the past decade or so is that TGF- β can play two different and opposite roles with respect to the process of malignant progression. During early stages of carcinogenesis, TGF- β acts predominantly as a potent tumor suppressor and may mediate the actions of chemopreventive agents such as retinoids and nonsteroidal anti-estrogens. However, at some point during the development and progression of malignant neoplasms, bioactive TGF- β s make their appearance in the tumor microenvironment and the tumor cells escape from TGF- β -dependent growth arrest. In many cases, this resistance to TGF- β is the consequence of loss or mutational inactivation of the genes that encode signaling intermediates. These include the types I and II TGF- β receptors, as well as receptor-associated and common-mediator Smads. The stage of tumor development or progression at which TGF- β -resistant clones come to dominate the tumor cell population in different types of neoplasm remains to be defined. The phenotypic switch from TGF- β -sensitivity to TGF- β -resistance that occurs during carcinogenesis has several important implications for cancer prevention and treatment. © 1999 Éditions scientifiques et médicales Elsevier SAS

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1. Introduction

In normal self-renewing tissues, whether or not a given cell enters the cell cycle and proceeds through cell division is largely dependent on the balance between the stimulatory (mitogenic) and inhibitory (antimitogenic) input signals it receives from its microenvironment. Transforming growth factor- β (TGF- β) is the prototypical microenvironmental regulatory molecule that signals cell cycle arrest. TGF- β acts primarily on epithelial and lymphoid cells and directly counteracts the effects of many different mitogenic growth factors [1].

A fundamental characteristic of malignant tumor cells is their ability to traverse the cell cycle in a manner that is independent from microenvironmental controls. In general, this autonomous proliferation of transformed cells appears to be driven by the constitutive activation of mitogenic signaling pathways such as, for example, the constitutive autocrine production of a mitogenic growth factor, or the activation of a growth factor receptor or downstream signaling intermediate. However, activation of a mitogenic pathway in and of itself is not sufficient for neoplastic transformation. Physiological countermeasures are invoked that limit the clonal expansion of the mutant cells, thereby protecting the organism against the out-

growth of a neoplastic clone (reviewed in [2]). These measures include, for example, the induction of cell senescence or apoptosis [2, 3]. In these cases, only cells that have acquired additional mutations that inactivate the senescence or apoptosis pathways (e.g., in p16^{INK4a}, p19^{ARF}, or p53) will be able to progress to the next step in neoplastic transformation [2–4].

Given its ability to inhibit cell cycle progression, activation of TGF- β within the microenvironment of transformed cells may represent yet another mechanism used to counteract the clonal expansion of mitogenically stimulated cells. If this is correct, these cells would have to overcome the inhibitory effects of TGF- β on cell proliferation in order to progress to frank malignancy. In this context, then, TGF- β can legitimately be viewed as a tumor suppressor [5, 6]. On the other hand, there is considerable experimental evidence that fully transformed malignant tumor cells themselves are often the primary source of bioactive TGF- β s. It is surmised that tumor-associated TGF- β provides neoplastic cells with a selective advantage by, for example, circumventing immune surveillance, inducing angiogenesis or facilitating invasion and metastasis. Whichever scenario applies in any particular tumor, the net result is that the presence of activated TGF- β selectively favors the clonal expansion of

TGF- β -refractory tumor cell populations. One would predict that these clones would be characterized by distinct genetic traits that allow them to escape from TGF- β -mediated growth control. In this chapter, I will discuss the extent to which existing experimental evidence supports this model, and discuss its implications for the design and application of therapeutic strategies for the prevention and treatment of cancer.

2. TGF- β 1 is a tumor suppressor

A number of transgenic mouse studies have provided strong evidence for the idea that one of the physiological roles of the TGF- β signaling pathway is to provide protection against malignant transformation. For example, transgenic mice that produce a constitutively active form of TGF- β 1 are resistant to DMBA-induced mammary tumor formation [7]. Furthermore, when these mice were crossed with mice that develop mammary tumors at a high rate because they overexpress the epithelial mitogen TGF- α , the incidence of mammary tumors in the offspring was markedly reduced [7]. Conversely, the absence of TGF- β 1 appears to promote tumor formation. Although targeted homozygous deletion of the TGF- β 1 gene results in midgestation loss due to defects in yolk sac vasculogenesis or death shortly postpartum due to a multisystemic inflammatory syndrome, primary skin keratinocytes isolated from TGF- β 1⁻ null embryos have been shown to be highly susceptible to malignant conversion by the *v-ras^{tsA}* oncogene [8–10]. Moreover, treatment of heterozygous (TGF- β 1^{+/-}) animals with various tumor induction protocols also resulted in a much higher number of malignant lung and mammary tumors [11] or pituitary adenomas [12] than in wild-type (TGF- β 1^{+/+}) animals. In some cases, loss of TGF- β expression may represent an early step in tumor progression in vivo. For example, preneoplastic colonic aberrant crypt foci in the rat have been noted to express lower levels of TGF- β than surrounding unaffected crypts [13]. Similarly, transfection of weakly tumorigenic colon carcinoma cells with anti-sense TGF- β suppressed endogenous TGF- β expression and dramatically increased the size of tumors formed by these cells in vivo [14, 15].

Based on these findings, one would predict that cells that are inherently less susceptible to TGF- β 's antiproliferative effects would also be predisposed to malignant transformation. This indeed appears to be the case. Several studies have been conducted using transgenic animals that carry a dominant-negative mutant TGF- β type II receptor (T β R-II) gene. These animals develop normally. Although they do not appear to be prone to spontaneous tumors [16], many of these animals readily develop malignant mammary and lung carcinomas when treated with the carcinogen DMBA [17]. A similar situation may exist in humans. We and others have recently described a variant of the TGF- β type I receptor (T β R-I) that lacks three of nine consecutive alanines at the N-terminus and that has a slightly diminished signaling capacity [18, 19]. Interestingly, the rare individuals who are homozygous carriers of this T β R-I variant appear to be at significantly higher risk

for the development of a variety of different types of malignant neoplasms [18, 19].

Besides targeted disruption of the T β R-II receptor, Zhu et al. reported recently that Smad3 mutant mice develop highly invasive and metastatic colorectal adenocarcinomas between four and six months of age [20]. Targeted homozygous deletion of the common-mediator Smad, Smad4, leads to embryonic lethality [21, 22]. However, in compound heterozygote mice that carry both adenomatous polyposis coli (APC) and Smad4 gene mutations, intestinal polyps develop into malignant tumors much more frequently than those in the simple APC mutant heterozygotes [21]. Furthermore, the reintroduction of Smad4 into Smad4 null carcinoma cells restores sensitivity to TGF- β in vitro and suppresses tumorigenicity in vivo [23–26].

In aggregate, these studies have provided overwhelming support for the idea that whether or not the initiating carcinogenic event will allow neoplastic clones to emerge is critically dependent on the presence or absence of active TGF- β in the microenvironment and on the integrity of the TGF- β signaling pathway in the target cell.

3. Production of TGF- β in association with human neoplasia

Since DeLarco and Todaro [27] first described the presence of TGF- β in conditioned medium of murine sarcoma-virus-transformed NIH 3T3 fibroblasts more than two decades ago, numerous RNA and DNA viruses and viral proteins, including cytomegalovirus, Epstein-Barr virus, and the human papillomavirus type 16 E6 protein, have been shown to directly activate the TGF- β 1 gene promoter and to induce TGF- β production by the cells they infect [28–34]. It may well be that the induction of TGF- β by oncogenic viruses represents a physiological response designed to counteract the effects of excessive cell proliferation. On the other hand, these viruses might induce TGF- β to provide the virus-infected cells with a selective advantage, for example by allowing them to evade immune surveillance. The fact that human papillomavirus-immortalized primary human keratinocytes are exquisitely sensitive to TGF- β early on, and only become refractory to TGF- β after a prolonged passage in culture, seems to favor the former possibility [35].

Besides virus-transformed cells, many human carcinoma cell lines have also been shown to secrete bioactive TGF- β s in vitro, although the molecular mechanisms remain to be elucidated [6, 36]. Moreover, in many cases, TGF- β s are produced by human tumors in vivo as well. For example, plasma levels of TGF- β are often elevated in patients with cancer, and the rate of urinary excretion of TGF- β appears to correlate with tumor burden [37–42].

In addition, numerous studies have shown that TGF- β s can be detected in tissue specimens from a variety of tumor types. In general, tumor tissue appears to express higher levels of TGF- β than the corresponding normal tissue, the association of TGF- β s with cancer is strongest in the most advanced stages of tumor progression, and the overexpression of TGF- β has a negative impact on prog-

nosis. For example, TGF- β 1, -2, and -3-specific mRNAs can be detected in the majority of primary breast cancers [43] and may be more abundant than in normal breast tissue [44]. In addition, immunostainable TGF- β 1, -2, and -3 are present in and around the majority of primary human breast carcinomas [45-49]. Furthermore, a significantly greater fraction of invasive carcinomas expressed immunodetectable TGF- β than in situ carcinomas [45], and the strongest staining was observed in invasive carcinomas with associated lymph node metastases [47, 50], suggesting that there may be a relationship between TGF- β production and tumor progression.

Increased expression of TGF- β 1, -2, and -3 mRNA and/or protein has also been noted in gastric carcinomas compared to normal gastric mucosa [51-55]. Interestingly, in gastric cancer, TGF- β is present not only in the epithelial compartment, but also in tumor stroma [52, 53]. The association appears to be particularly strong, with the diffuse or linitis plastica type as opposed to the 'intestinal' type of gastric cancer [51, 53, 56-60].

Several studies have indicated that TGF- β expression is associated with progression of colorectal cancers. For example, TGF- β 1 mRNA and protein expression in invasive colon cancers appears to be stronger than in adenomas, and higher in adenomas with dysplasia than in adenomas without dysplasia or in normal colon mucosa [61]. TGF- β staining was present in both the epithelial and stromal compartments of colorectal tumors [61]. Moreover, Picon et al. [62] showed that TGF- β 1 protein expression was higher in lymph node metastases than in the corresponding primary colon carcinoma. In one study, plasma TGF- β 1 levels were found to be elevated prior to resection of colorectal cancers, to decrease after surgery, and to correlate with the presence of TGF- β 1 in the tumor and with tumor stage [38].

TGF- β 1 mRNA levels also appear to be increased in prostate cancer tissue compared with benign prostatic hypertrophy and normal prostate [63]. In addition, extracellular TGF- β 1 expression detected by immunohistochemistry was significantly stronger in prostate cancer specimens than in cases of benign prostatic hypertrophy [64]. In a follow-up study, Eastham et al. [65] showed that lymph node metastases expressed less extracellular TGF- β than the corresponding primary tumors. The significance of this observation is unclear. Eklov et al. [66] showed that immunostainable TGF- β was present in both epithelial and stromal cell compartments of invasive prostate cancers. Interestingly, prostate cancer specimens were mostly negative for latent TGF- β binding protein (LTBP), whereas LTBP was expressed in normal prostate and in benign prostatic hyperplasia. This observation suggests the possibility that absence of LTBP may result in the constitutive activation of the TGF- β secreted by prostate cancer cells.

TGF- β 1 mRNA levels were increased in bladder carcinoma specimens compared with normal bladder mucosa [67]. However, expression was higher in low and intermediate grade tumors than in high grade tumors, and higher in superficial than in invasive tumors. Similarly, TGF- β 1 protein levels were found to be highest in super-

ficial and early stage invasive bladder cancer compared to normal urothelium as well as advanced invasive cancer [68]. These studies may indicate that TGF- β 1 production is induced as a compensatory response on the part of the host, or reflect the unique biology of superficial as opposed to invasive tumors.

In human endometrium, the glandular epithelium demonstrates a significant stepwise increase in the expression of all three TGF- β isoforms as it progresses from the normal proliferative endometrium to simple hyperplasia and on to complex hyperplasia. Interestingly, there appears to be no further increase in immunoreactivity as lesions progress from preneoplastic complex hyperplasia to carcinoma [69]. Moreover, there appears to be an excellent correlation between the intensity of active TGF- β staining in vivo and reduced responsiveness of primary cultures of the tumor cells to TGF- β in vitro (Leslie Gold, personal communication).

While normal epithelium of the uterine cervix expresses intracellular TGF- β 1, three fourths of in situ cancers, and less than half of invasive carcinomas show positive intracellular staining for TGF- β 1. On the other hand, two-thirds of stromal tissues associated with normal epithelium or with CIN stained for the extracellular form of TGF- β 1, while close to 95% of invasive cancer-associated stroma expressed TGF- β 1 [70]. These findings suggest that cervical carcinoma cells are increasingly subject to selection exerted by stromal, presumably bioactive, TGF- β 1 as they progress to invasive carcinoma.

It should be noted that the increased expression of TGF- β s is not limited to epithelial carcinomas. For example, one or more TGF- β isoforms are expressed in most osteosarcomas. Immunoreactivity for TGF- β 1 and TGF- β 3 is generally stronger than for TGF- β 2. Moreover, there appears to be a significant inverse correlation between increased immunoreactivity for TGF- β 3 (but not TGF- β 1 or -2) and disease-free survival of patients with osteosarcoma [71].

In aggregate, these studies suggest that the increased secretion and activation of TGF- β by human cancers most often occurs late during tumor progression, and seem to favor the hypothesis that this tumor-associated TGF- β somehow provides the tumor cells with a selective advantage and promotes tumor progression. However, the inverse may also occur. For example, mice that carry a dominant-negative mutant T β R-II transgene upregulate endogenous levels of biologically active TGF- β [72]. Thus, it is possible that, in certain cases, genetic inactivation of the TGF- β signaling pathway in the tumor cells precedes the high level TGF- β expression by the surrounding stromal cells.

Finally, several studies have suggested that TGF- β -overexpressing tumors may represent a particularly aggressive subset, and that the prognosis of patients whose cancers overexpress TGF- β s may be significantly worse than that of nonexpressors. For example, positive immunostaining for TGF- β 1 identified a subset of patients with colorectal cancer who were 18 times more likely to suffer a recurrence than nonexpressors, independently of the presence of nodal metastases and the degree of differentiation [73]. Interestingly, in one study, the absence of

TGF- β 1 mRNA expression predicted for response to immunotherapy and vice versa [74]. Similarly, the expression of TGF- β 1, -2, and -3 protein and mRNA in pancreatic carcinomas was inversely correlated with postoperative survival [75]. Similarly, increased immunoreactivity for TGF- β 3 predicts for a poor disease-free survival of patients with osteosarcoma [71]. The overall prognosis was also significantly worse for patients with pulmonary adenocarcinoma showing positive TGF- β 1, T β R-I, and T β R-II expression than for patients whose tumors failed to express all three proteins [76]. On the other hand, loss of expression of TGF- β receptors has been associated with poor prognosis in prostate cancer patients [77].

In contrast to other tumor types, patients with breast cancer with high levels of TGF- β 1 appear to have a longer disease-free interval with a significantly better probability of survival [78]. In one large study of close to 300 breast cancer cases, the expression of TGF- β 2 without concomitant expression of TGF- β 1 was predictive of a favorable disease outcome [48].

4. TGF- β provides a selective advantage

Several different mechanisms have been put forth to explain how tumor-associated TGF- β might endow neoplastic cells with a selective advantage. Tumor-cell-derived TGF- β s might facilitate invasion and metastasis, induce angiogenesis, or mediate the escape from immune surveillance. I will briefly review the experimental evidence in support of each of these mechanisms whereby TGF- β might promote tumor progression.

4.1. TGF- β facilitates tumor cell invasion and metastasis

Several *in vitro* studies have shown that the effects of TGF- β on cell-cell and/or cell-substrate interactions result in a greater propensity for invasion and/or metastasis. For example, human breast cancer cells that had been exposed to TGF- β *in vitro* were significantly more tumorigenic when they were subsequently injected into nude mice than cells that had not been pretreated with TGF- β [79]. In a comparable experiment, Arrick et al. [80] showed that cell lines that had been transfected with a TGF- β 1 expression vector displayed enhanced fibronectin mRNA and plasminogen activator synthesis and increased adhesiveness *in vitro* as well as increased tumorigenicity in nude mice. Moreover, Chinese hamster ovary (CHO) cells transfected with a TGF- β 1 gene gave rise to large numbers of metastatic colonies in the lungs after inoculation into nude mice, whereas control cells did not. In addition, the tumors derived from these transfected cells demonstrated marked angiogenesis [81].

4.2. TGFs may induce angiogenesis

During embryonic development, TGF- β 1 clearly plays a major role in vasculogenesis. Studies of TGF- β 1 or T β R-II null mouse embryos have clearly shown that an intact TGF- β pathway is required for the establishment and maintenance of blood vessel wall integrity [8, 10, 82]. Moreover, the vessel wall fragility seen in TGF- β 1 or T β R-II null mouse embryos is strikingly similar to the vascular

lesions seen in patients with hereditary hemorrhagic teleangiectasia, a syndrome caused by disruption of the endoglin (a homologue of the TGF- β type III receptor) or ALK-1 (a dual-specificity type I receptor) genes [83–85].

The role of TGF- β s in the process of tumor angiogenesis is less clear (see [86] for review). On the one hand, TGF- β 1 has been shown to induce angiogenesis in a number of different *in vivo* assays [86]. In some cases, TGF- β 's angiogenic activity could be blocked by local administration of neutralizing anti-TGF- β 1 antibody [86]. On the other hand, overexpression of TGF- β 1 in a tissue-specific manner *in vivo* (for example, in transgenic animals or by gene transfer) is not usually associated with increased angiogenesis [86]. An exception to this is the observation that TGF- β -transfected CHO cells inoculated into nude mice produced tumors with marked angiogenesis that could be blocked using anti-TGF- β 1 antibody [87]. One possible explanation for these discrepant observations is that, in most of the settings in which TGF- β appeared to induce angiogenesis, this was preceded by the influx of inflammatory cells [86]. Thus, TGF- β -associated angiogenesis *in vivo* may, in fact, be mediated by a complex interplay between TGF- β and other cytokines, such as vascular endothelial cell growth factor (VEGF) and FGFs.

Several immunohistochemical studies of human tumors provide circumstantial support for the idea that TGF- β may be involved in tumor angiogenesis. For example, in a series of primary breast cancers, the strongest immunostaining for TGF- β 2 was found in areas with the highest microvessel density, a measure of angiogenic activity [88]. Similarly, the microvessel density of VEGF-rich and TGF- β -positive epithelial ovarian cancers was significantly higher than that of VEGF-poor and TGF- β -negative tumors [89], and in gastric carcinomas, prominent immunoreactivity for TGF- β 1 was also associated with the depth of invasion and an increased microvessel count [90].

There are several different mechanisms whereby TGF- β might induce angiogenesis. First of all, TGF- β has dramatic effects on the growth and differentiation of microvascular endothelial cells (MECs) *in vitro* [91]. MECs in two-dimensional (2D) cultures proliferate rapidly and exhibit an undifferentiated phenotype. Addition of TGF- β 1 increases fibronectin expression and inhibits proliferation. In contrast, MECs cultured in three-dimensional (3D) type I collagen gels proliferate slowly and are refractory to the antiproliferative effects of TGF- β 1. In such 3D cultures, TGF- β 1 increases expression of fibronectin, urokinase plasminogen activator (uPA), and PAI-1, and promotes tube formation [92]. *In vivo*, TGF- β 's effects on angiogenesis may also be indirectly mediated by PAI-1. PAI-1 inhibits the conversion of plasminogen into the angiogenesis inhibitor, angiostatin, in a time- and dose-dependent manner, an effect that can be completely blocked by a neutralizing antibody to PAI-1 [93]. These findings suggest that TGF- β may promote angiogenesis by inducing PAI-1, thereby inhibiting angiostatin production. Furthermore, TGF- β might cooperate with VEGF and FGF2 in inducing angiogenesis. Thus, it may be that, *in vivo*, VEGF and FGF2 provide the primary mitogenic stimuli for MECs,

while TGF- β is the primary driver of endothelial cell migration and tube formation, similar to its effects in three-dimensional culture [94, 95].

4.3. Tumor-associated TGF- β causes immune suppression

Two types of experimental evidence support the notion that tumor-associated TGF- β allows tumor cells to escape from immune surveillance (reviewed in [96]): first, bioactive TGF- β is clearly a potent inhibitor of immune function, for example, by blocking the clonal expansion of activated lymphocytes [97, 98]. Conversely, TGF- β null mice develop a multisystem autoimmune disorder characterized by a deficiency of epidermal dendritic (Langerhans) cells and hyperactivation of most immune cell populations [8, 99–102]. Secondly, production of TGF- β by tumor cells prevents their lysis by tumor-specific cytotoxic T cells and natural killer (NK) cells [103]. For example, a highly immunogenic fibrosarcoma cell line transfected with TGF- β 1 failed to stimulate cytotoxic T lymphocytes, and was significantly more tumorigenic than the parental line *in vivo* [104]. Conversely, inhibition of tumor-associated TGF- β appears to enhance the host anti-tumor immune response. For example, treatment of mice bearing a large MOPC-315 plasmacytoma with a low dose of the anticancer drug L-phenylalanine mustard induces a potent CD8⁺ T-cell-mediated anti-MOPC-315 cytotoxic T-lymphocyte host response, and this immunity contributes to complete tumor eradication, probably because the production of TGF- β by MOPC-315 tumors decreased drastically as a consequence of the chemotherapy [105]. Similarly, TGF- β 2 anti-sense oligonucleotides appeared to reverse the immunosuppressive effects of human glioma cells on T lymphocytes *in vitro*, although they probably also counteract the autocrine TGF- β 2-mediated stimulation of glioma cell growth [106, 107]. A recent study showed that regression of experimental gliomas in rats could be achieved by expressing anti-sense TGF- β 2 mRNA. *In vitro* tumor cytotoxicity assays with lymph node effector cells revealed a three- to fourfold increase in lytic activity for the animals immunized with TGF- β anti-sense-modified tumor cells compared to immunizations with control vector-modified tumor cells [98]. Similar experiments using mammary or bladder carcinoma cells showed that expression of a TGF- β 1 anti-sense gene resulted in inhibition of TGF- β secretion, and a decreased capacity to inhibit alloantigen-specific cytotoxic T-cell responses *in vitro* [108, 109]. Furthermore, in both cases, expression of the anti-sense TGF- β 1 gene decreased the tumorigenicity of the carcinoma cells *in vivo* [108, 109]. These studies suggest that the approach of eliminating TGF- β -mediated immune suppression is a potentially useful strategy to enhance anti-tumor immune responses [108].

5. Activation of TGF- β in the tumor microenvironment

Defining the precise role of tumor-associated TGF- β *in vivo* is further complicated by the fact that its biological activity is dependent on activation within the extracellular environment. TGF- β 1 is secreted in a latent form consist-

ing of a homodimer of the mature 112-amino acid carboxy-terminal TGF- β 1 peptide in noncovalent association with a dimer of the processed N-terminal prosegment, called the latency-associated peptide (LAP) [110]. This latent TGF- β complex is unable to bind to TGF- β receptors unless the biologically active mature TGF- β is dissociated from the LAP [111]. Since TGF- β receptors are ubiquitously expressed [112], activation of TGF- β 1 by its release from the latent complex is the key event that regulates its biological activity *in vivo* [111]. Consequently, even though many tumors produce and secrete latent TGF- β into their microenvironment, this is not likely to be of major biological significance unless it is also accompanied by activation [110].

In *in vitro* systems, a significant fraction of the TGF- β that carcinoma cell lines secrete into tissue culture medium is biologically active. This seems to indicate that the tumor cells themselves induce its activation, although the precise mechanism remain to be elucidated [113–115]. Whether tumor cells are also capable of activating TGF- β *in vivo* remains to be clarified. Besides tumor cells being the primary source of TGF- β , it is also possible that environmental agents cause activation of latent TGF- β in the tumor stroma, such as occurs during the acute response to tissue injury. In an elegant series of experiments using antibodies that discriminate between the active and latent forms of TGF- β , Barcellos-Hoff et al. have demonstrated that active TGF- β was induced in normal breast stroma within hours of radiation exposure, and that this pattern did not revert to preirradiation conditions for at least seven days [116, 117]. Moreover, besides ionizing radiation, chemical tumor promoters such as phorbol esters and phenobarbital have also been shown to activate TGF- β [118–121].

Activation of latent TGF- β is almost certainly mediated primarily by thrombospondin 1 (TSP-1), a platelet alpha granule and extracellular matrix protein [122]. TSP-1 is able to activate latent TGF- β *in vitro* via a protease- and cell-independent mechanism [123–125]. Moreover, TSP-1 knockout mice are unable to activate TGF- β , strongly suggesting that TSP-1 is the principal physiological activator of TGF- β *in vivo* [122]. Interestingly, expression of the TSP-1 gene is induced by TGF- β action, suggesting the existence of a positive feedback loop that controls TGF- β activation [126]. Activation of latent TGF- β is mediated by two sequences present in the type 1 repeats of TSP1, a sequence (GGWSHW) that binds active TGF- β and potentially orients the TSP molecule and a second sequence (RFK) that activates latent TGF- β [127]. Peptides from the type I repeats of TSP-1 mimic the adhesive and growth inhibitory activities of the intact protein and specifically interact with heparin and activate latent TGF- β . However, these TSP-derived peptides antagonize endothelial cell growth and induce apoptosis of endothelial cells through their heparin-binding activity rather than through activation of latent TGF- β or increasing cell adhesion [128, 129]. Moreover, TSP-1 suppresses the tumorigenicity of transformed endothelial cells independently of its effect on TGF- β activation [130, 131]. This tumor suppressive activity may be mediated by a direct inhibition of tumor angiogenesis [132, 133]. Thus, these experimental

studies suggest that the interplay between TSP-1 and TGF- β adds yet another level of complexity to the regulation of normal and tumor-associated angiogenesis.

In summary, in some cases the appearance of bioactive TGF- β in the tumor microenvironment is associated with the initial oncogenic event, whereas in other cases, advanced invasive tumors themselves appear to produce excessive amounts of TGF- β . The biological consequences of TGF- β activation will depend on the responsiveness of the neoplastic cell population to the polypeptide. As long as the transformed cells are sensitive to TGF- β -dependent cell cycle arrest, activation of TGF- β probably helps limit the outgrowth of neoplastic clones and acts as a tumor suppressor. On the other hand, if the transformed cell population harbors any mutant cells that are refractory to TGF- β , microenvironments rich in bioactive TGF- β will selectively promote the clonal expansion of these particular tumor cell clones. Consequently, if the stroma surrounding a particular tumor contains bioactive TGF- β , it is reasonable to assume that the tumor cells themselves are refractory to TGF- β -mediated cell cycle arrest. Although this hypothesis remains to be formally tested, there is considerable evidence that many tumors are composed of TGF- β -resistant cells, and that this is the result of inactivation of the TGF- β signaling pathway.

6. Malignant cells are often refractory to TGF- β

Because it is difficult to measure the cellular response to TGF- β in an intact animal, the question whether and, if so, at which stage of tumorigenesis clonal expansion of TGF- β -resistant cells occurs *in vivo* has yet to be answered. However, the responses of human tumor cell lines to TGF- β *in vitro* have been studied extensively (reviewed in [134]). Cell lines derived from human carcinomas, melanomas, as well as lymphoid and myeloid malignancies are almost invariably refractory to TGF- β -mediated growth arrest *in vitro* compared to their untransformed precursors [35, 134, 135]. It is unlikely that *in vitro* selection accounts for the TGF- β resistant phenotype of tumor cell lines. For example, human papillomavirus-immortalized primary human skin keratinocytes become resistant to TGF- β only after prolonged cultivation [35]. Similarly, although primary cultures of ascites-derived ovarian cancer cells appear to be somewhat more responsive than established ovarian cancer cell lines, they are significantly less responsive than normal epithelial cells [136, 137]. Similarly, primary explants of endometrial carcinoma cells are refractory to TGF- β *in vitro* (Leslie Gold, personal communication). In contrast to these carcinoma-derived cells, primary explants of acute myeloid leukemia cells sometimes retain their responsiveness to TGF- β 1 [138].

A number of experimental studies have provided indirect evidence for the acquisition of TGF- β resistance at relatively late stages of carcinogenesis. Mice treated with carcinogens such as DMBA, followed by exposure to phorbol ester tumor promoters, first develop benign skin papillomas, and later well-differentiated invasive squa-

mous cell carcinomas (SCCs). Some of these lesions progress to poorly differentiated, aggressive, so-called spindle cell variant carcinomas [139]. While cell lines derived from the papillomas are almost invariably sensitive to TGF- β *in vitro*, many of the lines derived from the SCCs (particularly those of the spindle-cell type) are refractory to TGF- β [139, 140]. Similarly, early-passage immortalized human skin keratinocyte cell lines are typically sensitive to TGF- β , while TGF- β -resistant variants arise spontaneously after prolonged passage in culture, or more rapidly after the introduction of a Ha-*ras* oncogene [35, 141]. Furthermore, cell lines derived from *in situ* cervical neoplasias or from benign colonic adenomas are sensitive to TGF- β , whereas conversion to invasive carcinomas is associated with loss of TGF- β responsiveness in both cases [142–145]. Finally, T-cell lines derived serially from the same patients with cutaneous T-cell lymphoma (CTCL) as they progress from early-stage, indolent CTCL to advanced, tumor-stage CTCL gradually lose their sensitivity to TGF- β -mediated cell cycle arrest as a consequence of loss of expression and mutations of the T β R-II gene [146–148].

7. Molecular mechanisms of resistance of human tumors to TGF- β

Inactivation of the TGF- β pathway is the most likely cause of TGF- β resistance. Although it is difficult to ascertain the response of tumors to TGF- β *in vivo*, the increasing number of reports describing specific genetic mutations that result in functional inactivation of TGF- β signaling intermediates in cancer cells have provided incontrovertible evidence not only that the tumor cells in question are no longer responsive to TGF- β -mediated growth control, but also that these events contribute directly to neoplastic transformation. In principle, any one of the signaling intermediates could be the target for such mutational events [6, 149].

7.1. TGF- β receptors

The intracellular signals from all members of the TGF- β superfamily of proteins are transduced by a unique mechanism that involves ligand-specific pairs of transmembrane serine-threonine kinase receptors (reviewed in [150, 151]). In most cases, free ligand binds primarily to the type II receptor (R-II). Once ligand is bound to its R-II receptor, the appropriate member of the type I receptor (R-I) family is recruited into a ternary complex, typically in a heterotetrameric configuration with two R-II molecules and a single ligand dimer. The formation of this ternary complex allows the R-II kinase to phosphorylate specific serine residues located within the highly conserved GS domain immediately upstream of the R-I serine-threonine kinase domain. This activation of the R-I kinase is the necessary first step in transducing the signal downstream. Moreover, two specific amino acid residues (serine at position 172 and threonine at position 176) within the juxtamembrane domain of T β R-I are particularly important because mutation of either of these amino acids eliminates the effects of TGF- β on cell cycle control [152].

Thus, the R-I molecule is the primary transducer of TGF- β 's antiproliferative signal [112].

At this point, a limited number of studies have examined the molecular status of the T β R-I gene in human neoplasms. Rearrangement of the T β R-I gene with concomitant loss of mRNA expression has been noted in one particular TGF- β -resistant clone of the human prostatic carcinoma cell line, LNCaP [153]. Sensitivity to TGF- β could be restored by transfection of a wild-type T β R-I cDNA, suggesting that T β R-I inactivation was responsible for the TGF- β -resistant phenotype [153]. However, in a more recent study, these same investigators showed that responsiveness to TGF- β could also be restored by growing the cells in the appropriate concentration of dihydrotestosterone, suggesting that another receptor besides T β R-I mediates the effects of TGF- β in these cells [154]. These observations are intriguing but remain to be confirmed by other investigators.

Goggins et al. [155] recently reported two cases of homozygous deletion of the T β R-I gene among 109 pancreatic and biliary carcinomas examined. No point mutations were detected [155]. Furthermore, we [156] have recently identified a C-to-A transversion mutation resulting in a serine to tyrosine substitution at codon 387 (S387Y) of the T β R-I receptor gene in breast carcinomas. This is the first reported mutation in the T β R-I gene in human cancer. This T β R-I mutant, which has a diminished ability to mediate TGF- β -dependent effects on gene expression as compared to wild-type T β R-I, was present in two of 31 primary breast carcinomas and five of 12 associated lymph node metastases, suggesting that it is predominantly associated with metastatic lesions. Besides this somatic mutation, several groups [18, 19] have also recently identified a 9-bp in-frame germline deletion in exon 1 of T β R-I resulting in loss of three of nine consecutive alanine residues at the N-terminus of the receptor. Approximately 10% of the population appears to be heterozygous for this del(GGC)₃ variant [18, 19]. In two case-control studies, we showed that carriers of this del(GGC)₃ T β R-I variant allele may be at an increased risk for the development of cervical carcinoma [19]. Furthermore, the frequency of del(GGC)₃ homozygotes appears to be 5–10 times higher among cancer cases (leukemia, germ cell tumor, colon, cervix and bladder cancer) than among healthy donors [18, 19]. Thus, the homozygous state may be particularly strongly associated with cancer predisposition.

Guo et al. [157] have shown that T β R-I mRNA and protein levels were significantly reduced in primary as well as metastatic prostate cancer tissue compared to normal prostatic epithelium. Unfortunately, no structural analysis of the T β R-I gene was conducted in this study. The loss of immunostainable T β R-I in prostate cancer specimens appears to be associated with a higher Gleason score, clinical tumor stage, four-year survival rate, and serological recurrence rate after radical prostatectomy [77]. Whether loss of T β R-I expression is an independent prognostic marker in prostate cancer patients remains to be determined.

Several investigators have reported that the levels of T β R-I mRNA were markedly increased in pancreatic car-

cinoma specimens compared to normal pancreas, although there was no consistent correlation with T β R-I immunostaining and no structural analysis of the gene was conducted [158, 159]. Some pancreatic cancer cell lines express both receptors and are sensitive to TGF- β [160, 161], while others fail to express T β R-I and are refractory to TGF- β [162]. In the latter case, sensitivity to TGF- β could be restored by transfection with wild-type T β R-I [162]. Goggins et al. [155] recently described two cases of pancreatic carcinoma with a large intragenic homozygous deletion of the T β R-I gene, while a larger proportion of cases displayed a loss of Smad4 or a T β R-II mutation. Thus, inactivation of the T β R-I gene appears to be one of several mechanisms whereby the TGF- β signaling pathway becomes inactivated in pancreatic cancer.

In comparison with the T β R-I gene, there is considerably more evidence that the T β R-II gene becomes inactivated in a variety of human neoplasms. Retinoblastoma cells were perhaps the first to be noted to be refractory to TGF- β because of the complete absence of TGF- β binding sites [163]. Recently, Horie et al. [164] reported that these cells expressed T β R-I mRNA, whereas no T β R-II mRNA was detected. Mutational analysis failed to reveal any mutations within the coding region of the T β R-II gene. Transcriptional activation of the TGF- β -responsive genes was not seen by transfection of either receptor cDNA alone but could be induced by cotransfection of both T β R-II and T β R-I. These data suggest that the defect in the TGF- β response is caused primarily by the lack of T β R-II, but that T β R-I may be functionally inactivated in these cells as well. Unfortunately, no mutational analysis of T β R-I was conducted.

Homozygous gene losses, gross gene rearrangements and aberrant transcripts of the T β R-II gene have been encountered in gastric, breast and small-cell lung carcinoma cell lines [165–168]. Besides such large-scale genomic alterations of the T β R-II gene, two types of point mutations have been described in human tumors: First, between 70 and 90% of gastric and colorectal carcinomas and carcinoma cell lines derived from patients with hereditary or sporadic DNA mismatch repair deficiencies (the so-called RER⁺ phenotype) display mutations of specific microsatellite sequences within the T β R-II coding region that result in loss of its tumor suppressive activity [169–180]. These mutations apparently affect mRNA stability, as T β R-II mRNA levels are decreased. Furthermore, mutant T β R-II mRNA that does become translated presumably encodes a truncated protein. Transfection of RER⁺ gastric or colon carcinoma cells with wild-type T β R-II restores responsiveness to TGF- β and suppresses tumorigenicity [172, 181, 182]. About 20% of malignant gliomas have the RER⁺ phenotype, and the majority (70%) of those also carry T β R-II microsatellite mutations [183]. Interestingly, microsatellite mutations of the T β R-II gene are only rarely found in sporadic, nonRER⁺, gastric and colorectal cancers [173, 177, 179, 184–186]. Furthermore, the association between the RER⁺ phenotype and T β R-II mutations appears to be restricted to gastric and colorectal cancers and gliomas. Thus, T β R-II mutations have not been encountered in RER⁺ adenocarcinomas of the small intestine [187] or of the endometrium [170]. It

may be that inactivation of TGF- β signaling does not provide the same selective advantage to transformed cells in these tissues as it does in the stomach, colon or brain. Alternatively, it may be that TGF- β signaling is abrogated in these tumors by mutations in other signaling intermediates.

Besides the abovementioned microsatellite mutations in exon 3 of T β R-II, we have recently identified a novel G $\rightarrow$ T transversion in the same exon, which introduces a premature stop codon (E142Stop). This mutant presumably also encodes a truncated soluble exoreceptor [19]. In addition to nonsense mutations within 5' portion of the T β R-II gene, we and others have identified a number of different missense mutations in head and neck, gastric, colorectal and ovarian cancer cells which result in amino acid substitutions close to the C-terminal end of the T β R-II receptor serine-threonine kinase domain [177, 188–192]. Some of these mutations have been shown to result in loss of kinase activity and completely abolish the signaling activity of the T β R-II receptor protein [192, 193]. Thus, these mutations can be held directly responsible for the TGF- β -resistant phenotype of the tumor cells [192, 193]. In summary, cancer-associated mutations in the T β R-II gene appear to be clustered within two distinct regions: Nonsense mutations that introduce premature stop codons are found mainly within the extracellular juxtamembrane domain and missense mutations that result in loss of kinase activity are clustered at the C-terminus of the T β R-II kinase domain.

Inactivation of a tumor suppressor gene most often involves mutation of one allele followed by loss of the second allele [194]. Akiyama et al. [195] recently provided strong evidence that the T β R-II gene also obeys this rule. While inactivating mutations were found in one allele of the T β R-II gene in 57% of adenomas from patients with hereditary nonpolyposis colorectal cancer, 85% of invasive cancers carried T β R-II mutations with the concurrent loss of the second, wild type, allele [195]. Similarly, Grady et al. [196] found no T β R-II mutations in early RER⁺ adenomas, including all those with simple tubular or villous histology. The earliest T β R-II mutations detected were in regions of high-grade dysplasia and predominated in highly progressed adenomas that contained regions of frankly invasive adenocarcinoma. Thus, mutation of T β R-II is a late event in RER⁺ adenomas and correlates tightly with progression to cancer.

To address this same question, we [197] recently examined the frequency of loss of heterozygosity (LOH) of the two T β R genes in interphase nuclei from primary human breast carcinoma specimens using fluorescence in situ hybridization. Approximately half of the cases exhibited allelic losses of the T β R-I gene and 75% of the cases revealed losses of one or both T β R-II alleles. Thus, many primary invasive breast carcinomas appear to contain subpopulations of cells that have undergone allelic deletions of the T β R-I or -II genes. We are in the process of investigating whether the remaining T β R alleles carry intragenic mutations.

Finally, loss of T β R-II mRNA expression has been found in the majority of small cell lung cancers, in a significant proportion of esophageal tumors, and in a small number of

breast carcinoma cell lines [167, 168, 198, 199]. In most of these cases, no structural alterations within the gene have been found. However, Muñoz-Antonia et al. [200] have recently described a mutation within the T β R-II gene promoter that resulted in a significantly decreased level of T β R-II mRNA and protein expression in a squamous cell carcinoma cell line. In prostate cancer specimens, immunoreactivity for both types of T β R is either significantly reduced compared to the normal prostate, or totally absent [157]. The underlying mechanism remains unclear. These observations suggest that as yet unknown cellular factors that regulate T β R-II expression may be indirectly responsible for the TGF- β -resistant phenotype in some tumor types. However, even if an epigenetic mechanism is involved, it is clear that the lack of T β R-II not only results in TGF- β resistance, but also contributes to the malignant phenotype. This is best illustrated by several studies in which transfection of T β R-II into T β R-II-lacking tumor cell lines not only restored TGF- β -mediated cell cycle arrest and inhibited their anchorage-independent growth in vitro, but also suppressed their ability to form tumors in vivo [166, 167, 201, 202].

7.2. Postreceptor mediators of TGF- β signaling

Postreceptor events in TGF- β signaling are primarily mediated and regulated by homologues of the *Drosophila* mother-against-dpp (Mad) protein, so-called Smads [203–206]. In response to TGF- β binding, two receptor-associated Smads, Smad2 and Smad3, appear to transiently associate with the activated T β R-I receptor and to become phosphorylated in the process, presumably by the T β R-I kinase. Once activated, Smad2 and Smad3 form heteromeric complexes with the so-called common-mediator Smad, Smad4, followed by translocation of the entire complex to the nucleus [204, 207]. Smad4 binds DNA in a sequence-specific manner [208]. However, besides this Smad-binding element (SBE), Smad-responsive promoters must contain at least one additional protein recognition sequence that specifically binds accessory transcription factors present within the Smad complex. These include Smad3, or Smad2-associated FAST-1 (or-2) [206]. Target genes for TGF- β -dependent transcriptional activation in mammalian cells include INK4B [209], and WAF1 [210], which encode the cyclin-dependent kinase (CDK) inhibitors p15 and p21, respectively. The genes encoding the CDK tyrosine phosphatase, Cdc25A [211], and the proto-oncogene, *c-myc* [212], are repressed. Moreover, the expression of Cdk4 and cyclin E is downregulated [213, 214]. TGF- β 1's inhibition of cyclin/CDK activity, in turn, blocks the phosphorylation of the retinoblastoma protein (pRb), thereby preventing the activation of cell cycle genes by the transcription factor, E2F-1, resulting in cell cycle arrest.

Of the three Smad proteins involved in mediating the effects of TGF- β , two (Smad2 and Smad4) have clearly been associated with human cancer [215]. Interestingly, both of these genes are located on chromosome 18q21, a region that frequently undergoes allelic loss in a number of tumor types, including pancreatic, colorectal, breast, and head and neck carcinomas. Homozygous losses, nonsense- and missense mutations of the Smad2 gene

have been reported in 6–17% of colorectal carcinoma cell lines and primary tumors [216, 217]. In contrast, no structural alterations of this gene were encountered in early stage breast cancers, lung cancers or malignant sarcomas [216, 218]. Although the Smad3 gene has been mapped to chromosome 15q21, which undergoes allelic loss in several types of cancer [141, 219, 220], no structural alterations of Smad3 have been found in over 200 colon, breast, lung, or pancreatic carcinoma cell lines, primary tumors, or xenografts examined [221, 222]. This is a somewhat puzzling observation, as mice with a targeted deletion of Smad3 readily develop colon cancer [223]. These findings suggest that there may be partial functional redundancy between Smad2 and -3 in humans but not in mice.

Inactivation of Smad4 appears to occur more frequently than that of receptor-associated Smads. Homozygous losses, missense and nonsense mutations of Smad4 have been found in approximately 50% of pancreatic and biliary tract carcinomas and in 30% of sporadic colorectal carcinomas, but rarely in other tumor types [4, 23, 224–230]. Moreover, Howe et al. [231] have recently demonstrated that germline mutations of Smad4 are the proximal cause of the familial juvenile polyposis syndrome, an autosomal dominant disorder characterized by a predisposition to hamartomatous polyps and gastrointestinal cancers. The missense mutants of Smad4 found in pancreatic carcinoma are transcriptionally inactive [232]. Moreover, inactivation of the Smad4 gene abrogates signaling from TGF- β as well as activin [26]. These studies have provided unequivocal evidence that inactivation of Smad4 causes TGF- β unresponsiveness and a basis for understanding the role of this gene in tumor suppression.

In summary, there is rapidly mounting evidence that four out of the five main proteins involved in TGF- β signaling (T β R-I, -II, Smad2 and -4) can become inactivated in human tumors by genetic or epigenetic mechanisms. These findings strongly support the idea that inactivation of the TGF- β signaling pathway confers a selective advantage upon the tumor cells.

Because TGF- β appears to specifically antagonize the activity of a variety of mitogenic pathways, it is possible that the constitutive activation of such a mitogenic pathway would override the effects of TGF- β and so cause apparent phenotypic resistance to TGF- β . For example, the proto-oncogene, *c-myc*, plays an important role as a progression factor in early G1 prior to the initiation of DNA synthesis and is one of the genes that is directly repressed as a consequence of TGF- β action [212, 233]. Consequently, several investigators have tested the idea that cells that constitutively overexpress *c-myc* might be refractory to TGF- β -mediated cell cycle arrest [234, 235]. However, the results of these studies have been inconclusive. Moreover, sensitivity to TGF- β is not always accompanied by repression of *c-myc* [236]. In contrast to *c-myc*, overexpression of cyclin D1 causes nontransformed epithelial cells to be relatively resistant to growth inhibition by TGF- β 1 compared to appropriate controls [237]. Thus, under certain conditions, TGF- β -mediated cell cycle arrest can be overcome by cyclin overexpression.

Conversely, as TGF- β action results in an upregulation of a number of CDK inhibitors in the cell, one might expect loss of these CDK inhibitors to result in TGF- β resistance. However, even though the levels of p27, p21, and p15 are all increased in response to TGF- β 1, cells derived from knockout mice that lack either p21, p15 or p27 retain near complete sensitivity to TGF- β -mediated cell cycle arrest in vitro [238, 239] (G.P. Dotto and K. Polyak, personal communication). Thus, although inactivation of these CDK inhibitors is quite common in human neoplasms, these events in and by themselves do not result in TGF- β resistance.

It is interesting that certain subsets of cancer appear to be consistently associated with specific defects in the TGF- β signaling pathway. Particularly striking are the association between the RER⁺ phenotype and nonsense mutations of the T β R-II gene in malignant gliomas, and colorectal, and gastric cancers [173, 177, 179, 183–186], and the high frequency of allelic losses and mutations of Smad4, in pancreatic cancers [4, 226]. Perhaps these patterns reflect the exposure of these target organs to specific carcinogens.

Alternatively, inactivation of the TGF- β pathway may occur preferentially in the context of specific other oncogenic events. For example, there appears to be an inverse correlation between the presence of T β R-II and p53 gene mutations in RER⁺ gastric cancers, suggesting that these genetic lesions might affect the cellular phenotype in similar ways [179, 240]. A similar type of inverse relationship appears to exist between p53 mutation and inactivation of the TGF- β pathway in pancreatic cancer [4, 155]. In this disease, activation of the *Ki-ras* gene always precedes the inactivation of INK4a and p53, and Smad4 loss only seems to occur subsequent to both *Ki-ras* activation and inactivation of p16^{INK4a} [4]. Thus, loss of Smad4 (which results in TGF- β resistance) only confers a selective advantage in cells in which a mitogenic pathway has been activated (by *Ki-ras* mutation) and after they have escaped from senescence and/or apoptosis (by losing p16^{INK4a} and/or p53) [2, 3]. Although the precise mechanism is not well understood, cells expressing certain mutant forms of p53 are significantly less sensitive to TGF- β -mediated growth arrest [241, 242]. In addition, ectopic expression of the murine double minute 2 (MDM2) gene can cause TGF- β resistance by interfering with both p53 and Rb/E2F function [243]. Thus, inactivation of either p53 or TGF- β signaling intermediates may all confer the same phenotype of TGF- β -resistance.

8. Clinical implications

8.1. Chemoprevention

As summarized above, the available experimental evidence seems to indicate that TGF- β can play two different and opposite roles with respect to the process of malignant progression: It is able to inhibit the early steps in human carcinogenesis, while it may facilitate the progression of advanced tumors. We have seen that bioactive TGF- β present in the cellular microenvironment at the time of the initiating carcinogenetic event can act as a potent tumor

suppressor. This observation has important implications for the chemoprevention of cancer, for example in managing individuals who are at high risk for the development of malignant neoplasms. Such high-risk groups might benefit greatly from treatment with agents that can induce the secretion and activation of TGF- β s. The two most potent chemopreventive agents currently in clinical use include retinoids and the anti-estrogen, tamoxifen. Retinoids have been shown to dramatically reduce the incidence of second primary tumors in patients who had undergone definitive treatment for cancers of the upper aero-digestive tract [244, 247]. Similarly, studies of adjuvant tamoxifen in women with early stage breast cancer have demonstrated its protective effects with respect to the occurrence of contralateral primary breast cancers [248]. In addition, tamoxifen appears to limit the progression from in situ to invasive breast cancer [249]. Perhaps even more promising is the prospect that tamoxifen may be able to prevent the de novo development of primary breast cancer in women at high risk [250–252]. In the recently reported early results of the National Surgical Adjuvant Breast and Bowel Project-sponsored Breast Cancer Prevention Trial, tamoxifen was found to reduce the risk of invasive breast cancer by 49%, and the risk of noninvasive breast cancer by 50% [253].

What is particularly intriguing is the fact that the chemopreventive effects of both retinoids and tamoxifen may be mediated by TGF- β . Retinoic acid has been shown to induce the secretion and activation of TGF- β 1 or -2 by endothelial cells and epithelial cells of the upper aerodigestive tract, the skin, the cervix, and the prostate, in vitro as well as in vivo [35, 254–261]. Furthermore, the antiproliferative effects of retinoic acid on human papillomavirus-immortalized primary human keratinocytes correlate directly with the ability of retinoic acid to induce TGF- β secretion and the in vitro responsiveness of the cells to TGF- β [35]. Treatment of human estrogen receptor-positive MCF-7 breast cancer cells with tamoxifen in vitro was shown to induce the production and secretion of TGF- β by these tumor cells resulting in inhibition of their growth [262–264]. However, several other studies have failed to confirm these findings [265–267]. One intriguing possibility is that the antiproliferative effects of tamoxifen are mediated by induction of biologically active TGF- β s in nonneoplastic tumor-associated fibroblasts, as has been demonstrated in vitro [268–270]. A number of recent clinicopathological studies have suggested that tamoxifen can also induce the production of TGF- β s in vivo. For example, plasma levels of TGF- β 2 were increased in women treated with tamoxifen, and these elevations appeared to correlate with its anti-tumor effects [271, 272]. In addition, a number of investigators have examined the effects of tamoxifen treatment on the expression of TGF- β s in breast cancer specimens. Some of these studies found that tamoxifen induced TGF- β predominantly in stromal fibroblasts [273], which is in accord with in vitro observations [268]. Moreover, TGF- β 2 appears to be the predominant TGF- β isoform that is induced by tamoxifen [270, 271, 274]. Although none of these studies formally prove that TGF- β s mediate the effects of either retinoids or tamoxifen on tumor development, they

strongly suggest a causal relationship between the induction of TGF- β s in stromal cells and plasma, and the chemopreventive effects of these agents.

8.2. Treatment of advanced cancer

Although TGF- β may be able to prevent or control the initial outgrowth of neoplastic clones, we have seen that the tumor stroma of established invasive carcinomas often contains bioactive TGF- β and that, consequently, these tumors are likely to be refractory to the antiproliferative effects of TGF- β . Thus, under these conditions, treatments that induce the production and/or activation of TGF- β are unlikely to be beneficial. Moreover, such interventions may be detrimental if the tumor-derived TGF- β is providing the cells with a selective advantage either by facilitating angiogenesis, invasion and/or metastasis or by allowing them to escape from immune surveillance.

In these cases of advanced, TGF- β -resistant, cancers, therapeutic strategies should probably be aimed at inactivating TGF- β or at restoring tumor cell responsiveness to TGF- β . Fibrosis associated with chronic inflammation is mediated by the activation of TGF- β . Two different approaches to prevent fibrosis by inactivating TGF- β have been successfully tested in experimental model systems [275, 276]. These include the use of neutralizing antibodies to TGF- β [277] and the administration of the TGF- β -binding protein, decorin [276, 278]. Based on the idea that tumor-derived TGF- β allows tumors to escape from immune surveillance, a recent study showed that regression of experimental brain tumors in rats could be achieved by expressing anti-sense TGF- β 2 mRNA, which enhanced immune rejection [98]. Since then, this same anti-sense strategy has been used to induce regression of mammary and bladder tumors in vivo [108, 109]. In addition, competitive inhibitors of TGF- β binding to its receptors might be designed that would eliminate the selective advantage provided by tumor-derived TGF- β . Any approach aimed at neutralizing TGF- β might be applicable to the treatment of cancers associated with TGF- β activation. Finally, in cases in which tumors no longer respond to TGF- β because of inactivation of one of the two receptor genes, it may be possible to activate the signaling pathway downstream of the block, for example by using peptides or peptidomimetic drugs that mimic the activity of the Smad4-associated transcription complex [206].

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