Inhibition of Transforming Growth Factor-\(\beta\) Signaling in Human Cancer: Targeting a Tumor Suppressor Network as a Therapeutic Strategy

Swati Biswas, Tracy L. Criswell, Shizhen Emily Wang, and Carlos L. Arteaga

Transforming growth factor-\(\beta\) (TGF\(\beta\)) is both a tumor suppressor and tumor promoter. The loss or attenuation of TGF\(\beta\) signaling in epithelial cells and stroma is permissive for epithelial cell transformation, whereas the introduction of dominant-negative TGF\(\beta\) receptors into metastatic cancer cells inhibits epithelial-to-mesenchymal transition, motility, invasiveness, survival, and metastases. In addition, excess production and/or activation of TGF\(\beta\) by cancer cells can contribute to tumor progression by paracrine mechanisms involving modulation of the tumor microenvironment. These data provide a rationale in favor of the blockade of autocrine/paracrine TGF\(\beta\) signaling in human cancer with a therapeutic intent.

**TGF\(\beta\) Signaling and Developmental Functions**

The TGF\(\beta\) family comprises a superfamily of ligands that includes the TGF\(\beta\)s, activins, and bone morphogenetic proteins. TGF\(\beta\) ligands play functional roles in cell proliferation, functional differentiation, extracellular matrix production, cell motility, and apoptosis (1). They are secreted as small latent complexes composed of the active carboxy-terminal TGF\(\beta\) dimer linked noncovalently to a dimer of the latency-associated peptide. The large latent complex are the small latent complexes linked via disulfide bonds to the latent TGF\(\beta\)-binding protein. The latent TGF\(\beta\)-binding protein glycoproteins localize large latent complexes in the extracellular matrix in which the bulk of the TGF\(\beta\)s are sequestered. The release of active TGF\(\beta\) from matrix-associated latent complexes may require two steps: release of the complex from the extracellular matrix by proteolysis and subsequent activation by disruption of the noncovalent association between TGF\(\beta\) and the latency-associated peptide. This can be achieved by chemical, enzymatic, proteolytic, and hormonal mechanisms (2).

There are three mammalian TGF\(\beta\) isoforms, TGF\(\beta\)1, TGF\(\beta\)2, and TGF\(\beta\)3 exhibit similar functions *in vitro* (1). However, each seems to have distinct activities *in vivo* as evidenced by the phenotypes of mice lacking each of these ligands. Targeted inactivation of the Tgfb1 gene leads to hematopoietic and vasculogenic defects resulting in the death of approximately half of the embryos at 10 days of gestation (3). The embryos that survive succumb to a wasting syndrome and multiorgan necrosis due to inflammatory cells (4). TGF\(\beta\)2-null mice die in the perinatal period as a result of developmental abnormalities affecting the cardiopulmonary, urogenital, visual, auditory, neural, and skeletal systems (5). Mice lacking TGF\(\beta\)3 also die perinatally and exhibit abnormal lung and palate development (6, 7).

The TGF\(\beta\)s bind to a heteromeric complex of transmembrane serine/threonine kinases, the type I and type II receptors (T\(\beta\)RI and T\(\beta\)RII). These ligands also bind a large transmembrane proteoglycan referred to as the type III TGF\(\beta\) receptor, or betaglycan, whose role is to present ligand to T\(\beta\)RII (1). Following ligand binding to T\(\beta\)RII, T\(\beta\)RI is recruited to the ligand-receptor complex. This allows the constitutively active T\(\beta\)RII kinase to transphosphorylate and activate the T\(\beta\)RII kinase (8), which, in turn, phosphorylates the receptor-regulated Smad2 and Smad3 (Fig. 1). Smad2/3 then associate with Smad4 and translocate to the nucleus where they regulate the transcription of genes involved in cell cycle control and apoptosis (9). The inhibitory Smad7 can interact with T\(\beta\)RI and prevent the phosphorylation of effector Smads (10). In addition to Smads, other signaling pathways have been implicated in TGF\(\beta\) actions. These include the extracellular signal-regulated kinase, c-Jun NH2-terminal kinase, p38 mitogen-activated protein kinase (MAPK), phosphatidylinositol-3 kinase (PI3K), nuclear factor \(\kappa\)B, and Rho GTPases (11). The molecular mechanisms by which TGF\(\beta\) receptors engage non–Smad pathways remain to be fully characterized.

**Rationale for Inhibiting TGF\(\beta\) in Cancer**

Although TGF\(\beta\) was originally reported as an oncogene (12), early studies also showed that it is a potent inhibitor of epithelial cell proliferation (13). Mice with targeted disruption of TGF\(\beta\)1 and Smad genes as well as transgenic mice with tissue-specific expression of dominant-negative T\(\beta\)RII are prone to cancer development (reviewed in ref. 14). Inactivating mutations in the Tgfbri2 gene occur in both sporadic and inherited colon cancers with microsatellite instability (15); restoration of T\(\beta\)RII by transfection reverses transformation in certain colon cancer cell lines (16). Inactivating mutations in Smad2 and Smad4 are present in cancers of the gastrointestinal tract and pancreas (17). More recently, conditional inactivation of T\(\beta\)RII in mouse fibroblasts resulted in prostatic intraepithelial...
neoplasias and carcinomas of the forestomach (18). These data are consistent with the ability of TGFβ to maintain tissue architecture, inhibit genomic instability, and induce replicative senescence and apoptosis in nontransformed cells/tissues (14). Despite this evidence supporting a tumor suppressor role for TGFβ, exogenous TGFβ has never been shown to inhibit an established cancer in vivo nor has the administration of a TGFβ inhibitor resulted in spontaneous tumor development or the acceleration of an already established cancer in an animal model or in humans.

On the other hand, an excess production and/or activation of TGFβ by established tumor cells can promote cancer progression by mechanisms such as an increase in tumor neoangiogenesis and extracellular matrix production, up-regulation of peritumor proteases, and inhibition of immune surveillance mechanisms in the cancer host among others (reviewed in ref. 19). High TGFβ levels in tumors or in patients’ serum correlate with markers of a more metastatic phenotype and/or poor patient outcome (20–23). TGFβ can also induce an epithelial-to-mesenchymal transition and increase tumor cell invasiveness (24, 25). Re-expression of TβRII in colon cancer cells with low invasive potential restores tumor cells invasiveness (26). Forced expression of dominant-active Smad2 in squamous cancer cells results in enhanced tumor cell motility and metastatic dissemination (27). Finally, expression of dominant-negative TβRII in metastatic cancer cells prevents epithelial-to-mesenchymal transition and inhibits motility, tumorigenicity, and metastases (reviewed in ref. 19).

There is also evidence that the tumor suppressor versus oncogenic effects of TGFβ are contextual and/or depend on the temporal stage of cellular transformation. For example, the expression of a kinase-intact type I receptor (TβRI) mutant that is unable to bind Smad2/3 results in larger, more proliferative, less differentiated mammary tumors. However, expression of the same mutant in highly malignant mammary cells suppresses their ability to metastasize to the lungs (28). In a second example, transgenic mice engineered to lack TβRII in the mammary gland developed Polyomavirus middle T (PyVmtT) oncogene-induced mammary tumors with shorter latency and with more lung metastases compared with TβRII-expressing tumors (29). In these mice, the floxed TβRII allele was excised upon crossbreeding with mouse mammary tumor virus (MMTV)/Cre mice, where Cre is expressed in the mammary gland at puberty before mammary tumors are established. This report seems to contrast with another study of triple transgenic (MMTV/rtTA/C2 tet-op/TGFβ1S223/225/C2 MMTV/PyVmT) mice in which active TGFβ1 expression at tumor sites was controlled with doxycycline. In their study, 2 weeks of administration of doxycycline to 8-week-old mice with established mammary tumors markedly accelerated metastases and death (30). These data suggest that the net effect of TGFβ signaling in tumor progression may diverge as a function of the (early versus late) stage of transformation.

Additional examples support a promoter role for autocrine/paracrine TGFβ in late phases of transformation. Mice overexpressing active TGFβ1 in suprabasal keratinocytes develop fewer benign papillomas than controls. However, the few transgenic tumors which do develop acquire a spindle cell phenotype, overexpress TGFβ3, and metastasize (31). Overexpression of an active mutant of TβRII (Alk5) or active TGFβ1 in the mammary gland of transgenic mice accelerates metastases from Neu-induced mammary tumors (32–34). Finally, expression of
TβRII in tumors has been associated with shorter patient survival in several tumor types (35–37), suggesting that TGFβ signaling in cancer cells may select tumors with high metastatic behavior. Recent reviews have summarized a number of studies in which the inhibition of TGFβ by pharmacologic or genetic means inhibited tumor progression in animal models (19, 38, 39).

**TGFβ and Oncogenic Signaling Pathways**

Recent studies have identified oncogenic signaling pathways which are activated by TGFβ. For example, secreted TGFβ2 induces nuclear factor κB activity in cancer cells and RNA interference of TGFβ2 in LNCaP cells reduces nuclear factor κB activity and survival (40). TGFβ stimulates PI3K and Akt activities. Both TβRII and Alk5 associate with p85, the regulatory subunit of PI3K, and expression of constitutively active Alk5T204D markedly up-regulates PI3K catalytic activity (34, 41, 42). TGFβ- or active Alk5-mediated cell motility and protection from apoptosis are blocked by LY294002 (30, 43), dominant-negative Akt (44), dominant-negative c-Jun (45), or TβRI kinase inhibitors (34). Inhibition of PI3K has also been shown to reverse the fibroblastoid phenotype of Ras-transformed hepatocytes to an epithelial phenotype (46), further suggesting that PI3K signaling is an effector of the oncogenic function of TGFβ. Transgenic mice expressing an active mutant of Alk5 in the mammary gland exhibit increased PI3K activity in mammary epithelium as well as reduced apoptosis in terminal end buds and during (delayed) postlactational involution (34). Although MMTV/Alk5T204D mice did not develop mammary tumors, bigenic MMTV/Neu × Alk5T204D mice developed cancers that were markedly more metastatic than those occurring in MMTV/Neu mice (34).

Oncogenic signaling pathways activated by TGFβ can subvert Smad signaling and, thus, antagonize ligand-induced antiproliferative activity. For example, PI3K/Akt represses the induction of p21cip1 and, in doing so, inhibits the cytostatic action of TGFβ (47). Activated Akt can sequester Smad3 and prevent its phosphorylation, association with Smad4, and nuclear translocation, hence blocking TGFβ-induced apoptosis (48, 49). These data may explain in part how oncogenes attenuate the tumor suppressive action of TGFβ. How essential these non-Smad oncogenic pathways are for the effects of TGFβ on tumor progression remains to be determined.

TGFβ cooperates with activated Ras on the conversion of noninvasive to metastatic tumors and on the promotion of epithelial-to-mesenchymal transition (27, 50). Oncogenically activated Ras has been shown to inhibit TGFβ-mediated nuclear accumulation of Smad2/3, Smad-dependent transcription, and antimitogenesis (51). Ha-Ras abrogates TGFβ-mediated inhibition of proliferation by inducing MAPK-dependent proapoptotic degradation of Smad4 (52). TGFβ can activate Ras/MAPK signaling in transformed cells (53) and expression of a dominant-negative truncated Alk5 blocks the growth of Ras-transformed Ink4a−/− tumors in mice (54), suggesting that autocrine TGFβ is at least in part required for Ras-induced transformation in vivo.

TGFβ synergizes with other oncogenes that activate Ras/MAPK. For example, overexpression of active TGFβ1 or active Alk5 mutants in the mammary gland of bitransgenic mice also expressing neu/erbB2 accelerates metastases from oncogene-induced mammary cancers (32–34). A genetic modifier screen in nontumorigenic mammary epithelial cells identified TGFβ1 and TGFβ3 as molecules that cooperate with ErbB2 on inducing cell motility and invasion, which were blocked by inhibitors of MAPK (55). Inhibition of ErbB2 (HER2) with the HER2 neutralizing antibody trastuzumab or with MAPK inhibitors blocked the promigratory effect of TGFβ in HER2-overexpressing mammary epithelial cells (56), suggesting that oncogene function is required for TGFβ action. These data imply that (a) oncogenes that activate Ras/MAPK signaling engage and require TGFβ for tumor progression, and (b) tumors with activating Ras mutations or with active Ras/MAPK are attractive targets for the testing of TGFβ inhibitors.

**Therapeutic Inhibitors of TGFβ Signaling**

The main strategies for inhibition of TGFβ include compounds that interfere with the binding of ligand to TGFβ receptors, drugs that block intracellular signaling, and antisense oligonucleotides. These have been summarized in recent reviews (38, 39) and will be only mentioned in brief here. Lerdelimumab (CAT-152) and metelimumab (CAT-192) are recombinant human IgGs generated by phage display technology that blocks TGFβ1 and β2, respectively (57, 58). They are under development by Cambridge Antibody Technology (London, United Kingdom) and Genzyme Corporation (Framingham, MA). Because of the expression of multiple TGFβ isoforms in tumors, a panselective monoclonal antibody GC-1008 (also developed by CAT/Genzyme) is in early phase I testing in patients with cancer. The phase I studies of CAT-152 and GC-1008 have been conducted in patients with fibrotic disorders, however, the final reports for these are not yet available. In two studies, CAT-152 seemed to prevent subconjunctival scarring after glaucoma filtration surgery (57, 59). Recombinant fusion proteins containing the ectodomains of the type II and type III (beta/glycan) receptors have also been used to prevent ligand binding to TGFβ receptors. Soluble TβRII/Fc has shown potent antimitastatic activity in transgenic mice (60) but its clinical development in cancer is unclear at this time. Human recombinant TβRII has also shown antimitastatic and antiangiogenic cell activity (61) in preclinical models.

A second group of strategies is aimed at directly blocking the catalytic activity of TβRI. These include the small molecules SB-431542 and SB-505124 (GlaxoSmithKline, Collegeville, PA), SD-093 and SD-908 (Scios Inc., Fremont, CA), and LY2157299 (Lilly Research Laboratories, Indianapolis, IN), which are ATP-competitive inhibitors of the Alk5 kinase and whose structural properties have been reviewed recently (39). One difference (and potential advantage) of these compounds from the pharmacologically more stable ligand-specific antibodies is that they cross-react with other Alk5-homologous serine/threonine kinases such as Alk4 and Alk7, suggesting that they could mediate the activin-mediated activation of Smad2/3 (62–64). At the time of this writing, some of these compounds are just entering phase I investigation.

Finally, TGFβ antisense approaches are also in development in cancer. In preclinical studies using intracranial gliomas, a TGFβ2 antisense-modified allogeneic tumor cell vaccine was shown to inhibit TGFβ-mediated immune suppression and promote tumor rejection (65). Antisense Pharma (Regensburg, Germany) is developing two TGFβ-specific phosphorothioate antisense
oligonucleotides. AP-12009, against TGFβ1, has shown remarkable early clinical activity when delivered intratumorally by convection-enhanced delivery to patients with high-grade gliomas (66). AP-11014, against TGFβ1, is being developed for the treatment of non–small cell, colorectal, and prostate carcinomas (67).

At this point, therapeutic targeting of TGFβ signaling in human cancers has been endorsed by the scientific community. However, many questions remain about this enterprise as it applies to drug tolerability and/or toxicities, molecular and/or biochemical surrogate markers of TGFβ activation and drug-induced inactivation in situ, the molecular profile and/or tumor types most appropriate for investigational trials with TGFβ inhibitors, and scientifically based combinations that will include inhibitors of TGFβ signaling, among others.

References


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