Transforming growth factor-β: biology and application to cancer therapy

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SUMMARY

Transforming growth factor-β (TGF-β), an extensively investigated cytokine, plays a very important role in promoting the spread of cancers in the body, and can play a direct role in facilitating metastasis. Consequently, TGF-β is currently explored as a prognostic candidate biomarker of tumor invasiveness and metastasis. Therefore, in clinical scenarios involving increased TGF-β activity, attempts to decrease or abrogate TGF-β signaling could be used as a therapy for advanced or metastatic disease. It follows that TGF-β signaling offers an attractive target for cancer therapy. Several anti-TGF-β approaches, such as TGF-β antibodies, antisense oligonucleotides and small molecules inhibitors of TGF-β type 1 receptor kinase, have shown great promise in the preclinical studies. These studies, coupled with progressing clinical trials indicate that inhibition of TGF-β signaling may be indeed a viable option to cancer therapy. This review summarizes the TGF-β biology, screening cancer patients for anti-TGF-β therapy, and several strategies targeted against TGF-β signaling for cancer therapy. The next several years promise to improve our understanding of approaching cancer therapy by further evaluation of TGF-β signaling inhibitors for clinical efficacy. The complexity of TGF-β biology guarantees that many surprises lie ahead.

Key words: *Transforming Growth Factor beta; Signal Transduction; Neoplasms*

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INTRODUCTION

The transforming growth factor-βs (TGF-βs) are polypeptides that regulate several cellular functions, including cell growth and differentiation, motility, extra cellular matrix production, and immune function. TGF-βs have three mammalian isoforms, TGFβ-1, TGFβ-2, and TGFβ-3 each with distinct function *in vivo*. All three TGF-βs use the same receptor signaling system. During the past decade, there have been some important advances in the understanding of postreceptor TGF-β signaling pathways (1). The cascade of signal transduction events starts with the direct binding of TGF-β ligand to the constitutively active transmembrane TGF-β receptor type II (TβRII). The transduction signal is transmitted via either SMAD-mediated or mitogen activated protein kinase (MAPK) pathways that can cross-interact, and be modified by other pathways (2).

In SMAD-mediated pathway, TGF-β/TβRII complex recruits, binds and transphorylates the TGF-β receptor type I (TβRI), thereby stimulating its (serine/threonine) protein kinase activity. The activated TβRI receptor phosphorylates intracellular transducers Smad2 and Smad3, which form an oligomeric complex with the Smad4 protein. The Smad2/Smad4 and Smad3/Smad4 translocate into the nucleus and interact in a cell-specific manner with transcription factors to regulate specifically the transcription of a multitude of the TGF-β-responsive genes. TGF-β signaling is regulated by the level and duration of TβRII receptor activation (3). The MAPK pathway involves transcription factors such as cFOS/cJUN complexes, which mediate TGF-β autoinduction (4). Other molecular details of the MAPK pathway are yet to be elucidated (2).

TGF-β IN CANCER BIOLOGY

The TGF-β signaling pathways also have an important role in human carcinogenesis revealing dual function of TGF-β in this process i.e. tumor suppressive in early stages, and tumor promoting in advanced stages of the disease (2). In healthy tissue, premalignant, and early-transformed states, TGF-β might act mainly as an epithelial growth inhibitor. As cells progress along the neoplastic continuum, these regulatory mechanisms become compromised because of a loss of negative cell signaling or because of a fundamental change in the TGF-β switch. The net result of these pathophysiological changes is a loss of growth inhibition and concomitant stimulation of growth promotion in the process of tumor progression. Consequently, tumors that are further advanced generally express more TGF-β, which has been correlated with a more malignant phenotype and impaired clinical outcome. Therefore, a major challenge in this field is to define TGF-β molecular mechanisms of action in the process of carcinogenesis (3).

Given the stimulatory effect of TGF-β in the tumor progression, it follows that TGF-β plays a very important role in promoting the spread of cancers in the body, and can play a direct role in facilitating metastasis (5). Stepwise, the tumor promoting TGF-β biology proceeds as follows. On many different tumor systems excess TGF-β, produced by both tumor and stromal cells, has been shown to act in concert with oncogenes to stimulate tumor progression: by direct action on the tumor cell in stimulating cell migration, invasion and survival, and by the modulation of tumor microenvironment in stimulating angiogenesis, extracellular matrix remodeling and immunosuppression. In addition, emerging evidence has revealed that cFOS oncoprotein is constitutively expressed in advanced stages of cervical cancer (6) and it constitutes the major AP-1 binding protein required for TGF-β1 production in human colon carcinoma cells (7). Thus, cFOS may have utility as a target for blocking tumor-cell secreted TGF-β1, thereby suppressing the TGF-β action in human carcinoma (7). In general, the tumor promoter TGF-β pathway presents an attractive target for the development of cancer therapeutics that simultaneously attack the tumor and its microenvironment. This pathway exhibits considerable relevance to anti-TGF-β therapeutic drug design that may well have systemic effects on tumorigenesis (8).

SCREENING CANCER PATIENTS FOR ANTI-TGFβ THERAPY

An assessment of TGF-β1 overexpression in cancer patients may be an effective strategy in screening candidates for anti-TGF-β therapy since these patients frequently display metastasis and are most likely

to have tumors that have already overcome the growth-suppressing effects of this cytokine. The link between TGF-β overexpression, tumor TβRII receptor expression, and predisposition to tumor progression might provide one way to select the candidates for the therapy.

In recent years, numerous studies, including the data from our laboratory, have revealed elevated TGF-β1 levels in circulation of patients with advanced cancer stages, as detected by ELISA assay (3,5). Moreover, this elevation was frequently associated with poor prognosis, cancer recurrence, and mortality. Increased TGF-β1 levels are found in serum of patients with colorectal cancer, hepatocellular carcinoma, lung cancer, and metastatic melanoma (3). We have previously determined, for the first time, significantly elevated plasma TGF-β levels in prostate cancer (PC) patients with invasive disease (9). These findings have lead us to propose that plasma TGF-β concentration may be a new tumor marker attributed to the presence of invasive PC cells that may be used in the prognosis of invasive PC (9). Subsequently, confirmed by many laboratories, this marker has been proposed as one of the components of PC diagnostic multiplex panel. Indeed, a biomarker panel containing PSA, TGFβ, and, IL6SR has been recently utilized to develop a prostate cancer nomogram to predict the disease regression after radical prostatectomy (10). By extending this line of research to breast cancer (BC), we have subsequently confirmed and extended the evidence for the significantly elevated plasma TGF-β1 levels in advanced BC patients with a poor prognosis (11). Moreover, we have observed that elevated plasma TGF-ß1 levels correlate with decreased survival of metastatic BC patients (12).

Unfortunately, several methodological problems in TGF-β1 detection in circulation of BC patients prevent us from potentially including these biomarkers among the standard prognostic/predictive factors (13). The methodological obstacles pertain to a wide overlapping of circulating TGF-β1 levels between controls and BC patients analyzed at the time of surgery (9-12, 14-16). This may be due to time lapse necessary to detect TGF-β1 elevation in circulation and the use of heterologous normal blood samples as controls. To overcome the described difficulties, we have recently performed the study using post-operative metastatic axillary lymph nodes (ALN), an established prognostic factor of invasiveness and metastasis. Our results indicate that cytoplasmic extract of metastatic ALN tissue may be promising new specimen source to quantify TGF-β1 ovrexpression and thus provide an early biomarker of invasiveness for BC patients (17).

TARGETING TGF-β FOR CANCER THERAPY

To date, most of successful targeted anti-cancer therapies e.g. HER2 or VEGF-R, have been aimed at inhibiting oncogenes known to be mutationally activated in tumor cells. In contrast, the use of inhibitors of the TGF-β pathways may seem counter-intuitive, since activating mutations or genomic amplifications in genes encoding components in the TGF-β signaling pathway are only emerging (2-7). Nevertheless, given the stimulatory effect of TGF-β in the tumor progression, it follows that TGF-β offers an attractive target for clinical therapy. Initial efforts to develop TGF-β signaling antagonists focused on inhibition of TGF-β ligand binding to TβRII receptor by the use of soluble TGF-β binding proteins (decorin, fetuin). However, these inhibitors have not provided sufficient specificity for therapeutic purposes (8). Further advances in the field have generated new classes of inhibitors with greater specificity.

SPECIFIC TGF-β ANTAGONISTS

Three major classes of specific TGF-β antagonists with comparative advantages and disadvantages are presented in Table 1. They include (1) TGF-β monoclonal antibodies (MoAbs) that are typically used to disrupt TGF-β ligand binding to the TβRII receptor. The high degree of target specificity of MoAbs counterbalances inherent undesirable pharmaceutical properties, such as structural complexity, large molecular weight, and physiologic barriers to intratumoral uptake (Table 1); (2) TGF-β antisense oligonucleotides (ASO) as single strands of RNA (17-22 nucleotides in length) that are complementary to a chosen sequence of TGF-β mRNA. They prevent TGF-β protein translation of TGF-β mRNA strands through complementary nucleic acid hybridization. In general, because multiple copies of a protein are produced by each mRNA molecule, targeting mRNA rather than the protein itself is potentially a more efficient approach to modulate protein function by altering its levels (18). However, there remain several technical obstacles to the successful clinical application of this technology in terms of sequence design and delivery (Table 1); (3) Small molecules inhibitors (SMI), have been developed only recently since TβRI receptor kinase was not thought to be achievable as a target (19). These inhibitors are ATP mimetics designed to be specific for inhibition of phosphorylation of SMAD2 and SMAD3 by blocking TβRI receptor kinase site. The representative SMI compound is LY550410 and contains set of heteroaryl rings that have the key functionality necessary for potent binding to the kinase-domain ATP binding site (19). As typical for SMI, some cross-reactivity with other TGFβ-related ligand signaling pathways and other kinase domains has been observed (8). Nevertheless, what is lost in specificity, it is gained in terms of drug delivery *in vivo* (Table 1).

Table 1. Comparative advantages and disadvantages of three types of specific TGF-β antagonists (18)

Type	Advantages	Disadvantages
MoAbs*	Target specificity High binding affinity Long half-life (reduced frequency of dosing)	Large Mw (poor tissue penetration) Applicable only on extracellularly expressed target proteins
ASO	Target specificity High binding affinity	Off-target effects (sequence-dependent) Multiple administrations Special delivery systems (can't cross cellular membranes)
SMI	Pleiotropic targets Tissue penetration and delivery Availability of oral formulation	Cross-reactivity Multiple administrations More side effects

* Abbreviations: MoAbs, monoclonal antibodies; ASO, antisense oligonucleotides; SMI, small molecule inhibitors (of TβRI receptor kinase)

PRECLINICAL STUDIES WITH TGF-β ANTAGONISTS: ANIMAL MODELS

The described anti-TGF-β approaches have shown great promise in the preclinical studies (19-21). They have proven quite safe, and there is some powerful evidence highlighting the potency of inhibiting TGF-β signaling as a way of controlling tumor progression including metastasis (19). Most of these studies were performed using human xenografts (human tumors in athymic mice) and syngeneic tumors (human tumors in mice with intact immune system), which constitutively express TGF-β. Individual examples include: (1) Athymic nude mice inoculated with MDA-MB-231 human breast cancer cell line that showed reduced spleen natural killer (NK) cell activity, and also displayed metastasis in the lung (22). However, when these mice were treated with 2G7 IgG2b neutralizing antibody, NK activity was reestablished and intra-abdominal tumorigenesis and lung metastasis were suppressed (22); (2) The utility of antisense TGF-β for treatment of glioma (23), suggesting a mode of action via potentiation of the immune system; and (3) SMI pharmacodynamics and pharmacokinetics evaluation in rat and mouse models based on the ability of these small molecules to inhibit the TβRI receptor kinase (19).

The summary of extensive preclinical studies (19-21), which use various human xenografts (breast, prostate, colon, glioma, etc.), is presented in Table 2. The observed anti-tumor effects (20,21) produced by the described antagonists include dramatic increase in potency of immune response as well as suppression of tumor growth, tumor volume, invasiveness, angiogenesis, and metastasis (Table 2). Moreover, the preclinical success of small molecule inhibitors parallels that of MoAbs in cancer therapy. The SMI ability to modulate intracellular targets confers a distinct advantage over MoAbs. These versatile molecules are well suited to alter target intracellular proteins either qualitatively or quantitatively.

Table 2. Preclinical cancer therapy with specific TGF-β antagonists in human tumor xenografts (19,20)

Type	Human tumor xenografts	Common effect on tumor parameters
MoAbs*	breast, prostate, colon, renal cell carcinoma	↑ antitumor immune response, ↑ survival, ↓ tumor growth,
ASO	breast, prostate, colon, glioma, hepatocellular	↓ tumor volume, ↓ invasiveness,
SMI	breast, colon, glioma	↓ angiogenesis, ↓ metastasis.

* Abbreviations: MoAbs, monoclonal antibodies; ASO, antisense oligonucleotides; SMI, small molecule inhibitors (of TβRI receptor kinase)

CLINICAL TRIALS WITH TGF-β ANTAGONISTS

At present, large-molecule TGF-β antagonists, including MoAbs and ASO, have been predominantly used in clinical trials (8). Although most of them have been originally developed for the treatment of fibrotic disorder, they provide encouraging examples of the therapeutic potential of modulating TGF-β signaling in human disease and are indicative of the potential success of TGF-β signaling inhibition in cancer (19-21). The summary of ongoing clinical therapy trials with the TGF-β antagonists is described in Table 3.

Table 3. Summary of ongoing clinical therapy trials with TGF-β antagonists (19-21)

Type	Target	Drug	Ongoing clinical trials	Diagnosis
MoAbs*	TGF-β2	CAT 152	Phase III	Glaucoma
	TGF-β1	CAT 192	Phase II	Scleroderma
	pan-	GC-1008	Phase I	Idiopatic pulmonary fibrosis
ASO	TGF-β2	AP12009	Phase II	High-grade glioma
	TGF-β1	AP11014	None	

* Abbreviations: MoAbs, monoclonal antibodies; ASO, antisense oligonucleotides;

Several TGF-β-targeted human MoAbs have been designed and used to target TGF-β pathway activation. First MoAb, CAT-152 (Lerdelimumab) is a recombinant human IgG4 that neutralizes TGFβ-2 following glaucoma surgery. This MoAb is currently undergoing phase III trials for prevention of postoperative scarring following glaucoma surgery (Table 3), and it has been awarded Orphan Drug status in Europe. A second human antibody, CAT-192 (Metelimumab), is a recombinant human IgG4 that neutralizes TGFβ-1. It has also been awarded Orphan Drug status in Europe for the treatment of scleroderma and it is currently undergoing phase II trials (Table 3). However, due to the expression of multiple TGF-β isoforms in tumors, a pan-specific monoclonal antibody, GC-1008, is presently in the early phases of development as a drug therapeutic (19). This MoAb is undergoing phase I trials for idiopathic pulmonary fibrosis (Table 3).

To date, the anti-TGFβ drugs that have proceeded furthest in clinical trials for cancer are antisense oligonucleotides, particularly AP-12009 and AP-11014. Currently, AP12009 is being tested in a clinical phase II study for the systemic treatment of high-grade glioma (24). A total number of 24 patients were treated intratumorally with a single cycle (first study), a second cycle (second study) or up to 10 cycles (third study) of AP12009. The drug was considered safe and well tolerated. Overall, 7 patients out of 24 patients showed stable disease 28 days after start of treatment with AP12009. Furthermore, in two patients, long-lasting complete tumor remission was observed. In one of the patients, even the non-infused distant lesions in the contralateral hemisphere disappeared. The results were consistent with reversal of tumor-induced immunosuppression and the restoration of an effective antitumor immune response. The results suggest that the approach to block protein-mediated effects at the translation level by antisense oligonucleotides is superior to the employment of corresponding antibodies.

CONCLUSIONS AND OUTLOOK

TGF-β plays a very important role in promoting the spread of cancers in the body, and can play a direct role in facilitating metastasis. The potential to impede the metastatic potential of tumor cells while simultaneously having an impact on the tumor microenvironment, including angiogenesis, stromal activation and immunosuppression, provides a powerful rationale for evaluating TGF-β signaling inhibitors in cancer therapy. There is growing portfolio of drugs that target the TGF-β pathway and they may be used for cancer therapy, including both large and small molecule inhibitors. Since, TGF-β action is so context dependent within the cell and at the organismal level, it is likely that drug efficacy will depend very much on tumor type and clinical stage.

Ultimately, a deep understanding of the interacting networks of signal pathway that regulate TGF-β outcome in tumor and host cells should allow the judicial choice of drug combination for each specific tumor type. The next several years promise to improve our understanding of approaching

cancer therapy by further evaluation of TGF-β signaling inhibitors for clinical efficacy. The complexity of TGF-β biology guarantees that many surprises lie ahead.

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Conflict of interest

We declare no conflicts of interest.

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