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Review

TGF beta signalling and its role in tumour pathogenesis*

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Transforming growth factor beta (TGF-β) is a multifunctional cytokine involved in the regulation of cell proliferation, differentiation and survival/or apoptosis of many cells. Knock-out experiments in mice for the three isoforms of TGF-β have demonstrated their importance in regulating inflammation and tissue repair. TGF-β is implicated in the pathogenesis of human diseases, including tissue fibrosis and carcinogenesis. TGF-β receptors act through multiple intracellular pathways. Upon binding of TGF-β with its receptor, receptor-regulated Smad2/3 proteins become phosphorylated and associate with Smad4. Such complex translocates to the nucleus, binds to DNA and regulates transcription of specific genes. Negative regulation of TGF-β/Smad signalling may occur through the inhibitory Smad6/7. Furthermore, TGF-β-activated kinase-1 (TAK1) is a component of TGF-β signalling and activates stress-activated kinases: p38 through MKK6 or MKK3 and c-Jun N-terminal kinases (JNKs) *via* MKK4. In the brain TGF-β, normally expressed at the very low level, increases dramatically after injury. Increased mRNA levels of the three TGF-β isoforms correlate with the degree of malignancy of human gliomas. TGF-βs are secreted as latent precursors requiring activation into the mature form. TGF-β may contribute to tumour pathogenesis by direct support of tumour growth and influence on local microenvironment, resulting in immunosuppression, induction of angiogenesis, and modification of the extracellular matrix. TGF-β1,2 may stimulate production of vascular endothelial growth factor (VEGF) as well as plasminogen activator inhibitor (PAI-I), that are involved in vascular remodelling occurring during angiogenesis. Blocking of TGF-β action inhibits tumour viability, migration, metastases in mammary cancer, melanoma and prostate cancer model. Reduction of TGF-β production and activity may be a promising target of therapeutic strategies to control tumour growth.

Keywords: TGF beta signal transduction, Smad propteins, MAP kinases, tumour invasion, cancer therapy, RNA interference.

TGF-β is a multifunctional cytokine that regulates cell proliferation, differentiation and extracellular matrix production (Jennings & Pietenpol, 1998; Verrecchia & Mauviel, 2002). Deregulation of TGF-β expression or signalling has been implicated in the pathogenesis of a variety of diseases, including cancer and fibrosis. In the brain, TGF-β is expressed at a very low level that increases dramatically after injury (Lindholm *et al.*, 1992). Under physiological conditions TGF-β inhibits proliferation of normal astrocytes, but loses its growth-inhibitory potential towards gliomas, due to alterations in the expression of cell cycle inhibitors. There is growing evidence that in the later stages of cancer development TGF-β is actively secreted by tumour cells and does not merely act as a bystander but rather contributes to cell growth, invasion, and metastasis and decreases host-tumour immune responses, as depicted in Fig. 1 (Jennings & Pietenpol, 1998). The effects of distinct TGF-β isoforms depend on the type, differentiation state and physiological conditions of target cells (Bottner *et al.*, 2000). Despite the multitude of data regarding TGF-β expression in different brain tumours, the molecular mechanisms underlying the expression, signalling and role of TGF-β in pathogenesis of glioblastomas are still unknown. We summarize here data concerning molecular mechanisms of TGF-β activation, signalling, role in tumour pathogenesis and present a rationale for evaluating TGF-β signalling inhibitors as cancer therapeutics.

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Abbreviations: ERK1/2, extracellular signal-regulated kinase 1/2; JNK, c-Jun N-terminal kinase; LAP, latency associated peptide; LTBP, latent TGF-β binding protein; MAPK, mitogen activated protein kinase; MMP, matrix metalloproteinase; PAI-I, plasminogen activator inhibitor; shRNA, small hairpin RNA; TAK1, TGF-β-activated kinase-1; TGF-β, transforming growth factor beta; TβRI, Type I TGF-beta receptor; TβRII, Type II TGF-beta receptor; VEGF, vascular endothelial growth factor.

ACTIVATION OF TRANSFORMING GROWTH FACTOR β

TGF-β is produced and secreted *in vivo* as a latent complex, in which a dimer of mature growth factor is associated with the pro-peptide. The latent state prevents the cytokine from eliciting a response until certain physiological conditions occur or until the target cell is reached. TGF-β is synthesised as 55-kDa polypeptides, which dimerise shortly after production. Then the precursor molecule is cleaved in the Golgi apparatus by furine-like proteases to form small latent TGF-β (Dubois *et al.*, 1995). This complex contains the mature 25-kDa protein a non-covalently bound to the N-terminal pro-peptide called LAP — latency associated peptide. Such complex may be secreted, but it usually associates with latent TGF-β binding protein (LTBP) forming large latent TGF-β. LTBPs facilitate TGF-β secretion and address it to the extracellular matrix. Cellular recognition of extracellular matrix-associated LTBP and subsequent recognition of latent TGF-β are essential steps in its activation (Hyytiainen *et al.*, 2004). The latency proteins also contribute to the cytokine stability. Free TGF-β has a half life of about 2 min, whereas the latent form — 90 min.

In vivo TGF-β can be activated in an enzymatic process for example by plasmin, which concentrates at critical sites of the cell surface and is able to release mature cytokine, by cleavage of LAP (Grainger *et al.*, 1995). Thrombospondin-1 has been reported as another protein that activates TGF-β from small or large latent complexes and may cooperate with the plasmin-mediated process (Ribeiro *et al.*, 1999). Further proteins participating in TGF-β activation *in vivo* are: integrins, matrix metalloproteinases (MMP-2 and MMP-9) and calpains. Activation of latent TGF-β is a multi-step process and represents a regulatory stage, which translates into a tight control of active TGF-β formation.

INTRACELLULAR TGF-β SIGNALLING PATHWAYS

TGF-β binds tightly to Type II receptor (TβRII) first; this binding allows subsequent incorporation of Type I receptor (TβRI), forming a large ligand–receptor complex involving a ligand dimer and four receptor molecules. The Type I receptor requires activation by Type II to be functional and able to bind the ligand by itself. Simultaneous binding to

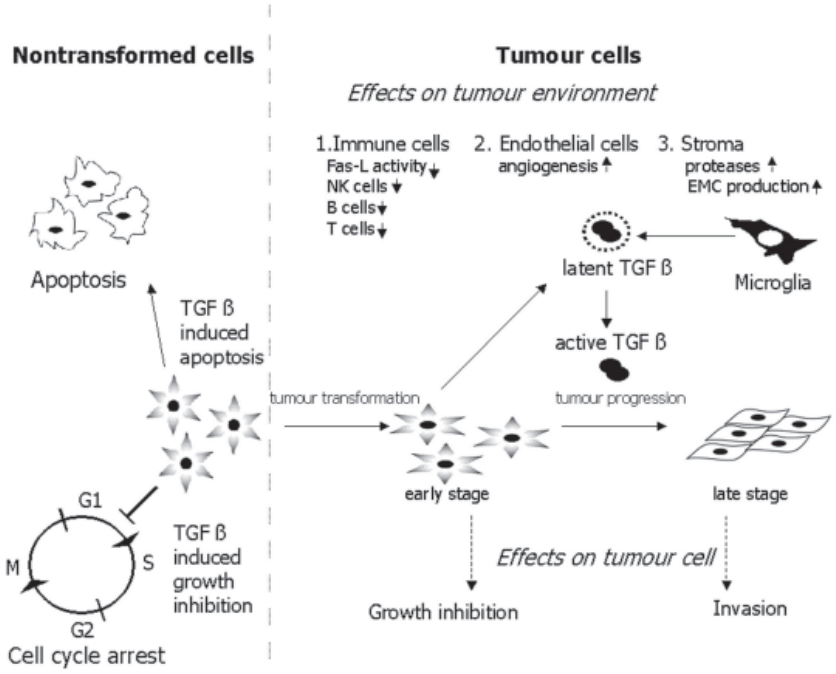

Figure 1. Possible multiple roles of TGF-β in tumour pathogenesis.

TGF-β can induce apoptosis or inhibit proliferation of nontransformed cells but loses its growth-inhibitory potential as cells progress to later stages of tumourigenesis. In the later stages of tumour development TGF-β is actively secreted by tumour cells or stromal cells and contributes to cell growth, invasion, metastasis, and decrease in host anti-tumour immune responses.

the extracellular domains of both types of the receptors by the dimeric ligand induces a close proximity and a proper conformation of the intracellular kinase domains of the receptors, facilitating the phosphorylation and subsequent activation of the Type I receptor (Shi & Massaque, 2003). Smad proteins are basic intracellular components of TGF-β signalling (Nakao *et al.*, 1997b). The MH2 (MAD-homology-2) domain is highly conserved among all Smad proteins and is responsible for receptor interaction, formation of homo- and heteromeric Smad complexes, and direct contact with the nuclear pore complex for shuttling to the nucleus. Phosphorylation of the two C-terminal serine residues in the SXS motif of the MH2 domain activates the receptor Smad (Souchelnytskyi *et al.*, 1997). After ligand binding, Smad2/3 is phosphorylated by an active form of Type I receptor (Piek *et al.*, 1999; Attisano & Wrana, 2002; Shi & Massaque, 2003) and associates with the common Smad4 to form a hetero-oligomeric complex which translocates to the nucleus (Fig. 2). Following translocation, it binds to a specific DNA sequence in the promoters of target genes to regulate their transcription. The transcriptional response to TGF-β depends also on the activity of Smad transcriptional partners. Most of the Smad partners identified to date (e.g., Fast1, Mixer, Jun/Fos, Runx, ATF3, E2F4/5) are highly responsive to different inputs.

The Smad signalling pathway may be negatively regulated by the inhibitory Smad6 and Smad7 (Nakao *et al.*, 1997a). Inhibitory Smad7 acts to oppose the signal mediated by Smads by forming stable associations with activated Type I receptors, thus preventing phosphorylation of receptor Smads and acting as a negative feedback regulator (Shi & Massaque, 2003). Moreover, transcriptionally activated by TGF-β signaling, Smad7 promotes the ubiquitination and degradation of the receptors *via* Smurf1/2 proteins. Smad6 competes with Smad1 for binding to Smad4 (Massague & Wotton, 2000; Derynck & Zhang, 2003).

Although the Smad pathway is widely represented in most of the cell types studied, additional pathways may be activated following treatment with TGF-β in specific contexts (Fig. 2). For example, activation of Ras, extracellular signal-regulated kinase 1/2 (ERK1/2), and c-Jun N-terminal kinase (JNK) by TGF-β signalling have been reported in primary intestinal epithelial cells and some breast cancer cell lines (for a review see Mulder *et al.*, 2000), whereas activation of protein kinase A contributes to TGF-β signalling in murine mesangial cells (Wang *et al.*,

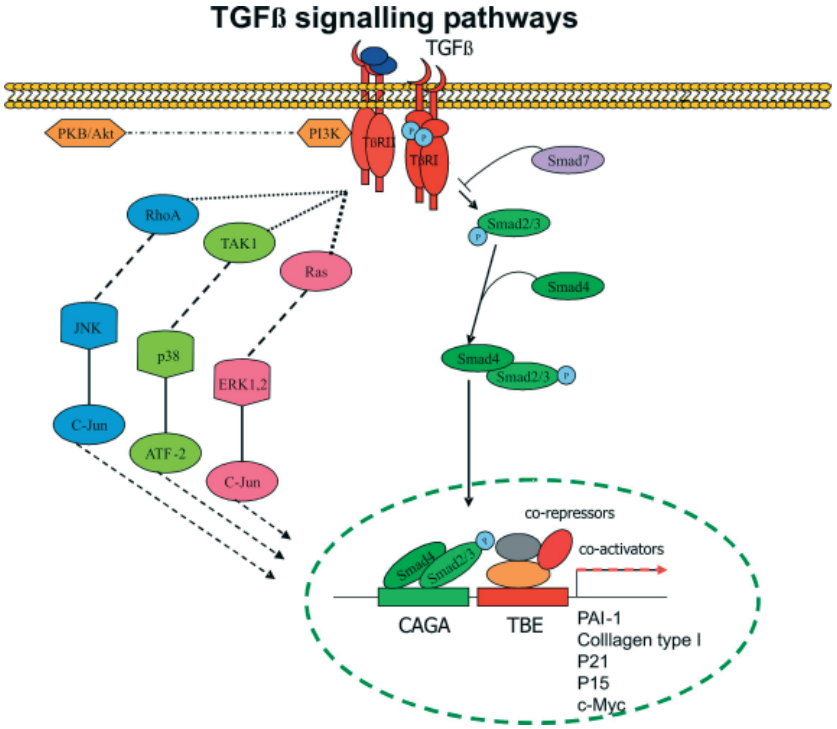

Figure 2. TGF-β signalling pathways.

TGF-β dimer binds to Type II receptors which leads to association with Type I receptors, conformational changes and activation of kinase domains of the receptors. Smad 2/3 proteins are cytoplasmic molecules which, after receptor activation, are phosphorylated by an active form of Type I receptor and associates with Smad 4. Hetero-oligomeric complex of Smad 2/3-Smad4 translocates to the nucleus and binds to specific DNA sequence in the promoters of target genes. The pathway is regulated by the activity of the inhibitory Smad7. TGF-β can activate several mitogen activated protein kinases (MAPKs), including extracellular signal-regulated kinases (ERKs), c-Jun N-terminal kinases (JNKs), and p38 MAPK. The interaction with these MAP kinases could regulate Smad transcriptional activity in a positive or a negative manner.

1998). TGF-β activated kinase-1 (TAK1), a member of the MEKK family and activator of JNK and p38 MAPK pathways (Yamaguchi *et al.*, 1995), was rapidly activated by TGF-β. Selective activation of p38 MAPK, with no apparent activation of JNK after TGF-β stimulation was reported for C2C12, Mv1Lu, and HaCaT cells (Hanafusa *et al.*, 1999; Karsdal *et al.*, 2003).

Recent studies suggest that different signalling modules may be responsible for a unique functions of the cytokine in epithelial cells. MEK, an inducer of Erk, appeared to be a major mediator of TGF-β1-dependent PAI-1 expression and cell motility in renal epithelial cells (Kutz *et al.*, 2001). TGF-β activates the Ras/MKK4/JNK1 signalling cascade, leading to induction of AP-1 activity, which, in turn, up-regulates uPAR (receptor of urokinase-type plasminogen activator) expression in nontransformed intestinal epithelial cells. Type II TGF-β receptor is required for activation of JNK1 and the resulting up-regulation of uPAR expression (Yue *et al.*, 2004). In mouse mammary epithelial cells, activation of p38 MAPK is required for TGF-β-induced apoptosis, epithelial-to-mesenchymal transition, but not growth arrest (Ungefroren *et al.*, 2003; Yoo *et al.*, 2003). TGF-β1 signalling potentiates staurosporine-induced apoptosis of renal epithelial cells by a Smad-independent, p38 MAP kinase-dependent mechanism (Dai *et al.*, 2003). In transformed human epidermal keratinocytes, TGF-β activates differently various MAPKs (Johansson *et al.*, 2000). p38 MAPK mediates TGF-β-induced expression of MMP-2 and MMP-9 in MCF10A human breast epithelial cells (Kim *et al.*, 2004).

REGULATION OF TGF-β EXPRESSION IN TUMOUR CELLS

The promoter regions of genes encoding three mammalian transforming growth factors-β (TGF-β1, 2, 3) show little similarity in sequence, indicating differential transcriptional regulation of the genes, whose protein products are functionally very similar. Inspection of the TGF-β1 and TGF-β3 promoters reveals the presence of several putative regulatory elements, including several Sp1 and AP-1 transcription factor binding sites. Promoter fragments of the TGF-β1 and TGF-β3 genes, but not of TGF-β2, were able to compete for binding of Sp1 to DNA oligomers containing consensus Sp1-binding sites (Geiser *et al.*, 1993).

An important property of TGF-β1 is an ability to activate its mRNA expression and thereby its own secretion (Van Obberghen-Schilling *et al.*, 1988; Kim *et al.*, 1990; Jennings *et al.*, 1991; Jachimczak *et al.*, 1996). The crucial role of the AP-1 transcription factor in the autocrine regulation of TGF-β1 expression has been demonstrated in human lymphoma cells and adenocarcinoma A-549 cells (Birchenall-Roberts *et al.*, 1990; Kim *et al.*, 1990). The authors identified three AP-1-binding elements responsible for the auto-induction. Antisense *c-jun* and antisense *c-fos* blocked autocrine TGF-β1-induced expression, confirming an involvement of these two components in the mechanism. This property of TGF-β1 may underlie the observed autocrine growth regulatory effect of the cytokine in glioma cells (Jennings *et al.*, 1991). Contribution of c-Jun N-terminal kinase (JNK) signalling to the control of TGF-β expression has been proposed after observing that JNK-deficient fibroblasts isolated from Jnk1–/– Jnk2–/– mice constitutively express TGF-β1. Complementation studies demonstrated that JNK is a repressor of TGF-β1 gene expression (Ventura *et al.*, 2004).

Some studies demonstrated that Cyclosporine A (CsA) induces increased expression of TGF-β *in vitro* as well as *in vivo*. CsA stimulated TGF-β 1 promoter-dependent transcription of CAT reporter gene in transiently transfected human A-549 cells as well as the synthesis of TGF-β mRNA in human T cells (Prashar *et al.*, 1995). Hojo *et al.* (1999) reported that CsA enhances the TGFβ production in A-549 adenocarcinoma cells that correlated with acquisition of a more aggressive phenotype and their enhanced invasiveness. Glucocorticoid dexamethasone causes a significant decrease in the basal and PMA-induced levels of TGF-β1 mRNA in glial cells but not in T cells (Batuman *et al.*, 1995).

Also post-transcriptional regulation of TGF-β1 expression has been reported in A-549 and PC-3 human prostate adenocarcinoma cells (Kim *et al.*, 1992). An increased level of the protein released to the culture medium does not always correlate with increased mRNA level of TGF-β1. It has been suggested that the 5′ untranslated region (UTR) of TGF-β1 mRNA containing a stem-loop element may contribute to enhanced mRNA stability.

ROLE OF TGF-β IN TUMOUR PATHOGENESIS

Alterations of TGF-β signalling pathways contribute to tumour risk

There is growing evidence that alterations in TGF-β signalling pathway components modify cancer risk. Approximately 14% of the general population carry TGFBR1*6A, a variant of the TGFBR1 gene that results in decreased TGF-β-mediated growth inhibition. Recent studies show that the overall cancer risk is increased by 70 and 19% among TGFBR1*6A homozygotes and heterozygotes, respectively (Kaklamani *et al.*, 2003). This suggests that TGFBR1*6A may contribute to the development of a large proportion of common forms of cancer and may become a target for cancer chemoprevention.

Mutational inactivation of TGFBR2 is the most common genetic event affecting the TGF-β signalling pathway and occurs in approx. 20–30% of all colon cancers (Biswas *et al.,* 2004). Using a mouse model that is null for Tgfbr2 in the colonic epithelium (Cre-lox inactivation of TGFIIR), the authors demonstrated that a loss of TGFBIIR expression in colon epithelial cells promotes the establishment and progression of azoxymethane-induced colon neoplasms, suggesting that TGFBR2 is a tumour suppressor gene in the colon. While decreased TGF-β signalling increases cancer risk, TGF-β secretion and activated TGF-β signalling enhance the aggressiveness of several types of tumours.

Role of TGF-β in tumour migration

The invasion of neoplastic cells into brain tissue is a pathologic hallmark of gliomas and contributes to the failure of current therapeutic modalities (surgery, radiation and chemotherapy). Glioma cells have the ability to invade as single cells through the unique environment of the normal central nervous system (CNS). The brain parenchyma has a unique composition, mainly hyaluronan and is devoid of rigid protein barriers composed of collagen, fibronectin and laminin. Proteases secreted during glioma progression degrade extracellular matrix allowing tumour cells to spread and diffusely infiltrate the brain parenchyma (Rao, 2003).

Exogenous TGF-β1 directly increases the motility of glioma cells by enhancing expression of collagen and subunit of $\alpha_{2,5}$, β_3 integrin, as well as by up-regulating the activity of metalloproteinases MMP-2, 9 at the cell surface of glioma cells (Wick *et al.,* 2001). Interaction between cell surface receptors such as integrins, and extracellular matrix components, for instance collagen, is essential for tumour metastasis and angiogenesis (Verrecchia & Mauviel, 2002). Additionally, increased enzymatic degradation of extracellular matrix proteins may facilitate tumour spread (Platten *et al.,* 2001; Wick *et al.,* 2001).

TGF-β1 as pro-angiogenic factor

Moreover, TGF-β1 may act as an angiogenic factor promoting neovascularization of the tumour. TGF-β1,2 stimulate production of vascular endothelial growth factor (VEGF), which is a major stimulus in the promotion of angiogenesis, as well as plasminogen activator inhibitor (PAI-I) (Benckert *et al.,* 2003; Sugano *et al.,* 2003). Both are involved in vascular remodelling which occurs during angiogenesis (Kaur *et al.,* 2004). TGF-β1 stimulates VEGF 164 *via* mitogen-activated protein kinase kinase 3 (MKK3) and activation of p38α and p38δ MAPK-dependent pathway in murine mesangial cells (Wang *et al.,* 2004). Some studies demonstrated that hypoxia and TGF-β signalling pathways can synergize in the regulation of VEGF gene expression at the transcriptional level and cooperate in the induction of the promoter activity of VEGF. This cooperation has been mapped on the human VEGF promoter within a region at –1006 to –954 that contains functional DNA-binding sequences for HIF-1 and Smads (Sanchez-Elsner *et al.,* 2001).

Role of TGF-β in tumour-mediated immunosuppression

TGF-β plays a crucial role in the escape of glioma from host immunity. The anti-tumour response in patients with glioma may be ineffective because of a lack of a specific tumour antigen and professional antigen presenting cells in the brain. TGF-β1 enhances this effect by inhibition of MHC class II expression on glioma cells, macrophages and microglia (Lee *et al.,* 1997; Dong *et al.,* 2001; Zagzag *et al.,* 2005). TGF-β1 exerts an immunosuppressive effect on all cells of the immune system (Jachimczak *et al.,* 1993; Beck *et al.,* 2001; Chen *et al.,* 2005). The main target of its action are T lymphocytes, which could develop into effector ($CD8^+$ CTL) or helper ($CD4^+$Th1 or Th2) cells. Although several cytokines have been reported to influence differentiation of naive T cells, TGF-β1 is the most effective inhibitor of their maturation. In the presence of this cytokine, $CD8^+$ failed to come CTL and $CD4^+$ did not achieve the Th1 or Th2 phenotype (Gorelik & Flavell, 2001). Th2 differentiation seems to be more sensitive to this effect. There is evidence, that TGF-β1 inhibits generation of cytotoxic $CD8^+$ T cell subpopulation, although the mechanism has not been clarified. TGF-β1 suppresses granzyme B and perforin expression that are crucial for the cytolytic action of cytotoxic lymphocytes (Smyth *et al.,* 1991).

There are contradictory results concerning the anti-proliferative effect of TGF-β1 on CTL (Inge *et al.,* 1992). Under some experimental conditions the effect induced by TGF-β1 was due to suppression of such immunostimulatory cytokine expression as INFγ and TNFα or down-regulation of IL-2-mediated proliferative signals (Ranges *et al.,* 1987). TGF-β1 can also abolish T cell activation by a negative effect on antigen presenting cells, such as dendritic cells. TGF-β1 can also suppress macrophages by down-regulation of TNFα, H_2O_2 and NO production. Moreover, TGF-β1 enhances the production of immunosuppressive IL-10 by macrophages (Maeda *et al.,* 1995). Additional targets of TGF-β1-mediated immunosuppression are natural killer (NK) and lymphokine activated killer cells (LAK), as well as neutrophils (Kuppner *et al.,* 1988).

Blocking of TGF-β1 signalling in the immune system cells led to enhanced anti-tumour response. Both thymoma and melanoma-derived cell lines

were eradicated by animals expressing a dominant negative TβRII under the control of a T-specific promoter (Gorelik & Flavell, 2001). In addition, CTLs transduced with a vector expressing dominant negative TβRII were resistant to the anti-proliferative and anti-cytotoxic effects of exogenous TGF-β1 in EBV-positive Hodgkin disease (Bollard *et al.*, 2002). Taken together, efforts to bypass TGF-β-mediated immunosuppression represent an attractive therapeutic strategy for the treatment of human cancers, both by directly increasing the efficacy of immunosurveillance and the efficacy of tumour immunotherapy.

DEVELOPMENT OF INHIBITORS OF TGF-β SIGNALLING

The TGF-β signalling pathway is emerging as an attractive target in cancer and it is predicted that inhibitors of this pathway will find their way into cancer clinical trials, leading to delays in tumour progression and improvement in overall survival. Blockade of TGF-β action inhibited mammary tumour cell viability, migration, and metastases (Muraoka *et al.*, 2002). Introduction of dominant negative TGF-β Type II receptors (TβRII) into these cells retards primary tumour and metastases formation and prevents epithelial-to-mesenchymal transition (EMT) (Oft *et al.*, 1998). Retrovirus-mediated introduction of a dominant negative TGFIIR to bone marrow cells led to generation of leukocytes capable of potent anti-tumour response and suppression of metastasis in melanoma and prostate cancer model (Shah *et al.*, 2002). The tumorigenicity of mouse thymoma was suppressed by soluble — TGFIIR therapy (Won *et al.*, 1999).

Table 1 summarizes recent therapeutic approaches targeting the TGF-β pathway. One strategy is based on blocking the interactions between the cytokine and its receptor. For example, an application of soluble TGFIIR or human α2-macroglobulin plasma protein that bind TGF-β isoform, limited the access of the cytokine to the receptor (Won *et al.*, 1999). Two humanized monoclonal antibodies: CAT-192 specific to TGF-β1 and CAT-152 against TGF-β2, are under clinical trial for treatment of fibrosis (Benigni *et al.*, 2003; Mead *et al.*, 2003). Positive results of these investigations will encourage application of this strategy to anti-cancer therapy.

The antisense oligonuclotide approach is represented by the AP-12009 molecule blocking TGF-β2 expression in tumour cells (Bogdahn *et al.*, 2004). Data obtained from phase I/II studies indicated a significant increase in survival time of glioblastoma patients that correlated with the reduction of tumour size by more than 80%. Additionally, a TGF-β1–specific antisense oligonuclotide (AP-11014) designed by the same company, Antisense Pharma, is under preclinical development for human non-small cell lung carcinoma, colorectal and prostate cancer. Recently, an effective strategy based on RNA interference was used to reduce TGF-β activity in malignant cells. Blockade of cytokine expression using siRNA against TGF-β inhibited tumour cell migration, invasiveness and restored anti-tumour immune response in a mouse model of glioma (Friese *et al.*, 2004). We have applied a similar strategy and developed vectors coding for small hairpin RNA which silence TGF-β receptor Type II gene expression by RNA interference in human glioblastoma cells. Transfection of glioblastoma cells with these vectors effectively diminished the expression of TGFβII receptor, abolished TGF-activated Smad signalling and reduced activation of the PAI-1 promoter (Wesolowska *et al.*, unpublished).

Another approach is aimed at directly blocking the catalytic activity of TGF receptor kinase. A group of competitive inhibitors of the ATP binding

Table 1. Development of inhibitors of TGF-β signalling

Agent	Type	Development stages	Company	References
Lerdelimumb CAT-152	TGF-β2 mAb	Phase III	Cambridge Antibody Technology	Mead *et al.*, 2003
Metelimumab CAT-192	TGF-β1 mAb	Phase II	Cambridge Antibody Technology	Benigni *et al.*, 2003
AP-12009	Oligonucleotide anti TGF-β2	Phase II	Antisense Pharma	Bogdahn *et al.*, 2004
AP-11014	Oligonucleotide anti TGF-β2	Preclinical	Antisense Pharma	Schlingensiepen *et al.*, 2004
LY550410 LY580276	Small molecule T β RI inhibitor	Preclinical	Lilly Research	Sawyer *et al.*, 2004
SB505124	Small molecule T β RI inhibitor	Preclinical	GlaxoSmithKline	DaCosta Byfield *et al.*, 2004
SB-431542	Small molecule T β RI inhibitor	Preclinical	GlaxoSmithKline	Hjelmeland *et al.*, 2004
SD-208	Small molecule T β RI inhibitor	Preclinical	Academic Institution	Uhl *et al.*, 2004

site of TGF receptor Type I kinase, such as LY550410, LY580276 and SB-505124, has been designed. Such compounds consist of domain with a hydrogen-bond acceptor (which may be imidazole core, pyrozole ring or quinoline scaffold) essential for blocking. Several studies demonstrated physiological efficacy of such molecules, as well as their kinase inhibitory activity (Sawyer *et al*., 2004; DaCosta Byfield *et al*., 2004). Another small-molecule inhibitor SD-208 appears to be a very potent antagonist of TGF-β receptor. The drug significantly prolonged the survival time of glioma-bearing mice. In the presence of the inhibitor, the immunogenicity of glioma cells was enhanced, while their migratory and invasion properties were diminished (Uhl *et al*., 2004).

Moreover, small-molecule inhibitors (such as SB-431542) have been developed that target downstream TGF-β signalling and block phosphorylation of Smad proteins, resulting in the inhibition of Smad nuclear translocation. TGF-β-mediated up-regulation of critical genes was abolished in human glioma cells by a treatment with SB-431542 (Inman *et al*., 2001; Hjelmeland *et al*., 2003). However, such an approach will not affect Smad-independent pathways.

A large-molecule antagonist of TGF-β signalling seems to be more selective and may have broader action than small-molecule inhibitors. Despite earlier predictions of severe toxicity, neutralizing antibodies to TGF-β are well tolerated and have potent anti-metastatic activity. Si/shRNAs are recognized as a new class of potential therapeutics against a wide range of diseases. Recent data obtained in several laboratories demonstrate the efficacy of systemically administered shRNA as a therapeutic strategy in experimental cancers. However, the success of this approach will largely depend on efficient delivery of shRNAs to tumour cells. Transfection of shRNA with lentiviral or adeno-associated vectors in cultured mammalian cells and in whole animals may be a promising approach in specific, efficient, and stable knockdown of various genes (An *et al*., 2003; Tiscornia *et al*., 2003; Grimm *et al*., 2005).

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