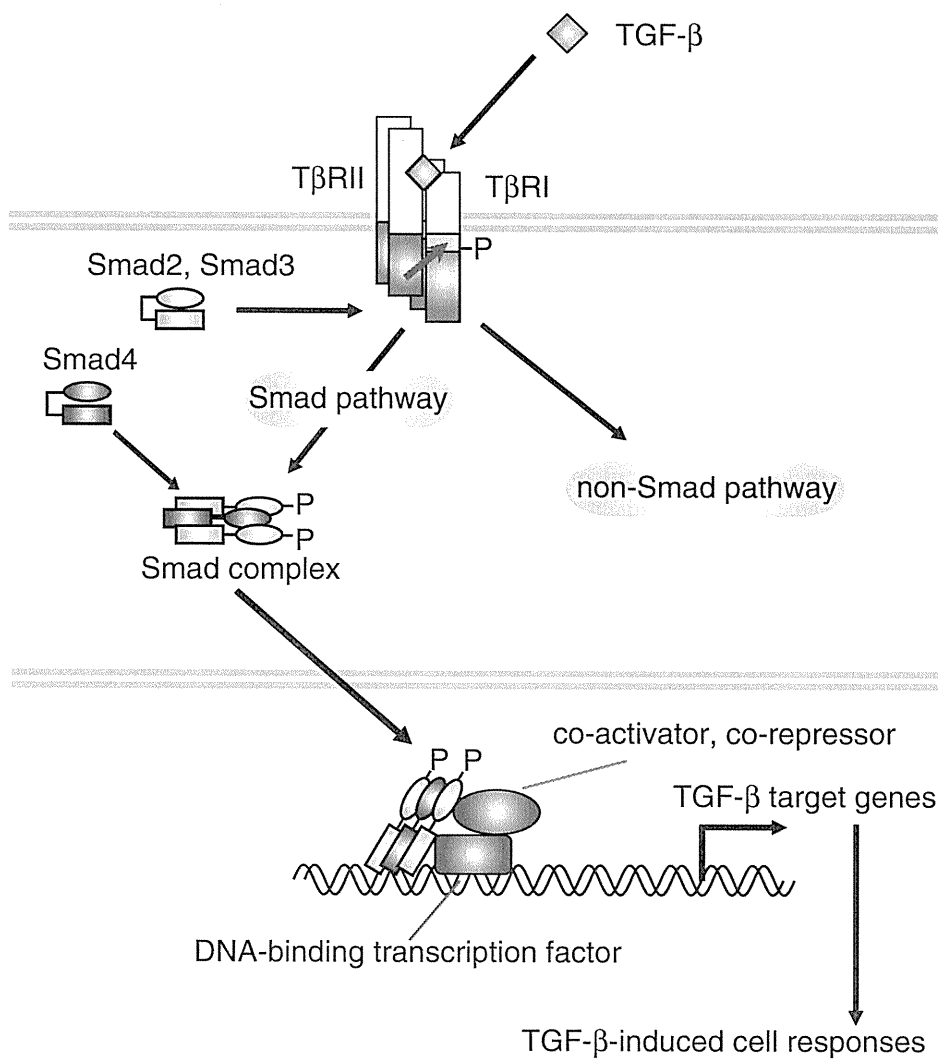

Fig. 1 Intracellular transforming growth factor-β (TGF-β) signal transduction. TGF-β signals are transduced by type II receptor (*TβRII*), type I receptor (*TβRI*), and their downstream Smad proteins (*Smad2-4*). Activated Smad complex interacts with DNA-binding transcription factors and co-activators/co-repressors and binds to the promoter regions of TGF-β target genes. Active TGF-β receptors also regulate signaling cascades other than the Smad pathway

cells. Smad4 is the only known Co-Smad in mammals. R-Smads consist of conserved Mad homology 1 (MH1) and MH2 domains, which are connected with a less-conserved linker region. The C-terminus of R-Smads has a characteristic SSXS (Ser-Ser-X-Ser) motif that is phosphorylated by active type I receptors. Smad4 contains MH1 and MH2 domains but lacks the C-terminal SSXS motif and, thus, is not phosphorylated by type I receptors. Smad complexes bind specific DNA sequences, namely 5′-AGAC-3′ or its reverse complement 5′-GTCT-3′, in the promoters or enhancers of target genes. They interact with other DNA-binding transcription factors, co-activators or co-repressors, and chromatin remodeling factors to the regulatory regions of target genes in order to regulate diverse TGF-β-induced cell responses. TGF-β stimulation also activates intracellular signals through non-Smad pathways, including mitogen-activated protein kinase, PI3K-Akt, and small GTPase pathways (Moustakas and Heldin 2005; Zhang 2009).

Context-dependent diversity of TGF-β-induced cell responses

At the core of this signaling pathway, TGF-β induces its membrane receptors directly to activate Smad proteins, which then form transcriptional complexes to control target genes. The aspect that makes this system complex is that these complexes activate or repress numerous target genes at the same time in a tightly regulated fashion. Furthermore, TGF-β stimulation induces numerous cell responses in a cellular context-dependent fashion (Roberts and Wakefield 2003; Bierie and Moses 2006). For example, TGF-β promotes cell proliferation in certain cellular contexts but inhibits it in most others (Ikushima and Miyazono 2010a). This cytokine plays crucial roles in the maintenance of the tumorigenic activities of some types of cancer stem cells (Ikushima et al. 2009; Peñuelas et al. 2009; Anido et al. 2010; Naka et al. 2010) but promotes the loss of tumorigenicity in others (Tang et al. 2007; Ehata et al.

2011). The cells making up one human body are all derived from a single cell, even if they are abnormal. However, they exhibit different responses to TGF-β because of slight but crucial differences. Moreover, even in the same type of cell, the cell responses mediated by TGF-β differ depending on environmental factors. Because of this inherent diversity, TGF-β-based therapeutic strategies are considered complex. Here, we discuss proposed or established mechanisms responsible for the chaotic diversity of TGF-β signaling.

Signal cross-talk

TGF-β is able to induce certain cell responses, under conditions including other types of signaling, but fails to induce the same responses without such signaling (Guo and Wang 2009). Cross-interaction with additional signaling is thus required for some TGF-β-induced cell responses (Fig. 2). Many signaling pathways have been reported to exhibit cross-talk with the TGF-β signaling pathway (Luo 2008; Zhang 2009); here, we discuss cross-talk with the Wnt, p53, and Ras signaling pathways.

Wnt signaling plays diverse roles in regulating numerous cell responses, including cell proliferation, differentiation, migration, and survival (Kestler and Kühl 2008; Logan and Nusse 2004). Canonical Wnt signaling is mediated by β-catenin, which functions as a transcription co-factor and is also essential for the formation of adherence junctions between cells through its interaction with cadherins. In the absence of Wnt, cytoplasmic β-catenin is degraded through glycogen synthase kinase (GSK)-3β-mediated serial phosphorylation and subsequent polyubiquitination, which keeps the Wnt pathway in an "OFF" state. The binding of Wnt ligand to its receptor Frizzled (Fz) and co-receptor LRP5/6 leads to GSK-3β inactivation and β-catenin stabilization. The cytoplasmic accumulation of β-catenin promotes its translocation into the nucleus, where it binds the lymphocyte enhancer factor/T-cell transcription factor (Lef/TCF) family of transcription factors and turns the Wnt pathway "ON". The most common format of cross-talk between the TGF-β and Wnt signaling pathways occurs in the nucleus, where the Smads and Lef/β-catenin synergistically regulate a set of shared target genes (Labbé et al. 2000, 2007; Hussein et al. 2003; Sasaki et al. 2003). These two pathways are also linked by protein interactions in the cytoplasm (Tang et al. 2008; Han et al. 2006; Liu et al. 2006; Edlund et al. 2005; Furuhashi et al. 2001).

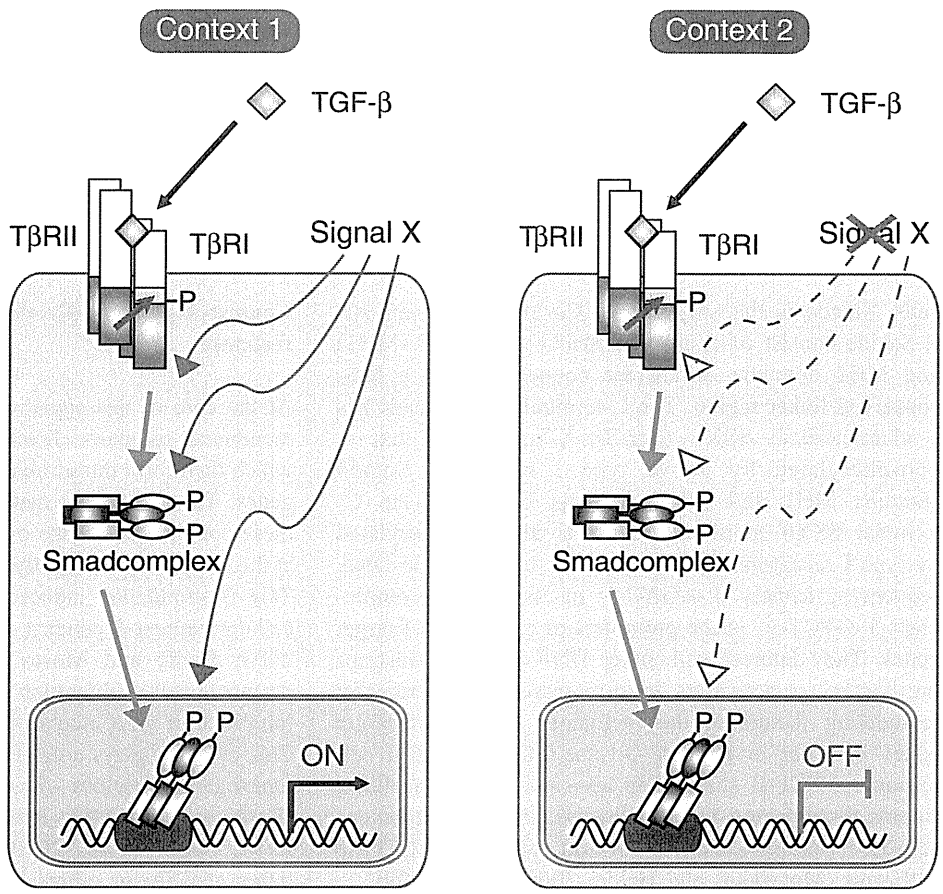

Fig. 2 "Signal cross-talk" model. In *Context 1*, but not in *Context 2*, *Signal X* is transduced in cells to modify downstream transducers of TGF-β signaling and induce a certain context-1-specific cell response

Perturbations of TGF-β signaling have been strongly implicated in cancer progression. TGF-β can play both tumor-suppressive and tumor-promoting roles and is now generally accepted to act as an anti-oncogenic factor in the early phase of tumorigenesis, although it can be converted to a pro-oncogenic factor during cancer progression (Roberts and Wakefield 2003; Bierie and Moses 2006). This switching of TGF-β from an anti-oncogenic factor to a pro-oncogenic factor might be induced by various mechanisms. Adorno et al. (2009) have reported that additional mutation of p53 plays a role in this switching. In the early stages of tumorigenesis, TGF-β inhibits the proliferation of tumor cells in concert with wild-type p53 as an anti-oncogenic factor. In contrast, in the later stages, Smad complexes function cooperatively with mutant p53 to abrogate the abilities of p63 to suppress sharp-1 and cyclin G2 expression and to inhibit metastasis. Indeed, the expression of mutant p53 in noninvasive tumor cells enhances the pro-invasive and migratory effects of TGF-β, whereas the suppression of mutant p53 expression in aggressive tumors impairs their ability to metastasize.

TGF-β induces epithelial-mesenchymal transition (EMT), in which epithelial cells acquire mesenchymal characteristics (Thiery et al. 2009). Some transcription factors, including Snail, Slug, Twist, δEF1/ZEB1, and SIP1/ZEB2, are induced by TGF-β signaling and regulate the expression of E-cadherin and other EMT-related genes. In certain cells, oncogenic Ras and TGF-β signaling pathways have been shown to induce EMT cooperatively (Oft et al. 1996, 2002). TGF-β alone can only weakly induce the expression of Snail and repress that of E-cadherin; however, oncogenic Ras signaling enhances the expression of Snail induced by TGF-β and synergistically induces EMT (Horiguchi et al. 2009).

In this fashion, TGF-β-induced cell responses can be determined by cooperatively acting signaling pathways.

Co-factors

Since the affinity of the activated Smad complex for the Smad-binding element (SBE) is insufficient to support an association with promoters of target genes, Smad complexes are associated with other DNA-binding transcription factors to regulate gene expression. Furthermore, the combination of the direct interactions of Smads with DNA and with sequence-specific DNA-binding transcription factors yields the selectivity of interaction between Smad complexes and the regulatory promoter sequences. Various families of transcription factors, such as the forkhead, homeobox, zinc-finger, activator protein 1, Ets, and basic helix-loop-helix (bHLH) families, serve as Smad partners (Ikushima et al. 2008; Koinuma et al. 2009a, b). The juxtaposition of an SBE at variable distances from the sequence, to which the Smad-interacting transcription factor binds, allows selection of a subset of promoter sequences to which the Smad transcription complexes bind with high affinity. Each Smad-cofactor combination targets a particular set of genes, which is determined by the presence of cognate binding sequence element combinations in the regulatory regions of target genes. Gene responses induced by TGF-β are thus classified by groups of genes that are simultaneously regulated by a common Smad-cofactor combination. A group of genes jointly controlled by a given Smad-cofactor complex is denoted a "synexpression group". Cells of different types or those exposed to different environments contain distinct repertoires of transcriptional partners for Smads and link their cellular context to their responses to TGF-β (Fig. 3).

A novel negative regulator of TGF-β signaling, human homolog of maternal Id-like molecule (HHM), has been demonstrated to suppress TGF-β signaling in a cell-response-selective fashion (Ikushima et al. 2008; Seto et al. 2009). Among the several cell responses induced by TGF-β, cell cycle arrest is repressed by HHM, but EMT is not. HHM bins to DNA-binding transcription factor Olig1 (oligodendrocyte transcription factor 1), a novel Smad-binding cofactor, and abrogates the binding of Olig1 to Smad proteins. Olig1 and R-Smads interact with each other on chromosomes and synergistically promote the expression of TGF-β target genes whose promoter regions have Olig1-binding sequence(s) and Smad-binding sequence(s) in close vicinity. HHM interferes with the interaction between Olig1 and the activated Smad complex and, as a consequence, inhibits the gene expression of the Olig1-Smad synexpression group at the transcriptional level. Since HHM interacts with some but not all Smad-binding transcription factors, HHM abrogates only a subset of Smad-cofactor complexes, including the Olig1-Smad complex. HHM thus inhibits TGF-β-induced cell responses, which are controlled by Smad-cofactor synexpression groups targeted by HHM, but fails to affect cell responses, which are regulated by Smad-cofactor synexpression groups not targeted by HHM.

The transcriptional cooperativity of Smad complexes with a variety of DNA-binding transcription factors thus creates marked complexity in the transcriptional regulation of target genes.

Genetic alterations

Although all cells except immune cells have nearly identical blueprints, or genomes, under physiological conditions, cancer cells have a variety of genetic alterations conferring survival advantage on them. Deletion or ampli-

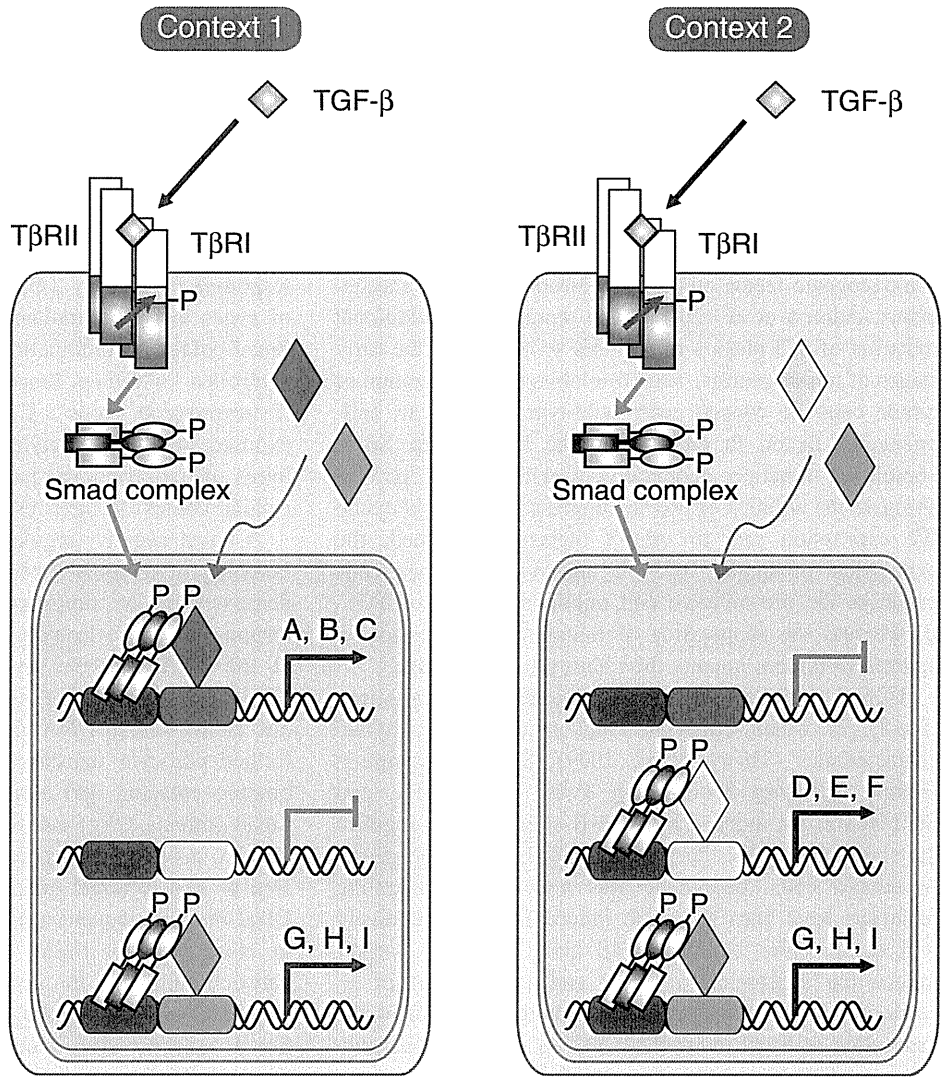

Fig. 3 "Cofactors" model. TGF-β target genes (*A–I*) are regulated by Smad proteins. Profiles of expression of cofactors of Smad proteins differ between *Context 1* and *Context 2*, resulting in different responses to TGF-β stimulation

fication of TGF-β target genes in cancer cells alters their responsiveness to TGF-β stimulation (Fig. 4). Although TGF-β up-regulates the expression of $p15^{Ink4b}$, one of the tumor suppressor genes, to inhibit cell proliferation (Hannon and Beach 1994), a subset of glioma cells sustains homozygous deletion of the $p15^{Ink4b}$ locus on chromosome 9p21 (Jen et al. 1994). Loss of $p15^{Ink4b}$ attenuates the anti-oncogenic effects of TGF-β, and glioma cells might benefit from host- and/or tumor-derived TGF-β stimulation.

Thus, genetic alterations of downstream genes modify the cell responses induced by TGF-β and contribute to the cellular context-specific plasticity of TGF-β signaling.

Epigenetics

Classical genetic processes are not sufficient to establish an organism. For proper development and cell functioning, epigenetic phenomena are absolutely required for the control of gene expression (Hirabayashi and Gotoh 2010; Ordovás and Smith 2010). In addition to genetic mechanisms, the gene expression and cell responses induced by TGF-β stimulation are regulated by epigenetic systems, including DNA methylation and post-translational histone modulation (Fig. 5).

DNA methylation is one of the most intensely studied epigenetic modifications in mammals and has a large impact on molecular pathophysiology and normal cell physiology (Esteller 2008; Suzuki and Bird 2008). Indeed, tumor cells are characterized by a different methylome from that of normal cells (Kulis and Esteller 2010). Interestingly, both hypo- and hypermethylation events can be observed in cancer. For instance, two cell-cycle-related genes, $p16^{INK4a}$ and $p15^{INK4b}$, undergo DNA methylation-mediated silencing in various types of cancer, leading to tumor development (Kulis and Esteller 2010).

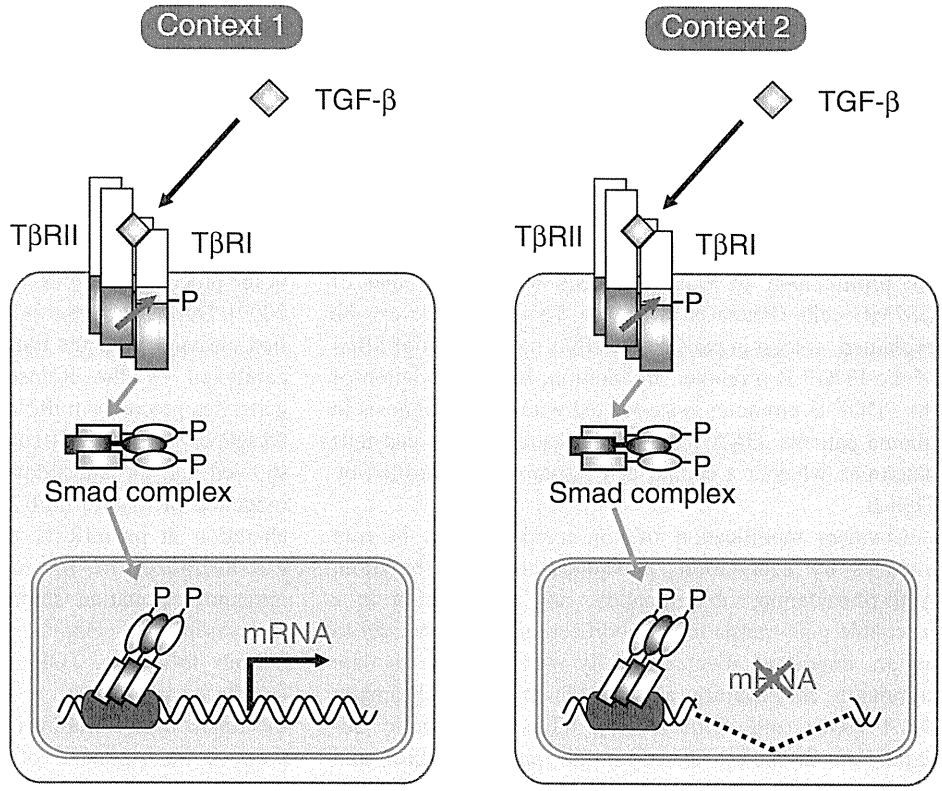

Fig. 4 "Genetic alterations" model. In *Context 1*, expression of a certain target gene is induced by TGF-β signaling. In *Context 2*, the gene is deleted at the chromosomal level, and TGF-β stimulation fails to induce its expression

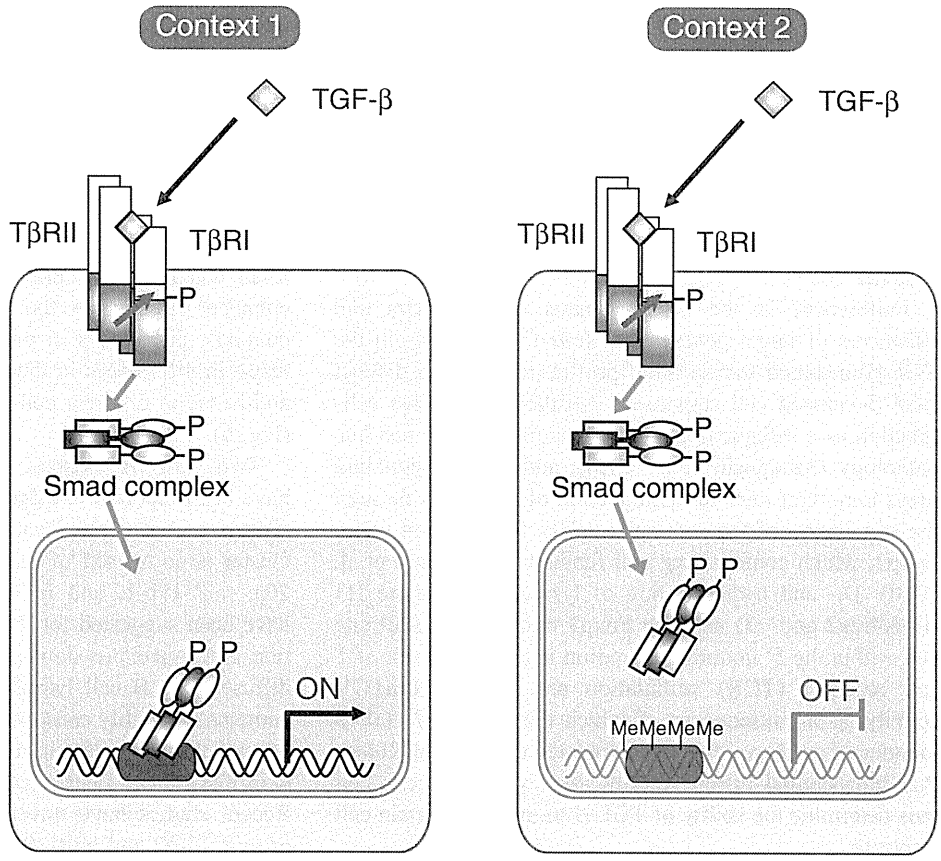

Fig. 5 "Epigenetics" model. In *Context 1*, promoter regions of certain TGF-β target genes adopt an "open conformation" and are exposed to the Smad complex. Conversely, in *Context 2*, promoter regions of the same target genes adopt a "closed conformation", and the Smad complex fails to access the Smad-binding elements. This difference results in differential responses to TGF-β stimulation

On the other hand, a global decrease in methylated CpG content contributes to genomic instability and to the activation of silenced oncogenes.

The regulation of gene expression by TGF-β can be affected by DNA methylation status. TGF-β induces platelet-derived growth factor-B (PDGF-B) expression in glioblastoma U373MG cells but fails to affect it in another glioblastoma cell line, U87MG cells. TGF-β thus induces the proliferation of U373MG cells but inhibits that of U87MG cells (Bruna et al. 2007). This difference can be explained, at least in part, by the DNA methylation of SBEs of the PDGF-B promoter. In addition, hypomethylation of the PDGF-B promoter is associated with poor prognosis in glioma patients. DNA methylation status in cells can thus determine whether a certain cell response is controlled by TGF-β.

Covalent modification of conserved residues in core histones by acetylation, phosphorylation, methylation, ADP-ribosylation, ubiquitination, and sumoylation is a reversible post-translational modification and is thought to be an important mechanism by which cells regulate chromatin accessibility and the function of chromatin DNA (Rice and Allis 2001). Thus, epigenetic de-regulation involving histone-modifying complexes and histone marks might be an important mechanism underlying the development and progression of diseases (Sawan and Herceg 2010). Furthermore, recent research has demonstrated that different types of cells might have specific patterns of histone modifications (histone modification signatures), which cause cellular context-dependent behaviors of cells (Lee et al. 2010). Indeed, the modification of histones varies drastically during tumorigenesis, and the disruption of many chromatin-modifying proteins is associated with the formation of various malignant tumors (Esteller 2007).

Differences in the histone status of promoters and enhancers of target genes might lead to alterations in the TGF-β-mediated transcription profile, resulting in distinct TGF-β-induced cell responses. Regulatory T (Treg) cells function as a safeguard against autoimmunity and immune pathology (Sakaguchi et al. 2010), and TGF-β signaling plays important roles in the induction of Treg cells through the stimulation of the expression of the transcription factor Foxp3, which confers Treg cell function (Yoshimura et al. 2010). Di- and trimethylation of lysine 4 of histone H3 (H3K4me2 and −3) near the Foxp3 transcription start site and within the 5' untranslated region is lost as a result of T cell receptor (TCR) stimulation and PI3K/Akt/mTOR activity, as a consequence of which the ability of TGF-β to induce Foxp3 expression is abrogated (Sauer et al. 2008). Post-translational histone modification status in cells can thus determine the ability of TGF-β to induce a certain cell response.

Non-coding RNA

Interactions of TGF-β signaling and non-coding RNA occur at various levels. microRNAs (miRNAs) are small non-coding RNAs that modulate diverse biological functions through the repression of target genes (Filipowicz et al. 2008; Winter et al. 2009). Recent studies have demonstrated that Smad complexes play a regulatory role in the processing of miRNA in the nucleus (Hata and Davis 2009). During the process of the maturation of miRNA, the first cleavage after the transcription of the miRNA gene is catalyzed by the RNase III enzyme Drosha, which generates precursor miRNA from primary miRNA (Davis-Dusenbery and Hata 2010). Davis et al. (2008, 2010) have showed that the knockdown of the R-Smads prevents the induction of mature miR-21 and pre-miR-21, although no alteration in pri-miR-21 transcription has been detected. Furthermore, co-immunoprecipitation and RNA-immunoprecipitation studies have confirmed that Smads are present in a complex with Drosha and the pri-miR-21 hairpin following TGF-β stimulation. The binding of Drosha to pri-miR-21 is also elevated following TGF-β treatment. These findings indicate that Smad complexes promote the association of Drosha with a subset of miRNA hairpins, resulting in the facilitation of the processing of the miRNAs, and that TGF-β can regulate gene expression not only through the direct transcriptional regulation of target genes, but also through miRNA processing.

Non-coding RNAs also contribute to the context-dependent diversity of TGF-β-induced cell responses (Singh and Settleman 2010). Cells of different cell types or cells exposed to different conditions express diverse repertoires of non-coding RNA (Lu et al. 2005), and TGF-β stimulation thus produces context-specific cell responses. Even when TGF-β stimulation activates promoter and/or enhancer regions to the same degree in two different contexts, differences in post-transcriptional regulation can result in differences in the levels of expression of proteins and hence in different cell responses to TGF-β stimulation (Fig. 6).

Two miRNA clusters, miR-17-92 and miR-106b-25, have been reported to affect the TGF-β signaling pathway (Petrocca et al. 2008; Ventura et al. 2009). The miR-17-92 cluster is composed of miR-17, miR-18a, miR-19a, miR-20a, miR-19b-1, and miR-92a-1. Tumor-promoting roles have been suggested for it based on its frequent amplification and overexpression in small-cell lung carcinoma and diffuse large B cell lymphoma. The miR-106b-25 cluster contains the highly conserved miR-106b, miR-93, and miR-25, which accumulate in different types of cancer, such as neuroblastoma, gastric cancer, and multiple myeloma. Recent studies have unveiled the functional involvement of miR-17-92 and miR-106b-25 clusters in TGF-β-induced

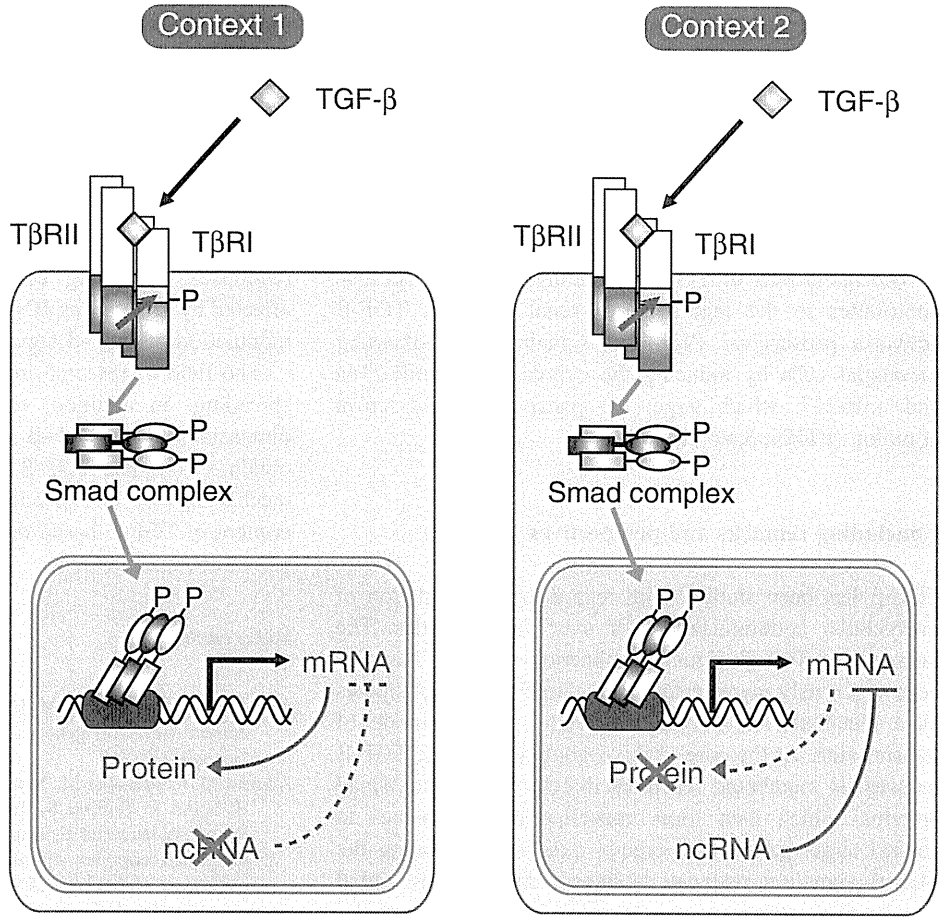

Fig. 6 "Non-coding RNA" model. In *Context 2*, transcribed mRNAs of TGF-β target genes are negatively regulated by non-coding RNA (*ncRNA*). In *Context 1*, such ncRNA is not expressed, resulting in the translation of the mRNAs

apoptosis and cell cycle arrest. They silence two main downstream effectors playing central roles in these cell responses: the pro-apoptotic gene Bim and the cyclin-dependent kinase inhibitor $p21^{Waf1}$. Furthermore, overexpression of miR-25 inhibits TGF-β-induced apoptosis, and overexpression of miR-106b and miR-93 prevents TGF-β-mediated cell cycle arrest. These reports indicate that the profiles of expression of miR-17-92 and miR-106b-25 clusters can determine whether TGF-β signaling has tumor-suppressive effects.

The miR-17-92 cluster is also involved in the post-transcriptional regulation of some of the regulatory components in TGF-β signaling. This cluster targets Smad4 and TβRII and, as a result, shuts down this signaling pathway (Dews et al. 2010; Mestdagh et al. 2010). In addition, enforced expression of miR-17-92 has been demonstrated to result in impaired gene activation by TGF-β in glioblastoma cells (Dews et al. 2010) and neuroblastoma cells (Mestdagh et al. 2010).

TGF-β-induced EMT, in which epithelial cells acquire mesenchymal characteristics, has been reported to be regulated by the miRNA-200 family (miR-200a, miR-200b, miR-200c, miR-141, and miR-429; Gregory et al. 2008; Korpal et al. 2008; Burk et al. 2008; Park et al. 2008). These miRNAs cooperatively interfere with expression of δEF1/ZEB1 and SIP1/ZEB2, which are transcriptional repressors of E-cadherin induced by TGF-β and involved in EMT. Manipulation of miR-200 family expression suppresses EMT and induces the opposite change, namely mesenchymal-epithelial transition. Since the levels of expression of the miR-200 family might vary from cell to cell, they determine, at least in part, at downstream gene levels whether TGF-β induces EMT. TGF-β has also been demonstrated to induce miR-155 expression through the Smad pathway, which in turn regulates epithelial plasticity by targeting RhoA and promotes TGF-β-mediated EMT as a result of the dissolution of tight junctions (Kong et al. 2008).

TGF-β-induced miRNAs also play important roles in cancer stem cells. TGF-β up-regulates miR-181 at the post-transcriptional level in breast cancer cells. miR-181 targets a tumor suppressor (ataxia telangiectasia mutaed, ATM) and maintains the breast cancer stem cell population (Wang et al. 2011).

PDGF-BB antagonizes the effects of TGF-β in certain cells, including smooth muscle cells, and Chan et al. (2010)

have reported that this antagonism is mediated in part via the function of miR-24. However, PDGF-BB induces the expression of miR-24, which in turn down-regulates Tribbles-like protein-3 (Trb3). Trb3 has been shown to induce the degradation of Smurf1 (Chan et al. 2007), and repression of Trb3 by miR-24 therefore results in the reduced expression of Smad proteins and the attenuation of TGF-β and bone morphogenetic protein signaling.

The interaction of TGF-β signaling and miRNAs also contributes to the regulation of renal function. TGF-β activates prosurvival PI3K-Akt signaling in glomerular mesangial cells by inducing the expression of miR-216a and miR-217, which target the phosphatase and tensin homolog (PTEN; Kato et al. 2009).

Concluding remarks and perspectives

TGF-β has been studied with regard to the regulation of intercellular communication for over three decades. The intracellular TGF-β signal transduction pathway has also been vigorously investigated, and a large number of studies have elucidated its simple but well-organized mode of transmission. At the core of this signaling pathway, TGF-β induces its membrane receptors directly to activate Smad proteins, which then form transcriptional complexes to control target genes. One crucial question concerning the TGF-β signaling pathway is how such a simple signal transduction pathway triggers multiple behaviors in cellular context-dependent fashion, i.e., how does TGF-β induce different responses in two different types of cells, despite their derivation from a single cell and possession of identical genetic makeup?

This question has been answered in part in terms of the classical frames: cross-interaction with other signaling pathways, different repertoires of Smad-binding transcription factors, and genetic alterations, especially in cancer cells. Nevertheless, the question remains largely unanswered, and recent research has added new frames to the field of intracellular TGF-β signal transduction.

The importance of epigenetic regulation in the development and maintenance of the human body is indicated by its disturbance in several types of diseases. Not surprisingly, gene expression and cell responses induced by TGF-β stimulation are regulated by epigenetic systems. Dynamic epigenetic changes determine an "open conformation" or "closed conformation" of chromatin status on TGF-β target genes; this is directly reflected in the induction of certain cell responses by TGF-β. Thus, differences in the epigenetic map can, at least in part, explain the cellular context-dependent diversity of TGF-β-induced cell responses.

Another new frame of intracellular signal transduction is its regulation by non-coding RNAs. The subtraction of transcribed mRNAs has added a novel paradigm to the regulation of TGF-β signal transduction, and recent research has demonstrated that interactions of TGF-β signaling and non-coding RNA occur at various levels. In addition to changes in non-coding RNA repertories by TGF-β stimulation at the transcriptional level, the TGF-β-Smad pathway is involved in the process of maturation of miRNAs. On the other hand, TGF-β-mediated cell responses, including cell proliferation and EMT, are affected by non-coding RNAs through direct and/or indirect modulation of TGF-β signaling.

The field of research into TGF-β signaling is thus still spreading. In addition, recent research has added new dimensions to the TGF-β field. Further work is needed to obtain a complete TGF-β map for the elucidation of the mechanisms of TGF-β-related diseases and for the development of TGF-β-based therapeutic strategies.

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REVIEW ARTICLE

Tumor-promoting functions of transforming growth factor-β in progression of cancer

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Abstract

Transforming growth factor-β (TGF-β) elicits both tumor-suppressive and tumor-promoting functions during cancer progression. Here, we describe the tumor-promoting functions of TGF-β and how these functions play a role in cancer progression. Normal epithelial cells undergo epithelial-mesenchymal transition (EMT) through the action of TGF-β, while treatment with TGF-β and fibroblast growth factor (FGF)-2 results in transdifferentiation into activated fibroblastic cells that are highly migratory, thereby facilitating cancer invasion and metastasis. TGF-β also induces EMT in tumor cells, which can be regulated by oncogenic and anti-oncogenic signals. In addition to EMT promotion, invasion and metastasis of cancer are facilitated by TGF-β through other mechanisms, such as regulation of cell survival, angiogenesis, and vascular integrity, and interaction with the tumor microenvironment. TGF-β also plays a critical role in regulating the cancer-initiating properties of certain types of cells, including glioma-initiating cells. These findings thus may be useful for establishing treatment strategies for advanced cancer by inhibiting TGF-β signaling.

Key words: *Angiogenesis, cancer-initiating cell, EMT, invasion, metastasis, TGF-β*

Introduction

Transforming growth factor-β (TGF-β) is a multifunctional regulator of cell growth, apoptosis, differentiation, and migration. TGF-β1 was originally discovered as a secreted protein that induces anchorage-independent growth in normal rat kidney NRK49F fibroblasts in the presence of epidermal growth factor (EGF) (1). TGF-β was shown to potently inhibit the proliferation of most cell types, including epithelial cells, endothelial cells, hematopoietic cells, and lymphocytes, and is widely known as a tumor suppressor. Studies investigating TGF-β signaling have revealed that perturbations of the TGF-β signaling pathway, such as mutations of TGF-β receptors or Smad proteins, lead to cancer progression and are related to poor prognosis of certain types of cancer. However, recent findings have shown that cancer cells become resistant to the growth inhibitory activity of TGF-β and that TGF-β facilitates invasion and metastasis of these cells both *in vitro* and *in vivo*.

Accumulating evidence has revealed that TGF-β plays a bidirectional role in cancer progression (2,3). TGF-β acts as a tumor suppressor by inhibiting cell growth through suppressing c-Myc expression and stimulating certain cyclin-dependent kinase inhibitors, including $p21^{WAF1}$ and $p15^{Ink4b}$, and by inducing cellular apoptosis through inducing DAP kinase, GADD45β, and Bim (4). Conversely, TGF-β functions as a tumor-promoting factor by stimulating extracellular matrix deposition and tissue fibrosis, perturbing immune and inflammatory function, stimulating angiogenesis, and promoting epithelial-mesenchymal transition (EMT).

In this review article, we discuss the tumor-promoting functions of TGF-β, particularly on EMT, on the basis of recent findings in our

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laboratory. We also describe the function of TGF-β in some cancer-initiating cells and discuss how inhibition of TGF-β signaling can be used for treating different types of cancer.

TGF-β family signaling

TGF-β binds to two different serine/threonine kinase receptors, TβRII and TβRI (5). Betaglycan, also known as the TGF-β type III receptor, facilitates binding of TGF-β (particularly TGF-β2 among the three isoforms of TGF-β) to TβRII. TβRII activates TβRI through phosphorylation of the Gly-Ser-rich (GS) domain of TβRI, which in turn phosphorylates and activates Smad2 and Smad3, receptor-regulated Smads (R-Smads) specific for TGF-β and activin signaling (Figure 1). Bone morphogenetic proteins (BMPs) activate another set of R-Smads, including Smad1, Smad5, and Smad8 (6). Activated Smad2 and Smad3 form complexes with Smad4, common partner Smad (co-Smad), and translocate into the nucleus. R-Smad/co-Smad complexes associate with various transcription factors (AP-2, Ets, and HNF-4α (7–9)) and transcriptional co-activators (p300, CBP, and GCN5) or co-repressors (p107, Ski, and SnoN) in the nucleus and regulate transcription of a wide spectrum of TGF-β target genes. Smad7, an inhibitory Smad (I-Smad), represses TGF-β signaling through multiple mechanisms; among these mechanisms, binding to activated type I receptors and competition with R-Smads for receptor binding play a major role in regulation of TGF-β signaling (10). c-Ski (also known as SKI) and the related SnoN (also known as SKIL) bind directly to Smad2/3 and Smad4 and function as transcriptional co-repressors by recruiting histone deacetylases and competing for binding with p300/CBP. C-Ski also disrupts formation of the R-Smads and

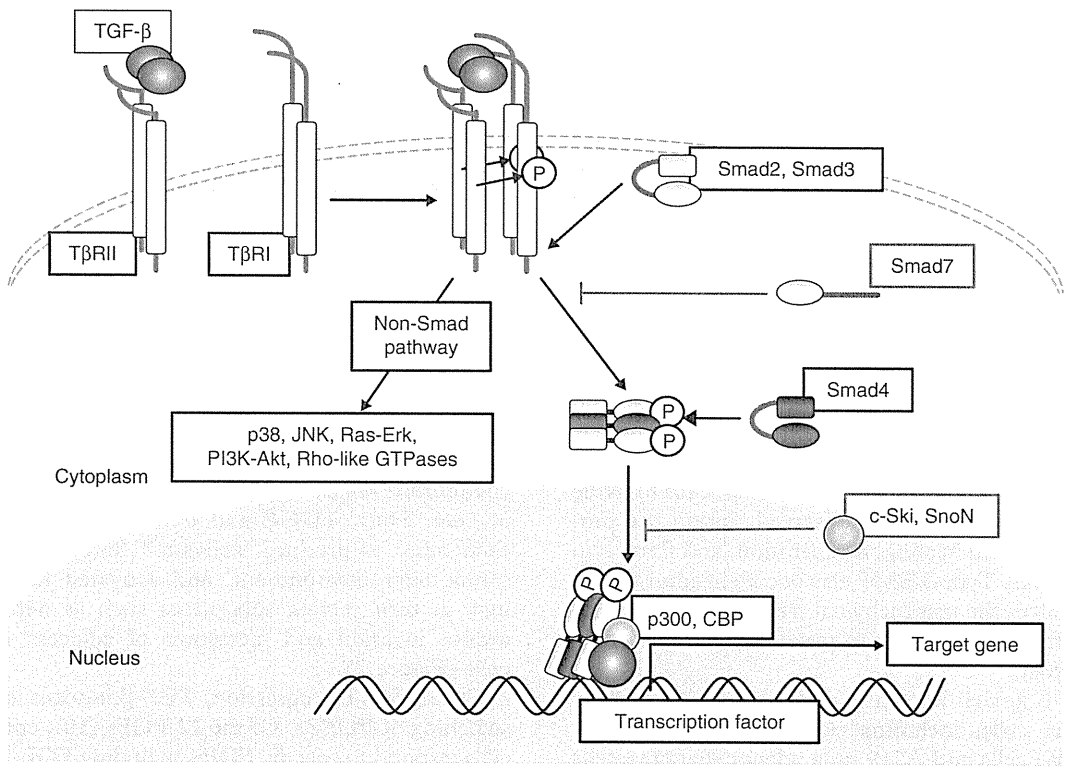

Figure 1. Schematic representation of TGF-β signal transduction pathways. TGF-β transduces signals through two different types of serine/threonine (and tyrosine) kinase receptors, termed TβRI and TβRII. Upon TGF-β binding, TβRI and TβRII form heterotetrameric complexes, and TβRII kinase transphosphorylates the juxtamembrane portion (GS domain) of the cytoplasmic region of TβRI. Phosphorylated TβRI transmits intracellular signaling through R-Smad phosphorylation. Smad2 and Smad3 are R-Smads phosphorylated by TβRI kinase and form heteromeric complexes with Smad4 (co-Smad). Smad complexes translocate into the nucleus and act as transcriptional regulators of target genes by interacting with other transcription factors and transcriptional regulators. Smad7 (I-Smad), which lacks the typical MH1 domain, interferes with the activation of R-Smads by interacting with TβRI and competitively prevents R-Smads from being phosphorylated by TβRI. TGF-β activates other intracellular signaling pathways in addition to Smads in order to regulate a wide array of cellular functions. These non-Smad pathways are activated by TGF-β receptors through phosphorylation or direct interaction.

co-Smad complex to inhibit TGF-β signaling (11). In addition to its involvement in Smad signaling pathways, TGF-β activates various non-Smad signaling pathways, including ERK, JNK, and p38 MAP kinases, phosphatidylinositol-3 kinase (PI3K)-Akt, and small GTPase pathways (12). TβRI functions as a dual-specificity kinase (tyrosine and serine/threonine kinase) and phosphorylates ShcA on tyrosine and serine residues to activate the MAP kinase pathway (13).

Induction of EMT

EMT is a differentiation switch through which epithelial cells differentiate into mesenchymal cells, and it occurs in the process of tissue morphogenesis during development, wound repair, and cancer progression in adult tissues (14,15). An early event of EMT includes disruption of tight junctions connecting epithelial cells and delocalization of tight junction proteins, such as ZO-1, claudin-1, and occludin. Early events of EMT also include disruption of adherence junctions, which contain E-cadherin and β-catenin, and reorganization of the actin cytoskeleton. Epithelial cells lose cell polarity and show spindle-like morphology with expression of various mesenchymal markers, including N-cadherin, fibronectin, and α-smooth muscle actin (α-SMA). Cell motility and invasive properties are enhanced in resulting mesenchymal cells.

EMT can be classified into three subtypes (16). Type 1 EMT occurs during development and includes the mesenchymal transition of primitive epithelial cells during gastrulation, generation of migrating neural crest cells from neuroepithelial cells, and formation of endocardial cushion tissue from cardiac endothelial cells. Type 2 EMT includes the transition of secondary epithelial (and endothelial) cells to tissue fibroblasts, which can be observed during the processes of wound healing, regeneration, and fibrosis in adult tissues. Type 3 EMT also occurs in adult tissues and involves the mesenchymal transition of epithelial carcinoma cells, leading to generation of metastatic tumor cells.

TGF-β is well known to induce EMT in various epithelial cells, including normal mouse epithelial NMuMG cells and A549 lung adenocarcinoma cells (17). Many transcription factors, including the two-handed zinc-finger factors δEF1 (also known as ZEB1) and SIP1 (ZEB2), the zinc-finger factors Snail (also known as SNAI1) and Slug (SNAI2), and the basic helix-loop-helix (bHLH) factors Twist and E12/E47, are induced by TGF-β signaling in a Smad-dependent fashion and play critical roles in EMT induction. Additionally, non-Smad signaling pathways activated by TGF-β and cross-talk with other signaling pathways, including fibroblast growth factor (FGF) and tumor necrosis factor-α (TNF-α) signaling, play important roles in EMT promotion.

Induction of EMT in tumor stromal cells by TGF-β

Epithelial cells in the tumor stroma undergo EMT (type 2 EMT) and play a critical role in cancer progression. We cocultured NMuMG cells with mouse mammary tumor JygMC(A) cells and found that NMuMG cells that have undergone EMT express α-SMA (18). The effect of the JygMC(A) cells was abolished by treatment with the TβRI inhibitor SB431542. Interestingly, when NMuMG cells were cocultured with the mouse mammary tumor cell line 4T1, NMuMG cells underwent EMT and produced mesenchymal cells with an activated fibroblastic phenotype, which lacked α-SMA expression. 4T1 cells produced TGF-β1 at a level comparable to that produced by JygMC(A) cells. When 4T1 cells were treated with FGF receptor 1 (FGFR1) inhibitor SU5402, α-SMA-positive NMuMG cells were detected, indicating that the loss of α-SMA expression is due to FGF(s) secreted from 4T1 cells. We have shown that treatment of NMuMG cells with TGF-β and FGF-2 prevents the production of mesenchymal cells expressing α-SMA and calponin by activating the MEK-ERK pathway. Interestingly, NMuMG cells that have undergone EMT following treatment with TGF-β and FGF-2 exhibit drastic morphological changes with marked actin reorganization, enhanced cell migration, and increased production of matrix metalloproteinases (MMPs), including MMP-9. Moreover, NMuMG cells treated with TGF-β and FGF-2 enhanced the invasion of cocultured breast cancer cells into collagen gels *in vitro*. Thus, TGF-β and FGF-2 co-operate with each other to produce 'activated' fibroblasts in the tumor microenvironment, and activated fibroblasts may in turn secrete substances such as MMPs to induce invasion and metastasis of adjacent cancer cells (Figure 2).

During EMT progression, TGF-β induces isoform switching of FGFRs. Of the 22 FGFs (19), epithelial cells respond to specific FGFs, including FGF-7 (also known as keratinocyte growth factor (KGF)), but not to FGF-2 (basic FGF) or FGF-4. However, cells that have undergone EMT become responsive to FGF-2 and FGF-4, but not to FGF-7 (18). We have shown that TGF-β-mediated EMT induces isoform switching of FGFRs through alternative splicing, following which expression of the IIIb isoform of FGFR decreased and that of the IIIc isoform increased.

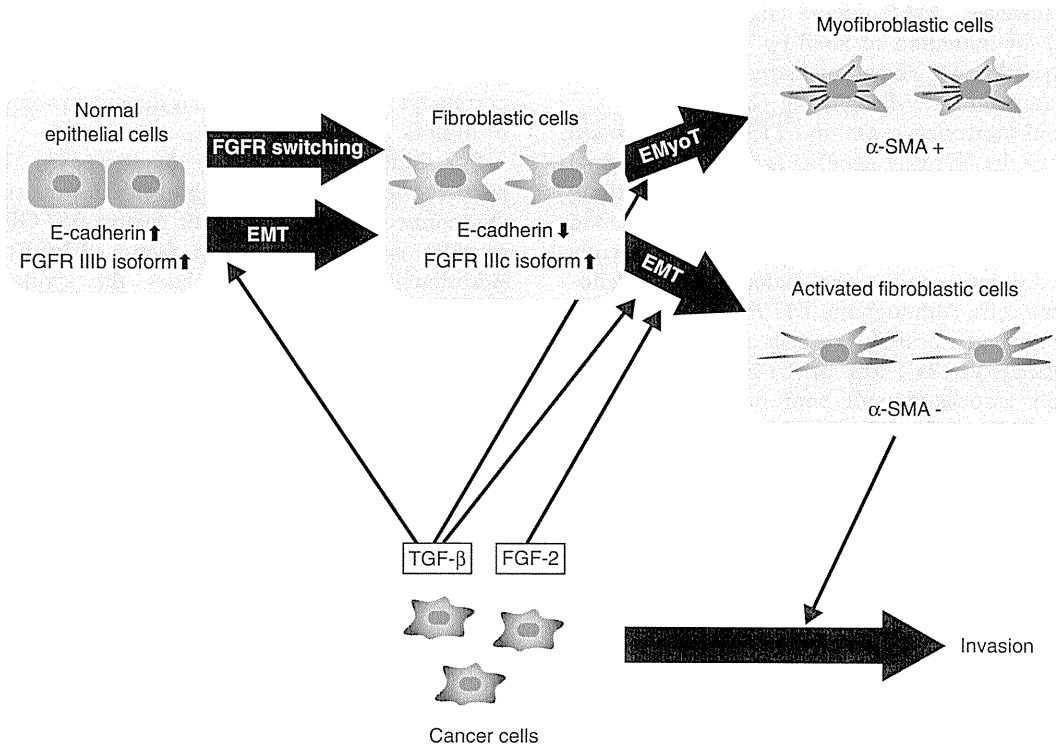

Figure 2. Schematic representation of EMT induction by TGF-β and FGF-2. 'Epithelial cells' differentiate into 'fibroblastic cells' through EMT induced by TGF-β and further differentiate into α-SMA-positive 'myofibroblastic cells' through epithelial-myofibroblastic transition (EMyoT). When FGF-2 is present in this process, FGF-2 induces differentiation of epithelial cells to 'activated fibroblastic cells'.

Exon array analysis showed that TGF-β alters a broad spectrum of splicing patterns by reducing the expression of epithelial splicing regulatory proteins (ESRPs) 1 and 2 (20). Warzecha et al. (21) recently reported that the ESRP-regulated splicing pathway is abrogated during EMT. We found that repression of the expression of ESRPs by TGF-β is mediated by up-regulation of the δEF1 family proteins δEF1 and SIP1, which suppress the transcription of ESRP(s) by binding to the ESRP promoter(s). Interestingly, the expression profiles of ESRPs were reciprocally correlated with those of δEF1 and SIP1 in human breast cancer cell lines as well as in tumor specimens. In addition to FGFRs, TGF-β induces alternative splicing of CD44, Mena, and CTNND1 (also known as δ-catenin or p120 catenin), which are reportedly involved in cancer progression. We have also shown that over-expression of ESRPs attenuates TGF-β-induced EMT and restores the expression of E-cadherin and some other epithelial phenotypes. Thus, ESRPs are downstream targets of TGF-β and serve as antagonists to EMT by regulating alternative splicing of specific genes involved in TGF-β-induced EMT.

Induction of EMT in cancer cells

EMT is observed in some transformed epithelial cells (type 3 EMT) to facilitate their invasive and metastatic properties. Type 3 EMT can be regulated by specific oncogenic and anti-oncogenic signals. We have shown that a zinc-finger transcription factor Snail is induced by TGF-β in pancreatic cancer Panc1 cells and plays a key role in EMT progression (22). Panc1 cells express active *K-ras*, and we found that induction of Snail by TGF-β is dependent on oncogenic Ras signals. Snail was strongly induced by TGF-β in Panc1 cells, but knock-down of Ras in Panc1 cells abolished Snail induction by TGF-β. Consequently, TGF-β failed to efficiently induce EMT in Panc1 cells in the absence of active Ras signaling. Exogenous expression of constitutively active Ras into HeLa cells resulted in marked induction of Snail by TGF-β, while induction of other direct targets of TGF-β, including Smad7 and PAI-1, was not enhanced by Ras signaling. MAP kinases have been reported to phosphorylate the linker region of Smad2 and Smad3, which both positively and negatively regulates TGF-β signaling

(23). However, MAP kinase signaling was not required for induction of Snail by TGF-β, and it is currently unknown which downstream signals of Ras co-operate with TGF-β signaling (22).

Thyroid transcription factor-1 (TTF-1, the protein product of the *NKX2.1* gene) is expressed in normal lung tissues and acts as a master regulator of lung morphogenesis (24). TTF-1 is primarily expressed in type II pneumocytes and Clara cells and frequently expressed in lung cancer cells, including lung adenocarcinoma cells. Although the *TTF1* gene is amplified in some lung adenocarcinoma cells and may function as an oncogene (25), loss of TTF-1 expression is reportedly associated with poor prognosis of lung carcinoma. Recently, Winslow et al. (26) reported that TTF-1 controls differentiation of lung carcinoma cells and limits their metastatic potential in mice with active K-Ras and inactive p53. Interestingly, we found that TTF-1 functions as a tumor-suppressor during EMT induction. TTF-1 is highly expressed in certain types of lung adenocarcinoma cell lines, including H441 cells and LC-2/ad cells, but not in A549 cells (27). A549 cells show a spindle-like phenotype and grow rapidly, while H441 cells show tight cell–cell junctions with cobblestone-like morphology and grow much more slowly than A549 cells. A549 cells express low levels of TTF-1 and E-cadherin, while H441 cells express high levels of TTF-1 and E-cadherin. We have further shown that exogenous expression of TTF-1 in A549 cells inhibits TGF-β-induced EMT, decreases MMP-2 activity, cell migration, and cellular invasive capacity, and restores the epithelial phenotype through high E-cadherin expression. Conversely, TGF-β induces the expression of Snail and Slug in A549 cells, and silencing of TTF-1 in H441 cells enhances TGF-β-mediated EMT. TTF-1 has been reported to interact physically with Smad3 (28) and may inhibit Smad3 function. We have also shown that TGF-β down-regulates TTF-1 expression in A549 cells and that TTF-1 inhibits the expression of TGF-β2, which is expressed in epithelial cells at the tip of the distal airway during lung morphogenesis. Thus, TTF-1 may exert a tumor-suppressive effect through antagonizing the effect of TGF-β. These findings indicate a functionally inverse relationship between TTF-1 and TGF-β signaling in the progression of lung adenocarcinoma through regulation of EMT.

TGF-β signaling in vascular tissues and angiogenesis

New blood vessel formation in tumor tissues (tumor angiogenesis) is essential for the growth and metastasis of tumor cells. Although TGF-β potently inhibits the growth of endothelial cells *in vitro*, it functions as a pro-angiogenic factor and stimulates angiogenesis *in vivo*. Increased expression of TGF-β is correlated to increased vascular density in some types of tumors.

For induction of tumor angiogenesis, TGF-β induces the expression of angiogenic factors, including connective tissue growth factor (CTGF) and vascular endothelial growth factor (VEGF) (29). Additionally, TGF-β stimulates the synthesis of MMP-2 and MMP-9 and down-regulates the expression of tissue inhibitors of metalloproteinase (TIMPs) in tumor tissues. Increased MMP activity leads to stimulation of migration and invasion of vascular endothelial cells, resulting in accelerated tumor angiogenesis.

However, TGF-β suppresses angiogenesis in certain types of tumors through reduced expression of some angiogenic factors or increased expression of angiogenic inhibitors. In diffuse-type gastric carcinoma, TGF-β induces the production of some angiogenic inhibitors, including thrombospondin-1 and TIMP-2, and perturbations of TGF-β signaling may thus lead to induction of angiogenesis and tumor growth *in vivo* (30,31).

In addition to induction of tumor angiogenesis, TGF-β acts on vascular endothelial cells and may disrupt cell–cell junctions and support the colonization of tumor cells to establish metastasis. Using endothelial cells derived from mouse embryonic stem (ES) cells, we showed that TGF-β suppresses the expression of claudin-5 and disrupts sheet formation *in vitro* (32). We also showed that TGF-β induces differentiation of certain endothelial cells into mesenchymal cells, resulting in the loss of tight cell–cell contacts *in vitro* (33). Moreover, through disruption of endothelial cell–cell junctions by inducing angiopoietin-like 4 (Angptl4) expression, TGF-β has been shown to increase the permeability of blood vessels and stimulate the trans-endothelial movement of cancer cells (34).

Acceleration of cancer metastasis by TGF-β signaling

TGF-β facilitates metastasis of certain types of cancer in advanced stages, including breast cancer (35). Inhibition of TGF-β signaling may thus be a potential strategy for preventing metastasis of advanced cancers. Though not discussed in detail in this review, TGF-β regulates tumor development by regulating immune functions (36,37). Wakefield and colleagues reported that inhibition of TGF-β function prevents the progression of breast cancer by enhancing various immune functions (38).

We have shown that Smad7, an I-Smad that inhibits TGF-β and BMP signaling, efficiently inhibits lung and liver metastasis of mouse breast cancer JygMC(A) cells (39). We subcutaneously inoculated JygMC(A) cells, which spontaneously metastasize to the lung, liver, and other organs in 3 to 4 weeks, in nude mice. Ten days after subcutaneous inoculation, adenoviruses containing Smad7 or LacZ were intravenously administered to the mice once weekly. Mice bearing JygMC(A) tumors and treated with LacZ adenovirus developed numerous metastases to the lung and liver, and all mice died by 50 days (median survival time, 41 days) after inoculation of JygMC(A) cells. In contrast, mice treated with Smad7 adenovirus showed a significant decrease in metastases of tumors in both the lung and liver, and the median survival time of Smad7-treated mice was 55 days. JygMC(A) cells treated with Smad7 showed increased expression of components involved in adherence and tight junctions, including E-cadherin, and decreased expression of mesenchymal markers, including N-cadherin. Smad7 also inhibited the migration and invasion of cells, indicating that Smad7 leads to prevention of the EMT process. Interestingly, Smad6, which preferentially inhibits BMP signaling, failed to show significant effects on the metastasis of JygMC(A) cells in nude mice, whereas c-Ski adenovirus showed effects similar to Smad7. Thus, inhibiting TGF-β signaling using Smad7 or c-Ski prevents the EMT process and eventually inhibits lung and liver metastasis of JygMC(A) cells (Figure 3).

In addition to preventing EMT, TGF-β appears to inhibit metastasis of JygMC(A) cells by some other mechanisms. Although TGF-β induces apoptosis of many different types of cells by inducing specific genes, it stimulates survival of certain types of cells in a context-dependent manner through activation of the PI3K-Akt signaling pathway. We have identified Dec1 (differentially expressed in chondrocytes 1, also known as SHARP2 and Stra13) as a downstream target of TGF-β–Smad signaling by DNA microarray analysis (40). Dec1 is a bHLH transcription factor, which is widely expressed in many tissues and overexpressed in certain types of cancer cells. Dec1 prevented the apoptosis of JygMC(A) as well as 4T1 cells, and a dominant-negative mutant of Dec1 suppressed lung and liver metastases of JygMC(A) cells in nude mice (Figure 3). Dec1 has been reported to induce the expression of an anti-apoptotic protein, survivin, in certain types of cells (41); however, we failed to show induction of survivin by TGF-β in JygMC(A) cells. Mechanisms of Dec1 induction of cell survival in JygMC(A) cells should be examined in the future.

We also found that inhibiting endogenous TGF-β signaling by a TβRI inhibitor, SB431542, induces the expression of the BH3-only protein, Bim (also known as Bcl2-like 11), in JygMC(A) and stimulates apoptosis in these cells (42). We showed that suppression of

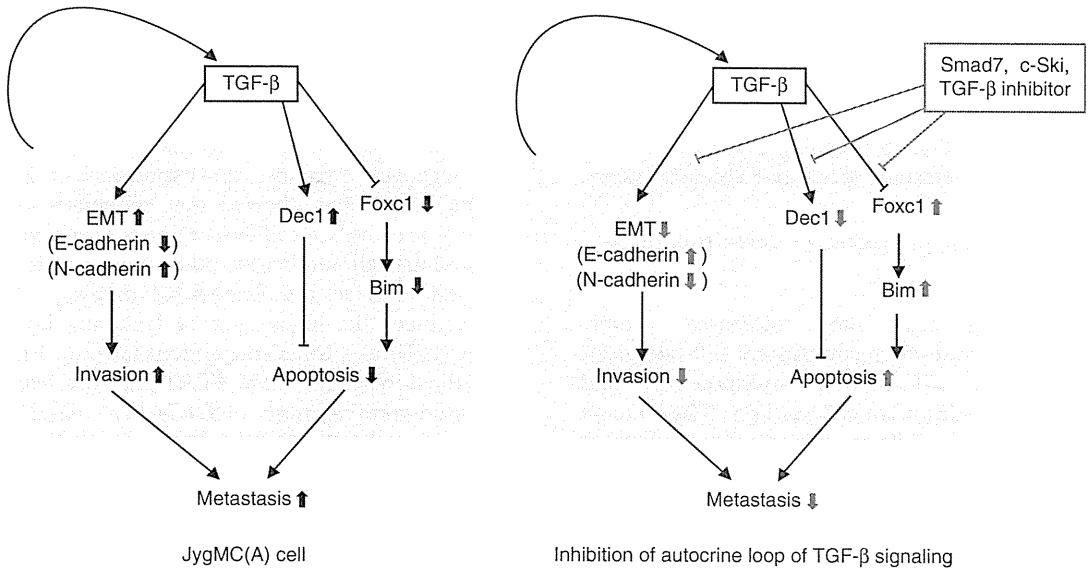

Figure 3. Mechanisms of TGF-β action on prevention of breast cancer metastasis using JygMC(A) cells. Endogenously activated autocrine loop of TGF-β regulates the expression of E-cadherin and N-cadherin by inducing EMT in JygMC(A) cells. Autocrine TGF-β also regulates the expression of various transcription factors, including Dec1 and Foxc1, and promotes the survival. Negative regulators of TGF-β signaling (Smad7, c-Ski, or TGF-β inhibitors) block these pathways and inhibit metastasis of JygMC(A) cells.

Bim expression by TGF-β is mediated by repression of a FOX family transcription factor, Foxc1, in JygMC(A) cells, thus suggesting an important role of the TGF-β–Foxc1–Bim axis in the survival of certain types of cells. Further studies are needed to determine whether the TGF-β–Foxc1–Bim axis is involved in lung and liver metastases of this type of cancer (Figure 3).

TGF-β also plays critical roles in bone metastasis, during which functional interaction between cancer cells and the bone microenvironment is important. *In-vivo* experimental models using intracardiac injection of cancer cells have been widely used to study the mechanisms of bone metastasis. Several studies revealed that TGF-β and its target molecules, such as parathyroid hormone-related protein (PTHrP) and interleukin-11 (IL-11), play critical roles in the development of bone metastasis of breast cancers (29,43), which occurs in a Smad-dependent fashion (44). PTHrP stimulates the expression of the RANK ligand (RANKL) in osteoblasts and induces differentiation of osteoclast precursors and resorption of bone. We studied the effects of a TβRI inhibitor, Ki26894, on bone metastasis in the human breast cancer cell line MDA-MB-231–5a-D (MDA-231-D), which is a highly metastatic variant of MDA-MB-231 cells. Ki26894 suppressed induction of PTHrP and IL-11 mRNA in MDA-231-D cells stimulated by TGF-β (45). When MDA-231-D cells were injected into the left ventricle of nude mice and treated with systemic administration of Ki26894 (treatment with Ki26894 was started 1 day before tumor cell inoculation), X-ray radiography showed that treatment with Ki26894 decreased bone metastasis of breast cancer cells and prolonged the survival of MDA-231-D-bearing mice compared to vehicle treatment. These findings suggest that inhibition of TGF-β signaling may be useful for preventing bone metastasis of advanced breast cancers.

TGF-β maintains stemness of certain cancer-initiating cells

Cancer-initiating cells show increased tumor-initiating ability and often exhibit stem cell-like properties such as self-renewal, multipotency, and expression of specific stem cell markers. The concept of cancer-initiating cells reveals a new strategy of therapy against intractable cancers, though it remains unclear how cancer-initiating cells can be specifically eradicated. It is important to investigate the differences between cancer-initiating cells and normal stem cells and to identify specific molecules to target cancer-initiating cells without affecting the function of normal stem cells. Recent studies have also revealed critical roles of TGF-β signaling in the maintenance of stem cell-like properties of certain cancer-initiating cells, including glioma-initiating cells (GICs) (46,47), breast cancer-initiating cells (48), and leukemia-initiating cells in chronic myeloid leukemia (CML) (49).

Glioma cells produce TGF-β1 and TGF-β2, and autocrine TGF-β signaling plays a pivotal role in maintaining the stem cell-like properties and tumorigenic activity of GICs (46,47). GICs obtained from patients with glioblastoma multiforme exhibit sphere-forming ability in a self-renewal medium containing EGF and FGF-2. Although TGF-β did not significantly affect the sphere-forming ability of GICs, a TβRI inhibitor, SB431542, efficiently reduced this ability in GICs. Moreover, SB431542 dramatically reduced the number of CD133-expressing cells and induced differentiation of GICs, leading to the appearance of cells expressing neural or glial cell markers. Analyses of TGF-β target genes using quantitative RT-PCR and by searching public datasets showed that TGF-β induces expression of the Sry-related HMG box (Sox) transcription factors Sox2 and Sox4. We showed that Sox4 is a direct target of Smad proteins activated by TGF-β and that it induces the expression of Sox2, which plays a critical role in the maintenance of GIC stemness. We also confirmed that in intracranial transplantation assays using immunocompromised mice, GICs pretreated with SB431542 showed decreased lethal potency. These results indicate that the TGF-β–Sox4–Sox2 pathway is essential for retaining the stemness of GICs, and inhibition of TGF-β signaling may be a potential method for treating glioma through targeting GICs (Figure 4).

Westermark and his colleagues (50) reported that another Sox family protein, Sox21, is expressed in glioma cells. Sox21 is an antagonizing partner of Sox2 and negatively regulates the expression of Sox2 in glioma cells. They showed that reduction in Sox2 expression using Sox2 siRNA or Sox21 over-expression reduced the cell number by inducing apoptosis.

In addition to the Sox4-Sox2 pathway, TGF-β also induces the expression of leukemia inhibitory factor (LIF) in a Smad-dependent fashion. LIF activates the downstream JAK-STAT pathway, leading to increased tumorigenesis of GICs (47). Anido et al. have shown that TGF-β inhibitors target GICs with high levels of CD44 and Id1 and that $CD44^{high}$/$Id1^{high}$ GICs are generally localized in the perivascular niche (51).

Conclusion and perspectives

As proposed by Roberts and Wakefield (2), it is now well known that TGF-β exhibits both positive and

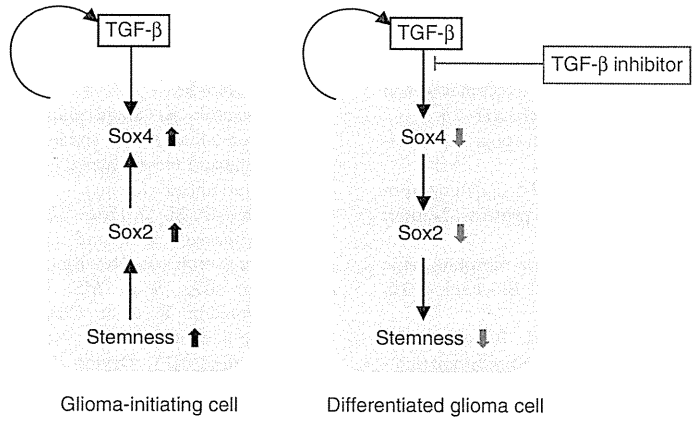

Figure 4. Effects of the TGF-β–Smad–Sox4–Sox2 axis on the maintenance of GIC stemness. TGF-β directly induces Sox4 expression. Subsequently, Sox4 promotes Sox2 expression, which plays significant roles in sustaining GIC stemness. TGF-β inhibitor blocks this TGF-β–Sox4–Sox2 axis, promotes GIC differentiation, and deprives these cells of their aggressiveness. Differentiated glioma cells (right panel) may be more sensitive to conventional chemotherapy and radiotherapy than undifferentiated GICs (left panel).

negative effects on cancer progression. The bidirectional roles of TGF-β can be observed at the molecular, cellular, and tissue levels. Although we described the positive effects of TGF-β on maintaining the stemness of cancer-initiating cells, TGF-β has also been shown to decrease the number of specific types of cancer-initiating cells, including diffuse-type gastric carcinoma cells (52). Moreover, TGF-β induces maintenance of stem cell-like properties of certain breast cancer-initiating cells (48), while suppression of the TGF-β pathway leads to an increase in breast cancer-initiating cells in other types of breast cancer cells (53), thereby suggesting that the response to TGF-β varies depending on the type of cancer-initiating cells.

Mani et al. (48) reported that TGF-β maintains stem cell-like properties of certain cancer-initiating cells through induction of EMT. They showed that normal and transformed mammary epithelial cells acquired stem cell-like properties with high tumorigenic activity when EMT was induced in these cells by TGF-β. Although we have not determined whether the sizes of cancer-initiating cell compartments are affected by EMT in the pancreatic carcinoma Panc1 cells and lung adenocarcinoma A549 cells described above, these findings suggest a functional connection between EMT and cancer-initiating properties of certain epithelial cells.

Recent findings based on genome-wide analyses of Smad-binding sites in some types of cells, which were performed using ChIP-sequencing analyses, revealed that the binding profiles of Smads differ remarkably depending on the cell types and are affected by interaction with transcription factors expressed in each cell type and by cell-specific differences in baseline chromatin accessibility patterns (7,9,54). It is thus possible that the response of cells to TGF-β may be differentially affected by coexisting transcription factors and chromatin assembly patterns. Further studies examining global gene expression profiles and genome-wide maps of protein binding sites or epigenetic marks using high-throughput sequencing may be valuable for elucidating the mechanisms of differential cellular responsiveness to TGF-β.

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