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REVIEW

TGF-β signal transduction spreading to a wider field: a broad variety of mechanisms for context-dependent effects of TGF-β

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Abstract Transforming growth factor (TGF)-β signaling is involved in almost all major cell behaviors under physiological and pathological conditions, and its regulatory system has therefore been vigorously investigated. The fundamental elements in TGF-β signaling are TGF-β ligands, their receptors, and intracellular Smad effectors. The TGF-β ligand induces the receptors directly to phosphorylate and activate Smad proteins, which then form transcriptional complexes to control target genes. One of the classical questions in the field of research on TGF-β signaling is how this cytokine induces multiple cell responses depending on cell type and cellular context. Possible answers to this question include cross-interaction with other signaling pathways, different repertoires of Smad-binding transcription factors, and genetic alterations, especially in cancer cells. In addition to these genetic paradigms, recent work has extended TGF-β research into new fields, including epigenetic regulation and non-coding RNAs. In this review, we first describe the basic machinery of TGF-β signaling and discuss several factors that comprise TGF-β signaling networks. We then address mechanisms by which TGF-β induces several responses in a cell-context-dependent fashion. In addition to classical frames, the interaction of TGF-β signaling with epigenetics and microRNA is discussed.

Keywords TGF-β · Smad · ALK5 · Epigenetics · microRNA

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Introduction

Cytokines are small secreted proteins that are produced by numerous types of cells and that play important roles in intercellular communication to maintain order in the organism. They elicit biological effects by binding to the extracellular domains of specific transmembrane receptors in the outer membrane of cells. Cytokines mediate intercellular communication via the regulation of cell growth and differentiation and are thus crucial for maintaining the homeostasis of multicellular organisms. Aberrant regulation of cytokine signaling can therefore result in various diseases.

The transforming growth factor (TGF)-β family is particularly prominent among these signals (Blobe et al. 2000; Feng and Derynck 2005; Massagué 2008). TGF-β signaling controls a diverse set of cellular processes, including cell growth, differentiation, apoptosis, survival, and specification of developmental fate, during embryogenesis and in mature tissues (Ikushima and Miyazono 2010a; Moustakas and Heldin 2009). To control TGF-β-induced cell responses, numerous factors tightly regulate this signaling pathway under physiological conditions (Ikushima and Miyazono 2010b; Bierie and Moses 2006). Loss of balance of TGF-β signaling thus leads to several pathological conditions, including malignant tumors, fibrotic diseases, and abnormal immune reactions (Levy and Hill 2006; Varga and Pasche 2009; Flavell et al.

2010). Indeed, studies of clinical samples indicate that a distortion of TGF-β signaling is one of the major causes of several disorders. Here, we first discuss the way that (1) cells translate TGF-β signaling into cellular responses, and (2) TGF-β signaling and TGF-β-induced cell responses are tightly controlled. Possible and/or established mechanisms of the context-dependent diversity of TGF-β-induced cell responses are also addressed. In addition, recent research on TGF-β signaling has spread into novel fields, including epigenetics and non-coding RNAs. Thus, we also mention the involvement of epigenetic regulation and non-coding RNAs in the classical TGF-β signaling pathway.

Extracellular regulation of TGF-β signaling

Effects of TGF-β are mediated by three TGF-β ligands: TGF-β1, TGF-β2, and TGF-β3 (Feng and Derynck 2005; Shi and Massagué 2003). Although each of these ligands is produced by distinct genes, they exhibit approximately 70%–80% sequence similarity. The TGF-β ligand is first synthesized as a dimeric pro-protein (pro-TGF-β), which is then cleaved to form the mature disulfide-bridged TGF-β dimer. The pro-peptide has high affinity for the cleaved mature TGF-β ligand, which is secreted from cells as a small latent complex (ten Dijke and Arthur 2007). Since TGF-β in this form does not have the ability to interact with its receptor, the pro-peptide is termed the latency-associated protein (LAP). The LAP dimer is also bound to the latent TGF-β binding proteins (LTBPs) by disulfide bonds, and the tri-molecular complex is termed the large latent complex (Rifkin 2005). The dissociation of TGF-β from the complex is a critical regulatory event and is achieved by integrin, shear force, thrombospondin-1 (TSP-1), some enzymes including plasmin, changes in pH, heat treatment, radiation, and other agents. Among the four different LTBPs, LTBP-1, 3, and 4 bind to small latent complexes and play key roles in targeting the large latent complex to the extracellular matrix, where active TGF-β is released by proteolytic cleavage. Although the synthesis of TGF-β is regulated by a variety of factors at the level of transcription and/or mRNA stability, the generation of active TGF-β from its latent form is also subject to regulation.

TGF-β receptors

Activated TGF-β ligands transduce their effects through TGF-β type I and II receptors (Ikushima and Miyazono 2010b; Wrana et al. 2008). The TGF-β type II receptor (TβRII) is the specific receptor for TGF-β ligands. Both type II and type I receptors are comprised of an N-terminal extracellular ligand-binding domain, a transmembrane region, and a C-terminal intracellular serine/threonine kinase domain. TGF-β has high affinity for TβRII, and upon binding the ligand, the type I receptor forms a heteromeric complex consisting of two of each receptor type and is activated by the type II receptor (Fig. 1). The type I, but not type II, receptors contain a characteristic GS domain, located N-terminal to the kinase domain. Activation of the type I receptor involves the phosphorylation of its GS domain by the type II receptor. Although activin receptor-like kinase 5 (ALK5), also known as TβRI, mediates TGF-β signal transduction in most types of cells, ALK1 and other type I receptors also transduce TGF-β signaling in certain cells, including endothelial cells (Goumans et al. 2003; Daly et al. 2008).

TGF-β is also able to interact with proteins called TGF-β type III receptors, which do not have intrinsic kinase activity (Bernabeu et al. 2009). Betaglycan is a membrane-anchored proteoglycan that facilitates binding of TGF-β2 to TβRII (Gatza et al. 2010). Endoglin, a glycoprotein expressed at high levels in endothelial cells, binds to TβRII and is thought to act as an accessory protein for the receptor complex (ten Dijke et al. 2008). Although the function of endoglin in TGF-β signaling is still controversial, mutations of it have been linked to hereditary hemorrhagic telangiectasia (McAllister et al. 1994; Abdalla and Letarte 2006). In addition, endoglin produced in a soluble form is associated with the pathogenesis of preeclampsia (Venkatesha et al. 2006). These findings indicate the central roles of endoglin in controlling vascular homeostasis.

Intracellular signal transduction through Smad proteins

Once the functional TGF-β receptor complex is formed, it regulates the activation of downstream signaling pathways. Although several substrates for the type I receptor kinases have been identified, the most important ones for the transduction of TGF-β stimulation are members of the Smad family proteins (Massagué et al. 2005; Schmierer and Hill 2007; Derynck and Zhang 2003). Phosphorylation and activation of the type I receptor enable the recruitment of receptor-regulated Smads (R-Smads). The type I receptor then phosphorylates R-Smads, allowing them to form hetero-oligomeric complexes with the common-partner Smad (Co-Smad) and to move into the nucleus. Of the five R-Smads in mammals, Smad2 and Smad3 are activated by the TβRII–ALK5 complex, whereas Smad1, Smad5, and Smad8 are activated by the TβRII–ALK1 complex. Interestingly, Liu et al. (2009) have recently reported that ALK5 can directly activate Smad1/5 in certain types of

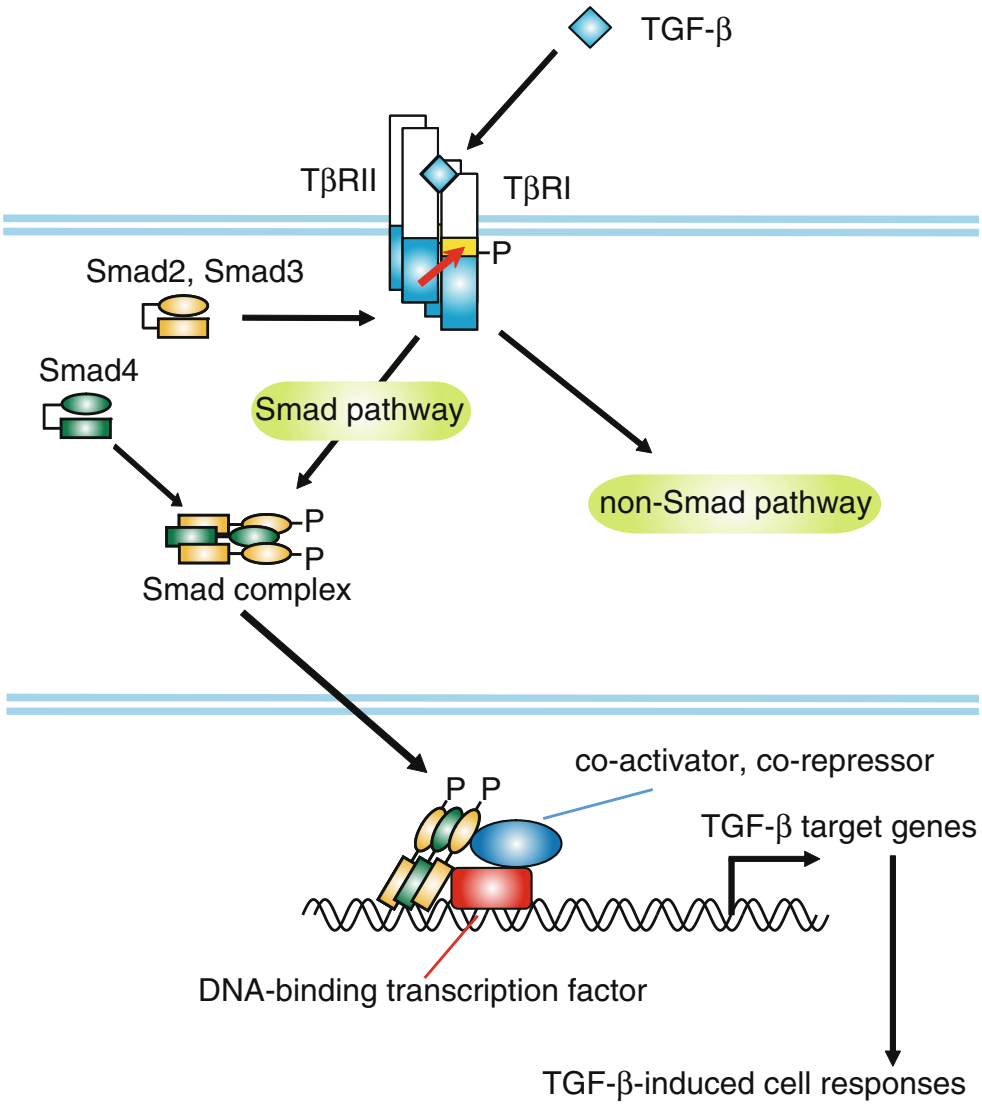

Fig. 1 Intracellular transforming growth factor-β (TGF-β) signal transduction. TGF-β signals are transduced by type II receptor (*TβRII*), type I receptor (*TβRI*), and their downstream Smad proteins (*Smad2-4*). Activated Smad complex interacts with DNA-binding transcription factors and co-activators/co-repressors 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).

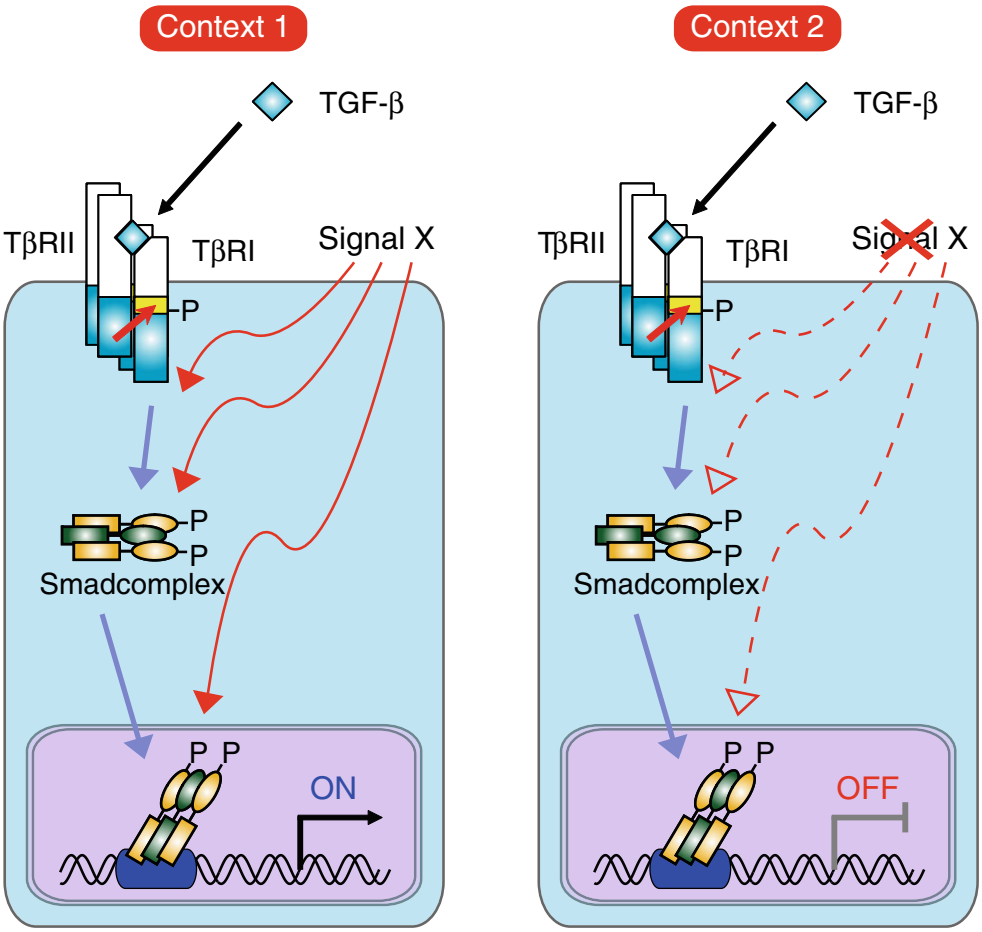

Fig. 2 "Signal cross-talk" model. In *Context 1*, but not in *Context 2*, *Signal X* is transduced in cells to modify downstream transducers of TGF-β signaling and induce a certain context-1-specific cell response

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-

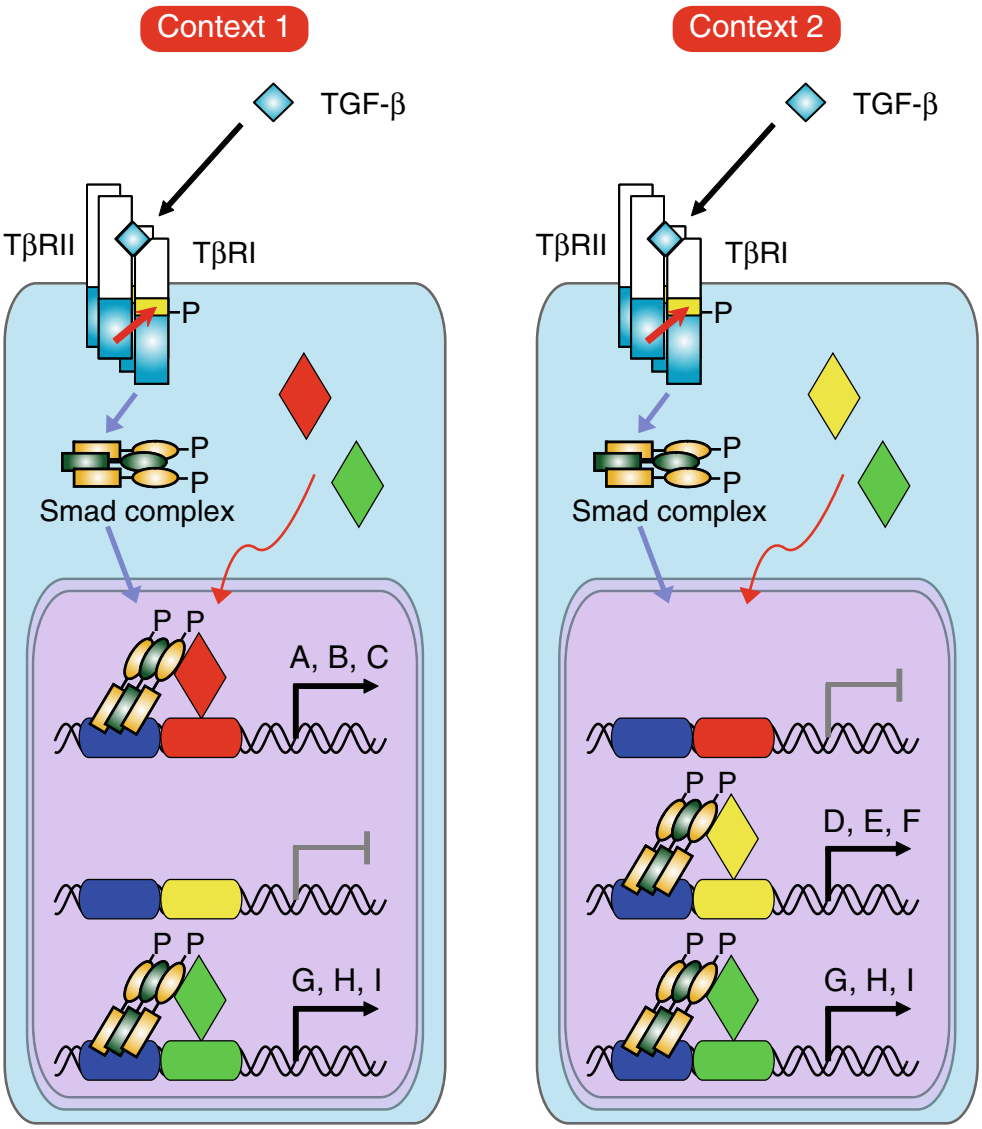

Fig. 3 "Cofactors" model. TGF-β target genes (*A–I*) are regulated by Smad proteins. Profiles of expression of cofactors of Smad proteins differ between *Context 1* and *Context 2*, resulting in different responses to TGF-β stimulation

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).

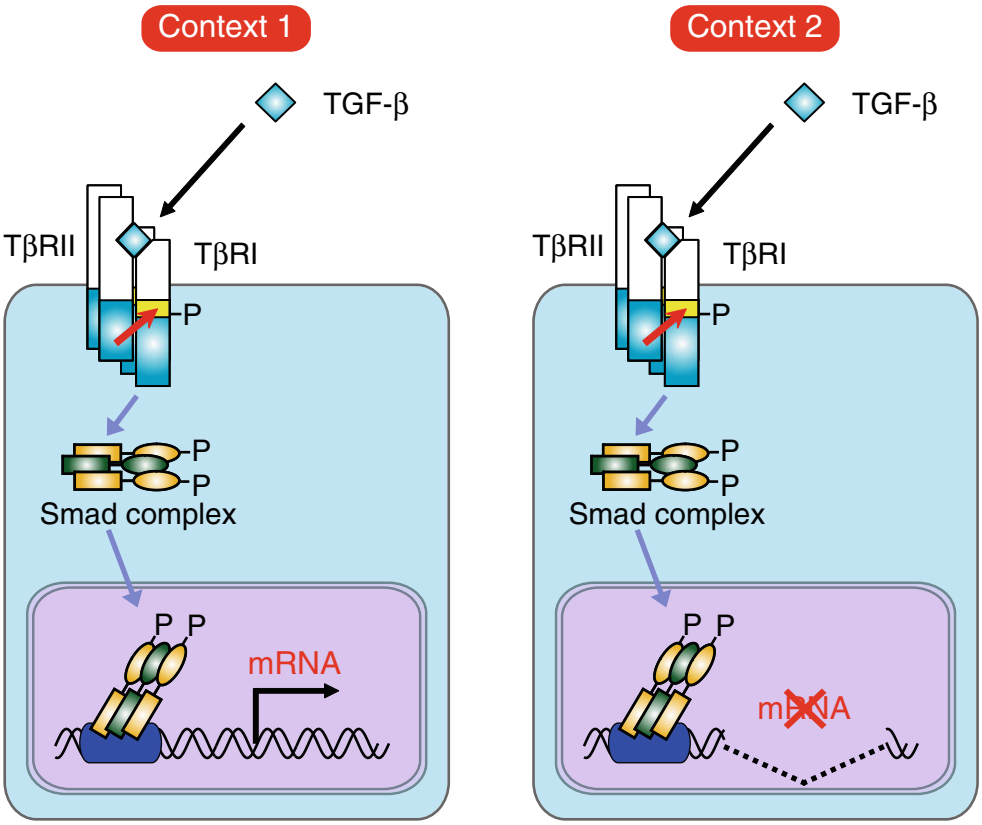

Fig. 4 "Genetic alterations" model. In *Context 1*, expression of a certain target gene is induced by TGF-β signaling. In *Context 2*, the gene is deleted at the chromosomal level, and TGF-β stimulation fails to induce its expression

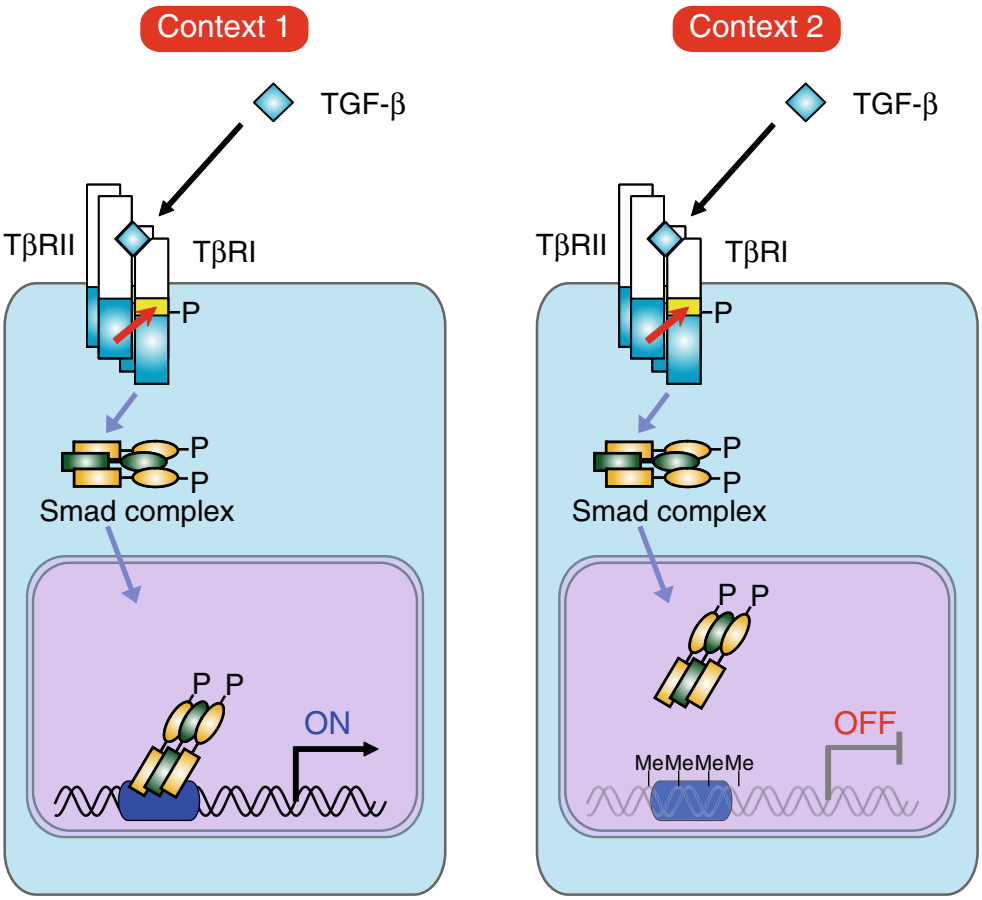

Fig. 5 "Epigenetics" model. In *Context 1*, promoter regions of certain TGF-β target genes adopt an "open conformation" and are exposed to the Smad complex. Conversely, in *Context 2*, promoter regions of the same target genes adopt a "closed conformation", and the Smad complex fails to access the Smad-binding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

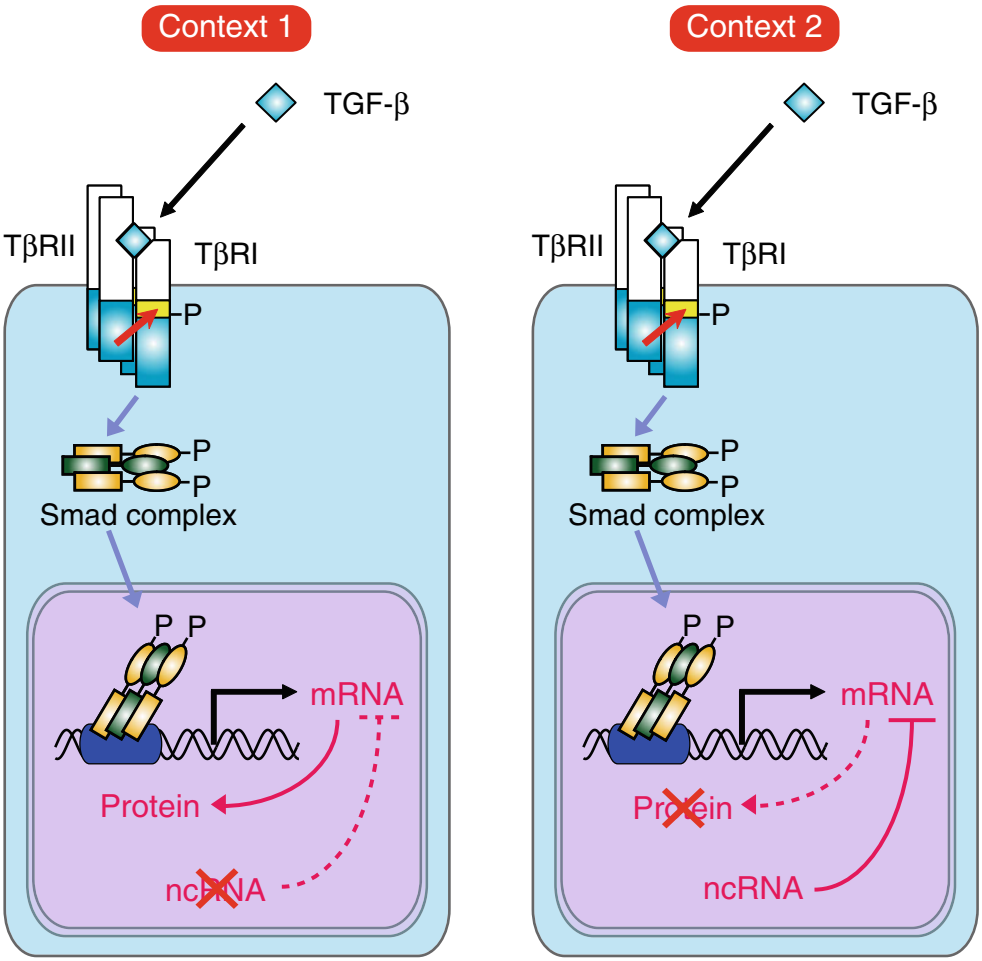

Fig. 6 "Non-coding RNA" model. In *Context 2*, transcribed mRNAs of TGF-β target genes are negatively regulated by non-coding RNA (*ncRNA*). In *Context 1*, such ncRNA is not expressed, resulting in the translation of the mRNAs

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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