[Frontiers in Bioscience 10, 1135-1145, May 1, 2005]

TRANSFORMING GROWTH FACTOR -BETA RECEPTOR SIGNALING IN CANCER

Satya Narayan, Thilakavathy Thangasamy and Ramesh Balusu

Department of Anatomy and Cell Biology and UF Shands Cancer Center, University of Florida, Gainesville, FL 32610, USA

TABLE OF CONTENTS

1. ABSTRACT

Transforming growth factor-beta (TGF-beta) is an ubiquitous cytokine that affects various biological processes, such as regulation of cell proliferation, immune responses, growth, differentiation, angiogenesis, and apoptosis of various cell types. The TGF-beta ligand initiates signaling by binding to and joining type I and II receptors of serine/threonine kinases. This review discusses the TGF-beta ligands and receptors, its positive and negative regulators in signaling, as well as its important roles with respect to tumor suppression and progression.

2. INTRODUCTION

Transforming growth factor-beta (TGF-beta) is a prototypic member of a large family of structurally related pleiotropic cytokines that exhibits a vital role in various biological processes such as regulation of cell proliferation, extra-cellular matrix production, apoptosis, angiogenesis and immune responses (1). Approximately 40 distinct TGF-beta members have been identified and they are generally divided into two subfamilies –the TGF-beta/activin/nodal family and BMP (bone morphogenetic protein)/GDF (growth and differentiation factor)/MIS (Mullerian inhibiting substance) subfamily as defined by sequence similarity and the specific signaling pathway they activate (2-4). The physiological effects of TGF-beta are manifested in a cell and context dependent manner and the cytokine has an important role as an antithetic in cells of different developmental lineages (5).

Improper signaling by TGF-beta has been implicated in a wide variety of human diseases, such as autoimmune disease (6, 7), tumorigenesis (8), and vascular disorders (9, 10). Due to its major role in cellular physiology, the specificity of TGF-beta is tightly regulated. TGF-beta can act as both tumor suppressor and as a significant stimulator of tumor progression, invasion and metastasis (11). As TGF-beta induces extra-cellular matrix accumulation, angiogenesis and immuno-suppression, it is reported to facilitate progression of tumors under certain conditions (12, 13). Depending on the cell type, TGF-beta is required for the maintenance of genomic stability, induction of replicative senescence and suppression of telomerase (14), and it appears to be important for prevention of early stage tumorigenesis. The tumor suppressor activity of TGF-beta pathway derives in part from its ability to inhibit growth of epithelial and lymphoid cells from which tumors arise. Recent studies indicate that TGF-beta exhibits pro-oncogenic effects that directly target the tumor itself in addition to indirect effects on the stromal compartment (15).

3. TGF-BETA LIGANDS AND RECEPTORS

TGF-betas are synthesized as latent high molecular weight complexes from producer cells and are activated by various mechanisms, including effects of plasmin and thrombospondin (16, 17). Latent TGF-beta can be activated by fibronectin receptor, alpha(v)-beta6, in squamous carcinoma cells (18). On the other hand, TGF-beta2 induces matrix metalloproteinases (MMPs) expressions and suppresses tissue matrix metalloproteinase inhibitor (TIMP)-2 expressions that promotes the invasion of U87MG and LN-229 glioma cells in a matrigel invasion

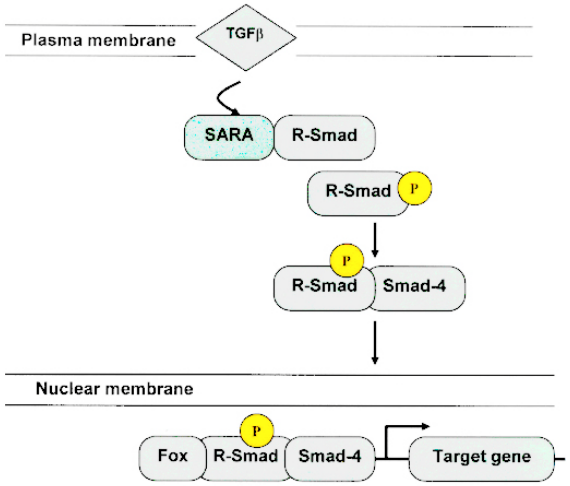

Figure 1. The basic TGF-beta/Smad signaling pathway. TGF-beta ligand binding with TGFbetaRII recruits TGFbetaRI into a tetrameric receptor complex resulting in trans-phosphorylation and activation of TGFbetaRI. After phosphorylation, the activated TGFbetaRI catalyzes phosphorylation of R-Smad, a receptor-regulated Smad. The resulting Smad complex moves into the nucleus and associates with transcriptional co-factors such as certain members of Forkhead box (Fox) family and other families of transcriptional factors). Finally, R-Smad complex binds to specific DNA-binding co-factors in target genes and activates transcription.

assay (19). Four isoforms of TGF-betas, TGF-beta1, -beta2, -beta3 and -beta4 are present and TGF-beta1, -beta2 and -beta3 are highly conserved in mammals. The amino acid sequences of all the three isoforms are 70-80% homologous. TGF-beta1 is a 25-kDa nonglycosylated homodimer with nine disulfide bonds and is expressed in endothelial (20), hematopoietic (21-23), and connective tissue cells (24). TGF-beta2 is expressed in epithelial and neuronal cells and TGF-beta3 primarily in mesenchymal cells (25). The TGF-beta ligands share a common set of sequence and structural features. The active form of TGF-beta cytokine is a dimer stabilized by an intra subunit disulfide. Each of the monomers comprises several extended beta strands interlocked by three conserved disulfide bonds that form a "cysteine knot" (26).

TGF-beta receptors are glycoproteins with serine/threonine kinase activity. Three types of TGF-beta receptors have been identified so far in mammalian cells by cell surface cross-linking experiments. They are type I (55 kDa), type II (75 kDa) and type III (200-400 kDa) based on their electrophoretic mobility (27-30). The type I (T-betaRI) and type II (T-betaRII) receptors have a similar domain structure – a relatively short extra-cellular domain, a single trans-membrane region and a long cytoplasmic region which consists almost entirely of kinase domain (31-34). The T-betaRI receptors contain a characteristic SGSGSG sequence known as the GS-domain, immediately N-terminal to the kinase domain that differentiates them from the T-betaRII receptor (35, 36). The T-betaRII receptor shares 30-40% amino acid identity within the kinase domain with other receptors of the TGF, whereas the T-betaRI receptors share 60-90% identity among their kinase domain.

The T-betaRII receptors and activin are able to bind their respective ligands independently, whereas the T-betaRI receptors require co-expression of T-betaRII receptors (37). More than 90% of hereditary non-polypotic colon cancers and 15% of microsatellite stable colon cancers had inactivating mutations in the T-betaRII gene (38). Somatic mutations in *TGF-betaR2*, the gene that encodes the T-betaRII receptor occurs most frequently in tumors with patient's hereditary non-polyposis colorectal cancer (HNPCC) (39). In these patients, a repeat stretch of adenines in the T-betaRII coding sequence is prone to mutation, owing to germ line defects in their capacity for DNA mismatch repair.

The type III receptor (T-betaRIII) is a trans-membrane 853-amino acid proteoglycan with a large extra-cellular domain containing both heparin sulfate and chondroitin sulfate chains, followed by a trans-membrane segment and a short cytoplasmic region which is rich in serines and threonines (40). The major function of T-betaRIII is to act as a modulator of ligand access to the signaling receptors. Mutations in TGF-beta receptors can cause hereditary hemorrhagic telangiectasia, primary pulmonary hypertension, persistent mullerian duct syndrome, hereditary nonpolyposis colon cancer (HNPCC) and juvenile polyposis syndrome.

4. SIGNALING THROUGH TGF-BETA RECEPTOR COMPLEX

Active TGF-beta binds to T-betaRI and T-betaRII, which is required for signaling. TGF-beta binds to T-betaRII and results in trans-phosphorylation of T-betaRI in the GS-domain. The phosphorylated T-betaRI becomes active and propagates the signal through phosphorylation of the Smad proteins. Smad-2 and -3 are activated *via* C-terminal phosphorylation by T-betaRI kinases and form heteromeric complexes with co-Smad, Smad-4 (39, 41-43). The phosphorylation occurs in the last two serine residues at the terminal Ser-Ser-x-Ser motif of R-Smads. Following phosphorylation of R-Smads by T-betaRI receptors, Smad oligomerization is thought to occur. The phosphorylated C-terminal tail of R-Smads interacts specifically with the L3-loop of another smad, which is sufficient to cause their oligomerization (44). Activated R-Smads then recruit co-Smads, forming a heterotrimeric complex that translocates to the nucleus and together with a co-activator or co-repressor and a DNA-binding co-factor regulate transcription of target genes (Figure 1) (45, 46).

In the nucleus, activated Smad complexes can directly recognize target genes with several copies of the Smad cognate sequence CAGAC to which they can bind through their Mad homology 1 (MH1)-domains (47). As most of the Smad responsive promoter elements contain only one copy of this motif, for effective recognition of such genes under physiological conditions, the Smad complex must incorporate additional DNA-binding co-

factors that recognize nearby sequences in the target promoter (48). Nuclear localization signals (NLS) in the MH1-domains of R-Smads play pivotal roles in translocation of Smads into the nucleus, whereas nuclear export signals in the MH2-domains of R-Smads and those in the linker regions of co-Smads are responsible for nuclear export of the complex (49-51). TGF-beta displays a high affinity for the ectodomain of the T-betaRII receptors and do not interact with the T-betaRI receptors (25). This adds in the incorporation of the T-betaRI, forming a large ligand receptor complex involving a ligand-dimer and four receptor molecules. The binding occurs at the far ends of the elongated ligand-dimer. This interaction creates certain concave patches which are supposed to be the binding site for the ectodomain of the T-betaRI. Because of its critical role in receptor activation, the GS-region serves an important regulatory domain for TGF-beta -signaling. Thus, T-betaRII is the primary component of ligand-binding, whereas receptor I is its substrate and a downstream component of the signal transduction pathway (52).

4.1. SMADS

Smad family members were first identified through genetic screens in flies and worms, and the family quickly grew to eight mammalian counterparts. The term "Smad" is derived from the fusion of Sma in *c. elegans* and Mads (Mothers against decapentaplegic) in drosophila (53). Smads are ubiquitously expressed throughout development and in all adult tissues, and many of them are produced from alternatively spliced mRNAs (54). Most of the effects of TGF-beta are mainly mediated through the TGF-beta receptor/Smad-signaling pathway. Smads are intracellular nuclear effectors of TGF-beta family members, and there are at least nine different Smads whose relative molecular masses range from 42-60 kDa. Ligand-induced activation of TGF-beta family members with intrinsic serine/threonine kinase activity triggers phosphorylation of TGF-beta receptor regulated Smads.

The Smad family can be divided into three distinct subfamilies - receptor regulated smads (R-Smads), common partner Smads (co-Smads), and inhibitory Smads (I-Smads) (55). Among the R-Smads, Smad-1, Smad-5 and Smad-8 are substrates for the bone morphogenetic protein (BMP) type I receptors and the orphan receptor activin receptor-like kinase 1 (ALK-1), while Smad-2 and Smad-3 are phosphorylated by TGF-beta or the activin type I receptor. The access of Smads to the receptors is restricted by an anchoring protein named SARA (Smad anchor for receptor activation) (56). R-Smads are bound to SARA, a Smad-2/Smad-3 interacting protein that contains a double zinc finger or FYVE-domain. SARA is anchored to the plasma membrane by phosphatidyl inositol-3-phosphate (PI3P). SARA forms a dimer in cells and anchors two molecules of unphosphorylated Smad-2/Smad-3 and preferentially binds to the MH2-domains of Smad-2 and Smad-3. When activated Smads become dissociated from SARA, then they are available for phosphorylation by the TGF-beta receptors (57). The co-Smad including Smad-4 and possibly Smad-10 are present in the cytoplasm and lack the ability to bind to or to be phosphorylated by TGF-beta receptors. I-Smads include Smad-6 and Smad-7 with Smad-6 mainly inhibiting BMP-signaling while Smad-7 is more specific to TGF-beta signal transduction. Smad-4 is a key partner of R-Smads proteins and was originally isolated as the product of tumor suppressor gene DPC4 (43). It recruits transcription co-activators such as CBP/p300 and migration inhibitory factor 1 (MIF1) to R-Smads and stabilizes the interaction between R-Smads and the co-activators (58, 59). The levels and activity of Smad-4 are affected both by the ubiquitin-proteosome system and by sumoylation (60-63). Smad-4 is a shared component in the TGF-beta/activin and BMP-signaling pathways. If the cellular pool of Smad-4 is limited, these two pathways may antagonize each other by competing for Smad-4 (64). Furthermore, TGF-beta -signaling is inhibited by lefty, a novel member of the TGF-beta super-family. Lefty inhibits the events that lie downstream from R-Smad phosphorylation, including heterodimerization of R-Smad proteins with Smad4 and nuclear translocation of the R-Smad.Smad4 complex (65).

R-Smads and co-Smads with about 500 amino acid sequences in length contain two conserved structural domains, the N-terminal Mad homology 1 (MH1) and C-terminal MH2-domains which are separated by a proline-rich linker region (66). The linker-region contains a number of phosphorylation sites crucial for cross-talk with other signaling pathways. A PY-motif, which specifically interacts with E3 ubiquitin ligases, plays a vital role in TGF-beta and BMP pathways. The L3-loop in the MH2 domain of R-Smads determines the specificity of the interaction with the T-betaRI receptors (45). The crystal structure of MH1-domain is a compact globular fold composed of four α helices, six short -beta strands and five loops. Additionally, the MH1-domain also has a beta hairpin structure made up of 11 amino acids through which MH1 can directly bind to DNA when activated Smads are trans-located to the nucleus (67). The MH1-domain is highly conserved among R-Smads and co-Smads. The MH1-domain regulates nuclear import and transcription by binding to DNA and interacting with other nuclear proteins (66). The MH2-domain is highly conserved among all the Smads. Its structure contains several α- helices and loops, which surround a -beta–sandwich (68). The MH2-domain regulates Smad oligomerization, recognized by T-betaRI receptors and interacts with cytoplasmic adaptors and several transcription factors.

Smad proteins are suggested to be tumor suppressors. Mutations in Smad genes have been reported in various carcinomas. Smad-4 was originally identified as a tumor suppressor gene lost in pancreatic cancers, *deleted in pancreatic cancer 4* (*DPC4*). About 50% of pancreatic cancers and 30% of colorectal cancers are caused by mutations in Smad-4 (69).

4.2. I–SMADS

Smads can positively regulate gene expression by recruiting co-activators such as CBP/p300 or negatively by recruiting histone deacetylases (HDACs) or co-repressors such as C-Ski and SnoN, which themselves associate with HDACs (70). Negative regulation of Smad-signaling occurs through multiple mechanisms including inhibitory

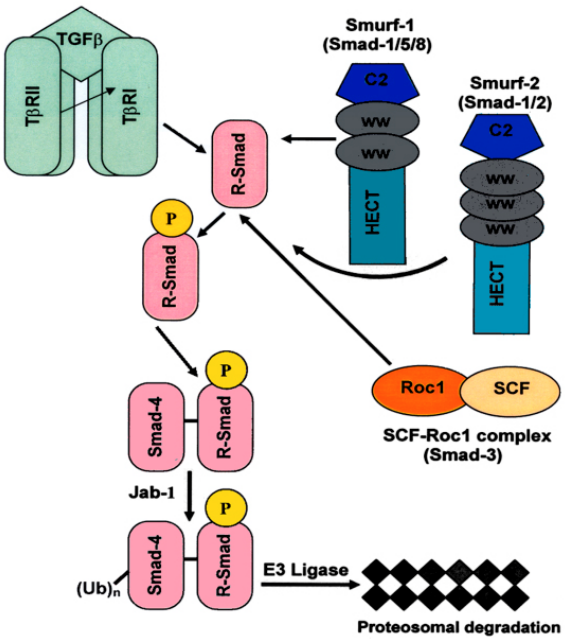

Figure 2. Ubiquitin-proteosome pathway dependent degradation of Smads. The ubiquitin-proteosome pathway regulates both the basal levels as well the turnover of Smads upon the activation of the signaling pathway. The degradationn of Smad is mediated at least in part by E3 ligases including Smurf-1, Smurf-2, SCF/Roc1. Jab-1 is the Jun-activating domain binding protein 1.

Smads (I-Smads) (71). I-Smads are mainly located in the nucleus and are unique in their action. I-Smads are structurally related to R-Smads and co-Smads, especially in the MH2-domains. However, their N-terminal regions are highly distinct from other Smads, and I-Smads inhibit the signaling activities induced by the TGF-beta super-family of proteins. I-Smads interact with T-betaRI receptors activated by T-betaRII receptor kinases (72). In addition to its inhibitory role in TGF-beta-signaling by preventing activation of R-Smads by the TGF-beta receptors, I-Smads also interact with R-Smads activated by the TGF-beta receptors and interfere with the complex formation of R-Smads with co-Smads (73). Inhibitory activities of I-Smads are facilitated by HECT type E3 ubiquitin ligases Smurf-1 and Smurf-2 (74, 75). The term Smurf is derived from Smad ubiquitin regulatory factors.

Smad-6, one of the I-Smads exerts inhibitory effects on signaling through other mechanisms than by competing with R-Smads for receptor interaction. Smad-6 competes with Smad-4 for heteromeric complex formation with activated Smad-1 and functions as a transcriptional co-repressor through interaction with a homeodomain transcription factor HOXC-8. It also associates with TGF-beta activated kinase (TAK) and interferes with BMP-induced p38 activation (76-78).

4.3. SKI

SnoN and ski are two closely related members of the family of nuclear oncoproteins that were identified on the basis of homology with V-Ski, the transforming protein of the Sloan-Kettering virus (79, 80). They play important roles in repressing Smad-mediated transcriptional activation in normal cells. Ski or SnoN antagonizes TGF-beta signaling through direct interactions with the co-Smad, Smad-4 and the R-Smads, Smad-2 or Smad-3 (81, 82). This results in the inability of the cell to induce growth inhibitory proteins following TGF-beta addition (83). High levels of Ski or SnoN are associated with many types of human cancer cells (84-87). Increased expression of Ski or SnoN causes oncogenic transformation of chicken and quail embryo fibroblasts (88). The Ski family of proto-oncogenes uses two separate sequence motifs to interact with the co-Smad, Smad-4 and the R-Smads, Smad-2, Smad-3, Smad-1 or Smad-5 (89, 90). Studies on crystal structure of a Ski fragment bound to Smad-4 indicates that Ski and the R-Smads compete for mutually exclusive binding to Smad-4 leading to the disruption of functional Smad-4/R-Smad complexes (91). The N-terminal region of C-Ski (16-40 amino acids) is the most important for the interaction with Smad-3 whereas the I-loop of C-Ski is responsible for the interaction with Smad-3. Binding of Ski to Smad-4 plays an essential role in Ski-mediated repression of TGF-beta signaling as well as Ski-mediated transforming activity (92).

5. PROTEOSOME AND SMAD SIGNALING

The ubiquitin-mediated proteosomal degradation pathway is a conserved cascade involved in the regulation of key cellular processes such as signal transduction, cell cycle progression, transcriptional regulation and endocytosis. The TGF-beta-signaling pathway is tightly regulated by the ubiquitin-proteosomal degradation system. In response to TGF-beta-mediated stimulation, Smads are also targeted for ubiquitination and degradation which ultimately results in termination of TGF-beta signals (Figure 2) (93).

Smurfs-1 and -2 are HECT type-E3 ubiquitin ligases, containing the N-terminal C2-domain followed by WW-domains and the C-terminal HECT-domain that interact with R-Smads and induce their degradation (94). The HECT-domain is responsible for the E3 ligase activities of Smurfs as they catalyze the transfer of the ubiquitin moiety to its target substrates. The WW-domains physically interact with the PY-motifs found in most R-and I-Smads. While Smurf-1 contains two WW-domains, Smurf-2 comprises three WW-domains, resulting from a 31-amino acid insertion downstream of the C2-domain (93). Smurfs induce degradation of R-Smads indirectly through the binding of R-Smads *via* I-Smads. The ubiquitination of Smad-2 is carried out by UbcH5b/c-E2 ubiquitin conjugating enzymes and Smurf-2-E3 ubiquitin ligase. Smurf-2 interacts with the ligand activated Smad-2 through its WW-domain and decreases the Smad-2 mediated-transcription (95). Proteosomal degradation of Smad-2 is likely to occur in the nucleus as suggested by preferential interaction of Smurf-2 with the phosphorylated Smad-2. Similar to Smad-2, the action of the ligand-activated Smad-3 is also ultimately terminated by its degradation via the ubiquitin-proteosome pathway. However, in this case, the SCF/Roc-1-E3 ligase complex

has been shown to be responsible for triggering the ubiquitination of phosphorylated Smad-3, after which Smad-3 is exported from the nucleus and undergoes proteosomal degradation in the cytoplasm (96).

The degradation of activated Smad-1 is reported to occur through a complex containing Smad-1, the ornithine decarboxylase antizyme (Az) and the 20S proteosome beta subunit, Hs-N3, which then targets Smad-1 for proteosomal degradation (97, 98). Ectopic expression of Jab-1 or Jun-activating domain-binding protein 1 mediated ubiquitination and degradation of Smad-4 (99). Smad-7 expression induced by TGF-beta leads to the association of Smad-7 with Smurf-2 in the nucleus and migration of the complex to the cytoplasm where it binds to the T-betaRI complex and induces its degradation (100, 101). The degradation of T-betaRI can be also mediated by Smurf-1, another member of HECT family of E3 ubiquitin ligases. Similar to Smurf-2, Smurf-1 is brought to ligand activated type I receptor by Smad-7 and mediates the ubiquitination and proteosome exerted degradation of the receptor (102). Smurfs induce poly-ubiquitination and degradation not only of Smad-7 but also of type I receptors and inhibit TGF-signaling through the down-regulation of the amount of receptors on cell surface. Smad-7 itself is also degraded via the proteosome when targeting the receptor complex for degradation.

Inactivation of the activities of phosphorylated R-Smads is crucial for controlling the extent of TGF-beta effects. Interestingly, although dephosphorylation of activated R-Smads by unidentified phosphatases is thought to contribute to the dissociation of the Smad complexes in the nucleus, growing evidence indicates the phosphorylated R-Smads are inactivated at least partially through the irreversible degradation via the ubiquitin-proteosome system. The ubiquitination of Smad-2 is carried out by UbcH5b/c E2 ubiquitin conjugating enzymes and Smurf-2 E3 ubiquitin ligase (103). Smurf-2 interacts with the ligand activated Smad-2 through its WW-domain and decreases the Smad-2 mediated transcription. Thus, the proteosome activity limits the function of Smad-2 in the TGF-beta signal transduction pathway. Proteosomal degradation of Smad-2 is likely to occur in the nucleus as suggested by the preferential interaction of Smurf-2 with the phosphorylated Smad-2.

Besides activating the Smad pathway, TGF-beta activates other transduction pathways including MAPK (mitogen activated protein kinase) (104), AP-1 (activated protein-1) (105, 106), TAK-1 (TGF-beta activated kinase-1) (107), and Jun-D (member of the AP-1 family transcription factors) (108) pathways. The Smad and TAK-1 pathways synergistically trans-activate transcription factor ATF-2 (109). After TGF-beta binds with the T-betaRII, a heteromeric complex with the T-betaRI is formed, activating the T-betaRI serine/threonine kinase which then phosphorylates Smad-3 (R-Smad). Smad-3 forms a heterotrimeric complex with Smad-4 (co-Smad) that trans-locates to the nucleus and binds ATF-2 and synergistically stimulates its trans-activating capacity (109). From these studies it has been concluded that ATF-2 plays a central role in TGF-beta signaling by acting as a common nuclear target of both Smad and TAK1 pathways.

6. ROLE OF TGF-BETA IN TUMOR SUPPRESSION AND TUMOR PROGRESSION

TGF-beta plays a biphasic role in cancer. In the earlier stages of carcinogenesis, it is known to act as a tumor suppressor probably by inhibiting the proliferation of non-transformed cells whereas in the later stages it is known to aid tumor progression by eliciting an epithelial to mesenchymal transition (8). TGF-beta receptors are known to act as tumor suppressors at early stages of carcinoma development. In tumor cells, TGF-beta receptors are often down-regulated or the TGF-beta receptor availability at the cell surface is impaired, and these defects are considered to help the cells to escape from the growth inhibitory properties of TGF-beta (110, 111).

TGF-beta1 is reported to induce a rapid and reversible epithelial-mesenchymal transition (EMT) in melanoma cells (112) and in *H-Ras* transformed mammary epithelial cells *in vitro* (113). Experimental animals with one inactivating allele of the gene encoding TGF-beta1 show increased susceptibility for developing carcinoma after exposure to carcinogen (114). Transfection of weakly tumorigenic colon carcinoma cells with antisense TGF-beta inhibited TGF-beta expression dramatically increased the size of tumors formed by these cells *in vivo* (115). T-betaRI mutations have been reported in ovarian carcinoma (116, 117), head and neck carcinoma (118), lymphoma (119) as well as in breast metastases (120, 121). Expression of wild type T-betaRII in colon or breast carcinoma cells, which lack a functional T-betaRII allele, has been reported to show the tumor suppressor role of the T-betaRII in these cells (122). Expression of T-betaRII strongly reduced tumor formation in nude mice by growth inhibition and by suppressing anchorage independence. Several studies on human tumors also indicate that TGF-beta has a vital role in angiogenesis and is suggested to promote angiogenesis by inducing PAI-1 and thereby inhibiting angiostatin (123).

7. APOPTOTIC AND CYTOSTATIC ROLE OF TGF-BETA

TGF-beta is a potent inducer of growth inhibition in several cell types. During cancer, a deregulation in the CDK2, CDK4 and CDK6 occurs through the G_1 phase of cell cycle. In G_1, the activation of CDK4/6 requires association with D-type cyclins whereas CDK2 is activated by cyclin E. One primary event that leads to TGF-beta induced growth arrest is the induction of expression of the CDK inhibitors $P^{15\ INK4B}$ and/or P^{21CIP1}. The inhibitor P^{21CIP1} interacts with complexes of CDK2 and cyclin A or cyclin E and thereby inhibits CDK2 activity, preventing progression of cycle (124). The $P^{15\ INK4B}$ of the INK4 family interacts with and inactivates CDK4 and CDK6 by disrupting their catalytic sites and by inhibiting cyclin binding (125). Induction of $P^{15INK4B}$ or $P^{21(WAF1/CIP1)}$ expression in response to TGF-beta is mainly due to Smad mediated transcriptional activation. TGF-beta down-regulates c-Myc to deprive cell of growth promoting

functions (126) as well as to facilitate the induction of CDKN2B and CDKN1A and therefore has an integrative role in the TGF-beta cytostatic program (125).

The apoptotic role of TGF-beta varies depending on the cell type. In various cell types, the TGF-beta inducible early response gene (TIEG1), a transcription factor is reported to inhibit proliferation and induce apoptosis in various cell types (127). Daxx adaptor protein has been proposed to mediate TGF-beta-induced apoptosis through its ability to interact with the T-betaII receptor (128). The levels of Smads are reported to have an influence on apoptosis. Increase in the levels of Smads-3 or Smad-4 induces apoptosis (129). Whereas Smad-7 can act either as an effector of TGF-beta induced cell death as reported in prostatic carcinoma cells (130) or can protect against cell death (131).

8. SUMMARY AND PERSPECTIVES

The TGF-beta receptor signal transduction pathway plays critical roles in many biological processes, including the development and progression of human tumors. Since TGF-beta receptors-mediated signaling pathway is complicated, it requires interaction with many functionally important molecules in either normal cells or tumor cells. Some of the important molecules which interact with TGF-beta receptor-signaling cascade are Smads, BMP, GDF, MIS, TIMP, SARA, CBP/p300, MIF1, ubiquitin ligases and Smurfs, to name a few. Any defect(s) in the molecules can cause the loss or gain of TGF-beta receptor signaling in normal or tumor cells. In some cases the proliferation of normal epithelial and lymphoid cells are strongly inhibited by TGF-beta, which is lost in cancer cells due to defective TGF-beta receptor-signaling. Interestingly enough, the altered TGF-beta receptor-signaling pathway can also stimulate proliferation of some cancer cells. This further suggests the complexity of the TGF-beta receptor-signaling pathway in normal and cancer cells. Thus, future research needs to be directed toward better understanding the function of various components of TGF-beta receptor-signaling pathways and to design approaches to interfere with these pathways – such as antibodies, RNA interference, chemotherapy and gene therapy.

9. ACKNOWLEDGEMENTS

We apologize to those colleagues whose work was not cited; instead, many of them have been accommodated in referenced review articles. We thank Mary Wall for proof reading of the manuscript.

10. REFERENCES

1. Kretschmer, A., K. Moepert, S. Dames, M. Sternberger, J. Kaufmann and A. Klippel: Differential regulation of TGF-beta signaling through Smad2, Smad3 and Smad4. *Oncogene* 22, 6748-6763 (2003)

2. Johnson, A. N. and S. J. Newfeld: The TGF-beta family: signaling pathways, developmental roles, and tumor suppressor activities. *Scientific World Journal* 2, 892-925 (2002)

3. Kulkarni, A. B., T. Thyagarajan and J. J. Letterio: Function of cytokines within the TGF-beta superfamily as determined from transgenic and gene knockout studies in mice. *Curr Mol Med* 2, 303-327 (2002)

4. ten Dijke, P., M. J. Goumans, F. Itoh and S. Itoh: Regulation of cell proliferation by Smad proteins. *J Cell Physiol* 191, 1-16 (2002)

5. Sporn, M. B: TGF-beta: 20 years and counting. *Microbes Infec* 1, 1251-1253 (1999)

6. Wahl, S. M. and W. Chen: TGF-beta: how tolerant can it be? *Immunol Res* 28, 167-179 (2003)

7. Tsuchida, K: Activins, myostatin and related TGF-beta family members as novel therapeutic targets for endocrine, metabolic and immune disorders. *Curr Drug Targets Immune Endocr Metabol Disord* 4, 157-166 (2004)

8. Sun, L: Tumor-suppressive and promoting function of transforming growth factor-beta *Front Biosci* 9, 1925-1935 (2004)

9. Goumans, M. J., F. Lebrin and G. Valdimarsdottir: Controlling the angiogenic switch: a balance between two distinct TGF-beta receptor signaling pathways. *Trends Cardiovasc Med* 13, 301-307 (2003)

10. van den Driesche, S., C. L. Mummery and C. J. Westermann: Hereditary hemorrhagic telangiectasia: an update on transforming growth factor-beta signaling in vasculogenesis and angiogenesis. *Cardiovasc Res* 58, 20-31 (2003)

11. Akhurst, R. J., and R. Derynck: TGF-beta signaling in cancer-a double-edged sword. *Trends Cell Biol* 11, S44-51 (2001)

12. Hahn, S. A., M. Schutte, A. T. Hoque, C. A. Moskaluk, L. T. da Costa, E. Rozenblum, C. L. Weinstein, A. Fischer, C. J. Yeo, R. H. Hruban and S. E. Kern: DPC4, a candidate tumor suppressor gene at human chromosome 18q21.1. *Science* 271, 350-353 (1996)

13. Miyaki, M., T. Iijima, M. Konishi, K. Sakai, A. Ishii, M. Yasuno, T. Hishima, M. Koike, N. Shitara, T. Iwama, J. Utsunomiya, T. Kuroki and T. Mori: Higher frequency of Smad4 gene mutation in human colorectal cancer with distant metastasis. *Oncogene* 18, 3098-3103 (1999)

14. Glick, A. B., W. C. Weinberg, I. H. Wu, W. Quan, and S. H. Yuspa: Transforming growth factor-beta1 suppresses genomic instability independent of a G1 arrest, p53, and Rb. *Cancer Res* 56, 3645-3650 (1996)

15. Huang, X. and C. Lee: Regulation of stromal proliferation, growth arrest, differentiation and apoptosis in benign prostatic hyperplasia by TGF-beta. *Front Biosci* 8, 740-749 (2003)

16. Miyazono, K. and C. H. Heldin: Latent forms of TGF-beta: molecular structure and mechanisms of activation. *Ciba Found Symp* 157, 81-89 (1991); discussion 157, 89-92 (1991)

17. Koli, K., J. Saharinen, M. Hyytiainen, C. Penttinen and J. Keski-Oja: Latency, activation, and binding proteins of TGF-beta. *Microsc Res Tech* 52, 354-362 (2001)

18. Thomas, G. J., I. R. Hart, P. M. Speight and J. F. Marshall: Binding of TGF-beta1 latency-associated peptide (LAP) to α(v)-beta6 integrin modulates behaviour of squamous carcinoma cells. *Br J Cancer* 87, 859-867 (2002)

19. Wick, W., M. Platten and M. Weller: Glioma cell invasion: regulation of metalloproteinase activity by TGF-beta. *J Neurooncol* 53, 177-185 (2001)

20. Pintavorn, P. and B. J. Ballermann: TGF-beta and the endothelium during immune injury. *Kidney Int* 51, 1401-412 (1997)

21. Keller, J. R., G. K. Sing, L. R. Ellingsworth and F. W. Ruscetti: Transforming growth factor-beta: possible roles in the regulation of normal and leukemic hematopoietic cell growth. *J Cell Biochem* 39, 175-184 (1989)

22. Ruscetti, F. W., C. Dubois, L. A. Falk, S. E. Jacobsen, G. Sing, D. L. Longo, R. H. Wiltrout and J. R. Keller: *In vivo* and *in vitro* effects of TGF-beta1 on normal and neoplastic haemopoiesis. *Ciba Found Symp* 157, 212-227 (1991); discussion 227-231 (1991)

23. Hu, X. and K. S. Zuckerman: Transforming growth factor: signal transduction pathways, cell cycle mediation, and effects on hematopoiesis. *J. Hematother. Stem Cell Res* 10, 67-74 (2001)

24. Weber, K. T., S. K. Swamynathan, R. V. Guntaka and Y. Sun: Angiotensin II and extracellular matrix homeostasis. *Int J Biochem Cell Biol* 31, 395-403 (1999)

25. Massague, J: TGF-beta signal transduction. *Annu Rev Biochem* 67, 753-791 (1998)

26. Daopin, S., K. A. Piez, Y. Ogawa and D. R. Davies: Crystal structure of transforming growth factor-beta 2: an unusual fold for the superfamily. *Science* 257, 369-373 (1992)

27. Lin, H. Y. and X. F. Wang: Expression cloning of TGF-beta receptors. *Mol Reprod Dev* 32, 105-110 (1992)

28. Cox, D. A: Transforming growth factor-beta 3. *Cell Biol Int* 19, 357-371 (1995)

29. Kolodziejczyk, S. M. and B. K. Hall: Signal transduction and TGF-beta superfamily receptors. *Biochem Cell Biol* 74, 299-314 (1996)

30. Dooley, S., B. Delvoux, B. Lahme, K. Mangasser-Stephan and A. M. Gressner: Modulation of transforming growth factor -beta response and signaling during transdifferentiation of rat hepatic stellate cells to myofibroblasts. *Hepatology* 31, 1094-1106 (2000)

31. Daopin, S., M. Li and D. R. Davies: Crystal structure of TGF-beta 2 refined at 1.8 A resolution. *Proteins* 17, 176-192 (1993)

32. Huse, M., Y. G. Chen, J. Massague and J. Kuriyan: Crystal structure of the cytoplasmic domain of the type I TGF-beta receptor in complex with FKBP12. *Cell* 96, 425-436 (1999)

33. Boesen, C. C., S. Radaev, S. A. Motyka, A. Patamawenu and P. D. Sun: The 1.1 A crystal structure of human TGF-beta type II receptor ligand binding domain. *Structure (Camb)* 10, 913-919 (2002)

34. Deep, S., K. P. Walker, Z. Shu and A. P. Hinck: Solution structure and backbone dynamics of the TGF-beta type II receptor extracellular domain. *Biochemistry* 42, 10126-10139 (2003)

35. Wieser, R., J. L. Wrana and J. Massague: GS domain mutations that constitutively activate T-betaRI, the downstream signaling component in the TGF-beta receptor complex. *EMBO J* 14, 2199-21208 (1995)

36. Saitoh, M., H. Nishitoh, T. Amagasa, K. Miyazono, M. Takagi and H. Ichijo: Identification of important regions in the cytoplasmic juxtamembrane domain of type I receptor that separate signaling pathways of transforming growth factor-beta *J Biol Chem* 271, 2769-2775 (1996)

37. Ebner, R., R. H. Chen, S. Lawler, T. Zioncheck and R. Derynck: Determination of type I receptor specificity by the type II receptors for TGF-beta or activin. *Science* 262, 900-902 (1993)

38. Markowitz, S., J. Wang, L. Myeroff, R. Parsons, L. Sun, J. Lutterbaugh, R. S. Fan, E. Zborowska, K. W. Kinzler, B. Vogelstein: Inactivation of the type II TGF-beta receptor in colon cancer cells with microsatellite instability. *Science* 268, 1336-1338 (1995)

39. Derynck, R., R. J. Akhurst and A. Balmain: TGF-beta signaling in tumor suppression and cancer progression. *Nat Genet* 29, 117-129 (2001)

40. Wang, X. F., H. Y. Lin, E. Ng-Eaton, J. Downward, H. F. Lodish and R. A. Weinberg: Expression cloning and characterization of the TGF-beta type III receptor. *Cell* 67, 797-805 (1991)

41. Attisano, L. and J. L. Wrana: Signal transduction by members of the transforming growth factor-beta superfamily. *Cytokine Growth Factor Rev* 7, 327-339 (1996)

42. Narayan, S. and D. Roy: Role of APC and DNA mismatch repair genes in the development of colorectal cancers. *Mol Cancer* 12, 41-55 (2003)

43. ten Dijke, P. and C. S. Hill: New insights into TGF-beta-Smad signalling. *Trends Biochem Sci* 29, 265-273 (2004)

44. Chacko, B. M., B. Qin, J. J. Correia, S. S. Lam, M. P. de Caestecker and K. Lin: The L3 loop and C-terminal phosphorylation jointly define Smad protein trimerization. *Nat Struct Biol* 8, 248-253 (2001)

45. Itoh, S., F. Itoh, M. J. Goumans and P. ten Dijke: Signaling of transforming growth factor-beta family members through Smad proteins. *Eur J Biochem* 267, 6954-6967 (2000)

46. Miyazono, K: Positive and negative regulation of TGF-beta signaling. *J Cell Sci* 113, 1101-1109 (2000)

47. Dennler, S., C. Prunier, N. Ferrand, J. M. Gauthier and A. Atf: c-Jun inhibits transforming growth factor-beta-mediated transcription by repressing Smad3 transcriptional activity. *J Biol Chem* 275, 28858-28865 (2000)

48. Siegel, P. M. and J. Massague: Cytostatic and apoptotic actions of TGF-beta in homeostasis and cancer. *Nat Rev Cancer* 3, 807-821 (2003)

49. Watanabe, M., N. Masuyama, M. Fukuda and E. Nishida: Regulation of intracellular dynamics of Smad4 by its leucine-rich nuclear export signal. *EMBO Rep* 1, 176-182 (2000)

50. Kurisaki, A., S. Kose, Y. Yoneda, C. H. Heldin and A. Moustakas: Transforming growth factor-beta induces nuclear import of Smad3 in an importin-beta1 and Ran-dependent manner. *Mol Biol Cell* 12, 1079-1091 (2001)

51. Xiao, Z. Q. and A. P. Majumdar: Increased *in vitro* activation of EGFR by membrane-bound TGF-α from gastric and colonic mucosa of aged rats. *Am J Physiol Gastrointest Liver Physiol* 281, G111-116 (2001)

52. Saltis, J: TGF-beta: receptors and cell cycle arrest. *Mol Cell Endocrinol* 116, 227-232 (1996)

53. Sekelsky, J. J., S. J. Newfeld, L. A. Raftery, E. H. Chartoff and W. M. Gelbart: Genetic characterization and cloning of mothers against dpp, a gene required for decapentaplegic function in Drosophila melanogaster. *Genetics* 139, 1347-1358 (1995)

54. Flanders, K. C., E. S. Kim and A. B. Roberts: Immunohistochemical expression of Smads 1-6 in the 15-day gestation mouse embryo: signaling by BMPs and TGF-betas. *Dev Dyn* 220, 141-154 (2001)

55. Miyazawa, K., M. Shinozaki, T. Hara, T. Furuya and K. Miyazono: Two major Smad pathways in TGF-beta superfamily signalling. *Genes Cells* 7, 1191-1204 (2002)

56. Wrana, J. L. and L. Attisano: The Smad pathway. *Cytokine Growth Factor Rev* 11, 5-13 (2000)

57. Wu, G., Y. G. Chen, B. Ozdamar, C. A. Gyuricza, P. A. Chong, J. L. Wrana, J. Massague and Y. Shi: Structural basis of Smad2 recognition by the Smad anchor for receptor activation. *Science* 287, 92-97 (2000)

58. Janknecht, R., N. J. Wells and T. Hunter: TGF-beta-stimulated cooperation of smad proteins with the coactivators CBP/p300. *Genes Dev* 12, 2114-2119 (1998)

59. Nishihara, A., J. I. Hanai, N. Okamoto, J. Yanagisawa, S. Kato, K. Miyazono and M. Kawabata: Role of p300, a transcriptional coactivator, in signalling of TGF-beta. *Genes Cells* 3, 613-623 (1998)

60. Lee, P. S., C. Chang, D. Liu and R. Derynck: Sumoylation of Smad4, the common Smad mediator of transforming growth factor-beta family signaling. *J Biol Chem* 278, 27853-27863 (2003)

61. Lin, X., M. Liang, Y. Y. Liang, F. C. Brunicardi and X. H. Feng: SUMO-1/Ubc9 promotes nuclear accumulation and metabolic stability of tumor suppressor Smad4. *J Biol Chem* 278, 31043-31048 (2003)

62. Lin, X., M. Liang, Y. Y. Liang, F. C. Brunicardi, F. Melchior and X. H. Feng: Activation of transforming growth factor-beta signaling by SUMO-1 modification of tumor suppressor Smad4/DPC4. *J Biol Chem* 278, 18714-18719 (2003)

63. Long, J., G. Wang, D. He and F. Liu: Repression of Smad4 transcriptional activity by SUMO modification. *Biochem J* 379, 23-29 (2004)

64. Candia, A. F., T. Watabe, S. H. Hawley, D. Onichtchouk, Y. Zhang, R. Derynck, C. Niehrs and K. W. Cho: Cellular interpretation of multiple TGF-beta signals: intracellular antagonism between activin/BVg1 and BMP-2/4 signaling mediated by Smads. *Development* 124, 4467-4480 (1997)

65. Ulloa, L. and S. Tabibzadeh: Lefty inhibits receptor-regulated Smad phosphorylation induced by the activated transforming growth factor-beta receptor. *J Biol Chem* 276, 21397-21404 (2001)

66. Moustakas, A., S. Souchelnytskyi and C. H. Heldin: Smad regulation in TGF-beta signal transduction. *J Cell Sci* 114(Pt 24), 4359-4369 (2001)

67. Shi, Y., Y. F. Wang, L. Jayaraman, H. Yang, J. Massague and N. P. Pavletich: Crystal structure of a Smad MH1 domain bound to DNA: insights on DNA binding in TGF-beta signaling. *Cell* 94, 585-594 (1998)

68. Shi, X., S. Bai, L. Li and X. Cao: Hoxa-9 represses transforming growth factor-beta-induced osteopontin gene transcription. *J Biol Chem* 276, 850-855 (2001)

69. Thiagalingam, S., C. Lengauer, F. S. Leach, M. Schutte, S. A. Hahn, J. Overhauser, J. K. Willson, S. Markowitz, S. R. Hamilton, S. E. Kern, K. W. Kinzler and B. Vogelstein:

Evaluation of candidate tumour suppressor genes on chromosome 18 in colorectal cancers. *Nat Genet* 13, 343-346 (1996)

70. Shi, Y. and J. J. Massague: Mechanisms of TGF-beta signaling from cell membrane to the nucleus. *Cell* 113, 685-700 (2003)

71. MiyazonoJ K., H. Suzuki and T. Imamura: Regulation of TGF-beta signaling and its roles in progression of tumors. *Cancer Sci* 94, 230-234 (2003)

72. Imamura, T., M. Takase, A. Nishihara, E. Oeda, J. Hanai, M. Kawabata and K. Miyazono: Smad6 inhibits signalling by the TGF-beta superfamily. *Nature* 389, 622-626 (1997)

73. Miyazono, K., P. ten Dijke and C. H. Heldin: TGF-beta signaling by Smad proteins. *Adv Immunol* 75, 115-157 (2000)

74. Murakami, G., T. Watabe, K. Takaoka, K. Miyazono and T. Imamura: Cooperative inhibition of bone morphogenetic protein signaling by Smurf1 and inhibitory Smads. *Mol Biol Cell* 14, 2809-2817 (2003)

75. Izzi, L. and L. Attisano: Regulation of the TGF-beta signalling pathway by ubiquitin-mediated degradation. *Oncogene* 23, 2071-2078 (2004)

76. Bai, S., X. Shi, X. Yang and X. Cao: Smad6 as a transcriptional corepressor. *J Biol Chem* 275, 8267-8270 (2000)

77. Itoh, S., F. Itoh, M. Goumans and P. ten Dijke: Signaling of transforming growth factor-beta family members through Smad proteins. *Eur J Biochem* 267, 6954-6967 (2000)

78. Bai, S. and X. Cao: A nuclear antagonistic mechanism of inhibitory Smads in transforming growth factor-beta signaling. *J Biol Chem* 277, 4176-4182 (2002)

79. Nomura, N., S. Sasamoto, S. Ishii, T. Date, M. Matsui and R. Ishizaki: Isolation of human cDNA clones of ski and the ski-related gene, sno. *Nucleic Acids Res* 17, 5489-5500 (1989)

80. Heyman, H. C. and E. Stavnezer: A carboxyl-terminal region of the ski oncoprotein mediates homodimerization as well as heterodimerization with the related protein SnoN. *J Biol Chem* 269, 26996-267003 (1994)

81. He, J., S. B. Tegen, A. R. Krawitz, G. S. Martin and K. Luo: The transforming activity of Ski and SnoN is dependent on their ability to repress the activity of Smad proteins. *J Biol Chem* 278, 30540-3057 (2003)

82. Xu, W., K. Angelis, D. Danielpour, M. M. Haddad, O. Bischof, J. Campisi, E. Stavnezer and E. E. Medrano: Ski acts as a co-repressor with Smad2 and Smad3 to regulate the response to type-beta transforming growth factor. *Proc Natl Acad Sci USA* 97, 5924-5929 (2000)

83. Liu, X., Y. Sun, R. A. Weinberg and H. F. Lodish: Ski/Sno and TGF-beta signaling. *Cytokine Growth Factor Rev* 12, 1-8 (2001)

84. Fumagalli, S., L. Doneda, N. Nomura and L. Larizza: Expression of the c-ski proto-oncogene in human melanoma cell lines. *Melanoma Res* 3, 23-27 (1993)

85. Zhang, F., M. Lundin, A. Ristimaki, P. Heikkila, J. Lundin, J. Isola, H. Joensuu and M. Laiho: Ski-related novel protein N (SnoN), a negative controller of transforming growth factor-beta signaling, is a prognostic marker in estrogen receptor-positive breast carcinomas. *Cancer Res* 63, 5005-5010 (2003)

86. Buess, M., L. Terracciano, J. Reuter, P. Ballabeni, J. L. Boulay, U. Laffer, U. Metzger, R. Herrmann and C. Rochlitz: Amplification of SKI is a prognostic marker in early colorectal cancer. *Neoplasia* 6, 207-212 (2004)

87. Fukuchi, M., M. Nakajima, Y. Fukai, T. Miyazaki, N. Masuda, M. Sohda, R. Manda, K. Tsukada, H. Kato and H. Kuwano: Increased expression of c-Ski as a co-repressor in transforming growth factor-beta signaling correlates with progression of esophageal squamous cell carcinoma. *Int J Cancer* 108, 818-824 (2004)

88. Boyer, P. L., C. Colmenares, E. Stavnezer and S. H. Hughes: Sequence and biological activity of chicken SnoN cDNA clones. *Oncogene* 8, 457-466 (1993)

89. Wang, W., F. V. Mariani, R. M. Harland and K. Luo: Ski represses bone morphogenic protein signaling in Xenopus and mammalian cells. *Proc Natl Acad Sci USA* 97, 14394-14399 (2000)

90. Frederick, J. P. and X. F. Wang: Smads "freeze" when they ski. *Structure (Camb)* 10, 1607-1611 (2002)

91. Xu, L., Y. Kang, S. Col and J. Massague: Smad2 nucleocytoplasmic shuttling by nucleoporins CAN/Nup214 and Nup153 feeds TGF-beta signaling complexes in the cytoplasm and nucleus. *Mol Cell* 10, 271-282 (2002)

92. Wu, J. W., A. R. Krawitz, J. Chai, W. Li, F. Zhang, K. Luo and Y. Shi: Structural mechanism of Smad4 recognition by the nuclear oncoprotein Ski: insights on Ski-mediated repression of TGF-beta signaling. *Cell* 111, 357-367 (2002)

93. Izzi, L. and L. Attisano L: Regulation of the TGF-beta signalling pathway by ubiquitin-mediated degradation. *Oncogene* 23, 2071-2078 (2004)

94. Komuro, A., T. Imamura, M. Saitoh, Y. Yoshida, T. Yamori, K. Miyazono and K. Miyazawa: Negative regulation of transforming growth factor-beta (TGF-beta) signaling by WW domain-containing protein 1 (WWP1). *Oncogene* 23, 6914-6923 (2004)

95. Zhang, F. and M. Laiho: On and off: proteasome and TGF-beta signaling. *Exp Cell Res* 291, 275-281 (2003)

96. Fukuchi, M., T. Imamura, T. Chiba, T. Ebisawa, M. Kawabata, K. Tanaka and K. Miyazono: Ligand-dependent degradation of Smad3 by a ubiquitin ligase complex of ROC1 and associated proteins. *Mol Biol Cell* 12, 1431-1443 (2001)

97. Gruendler, C., Y. Lin, J. Farley and T. Wang: Proteasomal degradation of Smad1 induced by bone morphogenetic proteins. *J Biol Chem* 276, 46533-46543 (2001)

98. Lin, Y., J. Martin, C. Gruendler, J. Farley, X. Meng, B. Y. Li, R. Lechleider, C. Huff, R. H. Kim, W.A. Grasser, V. Paralkar, T. Wang. A novel link between the proteasome pathway and the signal transduction pathway of the bone morphogenetic proteins (BMPs). *BMC Cell Biol* 3, 1-21 (2002)

99. Wan, M., X. Cao, Y. Wu, S. Bai, L. Wu, X. Shi, N. Wang and X. Cao: Jab1 antagonizes TGF-beta signaling by inducing Smad4 degradation. *EMBO Rep* 3, 171-176 (2002)

100. Kavsak, P., R. K. Rasmussen, C. G. Causing, S. Bonni, H. Zhu, G. H. Thomsen and J. L. Wrana: Smad7 binds to Smurf2 to form an E3 ubiquitin ligase that targets the TGF beta receptor for degradation. *Mol Cell* 6, 1365-1375 (2000)

101. Suzuki, C., G. Murakami, M. Fukuchi, T. Shimanuki, Y. Shikauchi, T. Imamura and K. Miyazono: Smurf1 regulates the inhibitory activity of Smad7 by targeting Smad7 to the plasma membrane. *J Biol Chem* 277, 39919-39925 (2002)

102. Ebisawa, T., M. Fukuchi, G. Murakami, T. Chiba, K. Tanaka, T. Imamura and K. Miyazono: Smurf1 interacts with transforming growth factor-beta type I receptor through Smad7 and induces receptor degradation. *J Biol Chem* 276, 12477-12480 (2001)

103. Lo, R. S. and J. Massague: Ubiquitin-dependent degradation of TGF-beta activated Smad2. *Nat Cell Biol* 1, 472-478 (1999)

104. Wenner, C. E. and S. Yan: Biphasic role of TGF-beta1 in signal transduction and crosstalk. *J Cell Physiol* 196, 42-50 (2003)

105. Wisdom, R: AP-1: one switch for many signals. *Exp Cell Res* 253, 180-185 (1999)

106. Hocevar, B. A. and P. H. Howe: Regulation of AP-1 activity by TGF-beta. *Methods Mol Biol* 142, 97-108 (2000)

107. Terada, Y., O. Nakashima, S. Inoshita, M. Kuwahara, S. Sasaki and F. Marumo: Mitogen-activated protein kinase cascade and transcription factors: the opposite role of MKK3/6-p38K and MKK1-MAPK. *Nephrol Dial Transplant* 14, Suppl 1, 45-47 (1999)

108. Yue, J., B. Sun, G. Liu and K. M. Mulder: Requirement of TGF-beta receptor-dependent activation of c-Jun N-terminal kinases (JNKs)/stress-activated protein kinases (Sapks) for TGF-beta up-regulation of the urokinase-type plasminogen activator receptor. *J Cell Physiol* 199, 284-292 (2004)

109. Sano, Y., J. Harada, S. Tashiro, R. Gotoh-Mandeville, T. Maekawa and S. Ishii: ATF-2 is a common nuclear target of Smad and TAK1 pathways in transforming growth factor-beta signaling. *J Biol Chem* 274, 8949-8957 (1999)

110. Knaus, P. I., D. Lindemann, J. F. DeCoteau, R. Perlman, H. Yankelev, M. Hille, M. E. Kadin and H. F. Lodish: A dominant inhibitory mutant of the type II transforming growth factor-beta receptor in the malignant progression of a cutaneous T-cell lymphoma. *Mol Cell Biol* 16, 3480-3489 (1996)

111. Kim, S. J., Y. H. Im, S. D. Markowitz and Y. J. Bang: Molecular mechanisms of inactivation of TGF-beta receptors during carcinogenesis. *Cytokine Growth Factor Rev* 11, 159-168 (2000)

112. Janji, B., C. Melchior, V. Gouon, L. Vallar and N. Kieffer: Autocrine TGF-beta-regulated expression of adhesion receptors and integrin-linked kinase in HT-144 melanoma cells correlates with their metastatic phenotype. *Int J Cancer* 83, 255-262 (1999)

113. Oft, M., K. H. Heider and H. Beug: TGF-beta signaling is necessary for carcinoma cell invasiveness and metastasis. *Curr Biol* 8, 1243-1252 (1998)

114. Tang, B., E. P. Bottinger, S. B. Jakowlew, K. M. Bagnall, J. Mariano, M. R. Anver, J. J. Letterio and L. M. Wakefield: Transforming growth factor-beta1 is a new form of tumor suppressor with true haploid insufficiency. *Nat Med* 4, 802-807 (1998)

115. Huang, F., E. Newman, D. Theodorescu, R. S. Kerbel and E. Friedman: Transforming growth factor-beta1 (TGF-beta1) is an autocrine positive regulator of colon carcinoma U9 cells *in vivo* as shown by transfection of a TGF-beta 1 antisense expression plasmid. *Cell Growth Differ* 6, 1635-1642 (1995)

116. Chen, T., J. Triplett, B. Dehner, B. Hurst, B. Colligan, J. Pemberton, J. R. Graff and J. H. Carter: Transforming growth factor-beta receptor type I gene is frequently mutated in ovarian carcinomas. *Cancer Res* 61, 4679-4682 (2001)

117. Baxter, S. W., D. Y. Choong, D. M. Eccles and I. G. Campbell: Transforming growth factor-beta receptor 1 polyalanine polymorphism and exon 5 mutation analysis in breast and ovarian cancer. *Cancer Epidemiol Biomarkers Prev* 11, 211-214 (2002)

118. Chen, T., W. Yan, R. G. Wells, D. L. Rimm, J. McNiff, D. Leffell and M. Reiss: Novel inactivating mutations of transforming growth factor-beta type I receptor gene in head-and-neck cancer metastases. *Int J Cancer* 93, 653-661 (2001)

119. Schiemann, W. P., W. M. Pfeifer, E. Levi, M. E. Kadin and H. F. Lodish: A deletion in the gene for transforming growth factor-beta type I receptor abolishes growth regulation by transforming growth factor-beta in a cutaneous T-cell lymphoma. *Blood* 94, 2854-2861 (1999)

120. Chen, T., D. Carter, L. Garrigue-Antar and M. Reiss: Transforming growth factor-beta type I receptor kinase mutant associated with metastatic breast cancer. *Cancer Res* 58, 4805-4810 (1998)

121. Schwirzke, M., S. Schiemann, A. U. Gnirke and U. H. Weidle: New genes potentially involved in breast cancer metastasis. *Anticancer Res* 19, 3A, 1801-1814 (1999)

122. Markowitz, S: TGF-beta receptors and DNA repair genes, coupled targets in a pathway of human colon carcinogenesis. *Biochim Biophys Acta* 1470, M13-20 (2000)

123. O'Mahony, C. A., D. Albo, G. P. Tuszynski and D. H. Berger: Transforming growth factor-beta1 inhibits generation of angiostatin by human pancreatic cancer cells. *Surgery* 124, 388-393 (1998)

124. Massague, J., S. W. Blain and R. S. Lo: TGF-beta signaling in growth control, cancer, and heritable disorders. *Cell* 103, 295-309 (2000)

125. Siegel, P. M. and J. Massague: Cytostatic and apoptotic actions of TGF-beta in homeostasis and cancer. *Nat Rev Cancer* 3, 807-821 (2003)

126. Wanzel M., S. Herold and M. Eilers: Transcriptional repression by Myc. *Trends Cell Biol* 13, 146-150 (2003)

127. Tachibana, .I, M. Imoto, P. N. Adjei, G. J. Gores, M. Subramaniam, T. C. Spelsberg and R. Urrutia: Overexpression of the TGF-beta-regulated zinc finger encoding gene, TIEG, induces apoptosis in pancreatic epithelial cells. *J Clin Invest* 99, 2365-2374 (1997)

128. Perlman, R., W. P. Schiemann, M. W. Brooks, H. F. Lodish and R. A. Weinberg: TGF-beta-induced apoptosis is mediated by the adapter protein Daxx that facilitates JNK activation. *Nat Cell Biol* 3, 708-714 (2001), (Erratum in): *Nat Cell Biol* 4, 179 (2002)

129. Atfi, A., M. Buisine, A. Mazars and C. Gespach: Induction of apoptosis by DPC4, a transcriptional factor regulated by transforming growth factor-beta through stress-activated protein kinase/c-Jun N-terminal kinase (SAPK/JNK) signaling pathway. *J Biol Chem* 272, 24731-24734 (1997)

130. Landstrom, M., N. E. Heldin, S. Bu, A. Hermansson, S. Itoh, P. ten Dijke and C. H. Heldin: Smad7 mediates apoptosis induced by transforming growth factor-beta in prostatic carcinoma cells. *Curr Biol* 10, 535-538 (2000)

131. Patil, S., G. M. Wildey, T. L. Brown, L. Choy, R. Derynck and P. H. Howe: Smad7 is induced by CD40 and protects WEHI 231 B-lymphocytes from transforming growth factor-beta -induced growth inhibition and apoptosis. *J Biol Chem* 275, 38363-38370 (2000)

Abbreviations: ALK, activin receptor-like kinase; BMP, bone morphogenetic protein; GDF, Growth and Differentiation factor; MH, Mad homology; MIF1, migration inhibitory factor 1; MIS, Mullerian Inhibiting Substance; MMP, matrix metalloproteinase; SARA, Smad anchor for receptor activation; Smad, human homologue of Sma genes from *Caenorhabditis elegans* and Mad (Mothers against decapentaplegic) gene from Drosophila; Smurf, Smad ubiquitin regulatory factors; TAK, TGF-beta activated kinase; TGF-beta, transforming growth factor-beta; T-betaR, transforming growth factor-beta receptor; TIMP, tissue matrix metalloproteinase inhibitor.

Key Words: Apoptosis, Ligands, Receptors, Ski, I-Smads, Smads, Transforming growth factor beta, Tumor suppressor

Send correspondence to: Dr Satya Narayan, UF Shands Cancer Center, College of Medicine, Academic Research Building, R4-216, PO Box 100232, University of Florida, Gainesville, FL 32610-0232, USA, Tel: 352-846-1148, Fax: 352-392-5802, E-mail: snarayan@ufscc.ufl.edu

http://www.bioscience.org/current/vol10.htm