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Roles of Smad3 in TGF-β Signaling During Carcinogenesis

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ABSTRACT: Signaling of transforming growth factor β (TGF-β) is mediated through a heteromeric complex of two types of transmembrane receptors and downstream intracellular proteins known as Smads. Alterations of TGF-β signaling underlie various forms of human cancer and developmental diseases. Human genetic studies have revealed both point mutations and deletions of *Smad2* or *Smad4* in several types of cancers. However, the role of Smad3 in tumorigenesis is not clear. Recent data indicate that Smad3 also functions as a tumor suppressor by inhibiting cell proliferation and promoting apoptosis. In addition, Smad3 is essential for TGF-β–mediated immune suppression, and it plays an important role in regulating transcriptional responses that are favorable to metastasis. Therefore, through regulating different transcriptional responses, Smad3 functions as both a negative and positive regulator of carcinogenesis depending on cell type and clinical stage of the tumor.

KEY WORDS: tumor suppressor, growth inhibition, apoptosis, metastasis, immune suppression, transcription

I. INTRODUCTION

Transforming growth factor-β (TGF-β) is the prototype of a large family of secreted polypeptide growth factors that regulate a multitude of cellular processes affecting proliferation, differentiation, and apoptosis.[1] In addition, TGF-β exerts a multifaceted influence on cancer pathogenesis. Among the six essential alterations of cell physiology that are required for malignant transformation,[2] TGF-β is involved in the insensitivity to antigrowth signals, evasion of apoptosis, induction of tumor angiogenesis, and tendency to promote tissue invasion and metastasis. A large number of genetic or epigenetic changes affecting various components of the TGF-β pathway have been reported in a spectrum of human hyperproliferative disorders and various forms of cancers.[1,3] Although it is a potent growth inhibitor in cultured cells, TGF-β is actually abundantly expressed in most human tumors, and high levels of TGF-β often bode poor prognosis.[4,5] This dichotomy complicated early attempts at direct use of TGF-β in cancer treatment and stressed a need for a thorough understanding of its signaling mechanism. Following the identification of membrane-bound TGF-β receptors, a group of transcription factors known as Smads was discovered in the late 1990s to mediate transcriptional responses of TGF-β and its related factors.[6,7] Among these, Smad2 and Smad3 are accredited with mediating TGF-β responses. Because of their close relatedness, these two proteins exhibit interchangeable functions in many signaling systems.[8,9] In this review, we discuss recent advances in the understanding of how TGF-β suppresses tumor formation in some instances but promotes tumor growth in others. We will focus on roles and mechanisms of Smad3.

II. MECHANISM OF TGF-β SIGNALING THROUGH SMAD3

It is now generally accepted that the plethora of biologic activities of TGF-β is universally initiated by the binding of the ligand to a heteromeric

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complex of two types of transmembrane receptors: TGF-β receptor type I (TGFBR1) and TGFBR2, each equipped with an intrinsic serine/threonine kinase activity.[7] Ligand occupancy causes an association between TGFBR2 and TGFBR1, which results in phosphorylation of TGFBR1 by the constitutively active TGFBR2. The phosphorylated TGFBR1 then triggers activation of Smad3 by phosphorylation at the C-terminal serine residues,[6,7] forcing Smad3 to dissociate from the membrane-bound receptors and translocate into the nucleus. In the nucleus, it binds DNA at a preferential sequence of GTCT or the reverse complement AGAC, called Smad-binding element (SBE).[10,11] However, the affinity of Smad3 for SBE does not support one on one binding *in vivo*; instead, Smad3 relies on cooperative binding with other transcription factors, including a common Smad4 that is essential for many Smad-mediated transcriptional responses. The Smad transcriptional complexes have the ability to either activate or repress transcription of a selected set of target genes depending on the nature of associated cofactors and the status of local chromatin structure in the context of signal-receiving cells. Now it is clear that this Smad-mediated signaling pathway is subject to control or function in conjunction with Smad-independent mechanisms, such as those governed by mitogen-activated protein kinases (MAPKs) or rho-like GTPases.[12,13] The Smad-independent, noncanonic receptor-signaling conduits can provoke cellular response to ligands on their own right or modulate Smad activity to custom fit signaling output to a particular need, further lending complexity to the control of TGF-β signaling.

II. SMAD3 PLAYS A MAJOR ROLE IN TGF-β–MEDIATED GROWTH INHIBITION

TGF-β is a potent growth inhibitor of cells of epithelial, neuronal, and hematopoietic origins, causing them to arrest at the G_1 phase of the cell cycle.[3,14] Key to this inhibition are the downregulation of protooncogene *c-Myc*[15,16] and the induction of $p15^{Ink4b}$ and/or $p21^{Cip1}$,[17–19] which are inhibitors of G_1-phase cyclin-dependent kinases (CDKs). TGF-β has long been recognized for an ability to rapidly downregulate *c-Myc* in various cell types,[15] but it was not understood until recently that this downregulation occurs at the level of transcription through a *cis*-acting TGF-β inhibitory element (TIE) in the *c-Myc* promoter.[20] TIE is composed of a repressive SBE (RSBE) and an overlapping E2F site, which are recognized by a tripartite complex involving Smad3/4, E2F4/5, and the transcriptional repressor p107.[20,21] There is evidence to suggest that this multimeric Smad3 complex is preformed in the cytoplasm, and upon TGF-β induction, traverses into the nucleus to engage in the repression of *c-Myc* transcription (Fig. 1).[21] RSBE is distinct from the more common SBE in both sequence and function,[20] and loss of recognition of RSBE by the Smads–E2F4/5–p107 complex underlies the resistance of breast cancer cells to TGF-β–mediated growth inhibition.[21]

A complex scenario is at play in the induction of CDK inhibitors triggered by TGF-β. Initial studies suggested that Smad3 and Smad2 activate the transcription of $p15^{Ink4b}$ in concert with Sp1 at a compound SBE–Sp1 site in the proximal promoter region[22] and attributed the inhibition of $p15^{Ink4b}$ transcription by c-Myc to the interference brought in by the interaction between c-Myc and the MAD homology 2 domain of Smad3 in the Smad/Sp1 complex.[23] Although later studies confirmed the contribution of Sp1, the TGF-β inducibility conferred by this proximal promoter region was nevertheless accredited to the sequences surrounding the transcription initiation site, which is recognized by the Myc-interacting zinc-finger protein Miz-1.[24] Recruiting c-Myc by Miz-1 to the $p15^{Ink4b}$ promoter interferes with activation of the $p15^{Ink4b}$ gene by TGF-β.[24,25] A similar mechanism also applies to the induction of $p21^{Cip1}$ by TGF-β.[26–29] Downregulation of *c-Myc* is prerequisite for induction of $p15^{Ink4b}$ and $p21^{Cip1}$ but is not sufficient.[30,31] A transactivation complex containing Smad3, Smad4, and the forkhead box O (FOXO) family of transcription factors is also required.[32,33] Smads and FOXO bind cooperatively to a distal promoter region in both $p15^{Ink4b}$ and $p21^{Cip1}$ promoters containing multiple copies of SBE that are flanked by the forkhead-binding element. Thus, formation of the Smad/FOXO complex in response to TGF-β stimulation delivers the final activation signal for induction of transcription of $p15^{Ink4b}$ and $p21^{Cip1}$ genes. Because FOXO proteins are targets of the phosphoinosi-

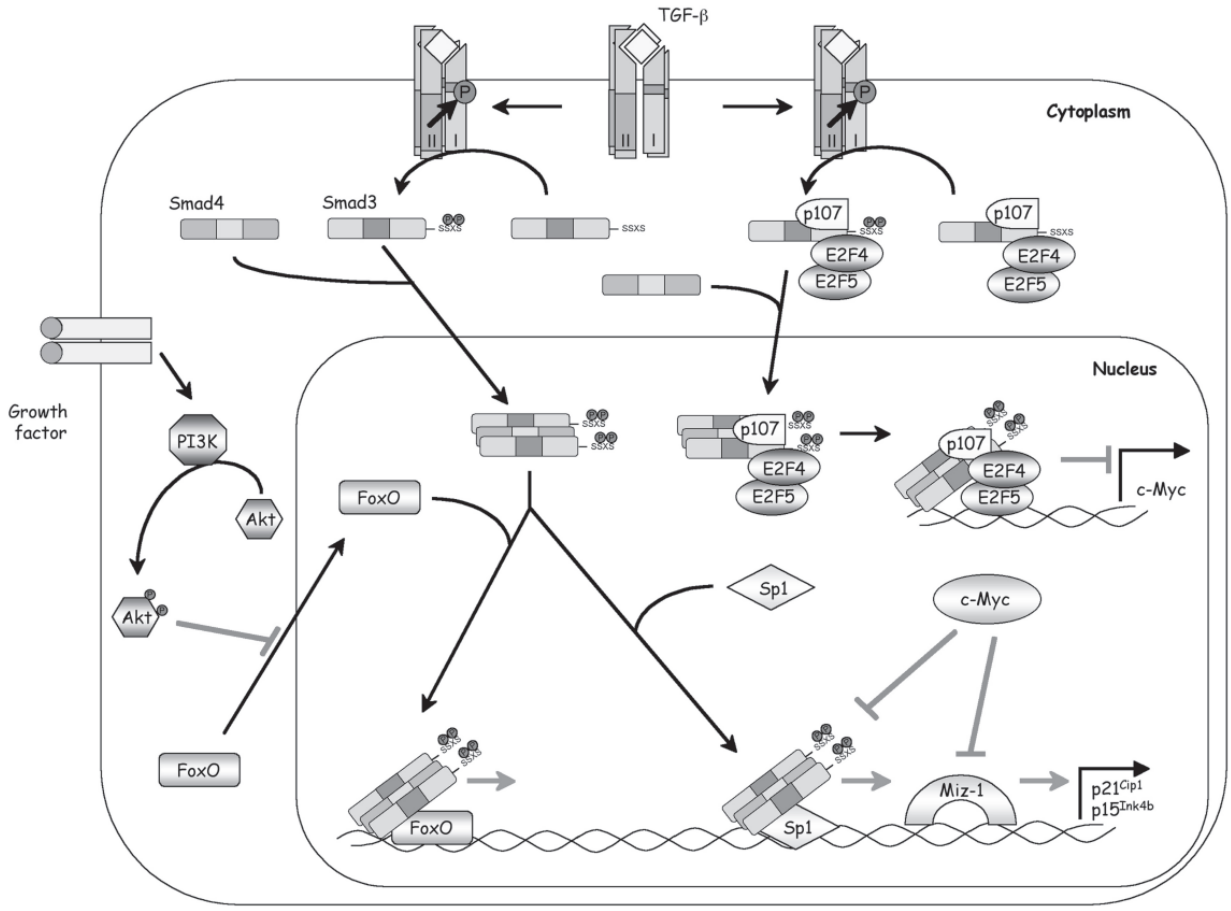

FIGURE 1. Mechanisms of Smad3 in TGF-β–induced antiproliferative transcriptional responses. In repressing the protooncogene *c-Myc*, a preexisting cytoplasmic complex containing Smad3, E2F4/5, and p107 likely begins to move into the nucleus in response to ligand stimulation and binds DNA at a RSBE/TIE site in the *c-Myc* promoter. Smad4 is recruited to this complex as well. In the induction of CDK inhibitors $p15^{Ink4b}$ and $p21^{Cip1}$, a different Smad3/4 complex cooperates with FOXO at a distal promoter element to activate transcription. The PI3K/Akt pathway has the ability to antagonize TGF-β/Smad3 signaling by inhibiting FOXO nuclear localization. The Smad3/4 complex also stimulates transcription in conjunction with Sp1 at a proximal promoter element. Finally, transcription of $p15^{Ink4b}$ and $p21^{Cip1}$ is directly repressed by c-Myc through its association with Miz-1 and/or Smad2/3 at the initiator site. Thus, attenuating c-Myc expression is a prerequisite for induction of $p15^{Ink4b}$ and $p21^{Cip1}$.

tide 3 kinase (PI3K)/Akt survival pathway, Akt can inhibit nuclear localization of FOXO proteins by phosphorylating them and thus barring them from target genes. This finding offered a mechanistic account for the antagonizing interaction between the TGF-β pathway and the PI3K/Akt pathway (Fig. 1).

Consistent with the essential role of Smad3 in the above transcriptional responses, depletion of Smad3 but not Smad2 by RNA interference sufficiently blocked TGF-β–mediated cell cycle arrest and growth inhibition.[34] Similar results were also obtained using primary T cells or hepatocytes isolated from Smad3-deficient mice.[35,36]

III. SMAD3 MUTATIONS IN HUMAN CANCER

The role of TGF-β signaling as a tumor suppressor pathway is best illustrated by the presence of mutations in genes encoding components of the TGF-β signaling pathway.[3,14] Mutations in TGFBR2 are frequently found in colon can-

cer,[37–39] gastric cancer,[39–42] glioma,[43] and non–small cell lung carcinoma,[44] whereas mutations in Smad4 have been found in more than 50% of pancreatic cancers[45] and in a subgroup of patients with juvenile polyposis syndromes.[46] Although less common, mutations in TGFBR1 have been observed in ovarian cancer,[47,48] metastatic breast cancer,[49] pancreatic cancer,[50] and T-cell lymphoma,[51] whereas mutations in Smad2 have been detected in a small portion of colorectal and lung cancers.[52–54] However, it is only most recently that a heterozygous missense mutation (R373H) in Smad3 was reported in colorectal cancer cell line SNU-769A, originated from a metastatic site in a lymph node.[55] This mutation is localized to the C-terminal domain of Smad3 that is involved in the interaction with TGFBR1, formation of Smad homomeric or heteromeric complex, and transcriptional activation. Although this mutation could functionally disable the Smad3 protein, its role in tumorigenesis is not clear because the same mutation is not present in the SNU-769B cell line, originated from a primary tumor site in the same patient. Moreover, a homozygous mutation in the *TGFBR2* gene is already present in both SNU-769A and SNU-769B cell lines.[55]

Despite the lack of inactivating mutations in human cancers, direct evidence supporting a tumor-suppressing role of Smad3 comes from the observation of the absence of Smad3 protein in T-cell acute lymphoblastic leukemia (ALL).[35] Interestingly, the loss of Smad3 protein in T-cell ALL is not due to mutations in the *Smad3* gene or alterations in the level of *Smad3* mRNA expression. Nevertheless, these results suggest that the level of Smad3 protein can be an important determinant in the suppression of tumorigenesis.

IV. TUMOR DEVELOPMENT IN SMAD3-DEFICIENT MOUSE MODELS

Smad3-deficient mice have been generated in three different laboratories with different strategies.[56–58] One of these lines, Smad3$^{exo2/exo2}$, was originally reported to develop colon carcinomas, including adenocarcinomas and metastatic carcinomas.[58] However, this phenotype was not seen in two other independently generated lines despite all of the lines being reported to have impaired TGF-β signaling.[56,57] This discrepancy could be due to differences in genetic background and/or in environmental factors associated with animal holding. For example, on the 129/Sv background, 100% of Smad3$^{exo2/exo2}$ mice had tumors by age 6 months,[58] whereas on the hybrid background of 129/Sv and C57BL/6, a less aggressive tumor phenotype was observed: tumor onset was delayed to 10 months with only 30% of mice developing tumors.[58] Moreover, a follow-up study indicated that, when maintained in a *Helicobacter*-free environment for up to 9 months, previously reported colon cancer phenotypes were not developed in Smad3$^{exo2/exo2}$ mice on the 129/Sv background; infection of the same mice with *Helicobacter* triggered colon cancer in 50% to 66% of the animals.[59] These results suggested that the original Smad3$^{exo2/exo2}$ mice may have been infected with *Helicobacter* or other colitis-inducing organisms.[58] Bacterial infection, including *Helicobacter* infection, can induce chronic inflammation, during which reactive oxygen species may arise,[59] ultimately resulting in DNA damage, genomic instability, and transformation.[60] Impairment of TGF-β–mediated immunosuppression has been documented in all three lines of Smad3-deficient mice,[56,57,59] which could contribute to increased inflammatory responses that predispose the animals to cancer formation.

Interestingly, although infection with *Helicobacter* restored the colon cancer phenotype in Smad3-deficient mice, it alone had no effect on another rodent intestinal cancer model, the Apc$^{min/+}$ mouse.[59] In contrast, Smad3 deficiency promotes tumorigenesis in the distal colon of Apc$^{min/+}$ mouse.[61] A mixture of adenomas and invasive carcinomas were observed in Apc$^{min/+}$ Smad3$^{exo2/exo2}$ mice at 2 months, exclusively in the distal colon, closely mimicking the familial adenomatous polyposis disease.[61] Transcriptional profiling revealed higher expression of several TGF-β activators in the normal distal mucosa than in the proximal mucosa, suggesting a stronger reliance on TGF-β–mediated growth control in the distal than in the proximal colon. Therefore, the absence of Smad3 could also predispose the colon epithelium toward neoplasia in the context of reduction of TGF-β–mediated antiproliferative signals.[61]

Furthermore, the studies using Smad3-deficient mice indicated that the loss of Smad3

alone is insufficient to initiate tumorigenesis.[59,61] Instead, a reduction in Smad3 increased the risk or tendency of tumorigenesis when associated with alterations in other factors that control cellular proliferation and apoptosis. This is best illustrated by the studies of Wolfraim et al.,[35] which showed that loss of one allele of Smad3 in mice impairs the inhibitory effect of TGF-β on the proliferation of normal T cells and works in tandem with the homozygous inactivation of $p27^{Kip1}$, a CDK inhibitor whose gene is frequently altered in human T-cell ALL, to promote T-cell ALL.

V. TUMOR-SUPPRESSING ROLE OF SMAD3 IN CHEMICALLY HEPATOCELLULAR CARCINOMA (HCC) MODELS

Perhaps more convincing evidence for a tumor-suppressing role of Smad3 stems from a recent study of chemically induced HCC in mice expressing *Smad3* transgenes specifically in the liver.[62] This study showed that enhancing Smad3 function through forced expression of wild-type or constitutively active Smad3 protected mouse livers from diethyl nitrosamine/phenobarbital-induced carcinogenic insult, whereas disrupting Smad3 function by dominant-negative mutant Smad3 aggravated the tumor load in the affected livers. It was also found that while the liver tumors lost response to the growth inhibition of TGF-β, they still succumbed to a high rate of apoptosis, particularly when the function of Smad3 was elevated. These results suggest that Smad3 is a tumor suppressor of mouse liver cancers and it does so by promoting TGF-β–mediated apoptosis.

Induction of apoptosis is a well-recognized mechanism of TGF-β to exert its tumor suppression function, but a detailed understanding of the chain of events from ligand occupying the receptor leading to the activation of apoptotic machinery is lacking. Several studies have indicated that overexpression of Smad3, but not Smad2, promotes TGF-β–induced apoptosis, whereas interfering with Smad3 function by dominant-negative mutants, RNA interference, or inactivation of the *Smad3* gene locus, protects against apoptosis,[36,62–64] suggesting that Smad3 is a physiologic mediator in this signaling process. One target of Smad3 in inducing apoptosis is *Bcl-2*, a key antiapoptotic inhibitor.[62] Upregulation of *Bcl-2* is one of the traits widely acquired by cancer cells to evade apoptosis,[65] and the significance of Bcl-2 as an anticancer target is reflected by the sheer number of clinical trials designed for various strategies of inactivating Bcl-2.[66,67] Smad3 can bind directly to a GC-rich element in the *Bcl-2* promoter, and the interaction between Smad3 and the *Bcl-2* promoter correlates with a repressed state of transcription of *Bcl-2 in vivo*.[62] Thus, by attenuating the level of the major apoptosis inhibitor Bcl-2, TGF-β/Smad3 signaling facilitates a permissive cellular context that is conducive to apoptosis. Besides Bcl-2, several other proteins, including death-associated protein kinase (DAPK),[68] growth arrest and DNA damage–inducible β (GADD45B),[69,70] and BH3-only protein Bim,[71] have also been identified as Smad3's targets in mediating the proapoptotic response of TGF-β (Fig. 2).

Interestingly, the mouse livers that ectopically expressed constitutively active mutant Smad3SD exhibited no phenotype even though it was clearly shown that Smad3SD was localized to the nucleus. This argues that C-terminal phosphorylation and nuclear translocation alone are probably not sufficient to render Smad3 fully active in inducing apoptosis.[62] The proapoptotic activity of Smad3 may require additional activation of other cytoplasmic kinase pathways *in vivo*. One of the candidates is p38 MAPK, which has been reported to phosphorylate Smad3 at the linker region.[72,73] More importantly, activation of p38 MAPK was shown to be essential to TGF-β–induced apoptosis in hepatocytes, mammary epithelial cells, and B cells.[62,74–77] Another kinase, Akt (protein kinase B), which can be activated in response to insulin and serum, has been shown to associate directly with Smad3. The interaction between Akt and Smad3 prevents Smad3 from being activated by TGF-β and translocating into the nucleus.[78,79] As such, Akt can decrease the activity of Smad3 and protect cells against TGF-β–induced apoptosis. In addition, like in the case of $p15^{Ink4b}$ and $p21^{Cip1}$ promoters, Akt could negatively regulate Smad3-dependent target genes that act in apoptotic response through FOXO (e.g., GADD45A and GADD45B).[33] A third protein, mTOR, which is a key mediator downstream from Akt, was also recognized to have an ability to

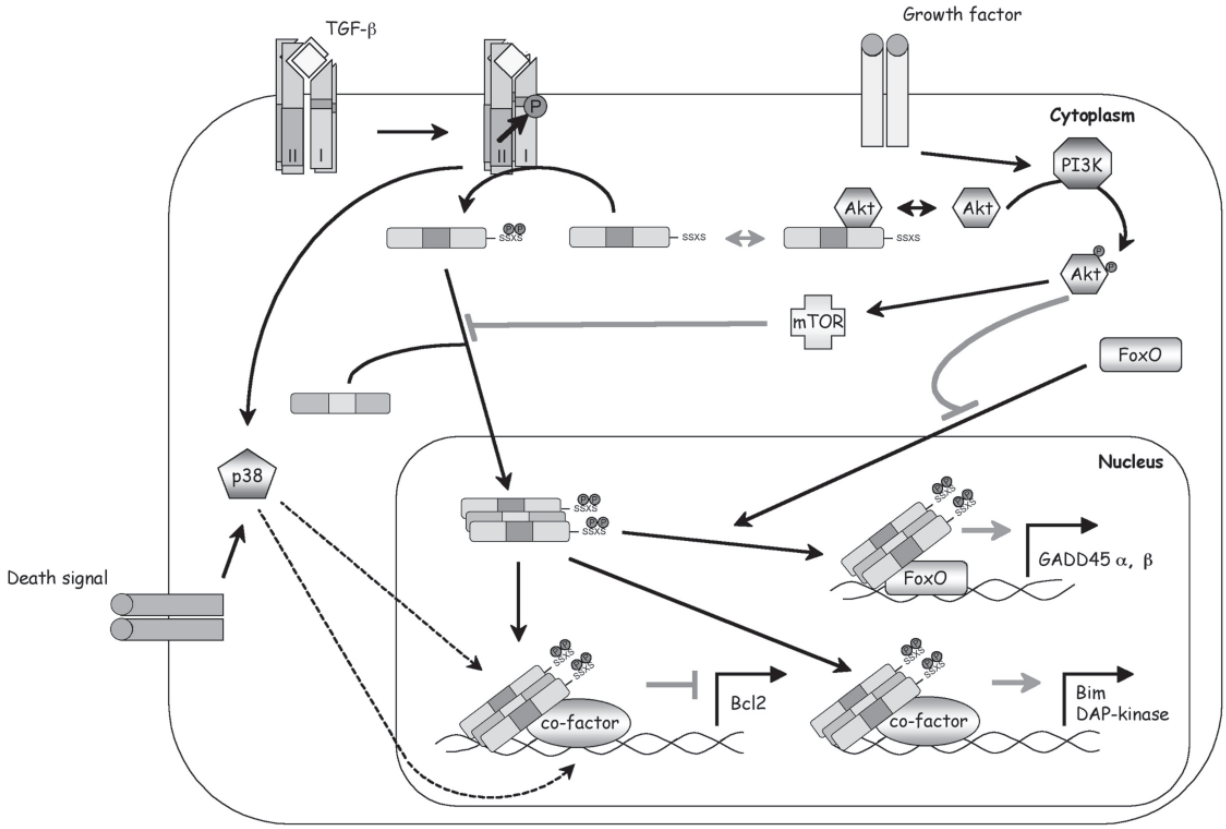

FIGURE 2. Mechanisms of Smad3 in TGF-β–mediated proapoptotic transcriptional responses. An activated Smad3/4 complex is required in the nucleus to activate the transcription of *GADD45* (along with *FOXO*) and *Bim* or DAPK and to repress *Bcl-2*. p38 MAPK is likely involved in the repression of *Bcl-2*, but the mechanism is not clear. Three mechanisms have been reported for the PI3K/Akt pathway to dampen the proapoptotic signaling of Smad3: Akt can sequester Smad3 in the cytoplasm by the binding of Smad3, activity of Smad3 can be inhibited by mTOR, or Akt may inhibit FOXO by sequestering it from participating in Smad3-mediated transcription of *GADD45*.

suppress Smad3 activation and inhibit the apoptosis induced by TGF-β.[80]

VI. ROLES OF SMAD3 IN PROMETASTATIC TRANSFORMATION

Although TGF-β has long been regarded as a tumor suppressor, it also promotes tumor progression during the late stages of cancer by suppressing immune surveillance, inducing epithelium to mesenchyme transition (EMT), and enhancing cell migration and transcription of factors favorable to metastasis (Fig. 3).[81–84] Again, Smad3 plays an indispensable role in these multifaceted pathway activities and thus can function as a tumor promoter in certain instances.

A. Smad3 is Essential for TGF-β–Mediated Immune Suppression

TGF-β–mediated immunosuppression may contribute to its tumor-suppressing role by inhibiting inflammatory responses upon bacterial infections that predispose animals to cancer formation as documented in Smad3-deficient mice[59]; it also stimulates tumor development and progression by escaping immune surveillance during the late stage of carcinogenesis.[85,86] It has been shown that secretion and expression of TGF-β by tumor cells downregulates major histocompatibility complex class II antigens and renders the tumor cell surface less immunogenic.[87,88] Moreover, TGF-β produced by a wide variety of noncancerous cells present at tumor sites also contributes to the local

suppression of immune function and the escape of tumor cells from immune surveillance. The inhibitory role of TGF-β–mediated immune suppression in tumor eradication has been demonstrated in various mouse tumor models. When challenged with TGF-β–producing tumors, mice that express a dominant-negative TGF-β receptor transgene in T cells were able to generate an immune response capable of eliminating tumors.[89] In another study, systemic neutralization of TGF-β activity in the EL4 thymoma cells by knocking down TGF-β expression through RNA interference or inhibiting TGF-β receptor activity restored T-cell cytotoxicity that was responsible for the antigen-specific tumor clearance *in vivo*.[84]

Analysis of Smad3-deficient mice revealed an essential role of Smad3 in mediating the immune-suppressing activity of TGF-β. This notion was supported by the finding that T cells in these mutant mice are constitutively activated and resistant to TGF-β–mediated growth inhibition.[56,57,59] Consistent with this observation, TGF-β–mediated inhibition in the production of several cytokines, such as interleukin 2 (IL-2), IL-13, IL-15, interferon γ (IFN-γ), and expression of CDK4 was lost in the Smad3-deficient primary T-cell cultures.[35,56,90,91] Furthermore, TGF-β has been shown to directly inhibit expression of five cytolytic gene products in cytotoxic T cells (CTLs): perforin, granzyme A, granzyme B, Fas ligand, and IFN-γ, which are collectively responsible for CTL-mediated tumor cytotoxicity.[84] In each of the above cases, Smad3 was shown to be crucial in mediating these TGF-β–mediated transcriptional responses.[84]

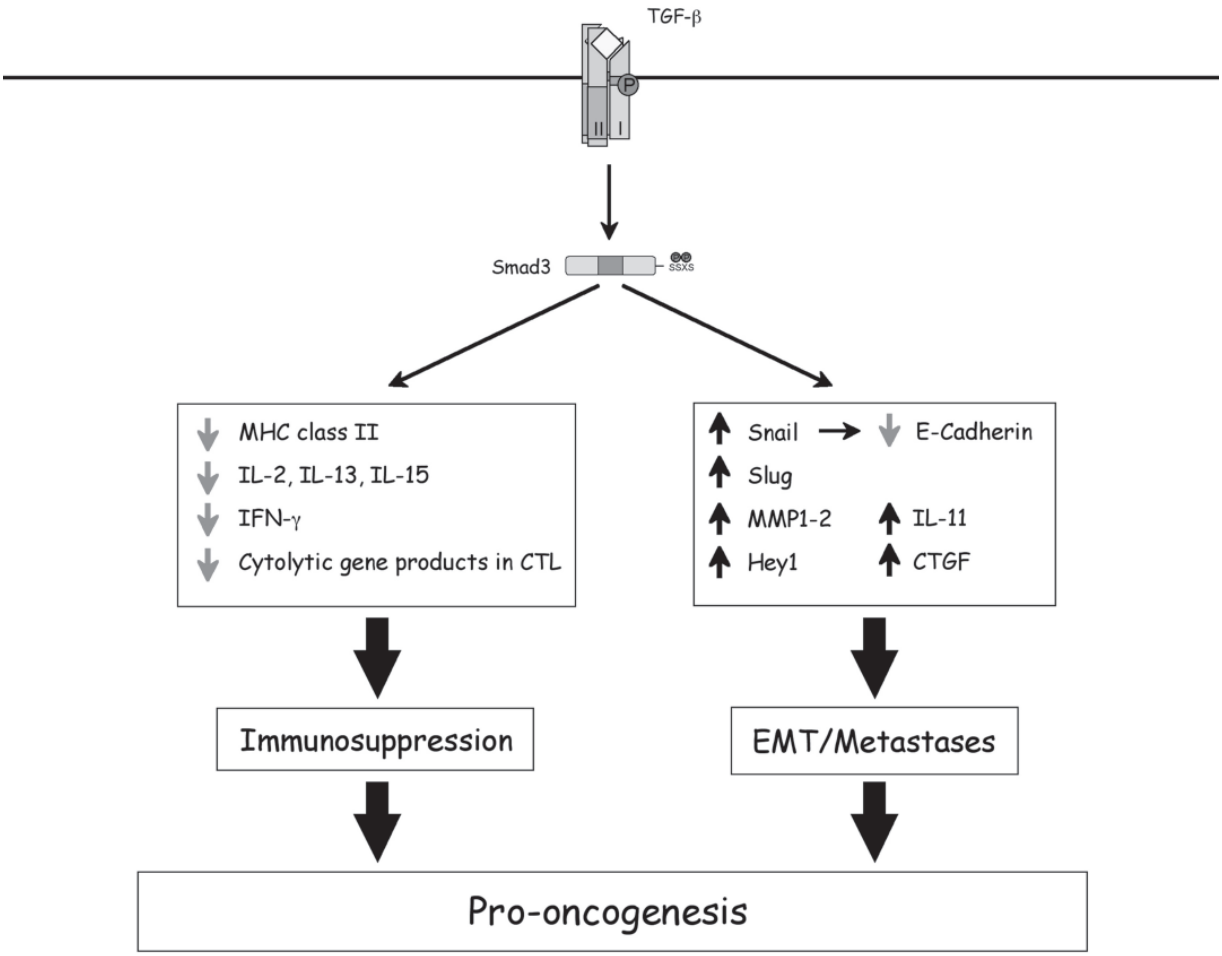

FIGURE 3. Mechanisms underlying prooncogenic activities of Smad3 during the late stages of carcinogenesis. Smad3 is essential for TGF-β–mediated immune suppression, and it regulates transcriptional responses that are favorable to EMT and metastasis. The induced genes are labeled with upward arrows and repressed ones with downward arrows.

B. Smad3 is Directly Involved In EMT

EMT is a process of delaminating mesenchymal cells from epithelium. It often associates with weaker cell–cell interactions and acquisition of motile and invasive properties of the cells that are prerequisite for progressing to advanced metastatic tumors.[92] TGF-β is a major player in EMT *in vivo*. The tumor-promoting role of TGF-β by inducing EMT at late stages of carcinogenesis is best exemplified in a study based on transgenic mice overexpressing TGF-β1 specifically in epidermis.[93,94] When subjected to a standard 7,12-dimethylbenz-α-anthracene–initiated and 12-O-tetradecanoylphorbol 13-acetate–promoted skin tumor induction protocol, these mice developed fewer benign tumors than controls, consistent with the potent growth inhibitory action of TGF-β. Nevertheless, despite fewer benign papillomas, they developed more carcinomas, and the carcinomas were more aggressive, with a noticeable increase in the incidence of overt fibroblastoid spindle tumors. Recent experiments with conditional transgenic mice have provided further support for the requirement of TGF-β activity in inducing tumor cell EMT *in vivo*.[94] Mice that overexpressed TGF-β1 in keratinocytes developed spindle carcinoma. In contrast, such tumor types were rare in mice that harbored an empty control vector, a dominant-negative TGFBR2, or TGF-β1 plus a dominant-negative TGFBR2.

Requirements of the Smad pathway in TGF-β–induced EMT of malignant keratinocytes and normal mammary epithelial cells were first demonstrated in experiments with constitutively active or dominant-negative forms of Smad2 and Smad3 in cultured cells.[95,96] Most prominently, a mutant TGFBR1 defective in Smad binding was shown to lack the ability to induce EMT in mammary epithelial cells,[76] indicating that Smad-dependent signaling is required for TGF-β–mediated EMT response. Direct evidence for a role of Smad3 stems from observations that the loss of Smad3 in mice blocked EMT in response to the injury of the lens, retina, and kidney *in vivo* or by exposure to exogenous TGF-β in organ culture.[97–99] Furthermore, TGF-β fails to induce EMT in primary tubular epithelial cells derived from kidneys of Smad3-deficient mice.[100]

At the molecular level, EMT is characterized by a transcriptional program shift marking the transition from epithelium to mesenchyme.[95,96,101,102] One of the transcriptional changes is the downregulation of E-cadherin.[102,103] Snail, a zinc-finger transcription factor known to act as a potent repressor of the E-cadherin gene,[104] is one of the immediate Smad3 target genes.[105] In Smad3-deficient cells, expression of Snail was ablated during TGF-β–mediated EMT initiated by injury of resident epithelial cells. In addition, Smad3 can directly or indirectly regulate transcription of a number of other genes involved in the EMT process, including epithelial and mesenchymal markers, extracellular matrix/cytoskeleton proteins, inhibitors of differentiation, and components of the Notch signaling pathway (including Krt1-19, Slug, N-cadherin, α smooth muscle actin, MMP1-2, Id1-3, and Hey1).[96,100,106]

C. Smad3-Mediated Transcriptional Response Fosters Metastasis in Humans

The increased expression and activation of TGF-β also affects the tumor microenvironment that permits tumor growth, invasion, and angiogenesis.[103,107–109] There is substantial evidence supporting the notion that excessive TGF-β production is associated with poor prognosis.[4,5] In mouse breast cancer models, TGF-β signaling promoted bone and lung metastasis.[110–112] Both Smad-independent and Smad–dependent signaling pathways contributed to the prometastatic role of TGF-β. In the case of the Smad pathway, it was observed that reduction in the signaling flux through the Smad2/3 pathway is sufficient to block metastases of oncogenically transformed or tumor-derived cells, whereas overexpression of Smad3 in these same cells increased the number and size of lung metastases.[113] It appears that Smad3 signaling is required for cells to either extravasate into the lung or proliferate in that tissue and induce an angiogenic response. Using the MDA-MB-231 human breast cancer cell line as a model system, *IL-11* and connective tissue growth factor (*CTGF*) have been identified among a group of 48 genes with expression elevated in MDA-MB-231 subpopulations selected *in vivo* for high bone metastatic activity.[114]

Enforced expression of IL-11 and CTGF increases the osteolytic bone metastatic activity in MDA-MB-231 xenografts. Both *IL-11* and *CTGF* are direct targets of TGF-β. TGF-β stimulation induces binding of Smad3 (and/or Smad2) and Smad4 to the relevant regions of the *IL-11* and *CTGF* promoters.[114] In the *IL-11* promoter, Smads also cooperate with AP1 transcription factors to enhance transcription.[82] Therefore, these Smad-dependent transcriptional responses could provide an advantage to cancer cells for osteolytic metastasis in a TGF-β–rich bone microenvironment.

V. CONCLUDING REMARKS

In the past few years, considerable progress has been made toward the understanding of the signaling networks and biologic function of TGF-β and Smad3 during carcinogenesis. It has been greatly appreciated that, like TGF-β, Smad3 could function as both a tumor suppressor and prometastatic factor. However, it is still not known how Smad3 switches from a tumor suppressor to a prometastatic factor during carcinogenesis. It is presumed that TGF-β/Smad3–mediated prometastatic responses can emerge once the pathway becomes uncoupled from the tumor suppressor effect. Finally, as we continue to advance our understanding of the function of Smad3 and how subtle perturbation of Smad3 function can result in pathologic situations, consideration should be paid to the development of practical and clinical approaches for pharmacologic interventions that target Smad3 to enhance or retain its growth-inhibiting and apoptosis-inducing effects but also inhibit its prometastatic activities.

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