

A New Switch for TGF β in Cancer

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Abstract

The TGF β cytokine plays dichotomous roles during tumor progression. In normal and premalignant cancer cells, the TGF β signaling pathway inhibits proliferation and promotes cell-cycle arrest and apoptosis. However, the activation of this pathway in late-stage cancer cells could facilitate the epithelial-to-mesenchymal transition, stemness, and mobile features to enhance tumorigenesis and metastasis. The opposite functions of TGF β signaling during tumor progression make it a challenging target to develop anticancer interventions. Nevertheless, the recent discovery of cellular contextual determinants, especially the binding partners of

the transcription modulators Smads, is critical to switch TGF β responses from proapoptosis to prometastasis. In this review, we summarize the recently identified contextual determinants (such as PSPC1, KLF5, 14-3-3 ζ , C/EBP β , and others) and the mechanisms of how tumor cells manage the context-dependent autonomous TGF β responses to potentiate tumor progression. With the altered expression of some contextual determinants and their effectors during tumor progression, the aberrant molecular prometastatic switch might serve as a new class of theranostic targets for developing anticancer strategies.

Introduction

TGF β signaling is a relatively simple membrane receptor to nuclear transcription activation pathway that plays critical roles in many biological events, such as embryonic stem cell self-renewal and differentiation, the homeostasis of differentiated cells, and the suppression of the immune system and cancer progression (1). The activated TGF β ligands bind to the TGF β type I and II transmembrane receptor complex, including a canonical type II receptor-mediated serine/threonine phosphorylation on type I receptor for activating downstream signaling modulators. Then, the phosphorylation-activated regulatory Smads (R-Smads, Smad2/3 in TGF β signaling) complex with Smad4 (Co-Smad) to translocate into the nucleus to alter gene expression. Because Smad2 does not bind to DNA, and the binding affinity of Smad3 and Smad4 is relatively weak, partnering with other transcription modulators is commonly observed in TGF β signaling to potentiate designated transcription (2, 3). Although the mammalian genome encodes 33 ligands, five type II and seven type I receptors, and eight Smad proteins of TGF β family members, with multi-layer modulation that might explain the complicated functions of TGF β signaling, the cellular contextual determinants that decide the opposite outcomes of TGF β responses remain elusive.

In normal and premalignant epithelial cancer cells, activation of TGF β signaling could promote cell-cycle arrest and apoptosis to sustain tissue homeostasis and to suppress aberrant cell growth

for preventing uncontrollable cell transformation and tumor progression. However, elevated TGF β expression and activation of TGF β receptor-initiated intracellular signaling in tumor microenvironments are observed to facilitate tumor metastasis in many cancer types. Pathologic TGF β is capable of promoting epithelial-to-mesenchymal transition (EMT) and stemness, leading to increased cancer cell growth and invasion, evasion of immune surveillance, cancer dissemination, and tumor metastasis (4, 5). Dichotomous TGF β functions have been suggested in different cellular contexts for determination of TGF β responses with increasing understanding of molecular details (6). This review will briefly summarize the recent progress of autonomous TGF β prometastatic switches during cancer progression and provide molecular insights to target the contextual determinant for developing anticancer strategies (Fig. 1).

TGF β /Smad-induced tumor-suppressive effects

In noncancerous epithelial cells at premalignant stages, TGF β secreted by tumor and stroma cells can induce cell-cycle arrest and promote apoptosis through increased expression of cyclin-dependent kinase (CDK) inhibitors 1A (CDKN1A or p15), 2B (CDKN2B or p21) and 1C (CNKN1C or p57) and apoptosis inducer death-associated protein kinase (DAPK; ref. 7), and thereby play a tumor-suppressive role (8, 9). Moreover, suppression of c-Myc expression is another indication of TGF β tumor-suppressive effects. c-Myc is a key transcriptional activator of cell proliferation. For example, in keratinocyte and mammary epithelial cells, TGF β induced cytostasis by upregulation of CDK inhibitor p15 via a Forkhead box (FoxO)/Smad complex and repression of c-Myc via an E2F4/5/Smad complex to avoid tumor progression (10, 11).

Cancer cells often bypass these cytostatic effects by inactivation of the key players along the TGF β signaling pathway and crosstalk with other oncogenic pathways. For instance, TGFBR1 is frequently mutated in colorectal cancer cells (12). Inactivating mutations in Smad2 were reported in hepatocellular carcinoma (HCC), colorectal cancer, and lung cancer. Smad4 mutations were detected in colorectal cancer and HCC, and it is highly deleted

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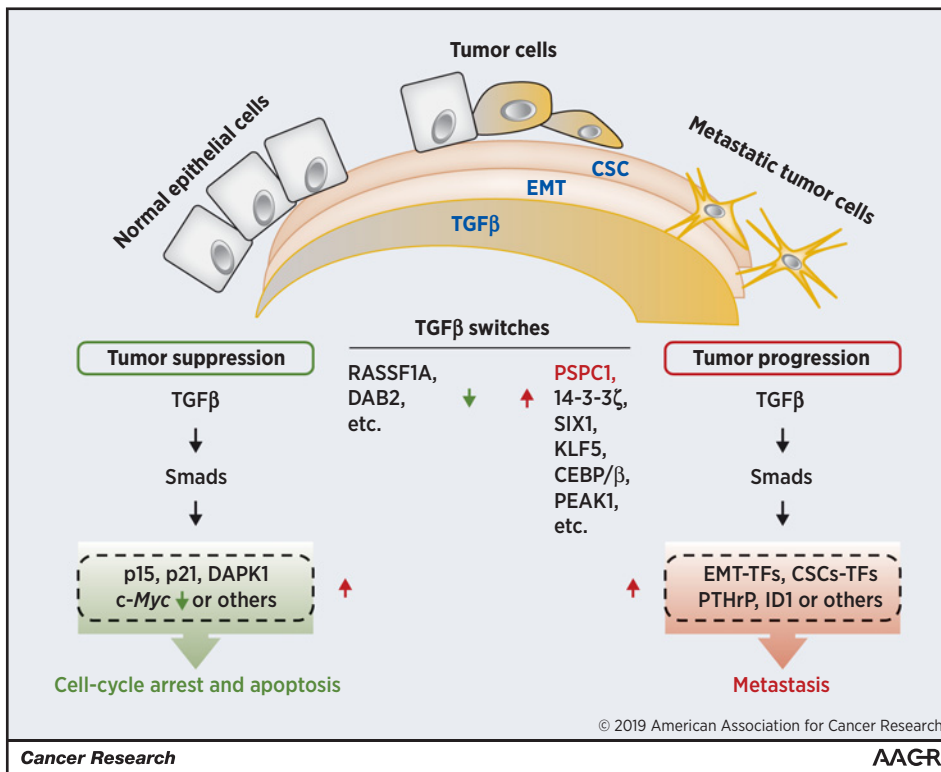


Figure 1. The dichotomous roles of TGFβ switches in tumor progression. In normal and premalignant cells, TGFβ promotes cell-cycle arrest and apoptosis via induction of tumor suppressors and *c-Myc* suppression. In late-stage tumor, the increased TGFβ activates downstream oncogenes, leading to EMT, CSC, and metastasis.

in pancreatic cancer (13, 14). Tumors that have lost core components of the TGFβ pathway have a growth advantage but are not highly invasive (15). In subgroups of advanced tumors of melanoma, glioma, and breast cancer, malignant cancer cells might have retained the TGFβ/Smad pathway, but remain resistant to TGFβ-induced cytostatic effects by acquiring oncogenic mutations in the noncanonical PI3K/AKT, RAS/MAPK, or p53 pathways (16). Collectively, cancer cells that lose the tumor-suppressive arm of the TGFβ pathway could still increase tumorigenicity via mutations on TGFβ pathway members or other cross-talk pathways to potentiate tumor growth and invasion.

Prometastatic arm of TGFβ/Smad signaling

Despite recognition of TGFβ/Smad signaling to inhibit cell proliferation and induce apoptosis of noncancerous cells, TGFβ1 mRNA is highly expressed within the tumor tissues of many cancer types, as evidenced by the searches of The Cancer Genome Atlas (TCGA) project of cBioPortal for Cancer Genomics (17). Elevated TGFβ1 expression within tumor tissue and plasma of patients correlated with advanced tumor stages and diminished patient survival in various cancer types. Moreover, functional and mechanistic studies indicated that increasing TGFβ1 expression is associated with malignant phenotypes of tumor progression, including EMT, cancer stemness, angiogenesis, tissue invasion, and metastasis (18, 19).

Subsequently, many of the TGFβ/Smad signaling-dependent pathways were identified. For instance, Smad-driven EMT enhances stemness and metastatic seeding in breast cancer (20, 21). TGFβ signaling in breast cancer is associated with seeding of lung metastasis, partly due to the SMAD-dependent expression of angiopoietin-like 4, which disrupts vascular endothelial cell-cell

junctions and enhances extravasation of circulating tumor cells (22). Upregulation of *PTH1LH*, *IL11*, *CTGF*, and *JAGGED1* is dependent on TGFβ/Smad signaling to enhance osteolytic metastasis in breast carcinoma (23, 24) and melanoma cells (25). Furthermore, TGFβ/Smad signaling not only induces activation of *SOX2*, *PDGFB*, and *LIF* to support glioblastoma stem cells (26, 27), but also provides the rationale for the clinical development of TGFβ inhibitors against glioma (28).

Cellular contextual determinants of TGFβ prometastatic switch

To clarify the mysterious insights of TGFβ dichotomous functions, major efforts focused on divergent Smad-interacting proteins because of the weak DNA sequence-binding specificity of Smads as well as tissue- and differentiation-specific expression patterns of Smad partners, including p53, members of the bHLH and FoxO, and zinc finger protein families (29, 30). Recent emerging efforts were made to identify key cellular contextual determinants that change their gene expression profiles during the TGFβ switch from tumor suppressive to prometastatic responses as potential theranostic targets for developing anticancer strategies (Table 1). On the basis of their acting mechanism in modulating the TGFβ1 dichotomous effect, we subgrouped these determinants into the following categories (Fig. 2).

Determinants participating in dichotomous TGFβ1 responses

PEAK1. The pseudopodium enriched atypical kinase 1 (PEAK1), which is an oncogenic nonreceptor tyrosine kinase controlling cellular migration and proliferation, was found to be upregulated in breast cancer, colon cancer, and pancreatic cancers (31, 32). PEAK1 was indicated as a molecular switch that regulates TGFβ1 response in different cellular context (33). High PEAK1 expression restricts TGFβ-induced growth arrest and potentiates

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Table 1. TGFβ contextual determinants in cancers

Determinants	Characteristics	Up/Downregulation	Expression in cancer type	EMT	CSCs	TGFβ		Mechanisms of TGFβ response	References
						suppressive	promoting		
Gain of function PSPC1	Transcription factor	Increase	Lung ^{a,b} , Breast ^{a,b} , Liver ^{a,b} , Colon ^b , Prostate ^b , Stomach ^b	Promotion	Promotion	-	+	Promotes TGFβ-induced EMT/CSCs and restricts TGFβ-mediated cytostasis	67
KLF5	Transcription factor	Increase/decrease	Lung ^a , Breast ^c , Cervical ^c , Prostate ^a	Promotion	Promotion	-	+	Partners with SMADs-induced SOX4 and promotes tumorigenesis in SMAD4 negative cells	65, 75-80
14-3-3ζ	Adaptor proteins	Increase	Lung ^a , Breast ^a , Liver ^a	Promotion	Promotion	+	+	Promotes bone metastasis through stabilizing Gli2/Smads complex and restricts cytostasis through destabilizing p53/Smads.	43, 44, 81-83
Six1	Transcription factor	Increase	Lung ^b , Breast ^a , Liver ^a , Ovary ^c ,	Promotion	Promotion	N.A	+	Promotes TGFβ-induced EMT through upregulating TGFBR1 expression	46-48, 84, 85
CEBP/β	Transcription factor	Increase/decrease	Liver ^a , Prostate ^b , Pancreatic ^a	Inhibition	Promotion	-/+	+	Induces cytostasis through activating FoxO-Smads-p15 and repressing E2F4/5-SMADs-c-MYC	11, 57, 86-90
PEAK1	Kinase	Increase	Breast ^b , Colon ^a , Pancreatic ^b	Promotion	Promotion	+	+	Restricts TGFβ-mediated cytostasis and promotes TGFβ-stimulated EMT in the presence of fibronectin	33, 91, 92
Loss of function RASSF1A	Scaffold proteins	Decrease	Lung ^d , Breast ^{a,c,d} , Brain ^d , Skin ^d , Bladder ^d , Kidney ^d	Inhibition	Inhibition	N.A	+	Restricts TGFβ-mediated transcription and invasion	53, 54, 93-98
DAB2	Adaptor proteins	Decrease	Bladder ^a , Oesophageal ^b , Colon ^a , Pancreatic ^c	Promotion/ inhibition	N.A	+	-	Promotes TGFβ-mediated cytostasis and restricts TGFβ-stimulated EMT	36, 99, 100

Abbreviation: N.A., not available.

^aIHC validation.

^bIn *silico* validation.

^cRNA validation.

^dBisulfite PCR validation.

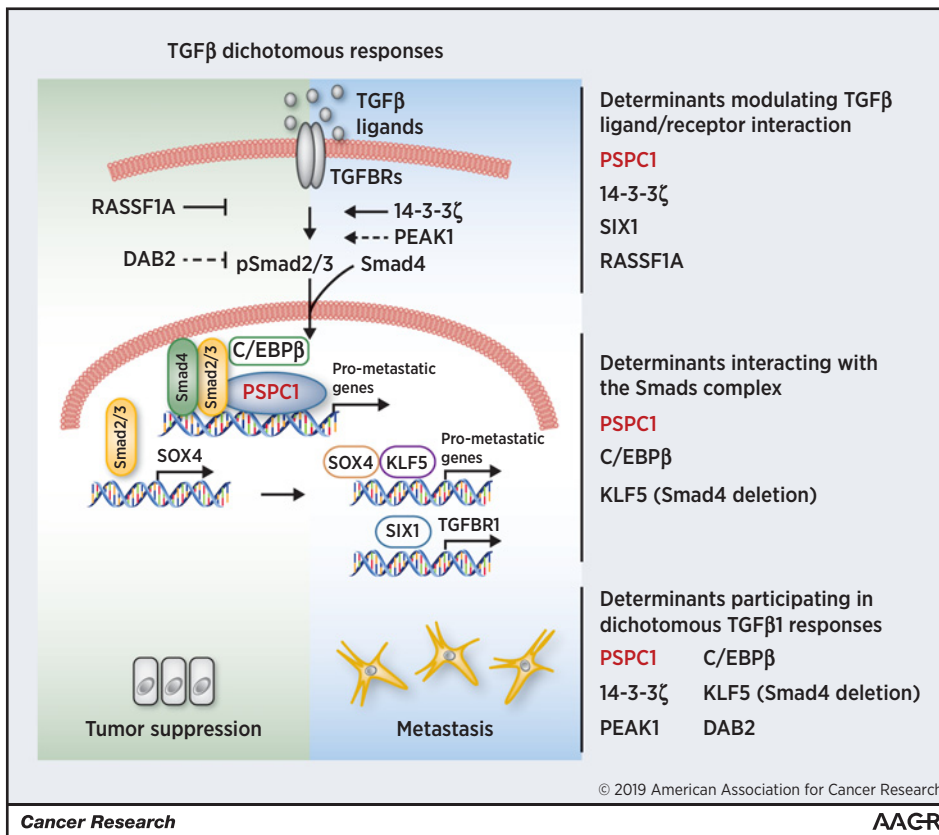


Figure 2. Mechanisms of contextual determinants of TGFβ dichotomous responses. Determinants were classified into three categories based on their molecular roles in participation of TGFβ dichotomous responses in tumor progression.

TGFβ-mediated EMT, cellular migration, and tumor metastasis with the presence of fibronectin. Mechanistically, PEA1K1 promotes the TGFβ-induced Src/MAPK signaling and canonical Smad2/3 pathway to convert TGFβ signaling from cytoysis toward a protumorigenic state. Moreover, PEA1K1 is necessary for TGFβ to induce Zeb1-mediated EMT under fibronectin/ITGB3 activation (34). Nevertheless, the detailed mechanisms of how PEA1K1 tyrosine kinase activity modulates the TGFβ/Smads signaling pathway in response to TGFβ treatments in precancerous and cancerous cells deserve further investigation for developing inhibitors to target PEA1K1. Together, PEA1K1 may function as a critical regulator in different TGFβ contexts and serve as a marker to determine TGFβ blockade response in target therapy.

DAB2. The disabled homolog 2 (DAB2) is a multiple adaptor protein that functions as a critical link of TGFBRs and Smads (35). DAB2 is downregulated in various cancer types and functions as a putative tumor suppressor (36, 37). Hypermethylation of the DAB2 promoter correlates with its low expression and predicts metastasis and poor clinical outcome in squamous cell carcinomas (SCC; ref. 38). Low DAB2 expression has been shown to restrict TGFβ-mediated cytoysis and facilitate TGFβ-induced EMT (38, 39). Mechanistically, DAB2 selectively represses TGFβ-mediated Smad2 activation. Downregulation of DAB2 switches TGFβ response from tumor suppression toward tumor promotion and facilitates cellular proliferation, motility, and tumor growth (38). However, other studies also indicated that DAB2 is induced under TGFβ1 stimulation and might be protumorigenic (40, 41). The elevated DAB2 is required for TGFβ-mediated adhesion, spreading, and EMT. The contradictory

results may be due to the different cellular contexts. Alternatively, tumor cells require both invasive ability and apoptosis resistance. The elevated DAB2 expression not only functions as a promoter of invasive and tumor formation but also protects tumor cells against apoptosis (40). Modulation of DAB2-interacting partners in both precancerous and cancerous cells to understand the switch of dichotomous TGFβ responses should be beneficial for developing inhibitors to improve cancer therapy.

Determinants modulating TGFβ ligand/receptor interaction

Because abundance and activity of TGFβ ligands, receptors, and regulators determine the responses of TGFβ signaling, aberrant expression of these switching determinants could alter TGFβ responses via regulating expression and functions of members in the TGFβ signaling pathway.

14-3-3ζ. 14-3-3ζ belongs to the 14-3-3 family proteins that mediate signal transduction as an adaptor protein by binding to phosphoserine-containing proteins. Aberrant 14-3-3ζ expression is detected in several human malignancies and predicts poor prognosis of patients with cancer (42). In breast cancer, overexpression of 14-3-3ζ promotes the TGFβ/Smads pathway through stabilizing TGFBR1 protein that leads to oncogenic ZFH1B/SIP-1 upregulation and loss of E-cadherin expression to promote EMT (43). Recent efforts by Xu and colleagues revealed that 14-3-3ζ promoted TGFβ-induced bone metastasis through fine-tuning the TGFβ/Hippo-YAP/Sonic Hedgehog-Gli2 pathway. Mechanistically, 14-3-3ζ was shown to disrupt the tumor-suppressing p53-Smads interaction by cytoplasmic sequestration of YAP1 and allowing the released Smads to

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complex with Gli2. 14-3-3 ζ drives the Smad–Gli2 complex to increase PTHrP expression and promote metastasis of breast cancer to bone (44). In contrast, TGF β induces p21 expression and cytostatic function in nonmalignant HMECs through the p53/Smad complex. Overexpression of 14-3-3 ζ inhibits the TGF β -induced cytostatic program in nontransformed human mammary epithelial cells by repressing 14-3-3 σ , p53, and p21 expression. Together, 14-3-3 ζ is indicated as the contextual determinant for TGF β /Smads responses by forming a complex with p53 in premalignant cells but with Gli2 in late-stage cancer cells to facilitate tumor metastasis. However, 14-3-3 ζ overexpression alone did not increase cellular invasive ability but required cooperation with other switching determinants to achieve the prometastatic switch. With contradictory roles of aberrant 14-3-3 family proteins in tumor progression, future studies of dysregulated 14-3-3 family proteins in modulating dichotomous TGF β signaling are warranted for clarifying and developing the suitable anti-TGF β inhibitors for clinical interventions.

SIX1. Aberrant expression of the sine oculis homeobox 1 (SIX1), which is a homeodomain-containing transcription factor, was frequently reported in various cancer tissues (45). SIX1 controls cell proliferation and regulates epithelial plasticity through stimulating EMT process (46–49). Expression of SIX1 is correlated with nuclear Smad3 to increase TGF β signaling and induce metastasis in breast cancer. SIX1 induces TGF β type I receptor (TGFBR1) expression and switches TGF β signaling toward pro-tumorigenicity (50). In addition, SIX1 increases miR-106b-25 miRNA cluster expression to target inhibitory Smad7, resulting in TGF β type I receptor upregulation and TGF β signaling activation, inducing EMT and tumor-initiating properties (51). These findings suggest that SIX1 shifts TGF β signaling from tumor-suppressive to tumor promoting. It remains unclear whether the SIX1 transcription factor could directly modulate Smad2/3 targets by switching cytostatic and tumor-progressive targets in precancerous and late-stage cancer cells, respectively, for better understanding of the molecular switch of TGF β signaling.

RASSF1A. RASSF1A (Ras association domain family 1 isoform A) functions as a tumor suppressor, and its inactivation is implicated in the development of many human cancers (52). RASSF1A plays critical roles in controlling YAP nuclear shuttling and restricting EMT and invasion (53). Recently, RASSF1A was reported to be involved in reprogramming of TGF β signaling. RASSF1A is recruited to activate TGFBR1, resulting in endogenous protein degradation by ITCH E3 ligase. The degradation of RASSF1A allows YAP1 to interact with Smad2 for promoting Smad2 nuclear translocation and transcription activation of TGF β -enhanced cellular invasion. Together, these results indicated that interactions of the TGF β and Hippo pathways modulated by RASSF1A are finely tuned determinations of cell fate and cancer progression (54). Although RASSF1A tightly regulated TGF β signaling, the molecular switch of TGF β dichotomous responses remains unclear. It should be warranted to investigate the TGF β -induced cytostatic mechanisms involved in programmed cell death mechanisms, such as ITCH E3 ligase-related autophagy, and to the switch of the TGF β /Smads regulatory targets of the RASSF1A/YAP/Smads axis in tumor progression in divergent cancer types.

Determinants interacting with the Smad complex

With aims to explain the TGF β dichotomous responses under the simple TGF β /Smad signaling pathway, the search for factors

that act as Smad2/3 partners and that bind to the CAGA box or Smad-binding element (SBE) involved in switches of mesoderm differentiation and tumor progression has been prioritized for decades.

C/EBP β . The CCAAT/enhancer binding protein β (C/EBP β) is a basic leucine zipper (bZIP) transcription factor that regulates gene expression to control cell proliferation, differentiation, inflammation, metabolism, oncogene-induced senescence, and tumorigenesis (55). C/EBP β has tumor suppressor and antiproliferation activities via complexing with the RB/E2F repressor to negatively regulate E2F target genes such as c-Myc, PCNA, and cyclin A2 (56). Depletion of C/EBP β promoted the TGF β response toward EMT and contributed to the evasion of the TGF β growth-inhibitory response (57). However, C/EBP β expression is also elevated and associated with a more aggressive subset of tumors (58–60). Interestingly, the elevated C/EBP β isoforms LIP:LAP ratio has not only been observed in malignant tumors associated with poor prognosis (61, 62) but also linked to defective TGF β -mediated cytostasis response in metastatic breast cancer (11). Mechanically, LAP2 associates with forkhead box protein FOXO and Smads to activate CDK inhibitor CDN2B and repress c-Myc expression. In contrast, the increased LIP expression in metastatic tumor cells antagonizes LAP2 activity and promotes proliferation by switching the TGF β responses toward p15 repression and c-Myc activation.

Although C/EBP β isoforms play key roles in the TGF β signaling shift, little is known about the molecular mechanisms of increasing the LIP/LAP ratio during tumor progression, especially the not significant increase of LIP/LAP ratio upon TGF β treatments (11). Nevertheless, in HER2-overexpressing breast cancer, the translational regulatory factor CUGBP1 is upregulated to favor the production of LIP (63). In mammary epithelial cells, IGF-1R/Akt signaling could upregulate LIP expression to increase the LIP/LAP ratio to counter anoikis (64). Future studies on how the TGF β modulation of the C/EBP β isoforms LIP/LAP ratio contributes to the TGF β signaling shift and the cross-talk of other oncogenic pathways leading to cancer metastasis are critical for developing TGF β inhibitors in combination with other drugs to improve therapy.

KLF5. In colon and pancreatic cancers, mutations in members of TGF β /Smads signaling pathway, especially Smad4 mutation or deletion, are frequently detected in tumor tissues. However, high concentrations of TGF β remain associated with tumor malignancy and poor prognosis in patients with gastrointestinal cancer. Recently, kruppel-like factor 5 (KLF5) was reported to be the contextual determinant to promote tumor progression in Smad4-deficient pancreatic cancer cells (65). KLF5 is a zinc finger transcription factor that controls cell proliferation, survival, and the tumor-initiating properties of cancer stem cells in multiple cancer types (66). In Smad4-positive pancreatic cancer cells, TGF β /Smad increased Snail-mediated EMT but reduced the oncogenic Sox4–Klf5 complex to increase the apoptotic Sox4 level, leading to lethal EMT. In contrast, Klf5 was shown to be the contextual determinant that converted the proapoptotic Sox4 to the oncogenic Klf5–Sox4 complex, thereby promoting Smad4-deficient PDAC tumorigenesis. It will be critical to extend the similar molecular switching mechanisms to other cancer types, especially malignant cancers with loss-of-function mutations in other members of TGF β /Smads signaling pathway.

PSPC1 is a new switch of dichotomous TGF β signaling

The contextual determinant of dichotomous TGF β responses confers molecular features to activate TGF β /Smads signaling and decide the cytostatic responses to precancerous cells and the tumor progressive responses to malignant cancer cells. The outcome for late-stage cancer patients is poor owing to high concentrations of TGF β cytokine in tumor tissues to promote tumor EMT, stemness, and metastasis. Paraspeckle component 1 (PSPC1) was identified owing to the integrated cancer genomic approaches with features of upregulated-PSPC1 expression in association with poor prognosis of patients with cancer, augmenting cancerous EMT and stemness, and potentiating TGF β signaling (67).

PSPC1. PSPC1, a known paraspeckle biomarker, is a putative transcription factor that belongs to the Drosophila behavior/human splicing (DBHS) family with limited knowledge about its role in cancer previously. Upregulated PSPC1 is correlated with advanced tumor stages and poor survival of patients with breast, lung, and liver cancers. Upregulated PSPC1 potentiates expression of mesenchymal markers, EMT transcription factors (EMT-TF), cancer stem-like cell transcription factors (CSC-TF), and c-Myc-related proliferation genes, thus promoting migration, invasion, spheroid formation, tumor formation, and metastasis. Moreover, upregulated PSPC1 could enhance expression and interact with phosphorylated-Smad2/3 to augment TGF β 1 autocrine signaling. PSPC1-potentiated tumor progression is dependent on canonical TGF β 1 signaling because treatment with a TGFBR1 inhibitor (SB431542) or Smad2/3 knockdown abolished PSPC1-enhanced expression of core EMT-TFs and CSC-TFs, leading to decreased cellular migration, invasion, and CSC populations.

To examine whether PSPC1 is the switching determinant of the dichotomous TGF β signaling, we integrated the PSPC1 transcriptome with TGF β -induced Smad2/3 target genes identified in chromatin immunoprecipitation (ChIP) array and found that PSPC1-upregulated and PSPC1-downregulated genes with Smad2/3-binding sites were subgrouped into gene signatures involved in cell growth and movement and in cell death and apoptosis, respectively. Therefore, PSPC1 is a putative candidate to serve as a contextual determinant to decide outcomes of TGF β 1 responses.

Indeed, ChIP experiments and target gene expression reveal that Smad2/3 preferentially binds at tumor suppressor gene promoters (p21, p57, and DAPK1) in PSPC1-deficient cancer cells, whereas the upregulated-PSPC1/Smad 2/3 transcription complex binds at key prometastatic gene promoters of EMT-TFs (Snail, Slug, and Twist) and CSC-TFs (Nanog, Oct4, and Sox2) in highly PSPC1-expressing cancer cells. Together, PSPC1 is the key determinant to switch the TGF β 1 signaling from cytostatic to highly aggressive responses in tumor progression. With validation of upregulation of the PSPC1/Smads/TGF β 1 axis in human breast and lung tumor tissues associated with poor patient prognosis, upregulation of PSPC1 is not only a master activator of prometastatic reprogramming, but also a theranostic target for developing antimetastasis reagents.

Conclusions and Perspectives

Anti-TGF β therapeutic drugs, including TGF β -neutralizing antibodies, peptide inhibitors, TGF β receptor kinase inhibitors, antisense oligonucleotides, and TGF β ligand traps were developed for the treatment of cancer, fibrosis, and other diseases with aberrant vascular symptoms (68–70). These inhibitors could reduce excessive TGF β ligands and/or abolish the TGF β signaling cascade for

therapeutic applications in oncology and the reduction of pathologic skin scarring. Despite TGF β cytokine playing important roles in divergent normal physiologic functions and cardiovascular toxicity detected in animals treated with TGF β inhibitors (71, 72), unexpected mild and reversible side effects were observed in human patients treated with appropriate dosing regimen of TGF β inhibitor (69, 73). Nevertheless, the low toxic feature of the TGF β inhibitor given to human cancer patients prompted a design of innovative bifunctional fusion protein targeting both immune checkpoint protein and TGF β with immunosuppressive mechanisms in the tumor microenvironment. As a result, cancer patients with solid tumors are being recruited in a phase I clinical trial (NCT02517398).

Another predictable difficulty for anti-TGF β therapeutic drugs in cancer treatment is the functional and phenotypic heterogeneity of cancer cells. In particular, heterogeneity of TGF β -induced stem-like cancer cells acquired advantages for uncontrollable cell proliferation, stemness, and resistance to anticancer therapeutics (74). Thus, targeting more specific oncogenic genes, such as contextual determinants of the TGF β prometastatic switch, without compromising the systemic homeostatic functions of TGF β would be a superior approach for cancer therapy.

Importantly, because tumor metastasis is the leading cause of death of patients with cancer and because targeting EMT and stemness provides limited benefits to prolong the life of patients with cancer, targeting the increasing members of contextual determinants of the TGF β prometastatic switches might become a new class of targets for developing drugs against cancers. In particular, the expression profiles of these contextual determinants during the prometastatic switch tend to be upregulated during cancer progression in tissue- and differentiation-specific gene expression manners, leading to increased drug specificity for reducing potential side effects. With genetic heterogeneity (e.g., mutation status of members of the TGF β /Smad signaling pathway) and tissue- and differentiation-specific expression profiles of TGF β -related metastatic driver genes in different cancer types, we speculated that other contextual determinants in modulating the TGF β prometastatic switch could be warranted for further exploration. Notably, with divergent mechanisms of these contextual determinants to direct and/or indirect modulations of dichotomous TGF β responses and the heterogeneity of cancer cells, combination therapy with other target or innovative therapy drugs could be beneficial for further investigation to prolong the life of patients with cancer.

Together, by uncovering other contextual determinants of TGF β /Smad signaling involved in different cancer types and malfunctions of human diseases including fibrosis, immune malfunctions, and various congenital diseases might be warranted for opening a new avenue for targeting aberrant TGF β /Smad signaling-related diseases to improve our health.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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