Review

Smad4-mediated TGF-β signaling in tumorigenesis

Guan Yang, Xiao Yang ✉

State Key Laboratory of Proteomics, Genetic Laboratory of Development and Diseases, Institute of Biotechnology, AMMS, Beijing 100071, P.R. China

✉ Correspondence to: Xiao Yang, Ph.D., Institute of Biotechnology, 20 Dongdajie, Beijing 100071, P.R. China. Tel/Fax: 86-10-63895937(O). E-mail: yangx@nic.bmi.ac.cn

Abstract

Transforming growth factor-β (TGF-β) family members exert their function via specific type I and type II serine/threonine kinase receptors and intracellular Smad transcription factors, including the common mediator Smad4. The dual effects of TGF-β signaling on tumor initiation and progression are cell-specific and yet to be determined under distinct contexts. A number of genetically manipulated mouse models with alterations in the TGF-β pathway genes, particularly the pivotal *Smad4*, revealed that these genes play crucial functions in maintaining tissue homeostasis and suppressing tumorigenesis. Loss of Smad4 plays a causal role in initiating squamous cell carcinomas of skin and upper digestive tract as well as adenocarcinomas of gastrointestinal tract. However, for some cancers like pancreatic and cholangiocellular carcinomas, Smad4 deficiency does not initiate the tumorigenesis but acts as a promoter to accelerate or synergize the development and progression of cancers that are started by other oncogenic pathways. Intriguingly, emerging evidences from mouse models have highlighted the important roles of non-cell autonomous effects of Smad4-mediated TGF-β signaling in the inhibition of oncogenesis. All these data have greatly deepened our understanding of molecular mechanisms of cell-autonomous and non-cell autonomous effect of Smad4-mediated TGF-β signaling in suppressing carcinogenesis, which may facilitate the development of successful therapies targeting TGF-β signaling for the treatment of human cancers.

Key words: TGF-β, Smad4, mouse model, tumorigenesis

Introduction

The tumorigenesis of all human cancers can be divided into a series of landmarks that are required to be overcome by a "cancer cell." First, the cells within a tissue undergo genetic or epigenetic alterations and acquire the potential to become malignant, whereby they undergo unregulated proliferation and recruit a blood supply, and finally, the cells invade and metastasize to other sites [1]. However, this is not a very favorable course for oncogenic cells because in addition to known cancer defense mechanisms such as DNA repair, there exists a dynamic and reciprocal struggle between the genetically altered cells and their microenvironment. Malignant cells must sub-vert the microenvironmental controls for survival; however, the tumor microenvironment, which includes extracellular matrix, blood vasculature, inflammatory cells, and fibroblasts, hinders the tumor development by the virtue of a network of soluble growth factors and cytokines within the stroma, which act in an autocrine and paracrine fashion. Any defection by the microenvironment during the anti-cancer battle may damage the equilibrium and result in a spectrum of dysfunctions, including cancer [2].

Among the pathways involving growth factors that serve as the mediators of tumorigenesis, the transforming growth factor-β (TGF-β) signaling

pathway has attracted much attention [3]. TGF-β plays a confirmed yet complicated role in directing the autonomous, local, and systemic cellular responses that together regulate the initiation, progression, and prognostic outcome of human cancers [4,5]. Other pathways altered in human cancer might contribute to the TGF-β-mediated regulation of tumorigenesis to some extent [6-13]. Unlike fibroblast growth factor, insulin-like growth factor, and epithelial growth factor, which mainly act as tumor promoters by influencing cell proliferation, TGF-β plays a dual role in tumorigenesis. During initiation and early progression of the tumor, TGF-β serves as a tumor suppressor by inhibiting proliferation and accelerating apoptosis, which is supported by the fact that loss or mutation of the members of the TGF-β signaling pathway in humans causes unregulated cell growth and eventually cancer. In late stages of tumor progression, elevated levels of TGF-β promote tumor formation by facilitating migration, invasion, angiogenesis, and evasion of the immune system, with its increased production being associated with poor prognosis for patients [3]. However, the "double-edged sword" of TGF-β exerts both cell-specific and context-dependent effects. For example, TGF-β not only inhibits the uncontrolled proliferation of epithelial, endothelial and hematopoietic cells, but also mediates tumor promotion predominately through the surrounding stroma other than the precancerous epithelial cells themselves [14]. Therefore, there is an urgent need to evaluate the mechanisms by which cell-specific and context-dependent responsiveness to TGF-β occurs in the fields of receptor expression, availability of downstream components, and establishment of crosstalk communication with other pathways.

Smads are the key intracellular mediators of transcriptional responses to TGF-β. In mammals, the 8 Smads are subdivided into 3 distinct classes: receptor-regulated Smads (R-Smads) comprising Smads 2 and 3 (transduce TGF-β signaling) and Smads 1, 5, and 8 (transduce bone morphogenetic protein (BMP) signaling); a common Smad called co-Smad4; and 2 inhibitory Smads (I-Smads), namely, Smads 6 and 7 [15]. Smad4 is the pivotal factor of the TGF-β pathway and functions as a key tumor suppressor. The germline mutation of *Smad4* gene causes Juvenile Polyposis Syndrome (JPS). Homozygous deletion or intragenic mutation of somatic *Smad4* gene frequently occurs in the carcinomas of the pancreas, gastrointestine, and skin. Dysregulated Smad4 expression is also usually found in few types of cancers [16,17]. The gene knockout and transgenic techniques of genetic manipulation have been used to generate a number of mouse models that faithfully recapitulate the initiation and progression processes of human cancers and deepen our understanding of molecular mechanisms of cancer physiopathology. The development of a conditional knockout mouse bearing a floxed *Smad4* allele [18] and a spectrum of appropriate tissue-specific Cre transgenic mice have been used to elaborate the function and the related molecular mechanisms of Smad4-mediated TGF-β signaling in maintaining tissue homeostasis and suppressing tumorigenesis (see Table 1).

Table 1. *Smad4*-deficient mouse models that recapitulate human tumorigenesis

Tumor type	*Smad4*-deficient cells	Phenotypic cells	With combined mutations
***Smad4* complete knockout mice**			
Tumors throughout the gastrointestinal tract	All type of cells	Gastrointestinal epithelial cells	Alone [51, 53] or with *Apc+/-* [50, 64, 65], *elf+/-* [58-60]
Tissue-specific *Smad4* conditional knockout mice			
Tumors throughout the gastrointestinal tract	T cells	Gastrointestinal epithelial cells	Alone [107]
Pancreatic ductal adenocarcinomas	Pancreatic progenitor cells	Pancreatic ductal epithelial cells	With *KrasG12D* [29, 31, 32] or *KrasG12D;Ink4a/ArfCo/Co* [29]
Skin squamous cell carcinomas	Keratinocytes	Keratinocytes	Alone [79, 80] or with *PtenCo/Co* [80]
Head and neck squamous cell carcinoma	Oral epithelial cells	Oral epithelial cells	Alone [82]
Esophagus and forestomach Squamous Cell Carcinoma	Esophageal and forestomach epithelial cells	Esophageal and forestomach epithelial cells	Alone or with *PtenCo/Co* [81]
Cholangiocellular carcinoma	Hepatocytes and bile duct epithelial cells	Bile duct epithelial cells	With *PtenCo/Co* [101]
Breast squamous cell carcinoma	Mammary epithelial cells	Mammary epithelial cells	Alone [102]
Keratocystic odontogenic tumors	Odontoblasts or HERS cells	Odontoblasts/HERS cells or HERS cells	Alone [108]

Cell-autonomous effect of Smad4-mediated TGF-β signaling in suppressing tumorigenesis

Pancreatic cancer

Genetic dysregulation of TGF-β signaling pathway is commonly observed in pancreatic cancer [19]. The alternative name of human *SMAD4*, i.e., *DPC4* (*deleted in pancreatic carcinoma, locus 4*), suggests the close relationship of loss of this gene with pancreatic cancer [20]. Several evidences support the role of *SMAD4* as a tumor suppressor gene in pancreatic tumorigenesis. Loss of heterozygosity (LOH) at 18q, where *SMAD4* gene is located, occurs in 90% of pancreatic carcinomas [21]. Homozygous deletion or intragenic inactivating mutations of *SMAD4* gene as well as the complete loss of SMAD4 protein expression are observed in 50% ductal adenocarcinomas [20], 34% invasive adenocarcinoma of the Vater ampulla [22], and 55% endocrine pancreatic carcinomas [23]. The expression level of SMAD4 protein is inversely associated with histopathological grades of pancreatic cancers [24]. Loss of SMAD4 expression has also been postulated as the indication of pancreatic origin in metastatic carcinoma [19]. However, some studies have also suggested that compromised TGF-β signaling may account for the progression of pancreatic cancer rather than the initiation step. Restoration of SMAD4 in a variety of SMAD4-null pancreatic tumor cell lines did not affect proliferation but inhibited pancreatic tumor invasion and angiogenesis [25]. However, the role of Smad4-mediated TGF-β signaling in pancreatic cancer progression and metastasis is controversial. For instance, high expression of TGF-β isoforms in human pancreatic ductal adenocarcinoma tissues correlates with the poor prognosis [26]. Patients expressing SMAD4 unexpectedly exhibit significantly worse outcomes and did not benefit from surgery [27]. One study showed that cells expressing SMAD4 showed an enhanced TGF-β-mediated epithelial-to-mesenchymal transition (EMT) [28,29]. These instances highlighted the tumor promoting role of SMAD4 in pancreatic carcinogenesis.

Recently, the dual role of Smad4 was established in a cohort of mouse models of human pancreatic cancer. Selective *Smad4* or *TGF-β type II receptor* (*Tgfbr2*) deletion in pancreatic epithelium had no detectable effect on pancreatic development or physiology, indicating an inculpable role of *Smad4* deficiency in initiating pancreatic tumorigenesis. However, when combined with activated Kras expression in mice, *Smad4* haploinsufficiency, loss of *Smad4* or

loss of *Tgfbr2* accelerated the progression of Kras-initiated neoplasms to high-grade tumors. These *in vivo* results favor the conclusion that Smad4 mediates the tumor inhibitory action of TGF-β signaling, predominantly at the progressive stage of tumorigenesis [29-31]. *Smad4* deficiency also markedly induces the development of tumors into adenocarcinomas in the event of *Ink4a/Arf* loss and Kras activation. Interestingly, however, the adenocarcinomas in *Pdx1-Cre;Kras^{G12D};Smad4$^{Co/Co}$;Ink4a/Arf$^{Co/Co}$* and *Pdx1-Cre;Kras^{G12D};Smad4$^{Co/Co}$;Ink4a/Arf$^{Co/+}$* mice exhibited greatly reduced proportion of sarcomatoid histology which is commonly presented in those of *Pdx1-Cre;Kras^{G12D};Ink4a/Arf$^{Co/Co}$* and *Pdx1-Cre;Kras^{G12D};Ink4a/Arf$^{Co/+}$* mice, while maintaining a differentiated histopathology [29,32]. This finding validates the observations that intact Smad4 facilitates EMT and TGF-β-dependent metastasis in human pancreatic cancers [33,34]. Although the above studies have not addressed the conundrum of the Smad4 switch from a tumor-suppressive to a tumor-promotion pathway in pancreatic cancer, Smad4-dependent inhibition of β-catenin degradation [35] and the activation of signal transducers and activators of transcription 3 (Stat3) [28] as well as the effects of stromal fibroblasts [36-38] may be involved. These experimental elaborations are regarded as a perfect paradigm in which molecular mechanisms of physiopathology in human diseases and mice models are reciprocally validated.

Gastroenterological tumor

Alimentary canal epithelial tumors with aberrant TGF-β signaling usually emerge as part of the JPS or in the form of sporadic gastric, intestinal, and colorectal adenocarcinomas [39-42]. LOH at 18q, homozygous deletion or intragenic mutations of *SMAD4* gene [3,16] as well as promoter hypermethylation [43,44] are widely observed in sporadic gastroenterological tumors. JPS is a rare autosomal dominant disorder characterized by a predisposition to hamartomatous polyps and cancers of the gastrointestinal and colorectal tract. This syndrome is caused by germline mutation of either *SMAD4* (15%–20%) or *bone morphogenetic protein receptor type IA (BMPR1A)* (20%–25%) [17,45-49]. Supporting evidence for *SMAD4* haploinsufficiency in tumor initiation and progression is provided by studies on heterozygous *Smad4$^{+/-}$* mice. Gastric, duodenal, and colonic polyps morphologically resembling those of human juvenile polypsosis develop in all aged *Smad4$^{+/-}$* heterozygous mice. LOH and malignant transformation are frequently observed at later stages of *Smad4$^{+/-}$* tumors [50-53]. Until recently, by using a

Sleeping Beauty system to generate transposon-based insertional mutations in the gastrointestinal epithelium of mice, Starr and his colleagues have generated mouse mutants by phenocopying the initiation and progression of human gastrointestinal tumors, and identified driver genes including *adenomatous polyposis coli (Apc), phosphatase and tensin homolog deleted on chromosome 10 (Pten), Bmpr1a*, and *Smad4* [54].

Lines of evidence indicate that *SMAD4* deficiency not only initiates gastroenterological carcinogenesis, but also functions during progression towards malignancy that commonly requires the compromise of other tumor suppressor pathways. Embryonic liver fodrin (ELF) is a crucial adaptor protein in TGF-β signaling and is required for Smad3 and Smad4 localization and signaling. Significant loss of ELF expression is often coupled with reduced SMAD4 expression in human gastric and colonic cancer tissues [55-57]. Similarly, a spectrum of early-onset gastrointestinal tumors ranging from oral to colonic linage develops in *elf*^{+/-};*Smad4*^{+/-} mutant mice, indicating a synergistic role of ELF and Smad4 in tumor suppression [58-60]. APC is a member of the WNT signaling pathway and the most commonly mutated gene in human colorectal cancer [61-63]. The in *cis* compound *Apc*^{+/-};*Smad4*^{+/-} gastrointestinal polyps develop into more malignant tumors as compared to the tumors in the simple *Smad4*[+/] or *Apc*^{+/-} heterozygotes [50,64]. Further studies indicated that the loss of Smad4-mediated TGF-β signaling in tumor epithelial cells induced the accumulation of immature myeloid cells through a CCL9/CCR1 chemotactic loop that promote tumor invasion [65].

Recently, multipotent intestinal stem cells that generate the entire epithelial structure are found to be located at the specific site of villus, and are likely to play the role of "cancer stem cells" during tumorigenesis [66-71]. Since BMP signaling plays an important role in the stem cell renewal function [72,73], it is of great importance to dissect the contribution of intestinal stem cell-specific TGF-β signaling pathway to the gastrointestinal carcinogenesis.

Squamous cell carcinomas in the skin and upper digestive tract

The epidermis of the skin, the mucosa of the oral cavity and esophagus comprise most of the stratified squamous epithelia of the body, and they share common ground on aspects of tissue genesis, differentiation and even oncogenic transformation. TGF-β is an important regulator of squamous epithelial cell development and the maintenance of tissue homeostasis. The biphasic role of TGF-β as both a tumor-suppressor and a tumor-promoter has been validated in mouse model overexpressing TGF-β1 in keratinocytes [74]. However, a majority of *in vivo* evidences have supported the concept that TGF-β signaling is primarily a tumor-suppression pathway with growth inhibitory effects. Keratinocytic Smad4 is the major transducer of TGF-β and BMP signaling, both of which exert their unique influences on epidermal biology [75-77]. In human skin squamous cell carcinomas, 57% samples exhibited LOH at the *SMAD4* locus. The incidence of loss of SMAD4 expression was high, particularly in poorly differentiated skin carcinomas [78]. Keratinocyte-specific loss of *Smad4* in mice resulted in spontaneous skin tumor formation at as early as 5 months of age, indicating that *Smad4* deficiency initiated squamous cell carcinoma formation. Notably, Smad4 has been shown to interact with the PTEN/Akt signaling pathway to repress skin tumor formation [79,80]. The synergistic role of Smad4 with PTEN in suppressing epidermal and esophageal tumorigenesis has further been confirmed in keratinocyte-specific *K5-Cre;Smad4*[Co/Co];*Pten*[Co/Co] mice. Smad4 and PTEN have been shown to suppress esophageal tumorigenesis through the cooperative induction of cell cycle inhibitors [81]. On the other hand, enhanced Smad4 binding to the Snail promoter likely contributes to Smad2 loss–associated Snail activation and EMT during skin carcinogenesis [78]. A very recent study has revealed the casual role of Smad4 loss in head and neck squamous cell carcinoma (HNSCC) development and progression. In either human HNSCC or mouse models in which *Smad4* was specifically deleted in the upper digestive tract, Smad4 downregulation occurred at the stage prior to tumor formation. Further analyses suggest that *Smad4* loss causes HNSCC formation and invasion which is largely due to defects in the Fanconi anemia/Brca DNA repair pathway, increased genomic instability and inflammation [82].

A number of studies have recently identified follicle stem cells that reside in a quiescent niche and can give rise to all skin epithelial lineages [83-85]. Our latest work has implicated that loss of Smad4 induces hyperactivation of follicle stem cells which is associated with skin squamous cell carcinoma formation in mice, and eventually results in the depletion of follicle stem cells, thereby indicating that Smad4 plays a pivotal role in follicle stem cell maintenance [86]. Increasing evidences suggest that the harmonization of TGF-β/BMP signaling with other pathways, including Sonic hedgehog, Wnt, Notch, and Akt signaling pathways is required for achieving balanced self-renewal and activation of multipotent follicle stem cells [86-93].

Other cancers

Cancers of the breast, liver, and prostate are among the most prevalent human cancers. However, the role of TGF-β signaling in the initiation and progression of these diseases is not as explicit as in pancreatic and colorectal cancers. Investigators have reported infrequent alteration of the *SMAD4* gene or its protein product in these cancers. For example, the LOH of 18q have been reported in these cancers, but *SMAD4* did not appear to be the target of inactivation [94]. Intragenic mutations of *SMAD4* were also rarely observed, particularly in liver and prostate cancers [95-99]. Most prostate cancers become resistant to the antiproliferative effects of TGF-β without defined mutations or deletions of the members of the Smad signaling pathway [100]. Tissue-specific ablation of *Smad4* in hepatocytes and bile duct epithelial cells causes neither discernable defects on liver development nor tumor formation [101]. Smad4 deletion in mammary epithelium gradually induced well-differentiated squamous cell carcinomas in all the mutant mice but with a long latency, and enhanced canonical Wnt signaling likely contributes to Smad4 loss–associated epithelial transdifferentiation during carcinogenesis [102]. These data revealed that, at least in prostatic epithelium and hepatocytes, the absence of Smad4 alone cannot drive the initiation of tumorigenesis, but may require the participation of other cancer-related genes, for example, a combined loss with *Pten* [101].

Non-cell autonomous effect of Smad4-mediated TGF-β signaling in suppressing tumorigenesis

The reasons for non-phenotype of some tissue-specific *Smad4* knockout mice are unclear, but may be interpreted as an outcome in which the malignant phenotype is held in check by the appropriate microenvironment [2]. In addition to genetically damaged cells, tumorigenesis is induced by an in situ tumor-favoring microenvironment normally comprising a complicated network of signals derived from many cell types.

It is being increasingly recognized that the neighboring cells in the microenvironment of the tumor may be the source of mutation, and thus the original cause of the tumor [14]. Compelling evidences have been derived from stromal cell-specific knockout mouse models. Conditional inactivation of the *Tgfbr2* gene in mouse fibroblasts unexpectedly resulted in intraepithelial neoplasia in the prostate and invasive squamous cell carcinoma of the forestomach, and both were associated with an increased abundance of stromal cells [103]. These *Tgfbr2*-deficient fibroblasts also promoted growth and invasion of co-transplanted mammary carcinoma cells [104,105]. Disruption of TGF-β signalling in T cells through transgenic expression of a dominant negative Tgfbr2 was shown to accelerate dextran sulfate sodium/azoxymethane-induced colon carcinogenesis [106]. A convincing study reveals that Smad4-mediated TGF-β signaling exerts a non-cell autonomous effect in tumorigenesis. Selective loss of Smad4-dependent TGF-β signaling in mouse T cells results in spontaneous epithelial cancers throughout the gastrointestinal tract, while no tumorigenesis is observed in 2 mouse models with the deletion of the *Smad4* gene restricted to the epithelial lineage. In addition, all heterozygotes of conditional mice showed a haploinsufficiency for *Smad4* in T cell lineage during tumorigenesis, supporting the hypothesis that a compromised TGF-β signaling in T cell contributes to the etiology of human FJP [107]. Our recent study has also suggested that epithelial tumorigenesis could largely be accounted to the wrong message sent by neighboring mesenchymal cells. Human keratocystic odontogenic tumors (KCOT) are benign uni- or multicystic intraosseous tumours of odontogenic origin with a high recurrence rate as well as a potential for aggressive behavior. Human KCOT usually harbor *PTCH1* or *PTCH2* mutations in the tumor squamous epithelium. However, odontoblast-specific *Smad4* knockout mice surprisingly exhibited 100% penetrance of odontogenic keratocysts resembling human KCOTs. The integrity of Smad4 remains unchanged within the KCOT entities. Further analysis revealed that the deletion of *Smad4* in odontoblasts, which changed the fate of odontoblasts, could also alter the fate of the neighboring Hertwig's epithelial root sheath (HERS) and epithelial rests of Malassez (ERM), which are genetically normal, thus leading to the formation of KCOT [108]. In these knockout models, tumor-promotion effects are mediated by the alteration of paracrine signals released by genetically manipulated cells into the microenvironment. Reduced expression of BMPs by *Smad4*-deficient odontoblasts may account for the ceaseless expansion of ERMs. Similarly, KCOTs that emerge from *Smad4*-deficient ERMs, which fail to receive TGF-β/BMP signals from odontoblasts, are frequently observed in the keratinocyte-specific *Smad4* knockout mice [108]. Therefore, the aforementioned mouse models have introduced a heuristic notion that in addition to the accumulation of somatic mutations in epithelial cells, genetic defects in stromal cells also contribute considerably to the development of epithelial tumors.

Conclusion and perspective

In vivo studies have revealed important physiological functions of Smad4-mediated TGF-β signaling in suppressing tumorigenesis via either cell-autonomous or non-cell autonomous mechanism. However, different experiments have apparently conflicting conclusions. These may largely be due to the fact that TGF-β has numerous and opposite effects on cells and the surrounding microenvironment; Smad4-mediated cellular responses to TGF-β signaling vary with extracellular matrix, ligand concentration, and cell type specific cofactors at different developmental stages. Investigating new components that could modify the Smad4-mediated TGF-β pathways and new targets that participate in the context-dependent effects of TGF-β signaling constitute the next step of the challenge. Increasing data have implicated that micro RNAs (miRNAs) play roles in TGF-β/Smad-pathway-induced tumor-suppressive effects [109,110]. Undoubtedly, miRNAs will be receiving more attention as the components of the TGF-β signaling pathway, and these might facilitate comprehensive understanding of the mechanisms underlying the function of TGF-β signaling in the suppression of tumorigenesis. Better understanding of the precise mechanisms that enable TGF-β and their downstream effectors to function in different cell types may facilitate the development of successful therapies targeting TGF-β signaling in the fight against cancers.

Acknowledgements

Conflict of Interest

The authors declare that there is no conflict of interest.

References

1. Hanahan D, Weinberg RA. The hallmarks of cancer. Cell. 2000; 100:57-70.
2. Bissell MJ, Labarge MA. Context, tissue plasticity, and cancer: are tumor stem cells also regulated by the microenvironment? Cancer Cell. 2005; 7:17-23.
3. Elliott RL, Blobe GC. Role of transforming growth factor Beta in human cancer. J Clin Oncol. 2005; 23:2078-93.
4. Bierie B, Moses HL. TGF-beta and cancer. Cytokine Growth Factor Rev. 2006; 17:29-40.
5. Stover DG, Bierie B, Moses HL. A delicate balance: TGF-beta and the tumor microenvironment. J Cell Biochem. 2007; 101:851-61.
6. Bian Y, Terse A, Du J, et al. Progressive tumor formation in mice with conditional deletion of TGF-beta signaling in head and neck epithelia is associated with activation of the PI3K/Akt pathway. Cancer Res. 2009; 69:5918-26.
7. Micalizzi DS, Christensen KL, Jedlicka P, et al. The Six1 homeoprotein induces human mammary carcinoma cells to undergo epithelial-mesenchymal transition and metastasis in mice through increasing TGF-beta signaling. J Clin Invest. 2009; 119:2678-90.
8. Northey JJ, Chmielecki J, Ngan E, et al. Signaling through ShcA is required for transforming growth factor beta- and Neu/ErbB-2-induced breast cancer cell motility and invasion. Mol Cell Biol. 2008; 28:3162-76.
9. Ragazzon B, Cazabat L, Rizk-Rabin M, et al. Inactivation of the Carney complex gene 1 (protein kinase A regulatory subunit 1A) inhibits SMAD3 expression and TGF beta-stimulated apoptosis in adrenocortical cells. Cancer Res. 2009; 69:7278-84.
10. Shi J, Wang DM, Wang CM, et al. Insulin receptor substrate-1 suppresses transforming growth factor-beta1-mediated epithelial-mesenchymal transition. Cancer Res. 2009; 69:7180-7.
11. Ueda Y, Wang S, Dumont N, et al. Overexpression of HER2 (erbB2) in human breast epithelial cells unmasks transforming growth factor beta-induced cell motility. J Biol Chem. 2004; 279:24505-13.
12. Uttamsingh S, Bao X, Nguyen KT, et al. Synergistic effect between EGF and TGF-beta1 in inducing oncogenic properties of intestinal epithelial cells. Oncogene. 2008; 27:2626-34.
13. Wu L, Derynck R. Essential role of TGF-beta signaling in glucose-induced cell hypertrophy. Dev Cell. 2009; 17:35-48.
14. Radisky DC, Bissell MJ. Cancer. Respect thy neighbor! Science. 2004; 303:775-7.
15. Shi Y, Massague J. Mechanisms of TGF-beta signaling from cell membrane to the nucleus. Cell. 2003; 113:685-700.
16. Miyaki M, Kuroki T. Role of Smad4 (DPC4) inactivation in human cancer. Biochem Biophys Res Commun. 2003; 306:799-804.
17. Waite KA, Eng C. From developmental disorder to heritable cancer: it's all in the BMP/TGF-beta family. Nat Rev Genet. 2003; 4:763-73.
18. Yang X, Li C, Herrera PL, et al. Generation of Smad4/Dpc4 conditional knockout mice. Genesis. 2002; 32:80-1.
19. Hruban RH, Adsay NV. Molecular classification of neoplasms of the pancreas. Hum Pathol. 2009; 40:612-23.
20. Hahn SA, Schutte M, Hoque AT, et al. DPC4, a candidate tumor suppressor gene at human chromosome 18q21.1. Science. 1996; 271:350-3.
21. Hahn SA, Seymour AB, Hoque AT, et al. Allelotype of pancreatic adenocarcinoma using xenograft enrichment. Cancer Res. 1995; 55:4670-5.
22. McCarthy DM, Hruban RH, Argani P, et al. Role of the DPC4 tumor suppressor gene in adenocarcinoma of the ampulla of Vater: analysis of 140 cases. Mod Pathol. 2003; 16:272-8.
23. Bartsch D, Hahn SA, Danichevski KD, et al. Mutations of the DPC4/Smad4 gene in neuroendocrine pancreatic tumors. Oncogene. 1999; 18:2367-71.
24. Wilentz RE, Iacobuzio-Donahue CA, Argani P, et al. Loss of expression of Dpc4 in pancreatic intraepithelial neoplasia: evidence that DPC4 inactivation occurs late in neoplastic progression. Cancer Res. 2000; 60:2002-6.
25. Duda DG, Sunamura M, Lefter LP, et al. Restoration of SMAD4 by gene therapy reverses the invasive phenotype in pancreatic adenocarcinoma cells. Oncogene. 2003; 22:6857-64.

26. Friess H, Yamanaka Y, Buchler M, et al. Enhanced expression of transforming growth factor beta isoforms in pancreatic cancer correlates with decreased survival. Gastroenterology. 1993; 105:1846-56.

27. Biankin AV, Morey AL, Lee CS, et al. DPC4/Smad4 expression and outcome in pancreatic ductal adenocarcinoma. J Clin Oncol. 2002; 20:4531-42.

28. Zhao S, Venkatasubbarao K, Lazor JW, et al. Inhibition of STAT3 Tyr705 phosphorylation by Smad4 suppresses transforming growth factor beta-mediated invasion and metastasis in pancreatic cancer cells. Cancer Res. 2008; 68:4221-8.

29. Bardeesy N, Cheng KH, Berger JH, et al. Smad4 is dispensable for normal pancreas development yet critical in progression and tumor biology of pancreas cancer. Genes Dev. 2006; 20:3130-46.

30. Ijichi H, Chytil A, Gorska AE, et al. Aggressive pancreatic ductal adenocarcinoma in mice caused by pancreas-specific blockade of transforming growth factor-beta signaling in cooperation with active Kras expression. Genes Dev. 2006; 20:3147-60.

31. Izeradjene K, Combs C, Best M, et al. Kras(G12D) and Smad4/Dpc4 haploinsufficiency cooperate to induce mucinous cystic neoplasms and invasive adenocarcinoma of the pancreas. Cancer Cell. 2007; 11:229-43.

32. Kojima K, Vickers SM, Adsay NV, et al. Inactivation of Smad4 accelerates Kras(G12D)-mediated pancreatic neoplasia. Cancer Res. 2007; 67:8121-30.

33. Helfman DM, Kim EJ, Lukanidin E, et al. The metastasis associated protein S100A4: role in tumour progression and metastasis. Br J Cancer. 2005; 92:1955-8.

34. Zavadil J, Bottinger EP. TGF-beta and epithelial-to-mesenchymal transitions. Oncogene. 2005; 24:5764-74.

35. Romero D, Iglesias M, Vary CP, et al. Functional blockade of Smad4 leads to a decrease in beta-catenin levels and signaling activity in human pancreatic carcinoma cells. Carcinogenesis. 2008; 29:1070-6.

36. Hwang RF, Moore T, Arumugam T, et al. Cancer-associated stromal fibroblasts promote pancreatic tumor progression. Cancer Res. 2008; 68:918-26.

37. Korc M. Pancreatic cancer-associated stroma production. Am J Surg. 2007; 194:S84-6.

38. Ohuchida K, Mizumoto K, Murakami M, et al. Radiation to stromal fibroblasts increases invasiveness of pancreatic cancer cells through tumor-stromal interactions. Cancer Res. 2004; 64:3215-22.

39. Coffey RJ, McCutchen CM, Graves-Deal R, et al. Transforming growth factors and related peptides in gastrointestinal neoplasia. J Cell Biochem Suppl. 1992; 16:111-8.

40. El-Rifai W, Powell SM. Molecular biology of gastric cancer. Semin Radiat Oncol. 2002; 12:128-40.

41. Matsuzaki K, Okazaki K. Transforming growth factor-beta during carcinogenesis: the shift from epithelial to mesenchymal signaling. J Gastroenterol. 2006; 41:295-303.

42. Walters JR. Recent findings in the cell and molecular biology of the small intestine. Curr Opin Gastroenterol. 2005; 21:135-40.

43. Grady WM, Markowitz SD. Genetic and epigenetic alterations in colon cancer. Annu Rev Genomics Hum Genet. 2002; 3:101-28.

44. Wang LH, Kim SH, Lee JH, et al. Inactivation of SMAD4 tumor suppressor gene during gastric carcinoma progression. Clin Cancer Res. 2007; 13:102-10.

45. Calva-Cerqueira D, Chinnathambi S, Pechman B, et al. The rate of germline mutations and large deletions of SMAD4 and BMPR1A in juvenile polyposis. Clin Genet. 2009; 75:79-85.

46. Friedl W, Uhlhaas S, Schulmann K, et al. Juvenile polyposis: massive gastric polyposis is more common in MADH4 muta-tion carriers than in BMPR1A mutation carriers. Hum Genet. 2002; 111:108-11.

47. Howe JR, Shellnut J, Wagner B, et al. Common deletion of SMAD4 in juvenile polyposis is a mutational hotspot. Am J Hum Genet. 2002; 70:1357-62.

48. Shikata K, Kukita Y, Matsumoto T, et al. Gastric juvenile polyposis associated with germline SMAD4 mutation. Am J Med Genet A. 2005; 134:326-9.

49. van Hattem WA, Brosens LA, de Leng WW, et al. Large genomic deletions of SMAD4, BMPR1A and PTEN in juvenile polyposis. Gut. 2008; 57:623-7.

50. Alberici P, Jagmohan-Changur S, De Pater E, et al. Smad4 haploinsufficiency in mouse models for intestinal cancer. Oncogene. 2006; 25:1841-51.

51. Takaku K, Miyoshi H, Matsunaga A, et al. Gastric and duodenal polyps in Smad4 (Dpc4) knockout mice. Cancer Res. 1999; 59:6113-7.

52. Taketo MM, Takaku K. Gastrointestinal tumorigenesis in Smad4 (Dpc4) mutant mice. Hum Cell. 2000; 13:85-95.

53. Xu X, Brodie SG, Yang X, et al. Haploid loss of the tumor suppressor Smad4/Dpc4 initiates gastric polyposis and cancer in mice. Oncogene. 2000; 19:1868-74.

54. Starr TK, Allaei R, Silverstein KA, et al. A transposon-based genetic screen in mice identifies genes altered in colorectal cancer. Science. 2009; 323:1747-50.

55. Katuri V, Tang Y, Marshall B, et al. Inactivation of ELF/TGF-beta signaling in human gastrointestinal cancer. Oncogene. 2005; 24:8012-24.

56. Mishra L, Katuri V, Evans S. The role of PRAJA and ELF in TGF-beta signaling and gastric cancer. Cancer Biol Ther. 2005; 4:694-9.

57. Tang Y, Katuri V, Dillner A, et al. Disruption of transforming growth factor-beta signaling in ELF beta-spectrin-deficient mice. Science. 2003; 299:574-7.

58. Katuri V, Tang Y, Li C, et al. Critical interactions between TGF-beta signaling/ELF, and E-cadherin/beta-catenin mediated tumor suppression. Oncogene. 2006; 25:1871-86.

59. Tang Y, Katuri V, Srinivasan R, et al. Transforming growth factor-beta suppresses nonmetastatic colon cancer through Smad4 and adaptor protein ELF at an early stage of tumorigenesis. Cancer Res. 2005; 65:4228-37.

60. Redman RS, Katuri V, Tang Y, et al. Orofacial and gastrointestinal hyperplasia and neoplasia in smad4+/- and elf+/-/smad4+/- mutant mice. J Oral Pathol Med. 2005; 34:23-9.

61. Aoki K, Taketo MM. Adenomatous polyposis coli (APC): a multi-functional tumor suppressor gene. J Cell Sci. 2007; 120:3327-35.

62. McCart AE, Vickaryous NK, Silver A. Apc mice: models, modifiers and mutants. Pathol Res Pract. 2008; 204:479-90.

63. Taketo MM, Edelmann W. Mouse models of colon cancer. Gastroenterology. 2009; 136:780-98.

64. Takaku K, Oshima M, Miyoshi H, et al. Intestinal tumorigenesis in compound mutant mice of both Dpc4 (Smad4) and Apc genes. Cell. 1998; 92:645-56.

65. Kitamura T, Kometani K, Hashida H, et al. SMAD4-deficient intestinal tumors recruit CCR1+ myeloid cells that promote invasion. Nat Genet. 2007; 39:467-75.

66. Barker N, Ridgway RA, van Es JH, et al. Crypt stem cells as the cells-of-origin of intestinal cancer. Nature. 2009; 457:608-11.

67. Barker N, van Es JH, Kuipers J, et al. Identification of stem cells in small intestine and colon by marker gene Lgr5. Nature. 2007; 449:1003-7.

68. Brabletz S, Schmalhofer O, Brabletz T. Gastrointestinal stem cells in development and cancer. J Pathol. 2009; 217:307-17.

69. Fodde R. The stem of cancer. Cancer Cell. 2009; 15:87-9.

70. Sato T, Vries RG, Snippert HJ, et al. Single Lgr5 stem cells build crypt-villus structures in vitro without a mesenchymal niche. Nature. 2009; 459:262-5.
71. Zhu L, Gibson P, Currle DS, et al. Prominin 1 marks intestinal stem cells that are susceptible to neoplastic transformation. Nature. 2009; 457:603-7.
72. He XC, Zhang J, Tong WG, et al. BMP signaling inhibits intestinal stem cell self-renewal through suppression of Wnt-beta-catenin signaling. Nat Genet. 2004; 36:1117-21.
73. Shroyer NF, Wong MH. BMP signaling in the intestine: cross-talk is key. Gastroenterology. 2007; 133:1035-8.
74. Cui W, Fowlis DJ, Bryson S, et al. TGFbeta1 inhibits the formation of benign skin tumors, but enhances progression to invasive spindle carcinomas in transgenic mice. Cell. 1996; 86:531-42.
75. Botchkarev VA. Bone morphogenetic proteins and their antagonists in skin and hair follicle biology. J Invest Dermatol. 2003; 120:36-47.
76. He W, Cao T, Smith DA, et al. Smads mediate signaling of the TGFbeta superfamily in normal keratinocytes but are lost during skin chemical carcinogenesis. Oncogene. 2001; 20:471-83.
77. Owens P, Han G, Li AG, et al. The role of Smads in skin development. J Invest Dermatol. 2008; 128:783-90.
78. Hoot KE, Lighthall J, Han G, et al. Keratinocyte-specific Smad2 ablation results in increased epithelial-mesenchymal transition during skin cancer formation and progression. J Clin Invest. 2008; 118:2722-32.
79. Qiao W, Li AG, Owens P, et al. Hair follicle defects and squamous cell carcinoma formation in Smad4 conditional knockout mouse skin. Oncogene. 2006; 25:207-17.
80. Yang L, Mao C, Teng Y, et al. Targeted disruption of Smad4 in mouse epidermis results in failure of hair follicle cycling and formation of skin tumors. Cancer Res. 2005; 65:8671-8.
81. Teng Y, Sun AN, Pan XC, et al. Synergistic function of Smad4 and PTEN in suppressing forestomach squamous cell carcinoma in the mouse. Cancer Res. 2006; 66:6972-81.
82. Bornstein S, White R, Malkoski S, et al. Smad4 loss in mice causes spontaneous head and neck cancer with increased genomic instability and inflammation. J Clin Invest. 2009; 119:3408-19.
83. Jaks V, Barker N, Kasper M, et al. Lgr5 marks cycling, yet long-lived, hair follicle stem cells. Nat Genet. 2008; 40:1291-9.
84. Morris RJ, Liu Y, Marles L, et al. Capturing and profiling adult hair follicle stem cells. Nat Biotechnol. 2004; 22:411-7.
85. Waters JM, Richardson GD, Jahoda CA. Hair follicle stem cells. Semin Cell Dev Biol. 2007; 18:245-54.
86. Yang L, Wang L, Yang X. Disruption of Smad4 in mouse epidermis leads to depletion of follicle stem cells. Mol Biol Cell. 2009; 20:882-90.
87. Demehri S, Kopan R. Notch signaling in bulge stem cells is not required for selection of hair follicle fate. Development. 2009; 136:891-6.
88. Horsley V, Aliprantis AO, Polak L, et al. NFATc1 balances quiescence and proliferation of skin stem cells. Cell. 2008; 132:299-310.
89. Kobielak K, Pasolli HA, Alonso L, et al. Defining BMP functions in the hair follicle by conditional ablation of BMP receptor IA. J Cell Biol. 2003; 163:609-23.
90. Kobielak K, Stokes N, de la Cruz J, et al. Loss of a quiescent niche but not follicle stem cells in the absence of bone morphogenetic protein signaling. Proc Natl Acad Sci U S A. 2007; 104:10063-8.
91. Nguyen H, Merrill BJ, Polak L, et al. Tcf3 and Tcf4 are essential for long-term homeostasis of skin epithelia. Nat Genet. 2009; 41:1068-75.
92. Rhee H, Polak L, Fuchs E. Lhx2 maintains stem cell character in hair follicles. Science. 2006; 312:1946-9.

93. Zhang J, He XC, Tong WG, et al. Bone morphogenetic protein signaling inhibits hair follicle anagen induction by restricting epithelial stem/progenitor cell activation and expansion. Stem Cells. 2006; 24:2826-39.
94. Yin Z, Babaian RJ, Troncoso P, et al. Limiting the location of putative human prostate cancer tumor suppressor genes on chromosome 18q. Oncogene. 2001; 20:2273-80.
95. Kawate S, Takenoshita S, Ohwada S, et al. Mutation analysis of transforming growth factor beta type II receptor, Smad2, and Smad4 in hepatocellular carcinoma. Int J Oncol. 1999; 14:127-31.
96. MacGrogan D, Pegram M, Slamon D, et al. Comparative mutational analysis of DPC4 (Smad4) in prostatic and colorectal carcinomas. Oncogene. 1997; 15:1111-4.
97. Xie W, Mertens JC, Reiss DJ, et al. Alterations of Smad signaling in human breast carcinoma are associated with poor outcome: a tissue microarray study. Cancer Res. 2002; 62:497-505.
98. Yakicier MC, Irmak MB, Romano A, et al. Smad2 and Smad4 gene mutations in hepatocellular carcinoma. Oncogene. 1999; 18:4879-83.
99. Zhong D, Morikawa A, Guo L, et al. Homozygous deletion of SMAD4 in breast cancer cell lines and invasive ductal carcinomas. Cancer Biol Ther. 2006; 5:601-7.
100. Wikstrom P, Stattin P, Franck-Lissbrant I, et al. Transforming growth factor beta1 is associated with angiogenesis, metastasis, and poor clinical outcome in prostate cancer. Prostate. 1998; 37:19-29.
101. Xu X, Kobayashi S, Qiao W, et al. Induction of intrahepatic cholangiocellular carcinoma by liver-specific disruption of Smad4 and Pten in mice. J Clin Invest. 2006; 116:1843-52.
102. Li W, Qiao W, Chen L, et al. Squamous cell carcinoma and mammary abscess formation through squamous metaplasia in Smad4/Dpc4 conditional knockout mice. Development. 2003; 130:6143-53.
103. Bhowmick NA, Chytil A, Plieth D, et al. TGF-beta signaling in fibroblasts modulates the oncogenic potential of adjacent epithelia. Science. 2004; 303:848-51.
104. Cheng N, Bhowmick NA, Chytil A, et al. Loss of TGF-beta type II receptor in fibroblasts promotes mammary carcinoma growth and invasion through upregulation of TGF-alpha-, MSP- and HGF-mediated signaling networks. Oncogene. 2005; 24:5053-68.
105. Cheng N, Chytil A, Shyr Y, et al. Transforming growth factor-beta signaling-deficient fibroblasts enhance hepatocyte growth factor signaling in mammary carcinoma cells to promote scattering and invasion. Mol Cancer Res. 2008; 6:1521-33.
106. Becker C, Fantini MC, Schramm C, et al. TGF-beta suppresses tumor progression in colon cancer by inhibition of IL-6 trans-signaling. Immunity. 2004; 21:491-501.
107. Kim BG, Li C, Qiao W, et al. Smad4 signalling in T cells is required for suppression of gastrointestinal cancer. Nature. 2006; 441:1015-9.
108. Gao Y, Yang G, Weng T, et al. Disruption of Smad4 in odontoblasts causes multiple keratocystic odontogenic tumors and tooth malformation in mice. Mol Cell Biol. 2009;29(21):5941-51
109. Huang S, He X, Ding J, et al. Upregulation of miR-23a approximately 27a approximately 24 decreases transforming growth factor-beta-induced tumor-suppressive activities in human hepatocellular carcinoma cells. Int J Cancer. 2008; 123:972-8.
110. Kong W, Yang H, He L, et al. MicroRNA-155 is regulated by the transforming growth factor beta/Smad pathway and contributes to epithelial cell plasticity by targeting RhoA. Mol Cell Biol. 2008; 28:6773-84.