[Frontiers in Bioscience S4, 749-766, January 1, 2012]

TGF-beta antiproliferative effects in tumor suppression

Stephan Christopher Jahn[1], Mary Elizabeth Law[1], Patrick Evan Corsino[1], Brian Keith Law[1]

[1]Department of Pharmacology and Therapeutics, Shands Cancer Center, University of Florida, Gainesville, FL 32610, United States

TABLE OF CONTENTS

1. ABSTRACT

The TGF-beta signaling pathway controls multiple functions of cancer cells and the surrounding stromal tissue. Some TGF-beta actions suppress cancer formation, while others contribute to tumor progression. Evidence supporting a tumor suppressive role for the TGF-beta/Smad signaling axis is presented here. These data are compiled from cell culture studies, animal models, analyses of human tumors, and investigations of polymorphisms of TGF-beta pathway components and their associated cancer risk. Therapeutic strategies for cancer treatment involving either restoring or potentiating TGF-beta tumor suppressive activities, or blocking TGF-beta tumor promoting functions are considered.

2. INTRODUCTION

Transforming growth factor-beta (TGF-beta) is the prototypic member of an evolutionarily conserved superfamily of cytokines that regulate nearly every aspect of organismal development and function, including cell differentiation, proliferation, apoptosis, bone morphogenesis, angiogenesis, immune system regulation, and muscularity (reviewed elsewhere (1-6)). TGF-beta signals through two conserved families of transmembrane receptor serine/threonine protein kinases. Receptor-mediated phosphorylation and activation of the Smad transcriptional regulators plays a central role in transducing the biological responses to TGF-beta superfamily cytokines.

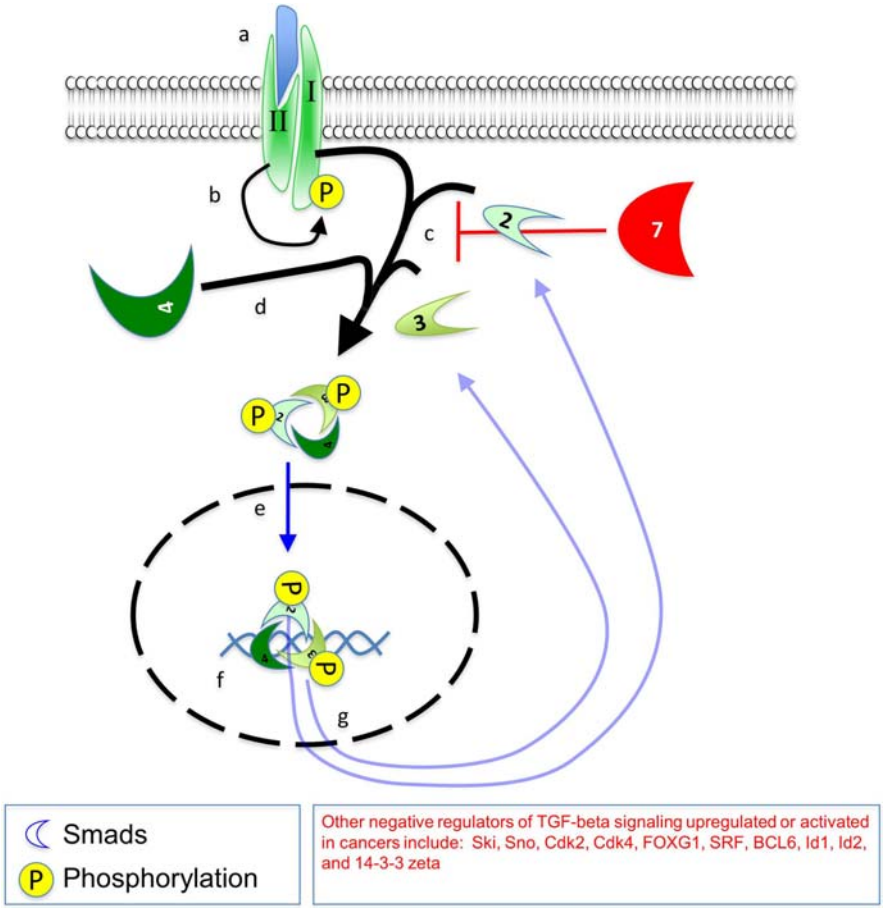

Figure 1. The TGF-beta/Smad signaling pathway. Binding of the TGF-beta ligand to the type II receptor (a) recruits the type I receptor to the complex. The type II receptor then phosphorylates and activates (b) the type I receptor. The type I receptor phosphorylates the receptor regulated Smads 2 and 3 (c). The R-Smads then bind to Smad4 (d), translocate to the nucleus (e), and bind to DNA and transcriptional coactivators and corepressors to control transcription (f). Smads become inactivated by dephosphorylation (g) and are exported out of the nucleus for additional rounds of receptor mediated phosphorylation. Elements downregulated or mutated in cancer are shown in green and elements overexpressed in cancer are shown in red.

TGF-beta inhibits the proliferation of nontransformed epithelial cells, but does not inhibit the division of certain carcinoma cell lines. This suggests that a normal function of TGF-beta is to restrain cell division. Since cancer is fundamentally a disease of uncontrolled cellular proliferation, and epithelial cells frequently secrete and respond to autocrine TGF-beta, the ability of TGF-beta to control cell replication was hypothesized to constitute a tumor suppressive mechanism (7-10). However, there is now a great deal of evidence indicating that depending on the molecular context within a given cell population and the microenvironment, TGF-beta can exhibit either tumor suppressive or tumor promoting activities. Several recent reviews focus on the tumor promoting actions of TGF-beta (11) as well as the numerous non-Smad signaling systems (12) whose effects are activated by TGF-beta. This review will summarize the data demonstrating TGF-beta anticancer activity obtained from *in vitro* experiments, studies of genetically modified animal models, analyses of mutations of TGF-beta pathway components discovered in human cancers, and the identification of polymorphisms of TGF-beta pathway components present in human populations and their association with tumorigenesis. Rational approaches for therapeutic targeting of the TGF-beta pathway to either restore its tumor suppressive functions in cancer, or to block TGF-beta tumor promoting activities are discussed.

3. TGF-BETA MEDIATED CELL CYCLE ARREST OF NONTRANSFORMED EPITHELIAL CELLS

In normal, non-transformed cells, TGF-beta acts as a potent suppressor of cell proliferation (7- 8). The signaling mechanisms involved in this process have been extensively studied, and although TGF-beta signaling interacts with many intracellular pathways, the primary pathway involves the Smad proteins (Figure 1). In classic, Smad-dependent TGF-beta signaling, the cascade begins with a precursor of TGF-beta being processed extracellularly by Furin-type enzymes (13). Upon activation by cleavage, the dimeric TGF-beta cytokine binds to the type II TGF-beta receptor (TGF-betaRII) on

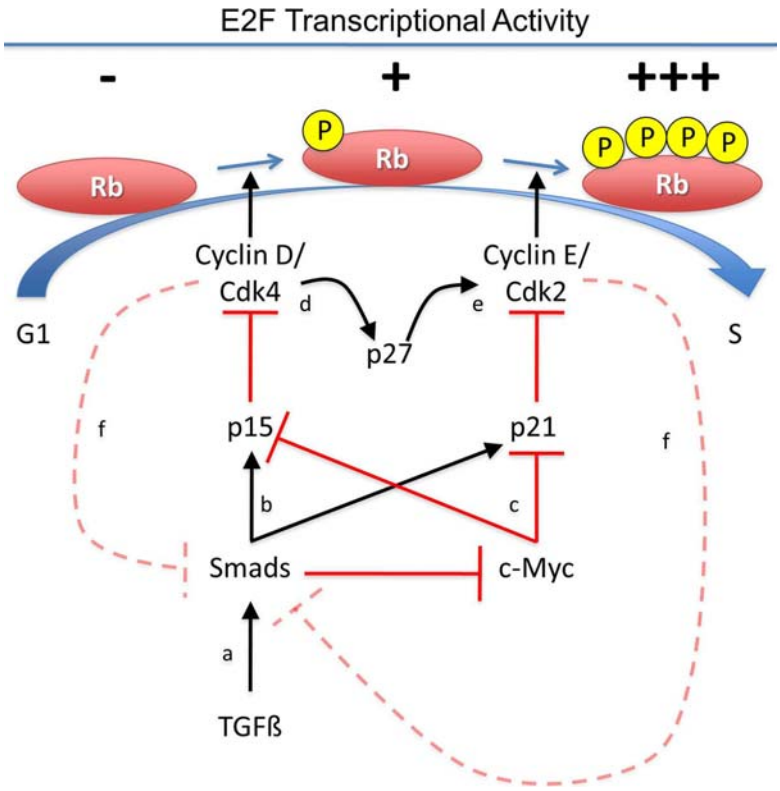

Figure 2. TGF-beta control of cell division. TGF-beta activates the Smad transcription factors (a) as detailed in Figure 1. The Smads activate the transcription of the Cdk inhibitors p15 and p21 (b). c-Myc antagonizes TGF-beta induction of p15 and p21, but TGF-beta represses the c-Myc promoter through a Smad-dependent mechanism (c). p15 inhibits the kinase activity of Cdk4 and displaces p27 from Cdk4 (d). The liberated p27, along with p21 that is transcriptionally upregulated by TGF-beta, inhibits Cdk2 kinase activity (e). Inhibition of Cdk4- and Cdk2-dependent phosphorylation of Rb and the related pocket proteins p107 and p130 maintains active repression of E2F-dependent genes and prevents the G1 to S-phase transition.

the cell surface. Once this interaction has occurred, the type I TGF-beta receptor (TGF-betaRI) is recruited to the complex. The close proximity of the type I and type II TGF-beta receptors results in the phosphorylation of the type I receptor by the type II receptor, thereby activating it. Activated TGF-betaRI recruits receptor-activated members of the Smad family (R-Smads) such as Smads 2 and 3, and subsequently phosphorylates them. In contrast, the bone morphogenetic proteins (BMPs) activate a distinct receptor complex containing different type I and type II receptors, and in this case the BMP type I receptor phosphorylates R-Smads 1 and 5. Phosphorylated R-Smads are then able to bind to the common mediator Smad, or co-Smad, Smad4. Once bound to the co-Smad, the R-Smads translocate to the nucleus where they, along with other nuclear co-factors, regulate gene transcription.

The core TGF-beta/Smad signaling system, as just described, is subject to regulation at multiple points. TGF-beta receptors and the Smad proteins are both subject to ubiquitin-mediated degradation in a ligand dependent manner (reviewed in (14)). Additionally, prolonged Smad-dependent transcriptional control requires continuous Smad nucleocytoplasmic shuttling to maintain their receptor-dependent phosphorylation and overcome the effects of phosphatase-dependent inactivation of Smad signaling (reviewed in (15)). These mechanisms can be altered in cancer cells as compared with normal cells and contribute to the loss of TGF-beta tumor suppressor function in cancers.

A primary mechanism by which TGF-beta uses Smad signaling to induce a cell cycle arrest is through the upregulation of Cyclin dependent kinase (Cdk) inhibitory proteins (Figure 2). Cdks are a family of proteins whose activity controls passage through the cell cycle (reviewed in (16-17)). Cdks 1, 2, 4 and 6, along with their respective Cyclin binding partners, phosphorylate a wide variety of substrates involved in cell cycle progression. There are two families of Cdk inhibitory proteins (CKIs), the INK4 family, which includes $p16^{ink4a}$, $p15^{ink4b}$, $p18^{ink4c}$, and $p19^{ink4d}$ (hereafter referred to as p16, p15, p18, and p19), and the Cip/Kip family, which includes $p21^{Cip1/WAF1}$, $p27^{Kip1}$, and $p57^{Kip2}$ (hereafter referred to as p21, p27, and p57) (18-19). The INK4 proteins inhibit Cdks 4 and 6, while the Cip/Kip family primarily inhibits Cdks 1 and 2, but still bind to Cdks 4 and 6. There are a number of means by which TGF-beta signaling can cause an upregulation of CKIs, in both a Smad-dependent and a Smad-independent manner.

Active nuclear Smad complexes can associate

with the transcription co-factor Sp1 and bind to the p15 promoter, causing an upregulation in p15 mRNA and protein levels. Increased p15 levels have at least two main consequences. Firstly, p15 binds to, and inhibits Cdk4 and Cdk6. Secondly, this binding to Cdks 4 and 6 results in the release of p21 and p27 from these complexes, allowing them to inhibit Cdks 1 and 2 (20). The Smad/Sp1 complex can also bind to the p21 promoter and upregulate p21 protein levels directly (21-22).

The oncoprotein c-Myc is a transcription factor that is able to both activate and repress certain genes. By interacting with Miz-1, c-Myc represses the transcription of p15, p21 and p27, among other genes (23-25). As this is the case, in order for TGF-beta to achieve its anti-proliferative effects, it must overcome this Myc induced repression. A complex containing Smad 3, E2F4/5, DP1, and p107 becomes activated upon stimulation by TGF-beta. This complex can bind to Smad4 in the nucleus and repress Myc expression by binding to its promoter (26). With Myc levels decreased, the Smad proteins are free to induce p15 and p21 expression.

Although Smad4 is the usual binding partner of the R-Smads, its presence is not absolutely necessary for upregulating p21. In Smad4 null cells, TGF-beta increases p21 levels, suggesting an alternative co-regulator of Smad activity (27). In another study, IKK-alpha, a kinase that regulates NF-kappaB, interacted with Smads 2 and 3, acting independently of Smad4 to control gene expression (28). The IKK-alpha/Smad complexes induced the expression of Mad1, Mad2, and Ovol1, all Myc antagonists.

Interestingly, like Smad4, Smads 2 and 3 are also not always necessary for upregulating p21 levels. Experiments performed in HaCaT human keratinocytes showed that the Map kinase pathway is required to increase p21 levels in response to TGF-beta (29). The MEK pathway was activated directly by the TGF-beta receptors. This led to an increase in the activity of Elk1, a transcription factor whose target genes include p21.

p53 also plays an important role in upregulating p21 in response to TGF-beta signaling. Smads 2 and 3 bind to p53, inducing p21 transcription in an Sp1 independent manner. Full transcription of p21 appears to require p53 activity, as p53 null cells do not respond as well to TGF-beta growth arrest signals (30). Recently, this pathway has been shown to become defective when p53 contains an *R175H* mutation (31). When this occurs, mutant p53 forms a complex with p63 and Smads 2 and 3. This complex not only fails to upregulate p15 or p21, but it also leads to increased invasion and metastasis.

TGF-beta can also induce cell cycle arrest independently of Smad activity. In cells treated with TGF-beta, protein kinase C-alpha (PKC-alpha) becomes activated. PKC-alpha then phosphorylates and activates S100C/A11, a Ca^{2+} binding protein (32). Upon activation, S100C/A11 translocates to the nucleus, activates Sp1, and induces p15 and p21 expression.

Although p21 and p15 expression is the primary means by which TGF-beta induces cell cycle arrest, it is not the only mechanism. TGF-beta stimulation in some cell types induces plasminogen activator inhibitor-1 (PAI-1) expression (33). Rather than regulating p21 or p15 expression, increased PAI-1 activity suppresses signaling through the protein kinase B/AKT pathway.

4. MECHANISMS BY WHICH CARCINOMAS BECOME RESISTANT TO TGF-BETA CYTOSTATIC ACTIONS

During neoplastic progression, tumor cells frequently acquire resistance to the cytostatic effects of TGF-beta, thereby enhancing the development and progression of malignancies. The mechanisms by which tumor cells become resistant to TGF-beta are varied and unique to specific cancer types. As discussed below, these mechanisms range from defects in the TGF-beta receptor to influences of the tumor microenvironment on TGF-beta sensitivity.

4.1. Mutation or loss of TGF-beta receptors

Cells frequently lose sensitivity to TGF-beta mediated growth suppression early in carcinogenesis, with the loss of TGF-beta responsiveness occurring at the level of TGF-betaRII in certain types of cancers. The mechanism of silencing TGF-betaRII covers the spectrum from genetic to epigenetic changes. The most common mutational loss of TGF-betaRII occurs in hereditary non-polyposis carcinoma (34-36). These tumors display microsatellite instability and a span of adenine nucleotides in the coding region of the TGF-betaRII gene that is subject to mutation, resulting in a truncated TGF-betaRII that does not activate downstream effectors (37). Interestingly, TGF-betaRII is mutated in >90% of colon cancers that display microsatellite instability and in 15% of microsatellite stable cancers (36, 38). In colon cancers that do not exhibit microsatellite instability, missense and inactivating mutations in the kinase domain of TGF-betaRII have been identified (34). Gastric cancers and gliomas with microsatellite instability also frequently display TGF-betaRII mutations (39-40). Furthermore, TGF-betaRII was mutated in 4% of pancreatic cancers and is often down-regulated in breast and lung cancers, and was undetectable at the protein level in 24% of prostate cancers (41). In addition, frameshift mutations in the polyadenine tract of the TGF-betaRII gene occur in some endometrial cancers (42).

Cancer cells also use epigenetic mechanisms to inactivate TGF-betaRII. Aberrant patterns of histone methylation and acetylation/deacetylation contribute to silencing of the gene encoding TGF-betaRII in breast, ovarian, and pancreatic cancer cell lines (43-44). CpG methylation in the promoter of the TGF-betaRII gene is present in human B-cell lymphoma cell lines that lack a functional TGF-betaRII on the cell surface (45). The TGF-betaRII promoter is also silenced in several different lung cancer cell lines. Three distinct patterns of histone modification indicative of progressive degrees of TGF-betaRII silencing were identified in these cell lines (46).

Furthermore, since the HDAC inhibitor Tricostatin A (TSA) induces TGF-betaRII expression in certain cell lines, it is thought that epigenetic silencing of TGF-betaRII occurs due to a compact nucleosome structure of the TGF-betaRII gene that is maintained by histone deacetylase and methyltransferase association (47).

In some cases, epigenetic silencing of the TGF-betaRII gene involves changes in promoter associated proteins. The activation of the TATA-less basal TGF-betaRII gene promoter is dependent on Sp1 binding to several sites within this promoter (47). Sp3 is a transcriptional repressor that also binds to this element, and there is evidence in certain cell lines that the ratio of Sp1/Sp3 affects whether the TGF-betaRII promoter is active or inactive (48-49).

Mutations in TGF-betaRI are less common than in TGF-betaRII, however TGF-betaRI mutations have been identified in ovarian, breast, head and neck cancers, and lymphomas (41). For instance, in human breast cancers a C to A transversion mutation resulting in a serine to tyrosine substitution at codon 387 (*S387Y*) of the TGF-betaRI gene was identified. This TGF-betaRI mutant is not as responsive to TGF-beta as compared with wild-type TGF-betaRI (50). Mutations of the TGF-betaRI signal sequence have been identified in B cell chronic lymphocytic leukemia. Although these mutant receptors were expressed at the cell surface and interacted normally with TGF-betaRII, their expression significantly reduced TGF-beta mediated gene expression (51). Reduced expression of TGF-betaRI has also been observed in the absence of mutation of the TGF-betaRI gene. For example, human papilloma virus (HPV)-19 oncogenes E6 and E7 have been found to repress TGF-betaRI gene expression in HPV-16 immortalized keratinocytes (52). Furthermore, hypermethylation of CpG islands in the 5′ region of the TGF-betaRI gene decreases its expression in human gastric cancer (53).

Loss of growth inhibitory signaling by TGF-beta is common in the context of Ras-transformation, which can alter the expression of TGF-betaRI and TGF-betaRII. *H-Ras^{G12V}* induces a time-dependent switch in the ratio of TGF-betaRII to TGF-betaRI expression in rat intestinal epithelial cells. Furthermore, the levels of TGF-betaRII mRNA are approximately 5-fold lower in the Ras-expressing cells as compared with control cells. While *H-Ras^{G12V}* expression does not affect the binding of TGF-beta to TGF-betaRII or TGF-betaRI, ligand-induced internalization of TGF-betaRI is suppressed, suggesting a mechanism for the loss of TGF-beta anti-proliferative effects in the Ras-transformed cells (54). RhoB also represses the expression of TGF-betaRII, and RhoB expression causes resistance of human pancreatic carcinoma cells to TGF-beta growth inhibition. A RhoB-responsive region was identified in the TGF-betaRII promoter that contains an AP-1 site. AP-1 binding to this site is strongly inhibited by RhoB, thus decreasing TGF-betaRII gene expression (55).

4.2. Aberrant Smad activity

Mutations and deletions of the genes encoding Smads have been identified. In pancreatic and colon cancers, regions of the human locus 18q21 encoding Smads 2 and 4 are frequently mutated or deleted (56-58). While mutation of Smad3 has not been identified in specific cancers, the levels of Smad3 are altered in some cancers, including human gastric cancers, and gastric cancer cells that lack Smad3 are no longer growth inhibited by TGF-beta (59-60). Loss of one allele of Smad3 impairs the growth inhibitory effect of TGF-beta on normal T cells and works in tandem with inactivation of p27 to promote T-cell leukemogenesis in mice (61). A characteristic of pediatric T-cell acute lymphoblastic leukemia is the loss of Smad3 (41).

Smad3 is inactivated by other mechanisms as well. Several corepressors inactivate Smad3. Serum response factor (SRF) acts as a nuclear repressor and associates with Smad3, thus repressing Smad transcriptional activity and causing resistance to the TGF-beta mediated cytostatic response (62). Two other transcriptional corepressors of Smad3 include Ski and Sno (a novel Ski-related gene). Both Ski and Sno repress the antiproliferative effects of TGF-beta, and overexpression of Ski and/or Sno correlates with more advanced stages of melanoma, esophageal, and colorectal cancers (63-68). BCL6 is a transcriptional corepressor that interacts with Smad3 and Smad4, disrupts the Smad-p300 interaction, and represses Smad4 activity. B lymphoma cells that overexpress BCL6 are refractory to TGF-beta growth inhibition, whereas knockdown of BCL6 expression restores TGF-beta mediated cell cycle arrest (69).

Forkhead box O (FoxO) transcription factors, which are under negative control by the PI3K pathway, are key partners of Smad3 and Smad4 for the transcriptional activation of the p21 gene (70). FoxG1 inhibits FoxO-Smad complexes. The combined action of FoxG1 and PI3K mediate resistance of human glioblastoma cells to TGF-beta mediated growth arrest (70). Furthermore, FoxG1 contributes to resistance to TGF-beta-induced growth arrest through inhibition of p21 expression in ovarian cancer (71). Another transcription factor that regulates sensitivity to TGF-beta is inhibitor of differentiation or DNA binding-1 (Id1), which is a helix-loop-helix transcription factor that lacks a DNA binding domain and acts as a dominant-negative regulator of basic helix-loop-helix transcription factors (72). Id1 modulates the sensitivity of prostate epithelial cells to TGF-beta-induced growth arrest and loss of Id1 sensitizes cells to TGF-beta mediated growth inhibition (73).

The transcription factor c-Myc activates genes required to promote G1-S phase transition and inhibits the expression of p15, a Cdk4 and 6 inhibitor (74). TGF-beta downregulates c-Myc through a Smad-dependent pathway and overexpression of c-Myc inhibits TGF-beta mediated growth suppression (75). Ovarian cancer cells become resistant to TGF-beta mediated growth arrest through the loss of c-Myc repression in a manner independent of Smad activation (76). Besides overexpression of c-Myc, overexpression of E2F1 also confers resistance to TGF-beta growth inhibition. Overexpression of E2F1 by adenovirus in mink lung epithelial cells confers resistance to TGF-beta

growth suppression (77).

The phosphorylation of the Smads also affects the ability of TGF-beta to induce cell cycle arrest. Cdk2- and Cdk4-mediated phosphorylation of Smad3 on Thr^{8}, Thr^{178}, and Ser^{212} reduces Smad3 transcriptional activity in nonhematopoietic cell lines, which leads to compromised p15 activation and c-Myc repression (78). Bone marrow myeloma cells are resistant to TGF-beta mediated growth suppression due to Smad2 phosphorylation on Thr^{8} by Cdk2 (79).

As mentioned above, *H-Ras*^{G12V} overexpression confers resistance to TGF-beta at the receptor level. Hyperactivation of Ras also inhibits Smad2 and 3 functions. SW480.7 colon carcinoma cells do not express Smad4 and contain activated K-Ras. Hyperactivation of Ras inhibits Smad2/3 nuclear localization by increasing their phosphorylation at MAPK sites (80). Furthermore, expression of activated *H-Ras*^{G12V} in intestinal epithelial cells decreases Smad4 nuclear translocation and TGF-beta-induced formation of Smads 2, 3, and 4 complexes, and inhibits TGF-beta mediated growth suppression. PD98059, an inhibitor of MEK, prevents the Ras-induced decrease in Smad4 expression and complex formation, suggesting that Ras represses TGF-beta signaling in a MAPK-dependent manner (81).

Mammary epithelial cells transformed with oncogenic H-Ras are no longer sensitive to TGF-beta-meditated growth inhibition. These cells display increased expression of 14-3-3 zeta and decreased expression of 14-3-3 sigma, as compared with the parental cells. It was found that 14-3-3 sigma is required for TGF-beta mediated growth suppression, whereas 14-3-3 zeta negatively modulates this growth inhibitory response. Furthermore, overexpression of 14-3-3 zeta increases the level of Smad3 that is phosphorylated at linker regions and cannot mediate the TGF-beta growth inhibitory response (82).

Increased levels of Smad7, which suppresses TGF-beta-induced growth arrest, may also decrease Smad responsiveness and lead to resistance of cancer cells to TGF-beta mediated growth suppression. Smad7 is often overexpressed in pancreatic cancers, which are generally resistant to the growth inhibitory effects of TGF-beta (83). Downregulation of Zinc-finger E-box binding homeobox 1, which binds phosphorylated Smads 2 and 3 to enhance TGF-beta signaling, combined with overexpression of Smad7 also contributes to the resistance of TGF-beta mediated growth suppression in adult T-cell leukemia/lymphoma (84).

COLO-357 pancreatic cells that are engineered to overexpress Smad7 become resistant to TGF-beta growth inhibition (85). Interestingly, Smad7 overexpression interferes with TGF-beta mediated attenuation of Cyclin A and B levels, inhibition of Cdk1 dephosphorylation and Cdk2 inactivation, up-regulation of p27, and the maintenance of Rb in a hypophosphorylated state. Smad7 also suppresses TGF-beta mediated inhibition of E2F activity, yet does not affect the phosphorylation of Smad2 or nuclear translocation or DNA binding of Smads 2, 3, and 4. Smad7 is, therefore, able to functionally inactivate Rb and de-repress E2F without interfering with Smad2 and 3 activation. Smad7 thus exerts Smad2, 3, and 4 independent actions in cancer cells that are resistant to TGF-beta mediated growth suppression (85).

Viral proteins also interfere with Smad activity and are responsible for resistance to TGF-beta mediated cytostatic effects. Hepatitis C virus (HCV) core variants isolated from liver tumors interact with Smad3 and inhibit the Smad3/4 transcription factor complex, suggesting that during chronic HCV infection, there is a selection of viral variants that promote cell transformation by providing resistance to TGF-beta antiproliferative effects (86). The human T-cell leukemia virus type I (HTLV-1) protein, Tax, has been proposed to contribute to leukemogenesis in adult T-cell leukemia. Tax interferes with the recruitment of CBP/p300 to Smad transcriptional complexes, which may account for the resistance of HTLV-1 infected T-cells to TGF-beta mediated growth arrest (87). The human papillomavirus (HPV) E7 oncoprotein interacts with Smad2, Smad3, and Smad4, and prevents Smad3 from binding to DNA. This may account for HPV-associated acquisition of resistance to TGF-beta growth inhibition (88).

Viruses use alternative mechanisms to induce resistance to TGF-beta mediated growth suppression as well. The Epstein-Barr virus encoded protein, latent membrane protein 1 (LMP1), confers resistance to TGF-beta mediated growth suppression. LMP1 activates NF-kappaB, which competes for a limited pool of transcriptional co-activators, thereby suppressing TGF-beta mediated transcriptional activity (89).

4.3. Dysregulation of cell cycle effectors

TGF-beta suppresses cell growth by downregulating components of the cell cycle, including Cyclin D1 and Cyclin E, and upregulating cell cycle inhibitors, including p15, p21, and p27 (90-91). Overexpression or activation of Cyclins or Cdks, or downregulation or inactivation of cell cycle inhibitors may ultimately lead to resistance of certain cancer cells to TGF-beta growth suppression.

Resistance to TGF-beta growth inhibition involves changes in Cyclin/Cdk/inhibitor complexes in certain cell types. The expression of Cyclin E is often increased in tumors, suggesting that it may contribute to abnormal growth and potentially to TGF-beta resistance. Along these lines, overexpression of Cyclin E is associated with increased resistance to TGF-beta mediated growth inhibition in two mammary epithelial cell lines (92). The overexpression of Cyclin D1 also contributes to TGF-beta resistance. For instance, overexpression of Cyclin D1 in several hepatocellular carcinoma cell lines is correlated with resistance to TGF-beta mediated growth inhibition, and suppression of Cyclin D1 expression with antisense Cyclin D1 in one of these cell lines partially overcame TGF-beta resistance (93). Primary keratinocytes derived from mice that overexpress Cyclin D1 are partially resistant

to TGF-beta mediated growth suppression (94).

Overexpression of Cdks also confers resistance to TGF-beta growth inhibition. Mink lung epithelial cells that overexpress Cdk4 are resistant to TGF-beta growth suppression (95). Cell lines derived from tumors engineered to express a constitutively active Cyclin D1-Cdk2 fusion protein secrete TGF-beta, yet are resistant to TGF-beta mediated antiproliferative effects, and mink lung epithelial cells stably expressing the Cyclin D1-Cdk2 fusion protein are also refractory to TGF-beta (96-97). These data indicate that constitutively active Cdk2 is sufficient to render cells resistant to TGF-beta cytostatic effects.

Activation of protein kinase B/AKT disrupts the actions of p27, which contributes to resistance to TGF-beta mediated growth suppression in human breast cancer cells (98-100). Thr^{157} is a phosphorylation site for Akt and is within the nuclear localization site of p27. Akt-mediated phosphorylation of p27 causes cytoplasmic localization and accumulation of p27, which leads to resistance of cells to TGF-beta mediated G1 growth arrest. Cytoplasmic localization of p27 in conjunction with Akt activation correlates with a poorer prognosis in breast cancer patients (98-100). The Thr^{157} p27 phosphorylation site is not conserved in rodents, however, revealing a potential limitation of the use of mouse models to study p27 function during tumorigenesis.

Regulation of p21 expression by microRNAs impairs TGF-beta-dependent cell cycle arrest. Two microRNAs, miR-106b and miR-93, suppress the ability of TGF-beta to increase p21 expression and inhibit cell cycle progression in gastric cancer cells (101). Regulation of p15 plays a role in resistance of melanoma cells to TGF-beta growth suppression. Id2 upregulation in melanoma cells, which is correlated with increased invasiveness, suppresses TGF-beta induction of p15, thus circumventing TGF-beta mediated inhibition of proliferation (102).

4.4. Tumor Microenvironment

Cross-talk between tumor cells and the surrounding stroma affects the ability of TGF-beta to mediate cytostatic activity. The autocrine and paracrine release of pro-inflammatory cytokines inhibits TGF-beta mediated growth suppression. For instance, interleukin-15 (IL-15) impairs TGF-beta cytostatic activity and Smad3-dependent TGF-beta signaling in human T lymphocytes. IL-15-mediated inhibition of Smad3 is associated with c-Jun-N-terminal kinase activation and is reversed by c-Jun antisense oligonucleotides, consistent with the inhibitory effect of phospho-c-Jun on Smad3-DNA complexes (103-104). Furthermore, tumor necrosis factor-alpha (TNF-alpha) interferes with Smad signaling through the induction of AP-1 components, which form complexes with Smad3 and prevent its binding to specific cis-elements. Additionally, Jun family members compete with Smad3 for binding to the co-activator p300, which may also explain suppression of Smad-dependent transcription by TNF-alpha (105).

Cyclooxygenase-2 is the key enzyme that converts arachidonic acid to several prostaglandins, including prostaglandin E2 (PGE2). PGE2 promotes cancer progression by increasing cell proliferation and angiogenesis and by inhibiting apoptosis (106-108). PGE2 was recently found to inhibit the cytostatic effect of TGF-beta by binding to the EP2 receptor during mammary tumorigenesis (109).

In certain types of liver cancer cells, TGF-beta simultaneously activates Smads and induces phosphorylation of cytosolic phospholipase A_2-alpha ($cPLA_2$-alpha) (110). While Smad activation inhibits tumor cell growth, phosphorylation of $cPLA_2$-alpha counteracts Smad-mediated inhibition of cell proliferation. $cPLA_2$-alpha increases the production of PGE2 for activation of the EP1 receptor and activates the eicosanoid receptor peroxisome proliferator-activated receptor-gamma, both of which counteract TGF-beta mediated growth suppression (110).

5. EVIDENCE FOR TGF-BETA ANTIPROLIFERATIVE EFFECTS FROM INTACT *IN VIVO* SYSTEMS

Understanding the role that TGF-beta signaling plays in inhibition of proliferation and tumor formation has been expanded greatly by studying polymorphisms that exist in the human population and the use of experimental animal models. In mouse models, all parts of the signaling cascade, including the ligand, the receptor, and the effector proteins have been manipulated to better understand their functions. The result has been strong *in vivo* evidence that TGF-beta signaling plays a crucial role in maintaining control over cell division.

5.1. Allelic variations in the TGF-beta signaling cascade

Plasma concentrations of TGF-beta are implicated in the pathogenesis of several diseases, including cancer, atherosclerosis, fibrotic disease and autoimmune disease (111-115). Polymorphisms of *TGF-beta1* are present within the human populations that affect TGF-beta levels. For instance, individuals with an Arg^{25} polymorphism in the *TGF-beta1* gene have increased circulating levels of TGF-beta and an increased predisposition to hypertension (116-117). Polymorphisms are also present in the *TGF-betaRI, TGF-betaRII* and *TGF-betaRIII* genes that have the potential to regulate the activity of these gene products. Polymorphisms of TGF-beta1 and TGF-betaRII, for instance, are associated with a lower risk of certain cancers, including esophageal squamous cell carcinoma (118).

5.2. The TGF-beta1$^{-/-}$ mouse

The use of a *TGF-beta1*$^{-/-}$ mouse to study the role of TGF-beta in growth suppression is complicated by the fact that these mice have a dysfunctional immune system, leading to organ failure and death approximately 20 days after birth (119). One method that has been used to obviate this problem is to knock out the gene in a severe combined immunodeficient (SCID) background. SCID mice are susceptible to *H. hepaticus* infection in the intestine, causing inflammation and a high rate of proliferation in the

intestinal epithelium (120). However, this only progresses to tumorigenesis in mice that also lack the TGF-beta1 gene (121). It was later shown that TGF-beta1 exerts its growth suppressive role in this model by blocking the recruitment of the NF-kappaB transcription factor to the interleukin-6 promoter (122).

A second tactic that has been used is to generate a heterozygous knockout, which results in animals that do not show the early death phenotype of the homozygous knockout. Mice heterozygous for the TGF-beta1 gene show increased cellular proliferation in the liver and lung, and show greater susceptibility to tumorigenesis in the presence of carcinogens than do wild type mice. These tumors retain their intact allele, indicating that TGF-beta1 is a haploinsufficient tumor suppressor (123).

5.3. Targeted expression of TGF-beta

Targeted over-expression of TGF-beta1 has provided a valuable source of data supporting the growth inhibitory effects of TGF-beta. Expression of transforming growth factor-alpha (TGF-alpha) in the mammary gland under the control of the mouse mammary tumor virus (MMTV) promoter results in breast tumor formation (124). However, co-expression of TGF-beta along with TGF-alpha greatly reduces the formation of tumors. The experiments also showed that TGF-beta expression blocks tumor formation induced by the carcinogen 7,12-dimethylbenz-[a]-anthracene (DMBA) (125). Similar studies have shown that mammary TGF-beta1 expression blocks cancer formation due to either *neu* expression (126) or MMTV infection (127). The effect has also been seen in a mouse model of skin cancer where animals expressing TGF-beta1 specifically in keratinocytes show a reduction in benign tumor formation following topical application of DMBA (128).

5.4. Expression of mutant receptors

Manipulation of the TGF-beta receptors *in vivo* is a valuable tool as the targeted expression of dominant negative and constitutively active receptors allows the examination of both over- and under-active signaling. Expression of a dominant negative type II TGF-beta receptor (*dnTGF-betaRII*) in the mammary epithelium causes the formation of spontaneous tumors (129), increased tumor formation after DMBA treatment (130), and decreases the latency of TGF-alpha induced mammary tumors (131). Likewise, a TGF-betaRII knockout specific to the mammary epithelium leads to spontaneous hyperplasia and decreased latency in polyomavirus middle T antigen induced tumor formation (132). Conversely, expression of constitutively active TGF-betaRI decreases the incidence of MMTV-*neu* tumors, and those that form show a greater latency of formation (126).

Dominant negative TGF-betaRII receptors have also been used to show this effect in locations other than the mammary epithelium. Epidermal expression of *dnTGF-betaRII* approximately doubles the rate of papilloma formation in response to the tumor promoter 12-*O*-tetradecanolyphorbol-13-acetate (TPA) (133). Expression of *dnTGF-betaRII* in the intestine results in greater azoxymethane induced carcinogenesis, while expression in the stomach increases tumor formation due to the bacterium *H. pylori* (134-135). Conditional knockout of TGF-betaRII in the liver also cooperates with TGF-alpha overexpression to induce hepatocellular carcinoma (136). The tumor suppressive effects of TGF-beta were also shown using mice with a pancreas-specific knockout of TGF-betaRII (137). These mice develop pancreatic ductal adenocarcinoma at a rate of 100% when active *K-Ras*^{G12D} is also expressed in a pancreas-specific manner, whereas the TGF-betaRII knockout itself showed no phenotype and *K-Ras*^{G12D} expression only produced low-grade lesions with a much longer latency on its own. To date, there has been one report that TGF-betaRI loss causes tumor formation. Conditional knockout of the type-I receptor in the neurons caused spontaneous tumor formation in the periorbital and perianal areas of mice (138).

5.5. Studies with altered Smad signaling

The downstream effector proteins of TGF-beta signaling, the Smads, have also been utilized in mouse models to demonstrate the growth inhibitory effects of TGF-beta. The results of these experiments have been highly dependent on which Smad is altered and, in the case of intestinal carcinogenesis, the strain of mouse used. Homozygous deletion of Smads 2, 4, and 5 is embryonic lethal (139-141), while Smad3$^{-/-}$ mice survive to adulthood. The original Smad3 knockout model showed an increased risk of colon cancer (142). Since that time, two additional Smad3 null mouse models have been developed and show no spontaneous tumor formation (143-144). The conflicting results may be due to the different mouse strains that were used (2).

Two separate lines of mice harboring heterozygous knockout of the genes encoding Smad2 and adenomatous polyposis coli (APC) have been generated, neither of which have shown an increased rate of colon carcinogenesis when compared to the APC heterozygote alone (145-146). Conversely, mice with heterozygous deletion of both APC and the co-Smad, Smad4, develop tumors that are more aggressive than those of the *APC*$^{-/+}$ mouse (147) and mice with heterozygous Smad4 deletion develop spontaneous gastrointestinal polyps after one year of age (148). Likewise, a conditional knockout of Smad4 in the mammary epithelium results in squamous cell carcinoma (149). These results suggest that Smad4 may play a critical role in the anti-proliferative effects of TGF-beta, while Smad2 does not. Alternatively, this may reflect the role of Smad4 as a common mediator that relays signals from multiple cytokines including the BMPs. It also appears that Smad3 plays an important role in certain genetic backgrounds, or in a manner that is dependent on the bacterial flora present.

5.6. The use of TGF-beta decoy receptors

A fusion of the TGF-betaRII extracellular domain to the constant fragment (Fc) of immunoglobulin, Fc:TGF-betaRII, has also been used quite extensively in mouse models. Expression of this decoy receptor induces tumor cell apoptosis, decreases invasive characteristics, and blocks lung metastasis in MMTV-polyoma virus middle T

antigen expressing mice (150). Similarly, expression of both Fc:TGF-betaRII and the *neu* proto-oncogene controlled by the MMTV promoter shows a reduced incidence of lung metastases when compared to mice expressing *neu* alone (151). Interestingly, in both studies, the decoy receptor had no effect on tumor latency or rate of incidence. The authors suggest that these data, which on the surface seem contradictory to other mouse models showing that lack of TGF-beta increases primary tumor formation, are explained by a selectivity that is inherent to this model (151). It is possible that the pro-invasive characteristics of TGF-beta are only seen when the ligand concentration *in vivo* is above a threshold value and that Fc:TGF-betaRII is able to reduce TGF-beta levels below that level but not low enough to block the tumor-suppressive effects. Although the process has not been well studied, TGF-beta has also been shown to exist in both active and inactive forms (152). If Fc:TGF-betaRII is selective towards one form or the other, either due to affinity or location, it could explain the observed results, which typify the complex and sometimes seemingly paradoxical role of TGF-beta in tumor development *in vivo*.

6. DIRECTIONS FOR FUTURE STUDIES

6.1. Abrogating TGF-beta signaling to block promotion of tumor invasion and metastasis

Many advanced human cancers retain a functional TGF-beta signaling pathway, although TGF-beta no longer arrests the cell cycle in these malignancies. With respect to breast cancer, while estrogen receptor (ER) positive tumors tend to exhibit decreased TGF-beta receptor expression, ER negative tumors frequently harbor an intact signaling pathway (153). In ER negative breast cancers this retention of TGF-beta signaling is thought to contribute to the invasive and metastatic properties that are characteristic of these tumors (154-156) and may confer increased cell survival (150). Despite these signaling responses, TGF-beta generally does not cause cell cycle arrest in ER negative tumors. These observations have led to the strategy of blocking TGF-beta signaling in advanced tumors in order to prevent autocrine or paracrine TGF-beta from promoting cancer invasion and metastasis. Approaches for abrogating TGF-beta signaling include the use of neutralizing antibodies, decoy receptors, and small molecule inhibitors of the serine/threonine kinase activity of the receptors (reviewed in (157-159)). Several studies indicate that these strategies can effectively block tumor metastasis in animal models, but convincing clinical evidence that these approaches significantly impact the progression of human cancer are lacking thus far. A recent article indicates that long-term treatment of tumor bearing mice with a TGF-beta receptor kinase inhibitor leads to the outgrowth of tumors with high levels of Smad phosphorylation that cannot be reversed by the kinase inhibitors (160). The authors cautioned against chronic treatment of patients with this class of drugs. Such treatment results in drug-resistant tumors were more aggressive, were associated with inflammation, and expression of markers consistent with an invasive phenotype and having undergone an epithelial to mesenchymal transition.

6.2. Restoring TGF-beta antiproliferative actions in tumors using rapalogs

Some tumor types, such as basal-like breast cancers, retain a subset of TGF-beta responses but are refractory to its cytostatic effects. This suggests that it may be possible to develop a pharmacological approach to restore or potentiate TGF-beta mediated cell cycle arrest. This approach has the advantage that cancers frequently secrete high levels of TGF-beta, and TGF-beta is a prominent component of the extracellular matrix and tumor microenvironment, so the putative therapeutic strategy would be to accentuate the anticancer effects of the preexisting TGF-beta ligand. Studies have demonstrated that the mTORC1 inhibitory rapamycin analogs, or "rapalogs," strongly cooperate with TGF-beta to arrest the proliferation of a wide array of cell lines (96, 161-166). This combined rapalog + TGF-beta effect correlated with inhibition of Cdk2 activity, an increase in p21 and p27 binding to Cdk2, and a corresponding decrease in Cdk2 association with complexes containing E2F4, p107, and p130. These changes in complex composition may also occur coincidently with Cdk2 translocation from the nucleus to the cytoplasm (164). It was proposed that TGF-beta and rapalogs cooperate to induce arrest of cancer cell proliferation by preventing Cdk2-dependent phosphorylation of p107 and p130, which actively repress E2F-dependent transcription in their hypophosphorylated states. Previous studies have shown that Cdk2-dependent phosphorylation of p107 and p130 requires the formation of transient but stable Cyclin/Cdk2/E2F4/DP1/pocket protein complexes (167). The extent to which potentiation of TGF-beta cytostatic action contributes to the anticancer activity of rapalogs *in vivo* is unknown at present.

Because of the plethora of biological activities attributed to TGF-beta, it may be difficult to completely extricate its tumor promoting effects from its tumor suppressive effects and to determine if in fact the TGF-beta axis is a useful therapeutic target in the treatment of cancer. It would be ideal if therapeutic strategies could be devised that simultaneously block TGF-beta tumor progression functions, while strengthening its tumor suppressive functions. Successful implementation of such a strategy will likely require sophisticated knowledge of the role of TGF-beta signaling in intact tumors encompassing all of the pertinent biological mechanisms. This strategy may need to be tailored to individual tumors in accordance with their particular pattern of oncogene activation and tumor suppressor gene inactivation. Despite these difficulties, the ability to therapeutically reprogram TGF-beta functions in tumors has the potential to significantly impact cancer therapy given the role of this factor in nearly every aspect of tumor initiation and progression including cell cycle control, chromosomal instability (168), apoptosis (169-171), tumor evasion of the immune response (172), and the epithelial to mesenchymal transition (173-175).

7. ACKNOWLEDGEMENTS

We thank Komen for the Cure (KG080510), the Florida Department of Health Bankhead-Coley Cancer Research Program (07BB-8 and 09BB-10), and the

National Institutes of Health/National Cancer Institute (CA93651) for research support. P. Corsino was supported by NIH/NCI T32 Training Grant in Cancer Biology CA09126.

8. REFERENCES

1. Krit Kitisin, Tapas Saha, Tiffany Blake, Nady Golestaneh, Merlyn Deng, Christine Kim, Yi Tang, Kirti Shetty, Bibhuti Mishra and Lopa Mishra: TGF-beta signaling in development. *Sci STKE*, 2007(399), cm1 (2007)

2. Katerina Pardali and Aristidis Moustakas: Actions of TGF-beta as tumor suppressor and pro-metastatic factor in human cancer. *Biochim. Biophys. Acta - Reviews on Cancer*, 1775(1), 21-62 (2007)

3. Aaron Johnson and Stewart Newfeld: The TGF-beta family: Signaling pathways, developmental roles, and tumor suppressor activities. *ScientificWorldJournal*, 2, 892-925 (2002)

4. Anita Roberts and Rik Derynck: Meeting report: Signaling schemes for TGF-beta. *Sci STKE*, 2001(113), pe43 (2001)

5. Garth Patterson and Richard Padgett: TGF-beta-related pathways. Roles in caenorhabditis elegans development. *Trends Genet.*, 16(1), 27-33 (2000)

6. Joan Massague: TGF-beta signal transduction. *Annu. Rev. Biochem.*, 67, 753-91 (1998)

7. Brian Carr, Izumi Hayashi, Earl Branum and Harold Moses: Inhibition of DNA synthesis in rat hepatocytes by platelet-derived type beta transforming growth factor. *Cancer Res.*, 46(5), 2330-4 (1986)

8. Gary Shipley, Mark Pittelkow, John Wille, Jr., Robert Scott and Harold Moses: Reversible inhibition of normal human prokeratinocyte proliferation by type beta transforming growth factor-growth inhibitor in serum-free medium. *Cancer Res.*, 46(4 Pt 2), 2068-71 (1986)

9. Robert Coffey, Jr., Gary Shipley and Harold Moses: Production of transforming growth factors by human colon cancer lines. *Cancer Res.*, 46(3), 1164-9 (1986)

10. Anton Goustin, Edward Leof, Gary Shipley and Harold Moses: Growth factors and cancer. *Cancer Res.*, 46(3), 1015-29 (1986)

11. Molly Taylor, Jenny Parvani and William Schiemann: The pathophysiology of epithelial-mesenchymal transition induced by transforming growth factor-beta in normal and malignant mammary epithelial cells. *J. Mammary Gland Biol. Neoplasia*, 15(2), 169-90 (2010)

12. Ying Zhang: Non-smad pathways in TGF-beta signaling. *Cell Res.*, 19(1), 128-39 (2009)

13. Justin Annes, John Munger and Daniel Rifkin: Making sense of latent TGF-beta activation. *J. Cell Sci.*, 116(2), 217-224 (2003)

14. Yasumichi Inoue and Takeshi Imamura: Regulation of TGF-beta family signaling by E3 ubiquitin ligases. *Cancer Sci*, 99(11), 2107-12 (2008)

15. Caroline Hill: Nucleocytoplasmic shuttling of smad proteins. *Cell Res.*, 19(1), 36-46 (2009)

16. Amit Deshpande, Peter Sicinski and Philip Hinds: Cyclins and Cdks in development and cancer: A perspective. *Oncogene*, 24(17), 2909-2915 (2005)

17. Irma Sánchez and Brian Dynlacht: New insights into Cyclins, Cdks, and cell cycle control. *Semin. Cell Dev. Biol.*, 16(3), 311-321 (2005)

18. Martine Roussel: The Ink4 family of cell cycle inhibitors in cancer. *Oncogene*, 18(38), 117-5317 (1999)

19. Charles Sherr and James Roberts: Inhibitors of mammalian G1 cyclin-dependent kinases. *Genes Dev.*, 9(10), 1149-1163 (1995)

20. Inga Reynisdottir, Kornelia Polyak, Antonio Iavarone and Joan Massague: Kip/Cip and Ink4 Cdk inhibitors cooperate to induce cell cycle arrest in response to TGF-beta. *Genes Dev.*, 9(15), 1831-45 (1995)

21. Aristidis Moustakas and Dimitris Kardassis: Regulation of the human p21/Waf1/Cip1 promoter in hepatic cells by functional interactions between sp1 and smad family members. *Proc. Natl. Acad. Sci. U. S. A.*, 95(12), 6733-8 (1998)

22. Katerina Pardali, Akirra Kurisaki, Anita Moren, Peter Ten Dijke, Dimitris Kardassis and Aristidis Moustakas: Role of smad proteins and transcription factor sp1 in p21(waf1/cip1) regulation by transforming growth factor-beta. *J. Biol. Chem.*, 275(38), 29244-56 (2000)

23. Joan Seoane, Celio Pouponnot, Peter Staller, Manuela Schader, Martin Eilers and Joan Massague: TGF-beta influences myc, miz-1 and smad to control the cdk inhibitor p15ink4b. *Nature Cell Biology*, 3(4), 400-408 (2001)

24. Siqin Wu, Cihan Cetinkaya, Maria Munoz-Alonso, Natalie Von Der Lehr, Fuad Bahram, Vincent Beuger, Martin Eilers, Javier Leon and Lars-Gunnar Larsson: Myc represses differentiation-induced p21cip1 expression via miz-1-dependent interaction with the p21 core promoter. *Oncogene*, 22(3), 351-360 (2003)

25. William Yang, Jian Shen, Min Wu, Marcello Arsura, Mark Fitzgerald, Zalman Suldan, Dong Kim, Claudia Hofmann, Stefania Pianetti, Raphaelle Romieu-Mourez, Leonard Freedman and Gail Sonenshein: Repression of transcription of the p27[kip1] cyclin-dependent kinase inhibitor gene by c-myc. *Oncogene*, 20(14), 1688-1701 (2001)

26. Chang-Rung Chen, Yibin Kang, Peter Siegel and Joan

Massagué: E2f4/5 and p107 as smad cofactors linking the TGF-beta receptor to c-myc repression. *Cell*, 110(1), 19-32 (2002)

27. Hideaki Ijichi, Motoyuki Otsuka, Keisuke Tateishi, Tsuneo Ikenoue, Takayuki Kawakami, Fumihiko Kanai, Yoshihiro Arakawa, Naohiko Seki, Kiyoshi Shimizu, Kohei Miyazono, Takao Kawabe and Masao Omata: Smad4-independent regulation of p21/waf1 by transforming growth factor-[beta]. *Oncogene*, 23(5), 1043-1051 (2004)

28. Pascal Descargues, Alok Sil and Michael Karin: Ikk[alpha], a critical regulator of epidermal differentiation and a suppressor of skin cancer. *EMBO J.*, 27(20), 2639-2647 (2008)

29. Patrick Pei-Chih Hu, Xing Shen, David Huang, Yueyi Liu, Christopher Counter and Xiao-Fan Wang: The mek pathway is required for stimulation of p21waf1/cip1 by transforming growth factor-beta. *J. Biol. Chem.*, 274(50), 35381-35387 (1999)

30. Michelangelo Cordenonsi, Sirio Dupont, Silvia Maretto, Alessandra Insinga, Carol Imbriano and Stefano Piccolo: Links between tumor suppressors: p53 is required for TGF-beta gene responses by cooperating with smads. *Cell*, 113(3), 301-314 (2003)

31. Maddalena Adorno, Michelangelo Cordenonsi, Marco Montagner, Sirio Dupont, Christine Wong, Byron Hann, Aldo Solari, Sara Bobisse, Maria Rondina, Vincenza Guzzardo, Anna Parenti, Antonio Rosato, Silvio Bicciato, Allan Balmain and Stefano Piccolo: A mutant-p53/smad complex opposes p63 to empower TGF-beta-induced metastasis. *Cell*, 137(1), 87-98 (2009)

32. Masakiyo Sakaguchi, Masahiro Miyazaki, Hiroyuki Sonegawa, Mariko Kashiwagi, Motoi Ohba, Toshio Kuroki, Masayoshi Namba and Nam-Ho Huh: Pkcalpha mediates TGF-beta-induced growth inhibition of human keratinocytes via phosphorylation of s100c/a11. *J. Cell Biol.*, 164(7), 979-984 (2004)

33. Roderik Kortlever, Jeroen Nijwening and René Bernards: Transforming growth factor-beta requires its target plasminogen activator inhibitor-1 for cytostatic activity. *J. Biol. Chem.*, 283(36), 24308-24313 (2008)

34. William Grady, Lois Myeroff, Sandra Swinler, Ashwani Rajput, Sam Thiagalingam, James Lutterbaugh, Aaron Neumann, Michael Brattain, Jay Chang, Seong-Jin Kim, Ken Kinzler, Bert Vogelstein, James Willson and Sanford Markowitz: Mutational inactivation of transforming growth factor beta receptor type I in microsatellite stable colon cancers. *Cancer Res.*, 59(2), 320-324 (1999)

35. Sanford Markowitz, Jing Wang, Lois Myeroff, Ramon Parsons, Luzhe Sun, James Lutterbaugh, Robert Fan, Elizabeth Zborowska, Kenneth Kinzler, Bert Vogelstein, Michael Brattain and James Willson: Inactivation of the type II TGF-beta receptor in colon cancer cells with microsatellite instability. *Science*, 268(5215), 1336-1338 (1995)

36. Ramon Parsons, Lois Myeroff, Bo Liu, James Willson, Sanford Markowitz, Kenneth Kinzler and Bert Vogelstein: Microsatellite instability and mutations of the transforming growth factor beta type II receptor gene in colorectal cancer. *Cancer Res.*, 55(23), 5548-5550 (1995)

37. Shi-Long Lu, Wen-Cong Zhang, Yoshimitsu Akiyama, Tadashi Nomizu and Yasuhito Yuasa: Genomic structure of the transforming growth factor beta type II receptor gene and its mutations in hereditary nonpolyposis colorectal cancers. *Cancer Res.*, 56(20), 4595-4598 (1996)

38. Swati Biswas, Patricia Trobridge, Judith Romero-Gallo, Dean Billheimer, Lois Myeroff, James Willson, Sanford Markowitz and William Grady: Mutational inactivation of tgf-betaR2 in microsatellite unstable colon cancer arises from the cooperation of genomic instability and the clonal outgrowth of transforming growth factor beta resistant cells. *Genes. Chromosomes Cancer*, 47(2), 95-106 (2008)

39. Yeun-Jun Chung, Ji-Min Song, Joo-Young Lee, Yong-Tae Jung, Eun-Joo Seo, Sang-Wook Choi and Mun-Gan Rhyu: Microsatellite instability-associated mutations associate preferentially with the intestinal type of primary gastric carcinomas in a high-risk population. *Cancer Res.*, 56(20), 4662-4665 (1996)

40. Shuichi Izumoto, Norio Arita, Takanori Ohnishi, Shoju Hiraga, Takuyu Taki, Naohiro Tomita, Masayuki Ohue and Toru Hayakawa: Microsatellite instability and mutated type II transforming growth factor-[beta] receptor gene in gliomas. *Cancer Lett.*, 112(2), 251-256 (1997)

41. Sonia Jakowlew: Transforming growth factor-beta in cancer and metastasis. *Cancer Metastasis Rev.*, 25(3), 435-457 (2006)

42. Trilok Parekh, Patricia Gama, Xie Wen, Rita Demopoulos, John Munger, Maria-Luisa Carcangiu, Michael Reiss and Leslie Gold: Transforming growth factor beta signaling is disabled early in human endometrial carcinogenesis concomitant with loss of growth inhibition. *Cancer Res.*, 62(10), 2778-2790 (2002)

43. Rebecca Hinshelwood, Lily Huschtscha, John Melki, Clare Stirzaker, Andrea Abdipranoto, Bryce Vissel, Timothy Ravasi, Christine Wells, David Hume, Roger Reddel and Susan Clark: Concordant epigenetic silencing of transforming growth factor-beta signaling pathway genes occurs early in breast carcinogenesis. *Cancer Res.*, 67(24), 11517-11527 (2007)

44. Satoshi Yamashita, Satoru Takahashi, Nathalie Mcdonell, Naoko Watanabe, Tohru Niwa, Kosuke Hosoya, Yoshimi Tsujino, Tomoyuki Shirai and Toshikazu Ushijima: Methylation silencing of transforming growth factor-baets receptor type II in rat prostate cancers. *Cancer Res.*, 68(7), 2112-2121 (2008)

45. Gang Chen, Paritosh Ghosh, Hiroshi Osawa, Carl Sasaki, Louis Rezanka, Jiandong Yang, Thomas O'farrell and Dan Longo: Resistance to TGF-beta 1 correlates with aberrant expression of TGF-beta receptor II in human B-cell lymphoma cell lines. *Blood*, 109(12), 5301-5307 (2007)

46. Hirotaka Osada, Yoshio Tatematsu, Nobuyoshi Sugito, Yoshitsugu Horio and Takashi Takahashi: Histone modification in the TGF-betaRII gene promoter and its significance for responsiveness to hdac inhibitor in lung cancer cell lines. *Mol. Carcinog.*, 44(4), 233-241 (2005)

47. Sanjib Chowdhury, Sudhakar Ammanamanchi and Gillian Howell: Epigenetic targeting of transforming growth factor beta receptor II and implications for cancer therapy. *Molecular and Cellular Pharmacology*, 1(1), 57-70 (2009)

48. Sudhakar Ammanamanchi and Michael Brattain: Sp3 is a transcriptional repressor of transforming growth factor-beta receptors. *J. Biol. Chem.*, 276(5), 3348-3352 (2001)

49. Sudhakar Ammanamanchi, Seong-Jin Kim, Lu-Zhe Sun and Michael Brattain: Induction of transforming growth factor-beta receptor type II expression in estrogen receptor-positive breast cancer cells through sp1 activation by 5-aza-2′-deoxycytidine. *J. Biol. Chem.*, 273(26), 16527-16534 (1998)

50. Taiping Chen, Darryl Carter, Laure Garrigue-Antar and Michael Reiss: Transforming growth factor beta type I receptor kinase mutant associated with metastatic breast cancer. *Cancer Res.*, 58(21), 4805-4810 (1998)

51. William Schiemann, Diana Rotzer, Waither Pfeifer, Edi Levi, Kanti Rai, Petra Knaus and Marshall Kadin: Transforming growth factor-[beta]-resistant B cells from chronic lymphocytic leukemia patients contain recurrent mutations in the signal sequence of the type I tgf-[beta] receptor. *Cancer Detect. Prev.*, 28(1), 57-64 (2004)

52. Melissa Hypes, Lucia Pirisi and Kim Creek: Mechanisms of decreased expression of transforming growth factor-beta receptor type I at late stages of HPV16-mediated transformation. *Cancer Lett.*, 282(2), 177-186 (2009)

53. Shin Kang, Bang Y, Y Im, Yang H, Lee D, Hwa Lee, Ho Lee, Noe Kim and Kim S: Transcriptional repression of the transforming growth factor-beta; type I receptor gene by DNA methylation results in the development of tgf-beta; resistance in human gastric cancer. *Oncogene*, 18(51) (1999)

54. Jianmin Zhao and Ronald Buick: Regulation of transforming growth factor beta receptors in H-Ras oncogene-transformed rat intestinal epithelial cells. *Cancer Res.*, 55(24), 6181-6188 (1995)

55. Jalila Adnane, Edward Seijo, Zhi Chen, Francisco Bizouarn, Martha Leal, Said M. Sebti and Teresita Muñoz-Antonia: Rhob, not rhoa, represses the transcription of the transforming growth factor beta type II receptor by a mechanism involving activator protein 1. *J. Biol. Chem.*, 277(10), 8500-8507 (2002)

56. Kolja Eppert, Stephen Scherer, Hilmi Ozcelik, Rosa Pirone, Pamela Hoodless, Hyeja Kim, Lap-Chee Tsui, Bharati Bapat, Steven Gallinger, Irene Andrulis, Gerald Thomsen, Jeffrey Wrana and Liliana Attisano: Madr2 maps to 18q21 and encodes a TGF-beta regulated mad related protein that is functionally mutated in colorectal carcinoma. *Cell*, 86(4), 543-552 (1996)

57. Stephan Hahn, Mieke Schutte, A. T. M. Shamsul Hoque, Christopher Moskaluk, Luis Da Costa, Ester Rozenblum, Craig Weinstein, Aryeh Fischer, Charles Yeo, Ralph Hruban and Scott Kern: Dpc4, a candidate tumor suppressor gene at human chromosome 18q21.1. *Science*, 271(5247), 350-353 (1996)

58. Sam Thiagalingam, Christoph Lengauer, Frederick Leach, Mieke Schutte, Stephan Hahn, Joan Overhauser, James Willson, Sanford Markowitz, Stanley Hamilton, Scott Kern, Kenneth Kinzler and Bert Vogelstein: Evaluation of candidate tumour suppressor genes on chromosome 18 in colorectal cancers. *Nat. Genet.*, 13(3), 343-346 (1996)

59. Brian Bierie and Harold Moses: Tgf-beta and cancer. *Cytokine Growth Factor Rev.*, 17(1-2), 29-40 (2006)

60. Sang-Uk Han, Heung-Tae Kim, Do Hwan Seong, Yong-Suk Kim, Yoon-Soo Park, Yung-Jue Bang, Han-Kwang Yang and Seong-Jin Kim: Loss of the Smad3 expression increases susceptibility to tumorigenicity in human gastric cancer. *Oncogene*, 23(7), 1333-1341 (2004)

61. Lawrence Wolfraim, Tania Fernandez, Mizuko Mamura, Walter Fuller, Rajesh Kumar, Diane Cole, Stacey Byfield, Angelina Felici, Kathleen Flanders, Thomas Walz, Anita Roberts, Peter Aplan, Frank Balis and John Letterio: Loss of Smad3 in acute T-cell lymphoblastic leukemia. *N. Engl. J. Med.*, 351(6), 552-559 (2004)

62. H Lee, C Yun, S Lim, B Kim, K Baik, J Kim, W Kim and S Kim: Srf is a nuclear repressor of Smad3-mediated TGF-beta signaling. *Oncogene*, 26(2), 173-185 (2006)

63. Shingo Akiyoshi, Hirofumi Inoue, Jun-Ichi Hanai, Kiyoshi Kusanagi, Nobuo Nemoto, Kohei Miyazono and Masahiro Kawabata: c-Ski acts as a transcriptional co-repressor in transforming growth factor-beta signaling through interaction with smads. *J. Biol. Chem.*, 274(49), 35269-35277 (1999)

64. Martin Buess, Luigi Terracciano, Juurgen Reuter, Pierluigi Ballabeni, Jean-Louis Boulay, Urban Laffer, Urs Metzger, Richard Herrmann and Christoph Rochlitz: Amplification of ski is a prognostic marker in early colorectal cancer. *Neoplasia*, 6(3), 207-212 (2004)

65. Minoru Fukuchi, Masanobu Nakajima, Yasuyuki Fukai,

Tatsuya Miyazaki, Norihiro Masuda, Makoto Sohda, Ryokuhei Manda, Katsuhiko Tsukada, Hiroyuki Kato and Hiroyuki Kuwano: Increased expression of c-ski as a co-repressor in transforming growth factor-beta signaling correlates with progression of esophageal squamous cell carcinoma. *Int. J. Cancer*, 108(6), 818-824 (2004)

66. Shannon Stroschein, Wei Wang, Sharleen Zhou, Qiang Zhou and Kunxin Luo: Negative feedback regulation of TGF-beta signaling by the snon oncoprotein. *Science*, 286(5440), 771-774 (1999)

67. Yin Sun, Xuedong Liu, Elinor Ng Eaton, William Lane, Harvey Lodish and Robert Weinberg: Interaction of the ski oncoprotein with smad3 regulates TGF-beta signaling. *Mol. Cell*, 4(4), 499-509 (1999)

68. Barbara Boone, Marc Haspeslagh and Lieve Brochez: Clinical significance of the expression of c-ski and snon, possible mediators in TGF-beta resistance, in primary cutaneous melanoma. *J. Dermatol. Sci.*, 53(1), 26-33 (2009)

69. Degang Wang, Jianyin Long, Fangyan Dai, Min Liang, Xin-Hua Feng and Xia Lin: Bcl6 represses smad signaling in transforming growth factor-beta resistance. *Cancer Res.*, 68(3), 783-789 (2008)

70. Joan Seoane, Hong-Van Le, Lijian Shen, Stewart Anderson and Joan Massagué: Integration of smad and forkhead pathways in the control of neuroepithelial and glioblastoma cell proliferation. *Cell*, 117(2), 211-223 (2004)

71. David Chan, Vincent Liu, R To, P Chiu, W Lee, K Yao, A Cheung and Hextan Ngan: Overexpression of foxg1 contributes to TGF-beta resistance through inhibition of p21waf1/cip1 expression in ovarian cancer. *Br. J. Cancer*, 101(8), 1433-1443 (2009)

72. Robert Benezra, Robert Davis, Daniel Lockshon, David Turner and Harold Weintraub: The protein Id A negative regulator of helix-loop-helix DNA binding proteins. *Cell*, 61(1), 49-59 (1990)

73. Kaijun Di, Ming-Tat Ling, Sai Tsao, Yong Wong and Xianghong Wang: Id-1 modulates senescence and TGF-beta1 sensitivity in prostate epithelial cells. *Biol. Cell*, 98(9), 523-533 (2006)

74. Beverley Warner, Stacy Blain, Joan Seoane and Joan Massague: Myc downregulation by transforming growth factor beta required for activation of the p15ink4b G1 arrest pathway. *Mol. Cell. Biol.*, 19(9), 5913-5922 (1999)

75. Mark Alexandrow, Masahiro Kawabata, Mary Aakre and Harold Moses: Overexpression of the c-myc oncoprotein blocks the growth-inhibitory response but is required for the mitogenic effects of transforming growth factor beta 1. *Proc. Natl. Acad. Sci. U. S. A.*, 92(8), 3239-3243 (1995)

76. Rae Baldwin, Hang Tran and Beth Karlan: Loss of c-myc repression coincides with ovarian cancer resistance to transforming growth factor beta growth arrest independent of transforming growth factor beta/smad signaling. *Cancer Res.*, 63(6), 1413-1419 (2003)

77. James Schwarz, Craig Bassing, Imre Kovesdi, Michael Datto, Mchael Blazing, Samuel George, Xiao-Fan Wang and Joseph Nevins: Expression of the e2f1 transcription factor overcomes type beta transforming growth factor-mediated growth suppression. *Proc. Natl. Acad. Sci. U. S. A.*, 92(2), 483-487 (1995)

78. Isao Matsuura, Natalia Denissova, Guannan Wang, Dongming He, Jianyin Long and Fang Liu: Cyclin-dependent kinases regulate the antiproliferative function of smads. *Nature*, 430(6996), 226-231 (2004)

79. Linda Baughn, Maurizio Di Liberto, Ruben Niesvizky, Hearn Cho, David Jayabalan, Joseph Lane, Fang Liu and Selina Chen-Kiang: Cdk2 phosphorylation of smad2 disrupts tgf-betaa transcriptional regulation in resistant primary bone marrow myeloma cells. *J. Immunol.*, 182(4), 1810-1817 (2009)

80. Maria Calonge and Joan Massagué: Smad4/dpc4 silencing and hyperactive ras jointly disrupt transforming growth factor-beta antiproliferative responses in colon cancer cells. *J. Biol. Chem.*, 274(47), 33637-33643 (1999)

81. Debabrata Saha, Pran Datta and R. Daniel Beauchamp: Oncogenic ras represses transforming growth factor-beta/smad signaling by degrading tumor suppressor smad4. *J. Biol. Chem.*, 276(31), 29531-29537 (2001)

82. Hye-Young Hong, Woo-Kwang Jeon, Eun-Jin Bae, Shin-Tae Kim, Ho-Jae Lee, Seong-Jin Kim and Byung-Chul Kim: 14-3-3 sigma and 14-3-3 zeta plays an opposite role in cell growth inhibition mediated by transforming growth factor-beta 1. *Mol. Cells*, 29(3), 305-309 (2010)

83. Jorg Kleeff, Toshiyuki Ishiwata, Haruhisa Maruyama, Helmut Friess, P. Truong, Markus Buchler, Dean Falb and Murray Korc: The TGF-beta; signaling inhibitor smad7 enhances tumorigenicity in pancreatic cancer. *Oncogene*, 18(39) (1999)

84. Shingo Nakahata, Satoshi Yamazaki, Hiroshi Nakauchi and Kazuhiro Morishita: Downregulation of zeb1 and overexpression of smad7 contribute to resistance to TGF-beta1-mediated growth suppression in adult T-cell leukemia/lymphoma. *Oncogene*, 29(29), 4157-4169 (2010)

85. Nichole Boyer Arnold and Murray Korc: Smad7 abrogates transforming growth factor-beta1-mediated growth inhibition in colo-357 cells through functional inactivation of the retinoblastoma protein. *J. Biol. Chem.*, 280(23), 21858-21866 (2005)

86. Nicole Pavio, Serena Battaglia, Delphine Boucreux, Bertrand Arnulf, Rodolphe Sobesky, Olivier Hermine and Christian Brechot: Hepatitis C virus core variants isolated from liver tumor but not from adjacent non-tumor tissue interact with smad3 and inhibit the TGF-beta pathway. *Oncogene*, 24(40), 6119-6132 (2005)

87. Naoki Mori, Mariko Morishita, Tomoo Tsukazaki, Chou-Zen Giam, Atsushi Kumatori, Yuetsu Tanaka and Naoki Yamamoto: Human T-cell leukemia virus type I oncoprotein tax represses smad-dependent transforming growth factor {beta} signaling through interaction with creb-binding protein/p300. *Blood*, 97(7), 2137-2144 (2001)

88. Dug Keun Lee, Byung-Chul Kim, Isaac Yi Kim, Eun-Ah Cho, Daniel Satterwhite and Seong-Jin Kim: The human papilloma virus E7 oncoprotein inhibits transforming growth factor-beta signaling by blocking binding of the smad complex to its target sequence. *J. Biol. Chem.*, 277(41), 38557-38564 (2002)

89. Naoki Mori, Mariko Morishita, Tomoo Tsukazaki and Naoki Yamamoto: Repression of smad-dependent transforming growth factor-beta signaling by epstein-barr virus latent membrane protein 1 through nuclear factor-kappab. *Int. J. Cancer*, 105(5), 661-668 (2003)

90. Tien Ko, Hong Sheng, David Reisman, E Aubrey Thompson and R. Daniel Beauchamp: Transforming growth factor-beta 1 inhibits Cyclin D1 expression in intestinal epithelial cells. *Oncogene*, 10(1), 177-84 (1995)

91. Craig Robson, Vincent Gnanapragasam, Ruth Byrne, Anne Collins and David Neal: Transforming growth factor-beta1 up-regulates p15, p21 and p27 and blocks cell cycling in G1 in human prostate epithelium. *J. Endocrinol.*, 160(2), 257-266 (1999)

92. Alessandro Sgambato, Yuichiro Doki, Ira Schieren and I Bernard Weinstein: Effects of cyclin e overexpression on cell growth and response to transforming growth factor beta depend on cell context and p27kip1 expression. *Cell Growth Differ.*, 8(4), 393-405 (1997)

93. Hyun-Soon Jong, Ho Lee, Tae Kim, Young-Hyuck Im, Jae-Won Park, Noe Kim and Yung-Jue Bang: Attenuation of transforming growth factor [beta]-induced growth inhibition in human hepatocellular carcinoma cell lines by cyclin D1 overexpression. *Biochem. Biophys. Res. Commun.*, 292(2), 383-389 (2002)

94. Luis Martinez, Yian Chen, Amy Pavone, Susan Fischer and Claudio Conti: Deregulated expression of cyclin D1 overrides antimitogenic signals. *Oncogene*, 19(2) (2000)

95. Mark Ewen, Hayla Sluss, Laura Whitehouse and David Livingston: TGF-beta inhibition of Cdk4 synthesis is linked to cell cycle arrest. *Cell*, 74(6), 1009-1020 (1993)

96. Anna Chytil, Mary Waltner-Law, Robert West, David Friedman, Mary Aakre, Dana Barker and Brian Law: Construction of a cyclin D1-Cdk2 fusion protein to model the biological functions of cyclin D1-Cdk2 complexes. *J. Biol. Chem.*, 279(46), 47688-47698 (2004)

97. Patrick Corsino, Bradley Davis, Mary Law, Anna Chytil, Elizabeth Forrester, Peter Nørgaard, Nicole Teoh and Brian Law: Tumors initiated by constitutive Cdk2 activation exhibit transforming growth factor beta resistance and acquire paracrine mitogenic stimulation during progression. *Cancer Res.*, 67(7), 3135-3144 (2007)

98. Jiyong Liang, Judit Zubovitz, Teresa Petrocelli, Rouslan Kotchetkov, Michael Connor, Kathy Han, Jin-Hwa Lee, Sandra Ciarallo, Charles Catzavelos, Richard Beniston, Edmee Franssen and Joyce Slingerland: PKb/AKT phosphorylates p27, impairs nuclear import of p27 and opposes p27-mediated G1 arrest. *Nat. Med.*, 8(10), 1153-1160 (2002)

99. Incheol Shin, Michael Yakes, Federico Rojo, Nah-Young Shin, Andrei Bakin, Jose Baselga and Carlos Arteaga: PKb/AKT mediates cell-cycle progression by phosphorylation of p27kip1 at threonine 157 and modulation of its cellular localization. *Nat. Med.*, 8(10), 1145-1152 (2002)

100. Giuseppe Viglietto, Maria Motti, Paola Bruni, Rosa Melillo, Amelia D'alessio, Daniela Califano, Floriana Vinci, Gennaro Chiappetta, Philip Tsichlis, Alfonso Bellacosa, Alfredo Fusco and Massimo Santoro: Cytoplasmic relocalization and inhibition of the cyclin-dependent kinase inhibitor p27kip1 by pkb/akt-mediated phosphorylation in breast cancer. *Nat. Med.*, 8(10), 1136-1144 (2002)

101. Fabio Petrocca, Rosa Visone, Mariadele Rapazzotti Onelli, Manisha Shah, Milena Nicoloso, Ivana De Martino, Dimitrios Iliopoulos, Emanuela Pilozzi, Chang-Gong Liu, Massimo Negrini, Luigi Cavazzini, Stefano Volinia, Hansjuerg Alder, Luigi Ruco, Gustavo Baldassarre, Carlo Croce and Andrea Vecchione: e2f1-regulated micrornas impair TGF-beta-dependent cell-cycle arrest and apoptosis in gastric cancer. *Cancer cell*, 13(3), 272-286 (2008)

102. Natalie Schlegel, Ossia Eichhoff, Silvio Hemmi, Sabine Werner, Reinhard Dummer and Keith Hoek: Id2 suppression of p15 counters TGF-beta-mediated growth inhibition of melanoma cells. *Pigment Cell & Melanoma Research*, 22(4), 445-453 (2009)

103. Franck Verrecchia, Charlotte Tacheau, Erwin Wagner and Alain Mauviel: A central role for the jnk pathway in mediating the antagonistic activity of pro-inflammatory cytokines against transforming growth factor-beta-driven smad3/4-specific gene expression. *J. Biol. Chem.*, 278(3), 1585-1593 (2003)

104. Mélika Benahmed, Bertrand Meresse, Bertrand Arnulf, Ullah Barbe, Jean-Jacques Mention, Virginie Verkarre, Matthieu Allez, Christophe Cellier, Olivier Hermine and Nadine Cerf-Bensussan: Inhibition of TGF-beta signaling by IL-15: A new role for IL-15 in the loss of immune homeostasis in celiac disease. *Gastroenterology*, 132(3), 994-1008 (2007)

105. Franck Verrecchia, Marcia Pessah, Azeddine Atfi and Alain Mauviel: Tumor necrosis factor-α inhibits transforming growth factor-beta /smad signaling in human dermal fibroblasts via AP-1 activation. *J. Biol. Chem.*, 275(39), 30226-30231 (2000)

106. Marius Hoper, Norbert Voelkel, Thomas Bates, Jenny Allard, Marilee Horan, David Shepherd and Rubin Tuder: Prostaglandins induce vascular endothelial growth factor in a human monocytic cell line and rat lungs via camp. *Am. J. Respir. Cell Mol. Biol.*, 17(6), 748-756 (1997)

107. Xiaojun Lu, Weilin Xie, David Reed, William Bradshaw and Daniel Simmons: Nonsteroidal antiinflammatory drugs cause apoptosis and induce cyclooxygenases in chicken embryo fibroblasts. *Proc. Natl. Acad. Sci. U. S. A.*, 92(17), 7961-7965 (1995)

108. Hongmiao Sheng, Jinyi Shao, Kay Washington and Raymond Dubois: Prostaglandin E2 increases growth and motility of colorectal carcinoma cells. *J. Biol. Chem.*, 276(21), 18075-18081 (2001)

109. Maozhen Tian and William Schiemann: Pge2 receptor ep2 mediates the antagonistic effect of cox-2 on TGF-beta signaling during mammary tumorigenesis. *FASEB J.*, 24(4), 1105-1116 (2010)

110. Chang Han, Jake Demetris, Youhua Liu, James Shelhamer and Tong Wu: Transforming growth factor-beeta (TGF-beta) activates cytosolic phospholipase a2alpha (cpla2alpha)-mediated prostaglandin e2 (pge)2/ep1 and peroxisome proliferator-activated receptor-gamma (PPAR-gamma)/smad signaling pathways in human liver cancer cells. *J. Biol. Chem.*, 279(43), 44344-44354 (2004)

111. Yasuhiro Shirai, Sumio Kawata, Nobuyuki Ito, Shinji Tamura, Kenji Takaishi, Shinichi Kiso, Hirofumi Tsushima and Yuji Matsuzawa: Elevated levels of plasma transforming growth factor-beta in patients with hepatocellular carcinoma. *Cancer Science*, 83(7), 676-679 (1992)

112. Vesna Ivanovich, Arnold Melman, Brian Davis-Joseph, Mira Valcic and Jan Geliebter: Elevated levels of TGF-beta 1 in patients with invasive prostate cancer. *Nat. Med.*, 1(4), 282-284 (1995)

113. David Grainger, Paul Kemp, James Metcalfe, Alexander Liu, Richard Lawn, Norman Williams, Andrew Grace, Peter Schofield and Anoop Chauhan: The serum concentration of active transforming growth factor-beta is severely depressed in advanced atherosclerosis. *Nat. Med.*, 1(1), 22-23 (1995)

114. Mitchell Anscher, William Peters, Herbert Reisenbichler, William Petros and Randy Jirtle: Transforming growth factor beta as a predictor of liver and lung fibrosis after autologous bone marrow transplantation for advanced breast cancer. *N. Engl. J. Med.*, 328(22), 1592-1598 (1993)

115. Ashok Kulkarni, Jerrold Ward, Linda Yaswen, Crystal Mackall, Steven Bauer, Chang-Goo Huh, Ronald Gress and Stefan Karlsson: Transforming growth factor-beta 1 null mice. An animal model for inflammatory disorders. *Am. J. Pathol.*, 146(1), 264-75 (1995)

116. Mohammed Awad, Ahmed El-Gamel, Phillip Hasleton, David Turner, Paul Sinnott and Ian Hutchinson: Genotypic variation in the transforming growth factor-[beta]1 gene: Association with transforming growth factor-[beta]1 production, fibrotic lung disease, and graft fibrosis after lung transplantation. *Transplantation*, 66(8), 1014-1020 (1998)

117. Francois Cambien, Sylvain Ricard, Alain Troesch, Christine Mallet, Laurence Generenaz, Alun Evans, Dominique Arveiler, Gerald Luc, Jean-Bernard Ruidavets and Odette Poirier: Polymorphisms of the transforming growth factor-beta1 gene in relation to myocardial infarction and blood pressure: The etude cas-temoin de l'infarctus du myocarde (ectim) study. *Hypertension*, 28(5), 881-887 (1996)

118. Guangfu Jin, Yimei Deng, Ruifen Miao, Zhibin Hu, Yan Zhou, Yongfei Tan, Jianming Wang, Zhaolai Hua, Weiliang Ding, Lina Wang, Wensen Chen, Jing Shen, Xinru Wang, Yaochu Xu and Hongbing Shen: TGF-beta1 and TGF-betaR2 functional polymorphisms and risk of esophageal squamous cell carcinoma: A case–control analysis in a chinese population. *J. Cancer Res. Clin. Oncol.*, 134(3), 345-351 (2008)

119. Marcia Shull, Ilona Ormsby, Ann Kier, Sharon Pawlowski, Ronald Diebold, Moying Yin, Ruth Allen, Charles Sidman, Gabriele Proetzel, Dawn Calvin, Nikki Annunziata and Thomas Doetschman: Targeted disruption of the mouse transforming growth factor-beta1 gene results in multifocal inflammatory disease. *Nature*, 359(6397), 693-699 (1992)

120. Sandra Engle, Ilona Ormsby, Sharon Pawlowski, Gregory Boivin, Joanne Croft, Edward Balish and Tom Doetschman: Elimination of colon cancer in germ-free transforming growth factor beta 1-deficient mice. *Cancer Res.*, 62(22), 6362-6366 (2002)

121. Sandr Engle, James Hoying, Gregory Boivin, Ilona Ormsby, Peter Gartside and Thomas Doetschman: Transforming growth factor beta1 suppresses nonmetastatic colon cancer at an early stage of tumorigenesis. *Cancer Res.*, 59(14), 3379-3386 (1999)

122. Dirk Haller, Lisa Holt, Sandra Kim, Robert Schwabe, R. Balfour Sartor and Christian Jobin: Transforming growth factor-beta1 inhibits non-pathogenic gram-negative bacteria-induced NF-kappab recruitment to the interleukin-6 gene promoter in intestinal epithelial cells through modulation of histone acetylation. *J. Biol. Chem.*, 278(26), 23851-23860 (2003)

123. Binwu Tang, Erwin Bottinger, Sonia Jakowlew, Kerri Bagnall, Jennifer Mariano, Miriam Anver, John Letterio and Lalage Wakefield: Transforming growth factor-beta1 is a new form of tumor suppressor with true haploid insufficiency. *Nat. Med.*, 4(7), 802-807 (1998)

124. Yasuhisa Matsui, Susan Halter, Jeffrey Holt, Brigid Hogan and Robert Coffey: Development of mammary hyperplasia and neoplasia in MMTV-TGFalpha transgenic mice. *Cell*, 61(6), 1147-1155 (1990)

125. Donald Pierce, Agnieszka Gorska, Anna Chytil,

Katherome Meise, David Page, Robert Coffey and Harold Moses: Mammary tumor suppression by transforming growth factor beta 1 transgene expression. *Proc. Natl. Acad. Sci. U. S. A.*, 92(10), 4254-4258 (1995)

126. Peter Siegel, Weiping Shu, Robert Cardiff, William Muller and Joan Massague: Transforming growth factor beta signaling impairs neu-induced mammary tumorigenesis while promoting pulmonary metastasis. *Proc. Natl. Acad. Sci. U. S. A.*, 100(14), 8430-8435 (2003)

127. Corrine Boulanger and Gilbert Smith: Reducing mammary cancer risk through premature stem cell senescence. *Oncogene*, 20(18), 2264-72 (2001)

128. Wei Cui, Deborah Fowlis, Sheila Bryson, Elizabeth Duffie, Hazel Ireland, Allan Balmain and Rosemary Akhurst: TGF-beta1 inhibits the formation of benign skin tumors, but enhances progression to invasive spindle carcinomas in transgenic mice. *Cell*, 86(4), 531-542 (1996)

129. Agnieszka Gorska, Roy Jensen, Yu Shyr, Mary Aakre, Neil Bhowmick and Harold Moses: Transgenic mice expressing a dominant-negative mutant type ii transforming growth factor-beta receptor exhibit impaired mammary development and enhanced mammary tumor formation. *Am. J. Pathol.*, 163(4), 1539-1549 (2003)

130. Erwin Böttinger, John Jakubczak, Diana Haines, Kerri Bagnall and Lalage Wakefield: Transgenic mice overexpressing a dominant-negative mutant type II transforming growth factor beta receptor show enhanced tumorigenesis in the mammary gland and lung in response to the carcinogen 7,12-dimethylbenz-[a]-anthracene. *Cancer Res.*, 57(24), 5564-70 (1997)

131. Agnieszka Gorska, Heather Joseph, Rik Derynck, Harold Moses and Rosa Serra: Dominant-negative interference of the transforming growth factor beta type II receptor in mammary gland epithelium results in alveolar hyperplasia and differentiation in virgin mice. *Cell Growth Differ.*, 9(3), 229-238 (1998)

132. Elizabeth Forrester, Anna Chytil, Brian Bierie, Mary Aakre, Agnieszka Gorska, Ali-Reza Sharif-Afshar, William Muller and Harold Moses: Effect of conditional knockout of the type II TGF-beta receptor gene in mammary epithelia on mammary gland development and polyomavirus middle T antigen induced tumor formation and metastasis. *Cancer Res.*, 65(6), 2296-2302 (2005)

133. Cindy Go, Ping Li and Xiao-Jing Wang: Blocking transforming growth factor beta signaling in transgenic epidermis accelerates chemical carcinogenesis. *Cancer Res.*, 59(12), 2861-2868 (1999)

134. Ki-Baik Hahm, K. M. Lee, Y. B. Kim, W. S. Hong, W. H. Lee, S. U. Han, M. W. Kim, B. O. Ahn, T. Y. Oh, M. H. Lee, J. Green and Seong-Jin Kim: Conditional loss of TGF-beta signalling leads to increased susceptibility to gastrointestinal carcinogenesis in mice. *Aliment. Pharmacol. Ther.*, 16, 115-127 (2002)

135. Swati Biswas, Anna Chytil, Kay Washington, Judith Romero-Gallo, Agnieszka Gorska, Pamela Wirth, Shiva Gautam, Harold Moses and William Grady: Transforming growth factor beta receptor type II inactivation promotes the establishment and progression of colon cancer. *Cancer Res.*, 64(14), 4687-4692 (2004)

136. Ji Yeon Baek, Shelli Morris, Jean Campbell, Nelson Fausto, Matthew Yeh and William Grady: TGF-beta inactivation and tgf-alpha overexpression cooperate in an in vivo mouse model to induce hepatocellular carcinoma that recapitulates molecular features of human liver cancer. *Int. J. Cancer*, 127(5), 1060-1071 (2010)

137. Hideaki Ijichi, Anna Chytil, Agnieszka Gorska, Mary Aakre, Yoshio Fujitani, Shuko Fujitani, Christopher Wright and Harold Moses: 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.*, 20(22), 3147-3160 (2006)

138. Yasuyuki Honjo, Yansong Bian, Koji Kawakam, Alfredo Molinolo, Glenn Longenecker, Ramanamurthy Boppana, Jonas Larsson, Stefan Karlsson, J. Silvio Gutkind, Raj Puri and Ashok Kulkarni: TGF-beta receptor I conditional knockout mice develop spontaneous squamous cell carcinoma. *Cell Cycle*, 6(11), 1360-6 (2007)

139. Michael Weinstein, Xiao Yang, Cuiling Li, Xiaoling Xu, Jessica Gotay and Chu-Xia Deng: Failure of egg cylinder elongation and mesoderm induction in mouse embryos lacking the tumor suppressor smad2. *Proc. Natl. Acad. Sci. U. S. A.*, 95(16), 9378-9383 (1998)

140. Xiao Yang, Cuiling Li, Xiaoling Xu and Chuxia Deng: The tumor suppressor smad4/dpc4 is essential for epiblast proliferation and mesoderm induction in mice. *Proc. Natl. Acad. Sci. U. S. A.*, 95(7), 3667-3672 (1998)

141. Hua Chang, Danny Huylebroeck, Kristin Verschueren, Qiuxia Guo, Martin Matzuk and An Zwijsen: Smad5 knockout mice die at mid-gestation due to multiple embryonic and extraembryonic defects. *Development*, 126(8), 1631-1642 (1999)

142. Yuan Zhu, James Richardson, Luis Parada and Jonathan Graff: Smad3 mutant mice develop metastatic colorectal cancer. *Cell*, 94(6), 703-714 (1998)

143. Michael Datto, Joshua Frederick, Lihua Pan, Anita Borton, Yuan Zhuang and Xiao-Fan Wang: Targeted disruption of smad3 reveals an essential role in transforming growth factor beta-mediated signal transduction. *Mol. Cell. Biol.*, 19(4), 2495-2504 (1999)

144. Gillian Ashcroft, Xiao Yang, Adam Glick, Michael Weinstein, John Letterio, Diane Mizel, Mario Anzano, Teresa Greenwell-Wild, Sharon Wahl, Chuxia Deng and Anita Roberts: Mice lacking smad3 show accelerated wound healing and an impaired local inflammatory response. *Nature Cell Biology*, 1(5), 260-266 (1999)

145. Kazuaki Takaku, Jeffrey Wrana, Elizabeth Robertson and Makoto Taketo: No effects of smad2 (madh2) null mutation on malignant progression of intestinal polyps in APCdelta716 knockout mice. *Cancer Res.*, 62(16), 4558-4561 (2002)

146. Toshiaki Hamamoto, Hideyuki Beppu, Hitoshi Okada, Masahiro Kawabata, Tadaichi Kitamura, Kohei Miyazono and Mitsuyasu Kato: Compound disruption of smad2 accelerates malignant progression of intestinal tumors in apc knockout mice. *Cancer Res.*, 62(20), 5955-5961 (2002)

147. Kazuaki Takaku, Masanobu Oshima, Hiroyuki Miyoshi, Minoru Matsui, Michael F Seldin and Makoto M Taketo: Intestinal tumorigenesis in compound mutant mice of both dpc4(smad4) and apc genes. *Cell*, 92(5), 645-656 (1998)

148. Kazuaki Takaku, Hiroyuki Miyoshi, Akihiro Matsunaga, Masanobu Oshima, Nobuya Sasaki and Makoto M. Taketo: Gastric and duodenal polyps in smad4 (dpc4) knockout mice. *Cancer Res.*, 59(24), 6113-6117 (1999)

149. Wenmei Li, Wenhui Qiao, Lin Chen, Xiaoling Xu, Xiao Yang, Dan Li, Cuiling Li, Steven Brodie, Michael Meguid, Lothar Hennighausen and Chu-Xia Deng: Squamous cell carcinoma and mammary abscess formation through squamous metaplasia in smad4/dpc4 conditional knockout mice. *Development*, 130(24), 6143-6153 (2003)

150. Rebecca Muraoka, Nancy Dumont, Christoph Ritter, Teresa Dugger, Dana Brantley, Jin Chen, Evangeline Easterly, L. Renee Roebuck, Sarah Ryan, Philip Gotwals, Victor Koteliansky and Carlos Arteaga: Blockade of TGF-beta inhibits mammary tumor cell viability, migration, and metastases. *J. Clin. Invest.*, 109(12), 1551-1559 (2002)

151. Yu-An Yang, Oksana Dukhanina, Binwu Tang, Mizuko Mamura, John Letterio, Jennifer Macgregor, Sejal Patel, Shahram Khozin, Zi-Yao Liu, Jeffrey Green, Miriam Anver, Glenn Merlino and Lalage Wakefield: Lifetime exposure to a soluble TGF-beta antagonist protects mice against metastasis without adverse side effects. *J. Clin. Invest.*, 109(12), 1607-1615 (2002)

152. John Munger, Harpel John, Gleizes Pierre-Emmanuel, Roberta Mazzieri, Irene Nunes and Daniel Rifkin: Latent transforming growth factor-beta: Structural features and mechanisms of activation. *Kidney Int.*, 51(5), 1376-82 (1997)

153. Carlos Arteaga, Atul Tandon, Daniel Von Hoff and C. Kent Osborne: Transforming growth factor beta: Potential autocrine growth inhibitor of estrogen receptor-negative human breast cancer cells. *Cancer Res.*, 48(14), 3898-904 (1988)

154. N. Dumont, A. V. Bakin and C. L. Arteaga: Autocrine transforming growth factor-beta signaling mediates smad-independent motility in human cancer cells. *J. Biol. Chem.*, 278(5), 3275-85 (2003)

155. Michail Shipitsin, Lauren Campbell, Pedram Argani, Stanislawa Weremowicz, Noga Bloushtain-Qimron, Jun Yao, Tatiana Nikolskaya, Tatiana Serebryiskaya, Rameen Beroukhim, Min Hu, Marc Halushka, Saraswati Sukumar, Leroy Parker, Karen Anderson, Lyndsay Harris, Judy Garber, Andrea Richardson, Stuart Schnitt, Yuri Nikolsky, Rebeccca Gelman and Kornelia Polyak: Molecular definition of breast tumor heterogeneity. *Cancer Cell*, 11(3), 259-73 (2007)

156. Vidya Ganapathy, Rongrong Ge, Alison Grazioli, Wen Xie, Whitney Banach-Petrosky, Yibin Kang, Scott Lonning, John Mcpherson, Jonathan Yingling, Swati Biswas, G. Rgregory Mundy and Michael Reiss: Targeting the transforming growth factor-beta pathway inhibits human basal-like breast cancer metastasis. *Mol Cancer*, 9, 122 (2010)

157. Dominique Bonafoux and Wen-Cherng Lee: Strategies for TGF-beta modulation: A review of recent patents. *Expert Opin Ther Pat*, 19(12), 1759-69 (2009)

158. Elisabeth Jones, Hong Pu and Natasha Kyprianou: Targeting TGF-beta in prostate cancer: Therapeutic possibilities during tumor progression. *Expert Opin Ther Targets*, 13(2), 227-34 (2009)

159. Anna Mourskaia, Jason Northey and Peter Siegel: Targeting aberrant TGF-beta signaling in pre-clinical models of cancer. *Anticancer Agents Med Chem*, 7(5), 504-14 (2007)

160. E. C. Connolly, E. F. Saunier, D. Quigley, M. T. Luu, A. De Sapio, B. Hann, J. M. Yingling and R. J. Akhurst: Outgrowth of drug-resistant carcinomas expressing markers of tumor aggression after long-term TGF-betaRI/II kinase inhibition with ly2109761. *Cancer Res.*, 71(6), 2339-49 (2011)

161. Bashier Osman, Anke Doller, Sayed Akool El, Martin Holdener, Edith Hintermann, Joseph Pfeilschifter and Wolfgang Eberhardt: Rapamycin induces the TGF-beta1/smad signaling cascade in renal mesangial cells upstream of mtor. *Cell. Signal.*, 21(12), 1806-17 (2009)

162. Kyung Song, Hui Wang, Tracy Krebs and David Danielpour: Novel roles of akt and mtor in suppressing TGF-beta/alk5-mediated smad3 activation. *EMBO J.*, 25(1), 58-69 (2006)

163. Henk Van Der Poel: Mammalian target of rapamycin and 3-phosphatidylinositol 3-kinase pathway inhibition enhances growth inhibition of transforming growth factor-beta1 in prostate cancer cells. *J. Urol.*, 172(4 Pt 1), 1333-7 (2004)

164. Kimberly Brown, Richard Roberts, Carlos Arteaga and Brian Law: Transforming growth factor-beta induces Cdk2 relocalization to the cytoplasm coincident with dephosphorylation of retinoblastoma tumor suppressor protein. *Breast Cancer Res*, 6(2), R130-9 (2004)

165. Henk Van Der Poel, Colleen Hanrahan, Hua Zhong and Jonathan Simons: Rapamycin induces smad activity in prostate cancer cell lines. *Urol. Res.*, 30(6), 380-6 (2003)

166. Brian Law, Anna Chytil, Nancy Dumont, Elizabeth Hamilton, Mary Waltner-Law, Mary Aakre, Cassondra Covington and Harold Moses: Rapamycin potentiates transforming growth factor beta-induced growth arrest in nontransformed, oncogene-transformed, and human cancer cells. *Mol. Cell. Biol.*, 22(23), 8184-98 (2002)

167. Brian Dynlacht, Ken Moberg, Jacques Lees, Ed Harlow and Liang Zhu: Specific regulation of e2f family members by cyclin-dependent kinases. *Mol. Cell. Biol.*, 17(7), 3867-75 (1997)

168. Adam Glick, Nicholas Popescu, Valarie Alexander, Hikaru Ueno, Erwin Bottinger and Stuart Yuspa: Defects in transforming growth factor-beta signaling cooperate with a ras oncogene to cause rapid aneuploidy and malignant transformation of mouse keratinocytes. *Proc. Natl. Acad. Sci. U. S. A.*, 96(26), 14949-54 (1999)

169. Caroline Freathy, David Brown, Ruth Roberts and Kelvin Cain: Transforming growth factor-beta(1) induces apoptosis in rat fao hepatoma cells via cytochrome c release and oligomerization of apaf-1 to form a approximately 700-kd apoptosome caspase-processing complex. *Hepatology*, 32(4 Pt 1), 750-60 (2000)

170. T. Haufel, S. Dormann, J. Hanusch, A. Schwieger and G. Bauer: Three distinct roles for TGF-beta during intercellular induction of apoptosis: A review. *Anticancer Res.*, 19(1A), 105-11 (1999)

171. Kiyoshi Yanagisawa, Hirotaka Osada, Akira Masuda, Masashi Kondo, Toshiko Saito, Yasushi Yatabe, Kenzo Takagi, Toshitada Takahashi and Takashi Takahashi: Induction of apoptosis by smad3 and down-regulation of smad3 expression in response to TGF-beta in human normal lung epithelial cells. *Oncogene*, 17(13), 1743-7 (1998)

172. Li Yang, Yanli Pang and Harold Moses: TGF-beta and immune cells: An important regulatory axis in the tumor microenvironment and progression. *Trends Immunol*, 31(6), 220-7 (2010)

173. Anurag Singh and Jeff Settleman: Emt, cancer stem cells and drug resistance: An emerging axis of evil in the war on cancer. *Oncogene*, 29(34), 4741-51 (2010)

174. Douglas Micalizzi, Susan Farabaugh and Heide Ford: Epithelial-mesenchymal transition in cancer: Parallels between normal development and tumor progression. *J. Mammary Gland Biol. Neoplasia*, 15(2), 117-34 (2010)

175. Aristidis Moustakas, Katerina Pardali, Annamaria Gaal and Carl-Henrik Heldin: Mechanisms of TGF-beta signaling in regulation of cell growth and differentiation. *Immunol. Lett.*, 82(1-2), 85-91 (2002)

Abbreviations: APC: adenomatous polyposis coli, BMPs: bone morphogenetic proteins, Cdk: Cyclin-dependent kinase, CKIs: Cdk inhibitory proteins, DMBA: 7,12-dimethylbenz-[a]-anthracene, *dnTGF-betaRII*: dominant negative type II transforming growth factor-beta receptor, ER: estrogen receptor, FoxO: forkhead box O, HCV: hepatitis C virus, HPV: human papilloma virus, HTLV-1: human T-cell leukemia virus type I, Id1: inhibitor of differentiation or inhibitor of DNA binding-1, Id2: inhibitor of differentiation or inhibitor of DNA binding-2, IL-15: interleukin-15, LMP1: latent membrane protein 1, MMTV: mouse mammary tumor virus, PGE2: prostaglandin E2, $cPLA_2$-alpha: phospholipase A_2-alpha, PAI-1: plasminogen activator inhibitor-1, PKC-alpha: protein kinase C-alpha, R-Smads: receptor-activated Smads, SCID: severe combined immunodeficient, SRF: serum response factor, TGF-alpha: transforming growth factor-alpha TGF-beta: transforming growth factor-beta, TGF-betaRI: type I transforming growth factor-beta receptor, TGF-betaRII: type II transforming growth factor-beta receptor, TNF-alpha: tumor necrosis factor-alpha.

Key Words: TGF-beta, Cell Cycle, Tumor Suppressor, Smad, Cyclin-dependent kinase, Review

Send correspondence to: Brian Law, University of Florida, ARB R5-152, 1600 SW Archer Rd, Gainesville, FL 3210, Tel: 352-392-3551, Fax: 352-392-9696, E-mail: bklaw@pharmacology.ufl.edu

http://www.bioscience.org/current/vol4S.htm